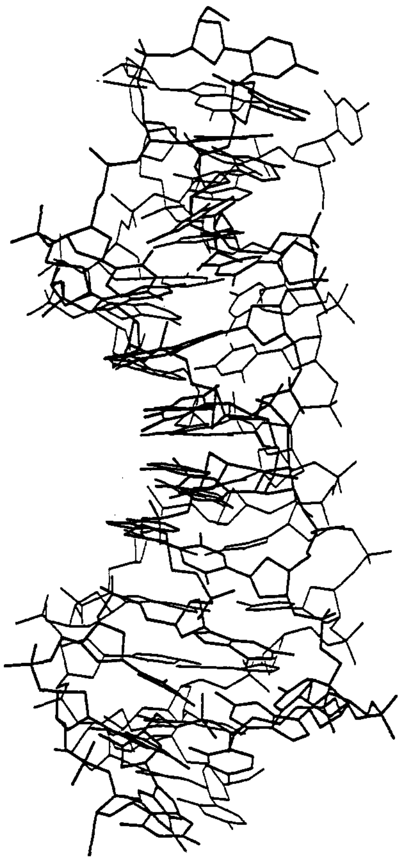

Figure 15. A comparison of two specific frames from the simulation. The energy of the two structures differs only by 0.3 kcal mol^{-1} but the rms deviation between them is 4.2 Å.

an initial B-DNA like structure to one in which the helix is severely distorted and kinked, with disrupted hydrogen bonds between base pairs. Similar disruptions of hydrogen bonding between Watson–Crick base pairs were also observed in the MD simulation by Beveridge et al.,[33] when specific constraints were not applied to the H-bonds.

The results presented here raise the question whether a longer MD simulation would result in a "unique" stable structure of DNA. Such a structure would be the energetic minimum around which the MD simulation would produce limited oscillations. Neither the present results nor the recently published reports[33] provide a definitive answer to this question. Additional experience with simulations performed with a variety of potential functions and boundary conditions and for more extended time periods will be required to approach such insight.

Notably, the theoretical work of Zhurkin et al.[49] suggests that besides the known A- and B-DNA families, several other structures are energetically possible. Their work demonstrates that the DNA is rigid only when the base pairs are fixed, for example, by the interaction of DNA with a protein. However, when the base pairs are allowed to move, different families of structural forms with similar energies can exist with possible continuous interconversions between them. Results from our simulations are compatible with the notion that very different DNA structures may have very similar energies (calculated within the AMBER force field), as is illustrated in Figure 15, which shows two specific frames from the simulation with rms difference of 4.20 Å, but a difference in energies of only 0.3 kcal mol^{-1}. Thus, it seems that a stabilization of a "unique" structure is not supported by the present MD simulations of DNA.

Acknowledgment. This work was supported in part by a grant from the U.S. Department of Energy (DOE), USDOE DE-FG02-88ER60675. H. Weinstein is recipient of a NIDA Research Scientist Award DA-00060. Computations were performed in part at the National MFE Computer Center of the DOE. The authors gratefully acknowledge the generous allocations of computer time at the University Computer Center of the City University of New York, and the Advanced Scientific Computing Laboratory at the Fredrick Cancer Center Research Facility of the National Cancer Institute (Laboratory for Mathematical Biology).

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Ab Initio Studies of Lipid Model Species. 1. Dimethyl Phosphate and Methyl Propyl Phosphate Anions

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Abstract: With the aim of developing force fields for lipid/membrane simulations, extensive ab initio calculations have been carried out on lipid model compounds. This contribution reports results from searching the potential energy surfaces of two simple model species, the dimethyl phosphate and methyl propyl phosphate anions. It is shown that most of the critical structural features of phospholipids found experimentally in crystals, in the gel and liquid crystal phases, and in solution are also seen from quantum mechanical calculations on these simple systems in the gas phase. Thus, a gauche-gauche conformation about phosphate is favored for the dimethyl phosphate anion, as is also seen experimentally for phospholipids (and nucleotides). Also, the CCOP torsional angle in the methyl propyl phosphate anion tends to be anti-periplanar or trans, as is the case in most of the structures of phospholipids in crystals and in solution. The vibrational spectra of various conformers of these species have been analyzed in detail. The results reveal some diagnostic frequencies for experimental determination of conformational preferences in structurally related phosphates.

Introduction

Bilayers of discrete lipid molecules, especially the phospholipids, are the primary structural components of both inter- and intracellular membranes in nearly all living organisms and are central to many types of cellular functions such as material transport across membranes and communication between cells. Typically the phospholipids are amphiphilic and consist of a positively charged group plus a phosphate moiety (the α chain) comprising

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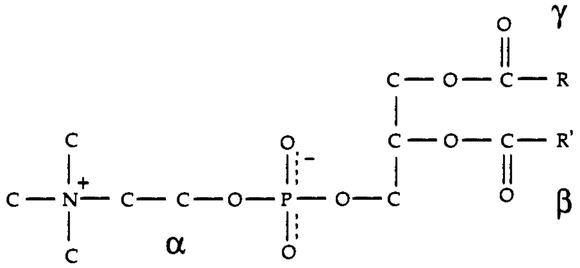
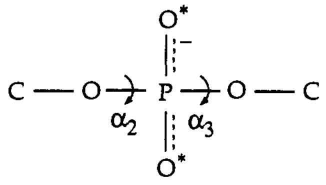
the hydrophilic head, with glycerol esterified to two fatty acids (β, γ chains) forming the hydrophobic tails. This is illustrated in I for the case of a phosphatidylcholine, one of the most common classes of phospholipids. (Here R and R′ are characteristic hydrocarbon chains.)



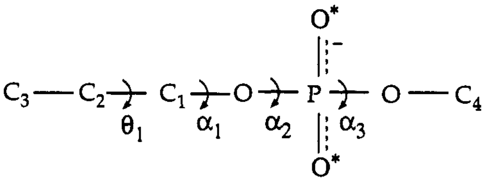
I

Recent years have witnessed a surge of computer-based molecular modeling of key lipid/membrane systems using molecular dynamics,[1] Monte Carlo simulation,[2] Brownian dynamics,[3] energy minimization,[4] and other simulation methods.[5] Despite the successes of these studies, for further progress on lipid/membrane simulation it is highly desirable to derive a quantitative description of their complete energy surfaces taking into account the unique features of individual phospholipids. This is particularly true if one is to study the interaction of lipids with proteins and other perturbants in membranes with atomic detail. One approach for constructing such models is the strategy of Maple, Dinur, and Hagler[6] to utilize ab initio potential energy surfaces in the derivation of force fields to enhance sparse experimental data, such as is generally found for these kinds of systems. Thus we have initiated extensive ab initio studies of lipid model compounds, and in this contribution we report results for two of the simplest ones, the dimethyl phosphate and methyl propyl phosphate anions. Our goal in this paper is 3-fold: (1) to provide theoretical results, such as relative energies and vibrational frequencies of differing conformers, that may be helpful in interpreting experimental studies on phospholipids, (2) to demonstrate that some peculiar structural features of phospholipids observed in crystals, in gel and liquid crystalline phases, and in solution are revealed by ab initio quantum mechanical calculations to exist in the isolated model compounds as well, and thus may be inherent properties of the molecule rather than induced on aggregation, and (3) to acquire pertinent ab initio data for developing a Class II force field[6] for ultimately modeling larger systems.

The dimethyl phosphate anion II is also a prototype for the phosphodiester linkage in nucleic acids, and in part for this reason there have been numerous theoretical and experimental studies on this species. (For a comprehensive review, see ref 7.) Interestingly, while theoretical studies on this molecule in the 1970s used relatively simple quantum mechanical methods and focused on the structure and energetics of isolated molecules,[8,9] (primarily due to limitations in computational resources) the few studies in the 1980s addressed only the solvation of the dimethyl phosphate anion.[7,10,11] (Early studies by Pullman et al. focused on hydration of the dimethyl phosphate anion by a few water molecules.[12] Despite its importance, to our knowledge no one has reexamined the structure and energetics of this system using higher levels of theory than those employed in the 1970s. Compared to the richness of studies on the dimethyl phosphate anion, both theoretical and experimental studies of the methyl propyl phosphate anion III are virtually nonexistent. This molecule may be taken as a prototype of either glycerophosphates or phosphatidylcholines (compare I and III) and also phosphatidylethanolamines. Thus, a comprehensive study on this molecule also should be profitable.



II



III

Selected vibrational frequencies of the dialkyl phosphate moiety have long been key experimental properties for probing the structures of biomolecules, especially the phospholipids, in terms of their preferred conformations as functions of their environments.[13] However, to our knowledge there has been no previous quantum-theoretical study of the vibrational spectra of dialkyl phosphates. Since these are also central to forming and testing force fields, we will consider the computed vibrational spectra (particularly infrared) in some detail.

We have employed ab initio theoretical methods to search the potential energy surfaces of the dimethyl phosphate and methyl propyl phosphate anions extensively. Analytical second derivatives were obtained to characterize all energy stationary points found. In this paper, attention will be focused on the structures, relative energies, and vibrational frequencies of the various conformations of these molecules. The results will be discussed in conjunction with both experiment and other theoretical studies.

Theoretical Methods and Choice of Basis Set

All calculations were performed on IBM RISC System/6000 workstations. The Hartree–Fock (HF) approximation, implemented in the GAUSSIAN 90[14] program as direct self-consistent-field methods, was employed for most of the calculations. Analytical first and second derivatives were determined for all energy extrema. Vibrational frequency assignments were based

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Table I. Comparison of Calculated and Experimental Geometries of the Dimethyl Phosphate Anion[a]

parameter	3-21G*	6-31G*	6-31+G*	experiment[b]
P–O*	1.473	1.470	1.474	1.490, 1.498 (1.52, 1.52; ±0.02)
P–O	1.634	1.642	1.639	1.536, 1.582 (1.59, 1.62; ±0.02)
C–O	1.435	1.393	1.396	1.425, 1.481 (1.44, 1.47; ±0.04)
O*PO*	124.6	124.9	124.6	117.2 (121.6; ±0.8)
OPO	98.2	99.3	99.8	104.8 (103.5; ±0.8)
POC	118.2	118.5	119.4	118.3, 123.0 (116.0, 118.0; ±2.0)
COPO	74.6	75.1	75.3	57.5, 62.4 (68.2, 71.6)

[a] Bond lengths in angstroms, angles in degrees. The structure is *gg* with C_2 symmetry. [b] From the crystal structure of dimethylammonium phosphate: Giarda, L.; Garbassi, F.; Calcaterra, M. *Acta Crystallogr.* **1973**, *B29*, 1826. Data in parentheses are from the crystal structure of barium diethylammonium phosphate (some uncertainties also given): Kyogoku, Y.; Iitaka, Y. *Acta. Crystallogr.* **1966**, *21*, 49. Note that both crystal structures are significantly distorted from perfect C_2 symmetry.

on the potential energy distributions,[15] with the symmetry internal coordinates defined according to recommendations of Pulay et al.[16] Single point calculations at the Hartree–Fock optimized geometries were carried out using the direct second-order Møller–Plesset perturbation method (MP2) implemented in the TURBOMOLE program[17] (core electrons frozen). TURBOMOLE was required for these MP2 calculations due to its much more efficient use of disk space when employing workstations, allowing us to carry out these calculations on these systems.

For systems as large as the phosphorylcholines that we will study in the next paper of this series, one must choose a basis set that is efficient as well as accurate in reproducing experimental quantities. We considered three candidates for basis sets: one with split valence plus polarization functions on phosphorus only (3-21G*);[18a,b] one with split valence with polarization on all atoms except hydrogen (6-31G*);[18c,d] and 6-31+G*, which is constructed by adding diffuse functions[19] to the 6-31G* basis set for the heavy atoms. Table I compares the predicted geometries for the global minimum of the dimethyl phosphate anion using these three basis sets and the HF method with the experimental crystal structure of dimethylammonium phosphate.[20] The 3-21G* predictions are very close to those from more extended basis sets, except that the calculated C–O bond length is about 0.03 Å too long. Excellent agreement is observed between the 6-31G* and 6-31+G* calculated geometries: the largest differences in the bond lengths and angles are only 0.005 Å and 1°, respectively. This shows that diffuse functions have a negligible effect on the geometry of phosphate anions, in agreement with other studies.[21] However, comparison of theoretical and crystal structures reveals significant differences. Specifically, the calculated P–O bond lengths are considerably longer than in the crystal state (by about 0.05–0.1 Å), while the P–O* and C–O distances (II) are notably shorter (by about 0.02–0.08 Å) than the experimental distances in the crystal (Table I). The predicted O*PO* and OPO bond angles and the COPO torsional angles are also significantly different from those observed crystallographically: by about 5° for bond angles and about 13–17° for torsional angles. It is likely that these relatively large discrepancies may be attributed to intermolecular forces in the crystal since the calculations were carried out, of course, on isolated molecules. These forces also affect the vibrational frequencies, as will be discussed below. In fact, the crystal structure[20] is considerably distorted from perfect C_2 symmetry, and the large observed differences between symmetrically equivalent bond lengths and angles (up to 0.05 Å in bond lengths and 5° in angles) are comparable to those between theory and experiment. Furthermore, this experimental structure is significantly different from that of an analogous compound, barium diethyl phosphate[22] (Table I), again demonstrating the effect of intermolecular forces on the geometry of dialkyl phosphate anions. Thus, since results obtained from the 6-31G* and 6-31+G* basis sets agree and past experience has demonstrated that more complete basis sets do not significantly improve results for similar systems,[23] we feel that the present theoretical results should be reliable, at least for gas-phase properties.

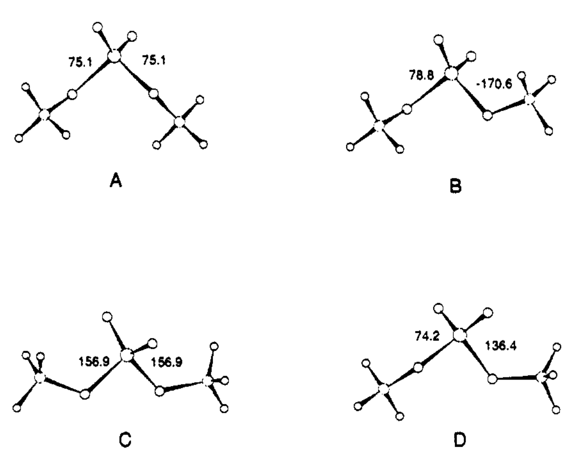

Figure 1. HF/6-31G* predicted geometries for the dimethyl phosphate anion. **A** is the global minimum and **D** is a transition state.

A more stringent test of the quality of the basis sets is the calculation of vibrational frequencies. Table II compares the harmonic vibrational frequencies of the dimethyl phosphate anion calculated using the three basis sets considered above (comparison of calculated frequencies with experiment is made in a later section). It is clear that the 3-21G* results are, in general, in qualitative agreement with those from 6-31G* and 6-31+G*, although the ordering of fundamentals 19 and 20 is changed and for a few fundamentals the frequencies differ by 30–70 cm^{-1}. On the other hand, the frequencies obtained from 6-31G* and 6-31+G* are in very good agreement and the ordering of all fundamentals is the same. The largest differences are found for the two P–O* stretching modes, vibrations 13 and 20. The 6-31+G* values are respectively 37 and 22 cm^{-1} lower. For all other vibrations the frequencies differ by no more than 10 cm^{-1} (Table II). Thus, it may be concluded that diffuse functions are not very important in predicting either the geometries or vibrational frequencies of the dimethyl phosphate anion. Further studies (vide infra) show that their effect on relative energies of differing conformers is also small. Therefore, as a compromise between accuracy and efficiency we chose the 6-31G* basis set for this and following studies. Table III reports the computed relative (electronic) energies, zero-point vibrational energies, free energies (including zero-point energies and the entropies and enthalpies of nuclear motion at 298 K), as well as the dipole moments for most of the species studied here.

Results and Discussions

1. Dimethyl Phosphate Anion. For this species we shall focus on the structure and relative energies of its differing conformers since its structure is critical in determining the head-group orientation of phospholipids I. We shall also discuss some characteristic vibrational frequencies that are helpful in discerning the

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Table II. Comparison of Calculated Harmonic Vibrational Frequencies (cm^{-1}) for the Global Minimum (with C_2 Symmetry) Using the Hartree–Fock Method and the 3-21G*, 6-31G*, and 6-31+G* Basis Sets[a]

mode	sym	3-21G*	6-31G*	6-31+G*	mode	sym	3-21G*	6-31G*	6-31+G*	mode	sym	3-21G*	6-31G*	6-31+G*
1	a	3284	3280	3275	12	a	1610	1626	1619	23	b	609	599	597
2	b	3284	3279	3274	13	b	1481	1434	1397	24	a	577	573	566
3	a	3230	3233	3234	14	a	1288	1330	1325	25	b	507	496	495
4	b	3228	3233	3233	15	b	1286	1327	1322	26	b	420	412	411
5	a	3184	3188	3188	16	a	1264	1300	1296	27	a	406	390	390
6	b	3182	3185	3186	17	b	1263	1299	1295	28	b	258	258	251
7	a	1711	1668	1659	18	a	1241	1244	1236	29	a	259	251	247
8	a	1708	1666	1656	19	b	1155	1228	1220	30	b	120	164	159
9	b	1673	1649	1640	20	a	1175	1203	1181	31	a	111	130	128
10	a	1673	1648	1639	21	b	845	804	799	32	b	78	99	107
11	b	1614	1628	1620	22	a	816	786	779	33	a	66	82	83

[a] For normal mode assignments see Table VI.

Table III. Potential Energies, Zero-Point Vibrational Energies, Relative Free Energies, and Dipole Moments of the Differing Conformers of the Dimethyl Phosphate and Methyl Propyl Phosphate Anions Calculated Using the HF/6-31G* Method

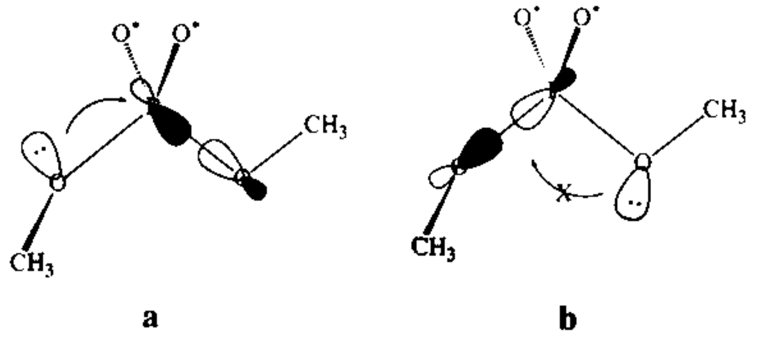
conformation	total electronic energy[a,b]	zero-point energy[a]	relative free energy[a,c]	dipole moment[d]
		Dimethyl Phosphate Anion		
A	0.00	63.89	0.00	5.763
B	1.15	63.76	1.13	5.027
C	2.78	63.62	2.74	2.670
D	1.70	63.69[e]	1.08[e]	4.709
		Methyl Propyl Phosphate Anion		
E	0.00	102.20	0.00	6.439
F	0.04	102.10	0.00	7.620
G	0.07	102.10	0.03	8.088
H	0.23	102.12	0.20	6.926
I	0.42	101.99	0.34	8.349
J	0.79	102.25	0.82	7.242
K	0.87	102.12	0.85	7.699

[a] In kcal/mol. [b] Relative to the lowest energy structure. For **A** the total electronic energy is −719.520867 hartrees, and for **E** it is −797.596931 hartrees. [c] Gibbs free energy at 298 K and 1 atm pressure relative to the most stable conformer of each species, in kcal/mol. [d] In debyes. [e] Contribution from the one imaginary frequency is ignored.

conformations of phospholipids using infrared and Raman spectra.

A. Structures. There are three low-energy conformers for this species. We have obtained the optimized structures and harmonic vibrational frequencies for each of them (Figure 1 and Table IV). The two COPO torsional angles may be used to classify the three structures (II). These are denoted α_2 and α_3 (II) in Sundaralingam's notation for torsional angles in lipids,[25] which we will employ in this study. Thus, structure **A** is *gg* ($\alpha_2 = \alpha_3 = 75.1°$) with C_2 symmetry, **B** is *gt* ($\alpha_2 = 78.8°$, $\alpha_3 = -170.6°$), and C is *tt* ($\alpha_2 = \alpha_3 = 156.9°$) with C_2 symmetry, where *g* stands for gauche and *t* for trans. It is interesting to note that the *tt* configuration has only C_2 symmetry instead of C_{2v} as one might expect. In fact, early empirical calculations[26] did predict a C_{2v} structure for *tt*. The HF/6-31G* results, however, indicate that it is actually the transition state between the two symmetrically equivalent *tt* energy minima and is only 0.05 kcal/mol higher in energy than structure C (Figure 1). This result was also verified by calculations using the HF/6-31+G* method. The distortion from C_{2v} to C_2, a change of 23.1° in the torsional angles, is apparently due to the repulsion between lone pairs of the two esterified oxygens since the OPO angle is very small (about 95° from HF/6-31G*). Note that the trans angle in the *gt* rotamer is also not 180°. These are examples of the unique kinds of geometric features that provide important information about the energy surface and that present a challenge for force fields which intend to reproduce these energy surfaces.

For bond lengths it may be seen from Table IV that the P–O bonds gauche to C–O bonds (1.642 Å as in *gg*) are slightly longer than those that are trans (1.635 Å as in *tt*, Table IV). The difference is more prominent in the *gt* conformer where both types of P–O bonds exist (1.627 vs 1.648 Å). This phenomenon may be explained by the anomeric effect.[27,28] When the P–O bond is gauche to the C–O bond (case **a** of IV), electron donation from the lone pair on the oxygen in C–O to the antibonding orbital of the P–O bond is feasible; on the other hand, when the P–O bond is trans to the C–O bond (case **b** of IV), such electron donation is unlikely since the lone pair overlaps both positive and negative lobes of this antibonding orbital. Thus, according to this rationale the more effective electron donation in case **a** reduces the bonding character of the P–O bond involved.



a b

IV

We also note that the reduced P–O bonding character in *gg*, as compared to *tt*, is somewhat compensated for by an increase in (double) bonding character of the P–O* bonds since these bonds in *gg* (1.470 Å) are slightly shorter than those in *tt* (1.476 Å, Table IV). This change in double bond character of the P–O* bonds may explain the O*PO* bond angle variation to be discussed below.

For bond angles, Gorenstein et al.[9] concluded from theoretical studies that there is a strong coupling between the COPO torsion and the OPO angles. This is supported by crystallographic studies, and our results again show this behavior. Thus, the OPO angle decreases (from 99.3° to 96.7° to 94.9°) on going from *gg* to *gt* or *tt* (Table IV), presumably due to reduced repulsion between methyl groups. However, the O*PO* angle also decreases (from 124.9° to 122.8° to 121.2°). This scissoring phenomenon is very similar to that seen in a structurally related molecule, dimethoxymethane.[9] But it is in contrast to the conventional bond angle antiscissoring effect observed in tetrahedral structures without the anomeric effect.[29] On the other hand, the larger O*PO* angle in *gg* may be understood from the increased double bond character of P–O* bonds in this conformer. Again, such trends present challenges for analytic functions designed to represent the energy surface of these functional groups.

B. Relative Energies. The HF/6-31G* energies show that the *gg* conformer is the most stable and the *gt* and *tt* conformers are,

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Table IV. HF/6-31G* Predicted Geometries of the Dimethyl Phosphate Anion[a]

parameter	A (*gg*)	B (*gt*)	C (*tt*)	D (TS)
P–O*	1.470	1.469, 1.477	1.476	1.474, 1.471
P–O	1.642	1.627, 1.648	1.635	1.633, 1.647
C–O	1.393	1.392, 1.394	1.393	1.392, 1.393
O*PO*	124.9	122.8	121.2	123.5
OPO	99.3	96.7	94.9	98.0
OPO*	108.6, 106.3	109.3, 107.4, 109.5, 107.9	110.4, 108.4	108.4, 107.7, 108.1, 108.0
POC	118.5	117.0, 119.1	117.6	119.1, 119.1
COPO	75.1	78.8, −170.6	156.9	74.2, 136.4

[a] Bond lengths in angstroms, angles in degrees. Structures **A** and **C** have C_2 symmetry. **D** is a transition state (TS).

Table V. Calculated Relative Energies of the Dimethyl Phosphate Anion (in kcal/mol)

method	A (*gg*)	B (*gt*)	C (*tt*)	D (TS)
HF/6-31G*	0.00	1.15	2.78	1.71
HF/6-31+G*	0.00	1.06	2.79	1.76
MP2/6-31G*//HF/6-31+G*	0.00	1.40	3.16	2.03
MP2/6-31+G*//HF/6-31+G*	0.00	1.45	3.66	2.26

respectively, 1.15 and 2.78 kcal/mol higher in energy (Tables III and V). Using the 6-31+G* basis set does not change the relative energies. For instance, this basis predicts the *tt* structure to be 2.79 kcal/mol higher than *gg*, which is virtually the same as the 6-31G* result. (This reinforces the above conclusion that diffuse functions are not important for this compound as far as for the properties concerned here.) Including electron correlation at the MP2 level of theory increases the relative energies of *gt* and *tt* by about 0.4–1.0 kcal/mol as compared to *gg* (Table V).

In the literature there are numerous experimental studies of the conformations of dialkyl phosphates,[7] and it has been established that the *gg* configuration about the phosphate is preferred in solution[25] and crystals.[9b] For example, a search of the Cambridge crystallographic data bank for structures with phosphodiester linkages[7,11a] showed 45 structures with the *gg* conformation, 6 with *gt*, and no structure with *tt*, consistent with the relative energies of the isolated molecules obtained theoretically. Searches by us and others[30] of crystal structures of lipids and related compounds showed an even stronger preference for *gg*: all but one of 22 reported structures are in *gg*. The only exception, cyclopentyl phosphatidylcholine, has the *gt* configuration, apparently due to strong steric repulsion between the cyclopentyl and choline groups when they are in the *gg* conformation.[31] The *tt* conformer is, again, not observed in any of these crystals.

Previous theoretical studies[8,9,32] of dimethyl phosphate all agree with the energy ordering obtained here. The magnitude of the energy difference between the *gg* and *gt* conformers, which is not known experimentally, has been estimated using minimal basis sets. The results vary from 7 to less than 1 kcal/mol, depending on whether (only partial) geometry optimization was carried out.[8,9] Our results should be more reliable since they were obtained using complete geometry optimization, much more complete basis sets (6-31G* vs STO-3G), and electron correlation (MP2) in evaluating the energies. Thus, we found that the energy differences between *gt* and *gg* are relatively small (1.45 kcal/mol according to the MP2/6-31+G*//HF/6-31+G* method). This is reasonable because some *gt* structures, albeit few, are observed in crystals as discussed above. In addition, it is interesting to note that while the crystal structure for the barium salt of diethyl phosphate anion[22] is in *gg*, that of the silver salt is not.[33] On the other hand, the relatively large energy difference between *tt* and *gg* (3.66 kcal/mol, MP2/6-31+G*//HF/6-31+G*) may well explain the absence of *tt* conformers in nucleic acids and phospholipids as discussed above. This preference for the gauche configuration in phosphates has again been attributed to an anomeric effect.[8,27,28]

The relatively small energy difference between the *gg* and *gt* conformers suggests that they may be in dynamic equilibrium in solution if the transition state between them is not too high in energy. We have located this transition state (**D** in Figure 1). The α_2, α_3 angles are 74.2° and 136.4°, respectively, essentially as one would expect. This structure is 1.71 kcal/mol higher in energy than the global minimum **A** and 0.56 kcal/mol higher than **B** (HF/6-31G*). Thus, the *gt* structure should be accessible in solution. There is experimental evidence that does suggest so,[34] although such an interpretation has been disputed.[7,26]

Comparison of the relative free energies of the four conformers with their dipole moments, shown in Table III, shows that stabilities and dipole moments are not correlated, contrary to the close correspondence found for neutral species such as dimethyl phosphonate.[23b] However, the large dipole moment differences suggest that there may be significant effects of polar solvents or other media in the relative conformational stabilities.

We have also calculated the C_{2v} structure with the C–O and P–O bonds eclipsed and found it to be very high in energy (19.60 kcal/mol higher than the *gg* minimum, HF/6-31G*). It is not a transition state according to HF/3-21G* calculations. Thus, interconversion of the two symmetrically equivalent *gg* minima in solution is not likely to go through this structure, although the interconversion could be achieved through the *gt* intermediate.

Finally, it should be noted that in phospholipid bilayers such as in biomembranes, a *gg* configuration about the phosphate is required for positively charged head groups such as choline and ethanolamine to be parallel to the plane of the membrane[35] (in order to maximize favorable electrostatic interactions between neighboring lipids in the monolayer). Our results show that this requirement is an intrinsic structural property of the isolated dimethyl phosphate anion as well.

C. Vibrational Frequencies. The harmonic vibrational frequencies for all three minima were determined analytically. Table VI gives the complete theoretical vibrational spectrum for the global minimum (*gg*) conformer of dimethyl phosphate together with the reported experimental frequencies for barium dimethyl phosphate in aqueous solution.[26] As can be seen, the agreement is moderate: theoretical values are 11–18% higher than experimental ones, except for P–O stretches for which the two sets of data are very close. In particular, the calculated P–O* symmetric stretching frequency is lower than those for the C–O stretches (1203 vs 1244 and 1228 cm^{-1}), while experiment indicates otherwise. The HF/6-31G* method also underestimates the P–O asymmetric stretching frequency as compared to experiment. The discrepancy between theoretical and experimental frequencies is consistent with that observed for geometries, as discussed in the previous section. Thus, the calculated C–O distance is shorter than that observed in the crystal and its corresponding stretching frequencies are higher. Similarly, the relatively long calculated

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(33) Hazel, J. P.; Collin, R. L. *Acta Crystallogr.* **1972**, *B28*, 2951. The published α_2 and α_3 values are 68° and 125°, respectively. However the α_3 torsion involves the C_3 atom, whose position does not seem to be well determined. For instance, the *single* bond lengths for C_3–C_4 and C_3–O_2 are 1.353 and 1.138 Å, respectively.

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Table VI. HF/6-31G* Computed and Experimental[a] (in parentheses) Vibrational Spectra for the Global Minimum of the Dimethyl Phosphate Anion[b]

mode	sym	frequency	intensity	assignment (%)
1	a	3280	61	CH sym stretch (100)
2	b	3279	50	CH asym stretch (100)
3	b	3233	206	CH asym stretch (100)
4	a	3233	2	CH sym stretch (100)
5	a	3188	124	CH sym stretch (100)
6	b	3185	81	CH asym stretch (100)
7	a	1668	1	$\mathrm{CH_3}$ asym deform (89) + $\mathrm{CH_3}$ rock (7)
8	b	1666	1	$\mathrm{CH_3}$ asym deform (90) + $\mathrm{CH_3}$ rock (7)
9	b	1649	1	$\mathrm{CH_3}$ asym deform (86) + $\mathrm{CH_3}$ sym deform (7)
10	a	1648	2	$\mathrm{CH_3}$ asym deform (91) + $\mathrm{CH_3}$ rock (5)
11	b	1628	22	$\mathrm{CH_3}$ sym deform (89) + $\mathrm{CH_3}$ asym deform (6)
12	a	1626	1	$\mathrm{CH_3}$ sym deform (93) + C–O sym stretch (3)
13	b	1434 (1225)	367 (vs)	P–O* asym stretch (92) + $\mathrm{PO^*_2}$ rock (6)
14	a	1330 (1190)	23 (w)	$\mathrm{CH_3}$ rock (85) + P–O sym stretch (4)
15	b	1327	28	$\mathrm{CH_3}$ rock (89) + P–O asym stretch (3)
16	a	1300	1	$\mathrm{CH_3}$ rock (94)
17	b	1299	4	$\mathrm{CH_3}$ rock (96)
18	a	1244 (1058)	82 (vs)	C–O sym stretch (84) + P–O sym stretch (6)
19	b	1228 (1038)	282 (vs)	C–O asym stretch (88) + P–O asym stretch (5)
20	a	1203 (1087)	330 (vs)	P–O* sym stretch (85) + P–O sym stretch (7)
21	b	804 (820)	386 (s)	P–O asym stretch (69) + $\mathrm{PO^*_2}$ wag (22) + COP bend (5)
22	a	786 (764)	114 (w)	P–O sym stretch (73) + $\mathrm{PO^*_2}$ sciss (8) + sym C–O stretch (8)
23	b	599	44	$\mathrm{PO^*_2}$ rock (43) + $\mathrm{PO^*_2}$ wag (27) + COP bend (23)
24	a	573	59	$\mathrm{PO^*_2}$ sciss (50) + COP bend (19) + $\mathrm{PO_2}$ sciss (17)
25	b	496	34	$\mathrm{PO^*_2}$ wag (58) + $\mathrm{PO^*_2}$ rock (23) + asym P–O stretch (10)
26	a	412	13	$\mathrm{PO^*_2}$ twist (57) + COP bend (31) + $\mathrm{PO^*_2}$ sciss (7)
27	a	390	1	$\mathrm{PO_2}$ sciss (53) + $\mathrm{PO^*_2}$ sciss (40)
28	b	258	6	COP bend (66) + $\mathrm{PO^*_2}$ rock (30)
29	a	251	5	COP bend (48) + $\mathrm{PO^*_2}$ twist (38)
30	b	164	2	$\mathrm{CH_3}$ rotation (62) + OPOC torsion (29) + COP bend (7)
31	a	130	0	$\mathrm{CH_3}$ rotation (82) + COP bend (8) + $\mathrm{PO_2}$ sciss (6)
32	b	99	1	OPOC torsion (59) + $\mathrm{CH_3}$ rotation (37)
33	a	82	6	OPOC torsion (87) + $\mathrm{PO_2}$ sciss (6)

[a] Experimental data is for barium dimethyl phosphate in aqueous solution: Shimanouchi, T.; Tsuboi, M.; Kyogoku, Y. *Adv. Chem. Phys.* **1964**, *7*, 435. [b] Frequencies in $\mathrm{cm^{-1}}$ and IR intensities in km/mol. The theoretical assignment is based on the potential energy distribution.

Table VII. Theoretical Vibrational Spectrum for the *gt* Conformer of the Dimethyl Phosphate Anion Calculated Using the HF/6-31G* Method[a]

freq	int	assignment (%)
1415	370	P–O* asym stretch (93) + $\mathrm{PO^*_2}$ rock (5)
1252	80	C–O sym stretch (84) + P–O sym stretch (5)
1226	410	C–O asym stretch (81) + P–O asym stretch (5)
1198	210	P–O stretch (78) + C–O stretch (9)
830	326	P–O sym stretch (67) + $\mathrm{PO^*_2}$ wag (18)
789	202	P–O asym stretch (74) + $\mathrm{PO^*_2}$ wag (5) + $\mathrm{PO^*_2}$ sciss (5)
594	41	$\mathrm{PO^*_2}$ wag (33) + $\mathrm{PO^*_2}$ rock (27) + COP bend (22)
554	41	$\mathrm{PO^*_2}$ wag (33) + $\mathrm{PO^*_2}$ sciss (27) + $\mathrm{PO^*_2}$ rock (17)
517	30	$\mathrm{PO^*_2}$ rock (41) + $\mathrm{PO^*_2}$ sciss (37) + P–O stretch (9)
405	17	$\mathrm{PO^*_2}$ twist (40) + COP bend (18) + $\mathrm{PO^*_2}$ sciss (16) + $\mathrm{PO_2}$ sciss (12)
389	6	$\mathrm{PO_2}$ sciss (43) + $\mathrm{PO^*_2}$ sciss (23) + $\mathrm{PO^*_2}$ twist (19)
259	4	COP bend (64) + $\mathrm{PO^*_2}$ twist (11)
234	6	COP bend (46) + $\mathrm{PO_2}$ sciss (22) + $\mathrm{PO^*_2}$ twist (12)
84	3	OPOC torsion (56) + $\mathrm{CH_3}$ rotation (31)
46	6	OPOC torsion (82) + $\mathrm{PO^*_2}$ twist (10)

[a] Frequencies in $\mathrm{cm^{-1}}$ and IR intensities in km/mol. The assignment is based on the potential energy distribution. Vibrations associated with methyl groups are omitted.

P–O distance is in accordance with the relatively low predicted stretching frequency. Since the HF method would be expected to underestimate the P–O distance and overestimate the stretching frequency, a large part of these discrepancies is likely due to the differences between the in vacuo and condensed-phase environments (as discussed above). It may be noted that the reported experimental frequencies for barium dimethyl phosphate are the same as those often used to characterize phospholipids[36,37] and nucleic acids.[38]

Table VIII. Theoretical Vibrational Spectrum for the *tt* Conformer of the Dimethyl Phosphate Anion Calculated Using the HF/6-31G* Method[a]

sym	freq	int	assignment (%)
b	1396	367	P–O* stretch (94) + $\mathrm{PO^*_2}$ rock (5)
a	1264	8	C–O stretch (82) + P–O stretch (4)
b	1215	551	C–O stretch (87) + P–O stretch (6)
a	1198	191	P–O* stretch (83) + P–O stretch (8)
b	827	400	P–O stretch (72) + $\mathrm{PO^*_2}$ wag (23)
a	814	133	P–O stretch (68) + $\mathrm{PO_2}$ sciss (7) + $\mathrm{PO^*_2}$ sciss (6)
b	610	36	$\mathrm{PO^*_2}$ wag (45) + COP bend (27) + $\mathrm{PO^*_2}$ rock (14)
b	529	47	$\mathrm{PO^*_2}$ rock (75) + $\mathrm{PO^*_2}$ wag (17)
a	525	29	$\mathrm{PO^*_2}$ sciss (40) + P–O stretch (8)
a	405	0	COP bend (34) + $\mathrm{PO^*_2}$ twist (29) + $\mathrm{PO_2}$ sciss (22)
a	362	0	$\mathrm{PO^*_2}$ twist (49) + $\mathrm{PO_2}$ sciss (27) + $\mathrm{PO^*_2}$ sciss (16)
b	285	28	COP bend (77) + $\mathrm{PO^*_2}$ wag (14)
a	210	7	$\mathrm{PO_2}$ sciss (47) + COP bend (29)
a	30	2	OPOC torsion (49) + COP bend (24)
b	17	13	OPOC torsion (82) + COP bend (22)

[a] Frequencies are in $\mathrm{cm^{-1}}$ and IR intensities in km/mol. The assignment is based on the potential energy distribution. Vibrations associated with methyl groups are omitted.

Table IX. Diagnostic Frequencies (in $\mathrm{cm^{-1}}$), Range, and Average IR Intensities (km/mol) for Determining the Conformations of Dialkyl Phosphate Groups, Based on HF/6-31G* Results for Dimethyl Phosphate

normal mode/conformer	A (*gg*)	B (*gt*)	C (*tt*)	range	intensity[b]
P–O* asym stretch	1434	1415	1396	38	368
C–O sym stretch	1244	1252	1264	20	57
P–O sym stretch	786	789	814	28	150
P–O asym stretch	804	830	827	26	371
$\mathrm{PO^*_2}$ wag	496	594/554[a]	610	114	37

[a] Both assignments are plausible (see Table VII). [b] Averaged over all conformers.

Tables VII and VIII give the vibrational spectra for the other two local minima (vibrations associated with the methyl hydrogens

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omitted). Comparison of data in Tables VI, VII, and VIII immediately shows a few diagnostic vibrations (summarized in Table IX) that may be useful for determining the conformations of dialkyl phosphate groups. Specifically, as one goes from *gg* to *gt* to *tt*, the P–O* asymmetric stretching frequency decreases appreciably from 1434 to 1415 to 1396 cm^{-1}, while the C–O symmetric stretching frequency increases from 1244 to 1252 to 1264 cm^{-1} (Table IX). But more useful vibrations in discerning the conformations of phospholipids are the $\mathrm{PO^*_2}$ wagging and P–O stretching modes. Casal et al.[39] concluded that the $\mathrm{NH_4^+}$, $\mathrm{Li^+}$, and $\mathrm{Na^+}$ salts of phosphatidylserines are in *gg* since the $\mathrm{PO^*_2}$ wagging is observed at 541 cm^{-1} and no absorption is found around 665 cm^{-1}. They also concluded that the $\mathrm{Ca^{2+}}$ salt may be predominantly *tt* since a moderately strong absorption is found at 664 cm^{-1} for $\mathrm{PO^*_2}$ wagging and a strong peak is observed at 825 cm^{-1} for asymmetric P–O stretching. Our results support most of their conclusions: (1) the calculated frequency for $\mathrm{PO^*_2}$ wagging in *gg* (496 cm^{-1}) is indeed distinctively smaller than that in *gt* (594 cm^{-1}) or *tt* (610 cm^{-1}, Table IX). The corresponding experimental value[39] (541 cm^{-1}) is higher, probably due to the presence of counterions which coordinate to $\mathrm{PO^*_2}$, limiting its freedom to distort; (2) the calculated P–O asymmetric stretching frequency for *gg* (804 cm^{-1}) is appreciably lower than those of the other two (830 and 827 cm^{-1}). However, it appears Casal et al. switched the symmetric and asymmetric stretching frequencies for the three conformers (compare Table I of ref 38 with their source, Figure 29 of ref 25). As a result, they apparently made the assumption that the P–O symmetric stretching in *tt* is lower than that in *gt* or *gg* rather than higher, and they assigned the conformation of the calcium phosphatidylserines to *tt* since the observed peak at 737 cm^{-1} is low compared to that of the lithium salt. Our HF/6-31G* calculations agree with the empirical calculations of Shimanouchi et al.[26] that the P–O symmetric stretching frequency for *tt* (814 cm^{-1}) is higher than those of the other two conformers (786 and 789 cm^{-1}). Thus, we suggest that the 737 cm^{-1} band, which is lower but close to 757 cm^{-1} for the same vibration in the lithium salt, indicates that the $\mathrm{Ca^{2+}}$ salt may be *gt* instead of *tt*. In the next section we shall show that the observed peak at 664 cm^{-1} for the calcium phosphatidylserines does not necessarily imply a *gt* or *tt* configuration and that an alternative interpretation is possible.

2. Methyl Propyl Phosphate Anion. We shall adopt the same approach as for dimethyl phosphate anion. That is, we will discuss the structure and energetics first. Then we shall study the vibrational spectra of various conformers and suggest some characteristic frequencies that may be used in determining the conformations of this and related molecules.

A. Structure and Energetics. Since the above discussion of the dimethyl phosphate anion has clearly established that conformers which are *gg* about the phosphate moiety are the most stable, we shall initially focus our attention only on the remaining two torsions for the methyl propyl phosphate anion. In Sundaralingam's notation for lipid structures,[25] the torsions about the C–O and C–C bonds are denoted as α_1 and θ_1 as in III (or Sundaralingam's α_4 and α_5, if one views this species as a prototype for the choline in phosphatidylcholines—I). Each of the α_1 and θ_1 can adopt three likely values according to the Newman projection: *–sc* (for left-side syn-clinal), *+sc* (right-side syn-clinal), and *ap* (anti-periplanar). These correspond to torsional angles in the vicinities of ±60° and ±180°. (The eclipsed structures near ±120° will be termed *ac* for anti-clinal.[35]) Thus, there may be nine possible energy minima corresponding to these individual possibilities. We found seven[40] (denoted by circles in Figure 2, which summarizes the search strategy and results), while the other two (α_1, θ_1) arrangements (*+sc,–sc*) and (*–sc+sc*) are not structurally stable. Figure 3 illustrates the two conformers with lowest energies (**E** and **F**, (*–ac,–sc*) and (*ap,+sc*)).

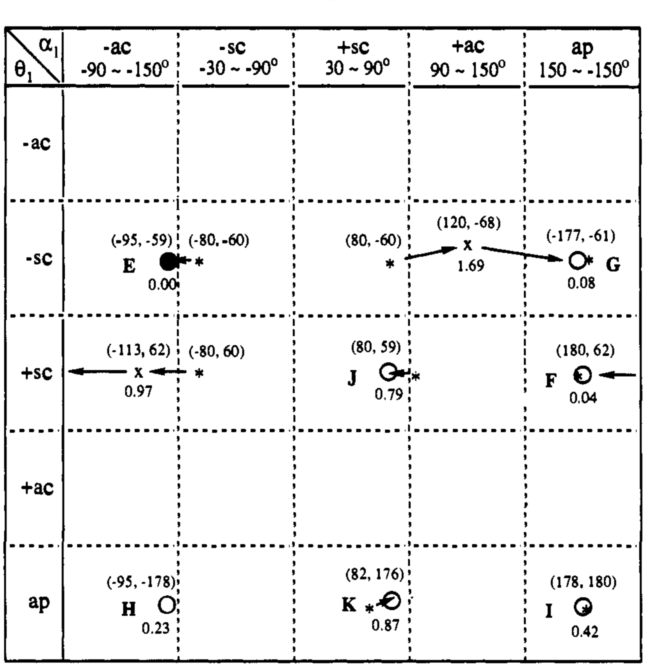

Figure 2. Values of (α, θ) at seven minima found for the methyl propyl phosphate anion obtained from HF/6-31G* method. Asterisks represent starting geometries, x stands for a selected point in the geometry optimization, circles denote energy minima, and the filled circle is the global minimum. Values in parentheses are coordinates, while relative energies are shown below the locations of the respective geometries.

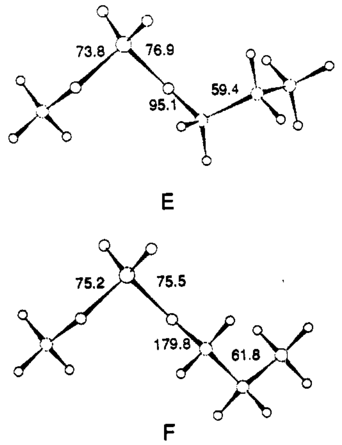

Figure 3. HF/6-31G* predicted geometries for the two lowest energy conformers of the methyl propyl phosphate anion.

All seven of the minimal energy structures we identified (*gg* about the phosphate) show bond lengths and bond angles very similar to those in the *gg* dimethyl phosphate anion, so they will not be enumerated in detail. Among the internal coordinates the two species have in common, individual bond lengths are within 0.002 Å and angles within 0.2°, except for the COP angle which varies from 118.6° to 121.1° between conformers. The only notable correlation among geometric parameters for methyl propyl phosphate is found for the CCO bond angle and the α_1 torsion: it is about 109° when α_1 is *ap* but about 111–113° for other values of α_1.

Figure 2 also shows the starting geometry of each optimization that crossed a grid boundary. Arrows are used to indicate the "path" of geometry optimization and "x" means a selected point in the optimization, usually with small gradients. Obviously, the geometry optimization crossed grids only horizontally, indicating that the α_1 angle has preferred ranges. Indeed, out of six possible conformers with α_1 being in *+sc* or *–sc*, only two of them are energy minima, and these exhibit the highest relative energies. The other four end up either in roughly the ±*ac* or the *ap* region (Figure 2). Experimental crystal structure determinations show an even stronger preference for α_1 (or α_4) in the ±*ac*/*ap* region.

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(39) Casal, H. L.; Mantsch, H. H.; Paltauf, F.; Hauser, H. *Biochim. Biophys. Acta* **1987**, *919*, 275.

(40) The α_2 angles in structures **J** and **K** are not gauche and are -96.0° and -91.2°, respectively.

Table X. HF/6-31G* Calculated Harmonic Vibrational Frequencies (in cm^{-1}) and IR Intensities (in Parentheses, in km/mol) of Important Vibrations in Different Conformations of the Methyl Propyl Phosphate Anion

	conformer						
	E	**F**	**G**	**H**	**I**	**J**	**K**
mode	($\alpha_1 = -ac$, $\theta_1 = -sc$)	($\alpha_1 = ap$, $\theta_1 = +sc$)	($\alpha_1 = ap$, $\theta_1 = -sc$)	($\alpha_1 = -ac$, $\theta_1 = ap$)	($\alpha_1 = ap$, $\theta_1 = ap$)	($\alpha_1 = +sc$, $\theta_1 = +sc$)	($\alpha_1 = +sc$, $\theta_1 = ap$)
P–O* asym stretch	1433 (304)	1437 (325)	1435 (350)	1432 (321)	1439 (190)	1431 (303)	1430 (347)
P–O* sym stretch	1204 (218)	1204 (245)	1203 (301)	1198 (328)	1202 (351)	1199 (235)	1199 (332)
C–O sym stretch	1255 (54)	1260 (73)	1262 (66)	1246 (46)	1251 (57)	1264 (60)	1255 (79)
C–O asym stretch	1234 (206)	1236 (174)	1236 (176)	1231 (179)	1237 (117)	1233 (220)	1232 (242)
C_2–C_3 stretch	1083 (52)	1090 (54)	1091 (54)	1107 (10)	1112 (7)	1082 (55)	1105 (15)
C_1–C_2 stretch	930 (8)	940 (7)	941 (7)	960 (8)	970 (12)	927 (13)	959 (10)
P–O asym stretch	799 (334)	803 (271)	803 (270)	805 (337)	861 (262)	799 (300)	801 (305)
P–O sym stretch	769 (151)	791 (103)	788 (88)	791 (100)	795 (228)	764 (166)	786 (138)
PO^*_2 rock	608 (43)	507 (24)	504 (26)	616 (32)	527 (32)	617 (34)	617 (25)
PO^*_2 sciss	579 (62)	583 (50)	585 (50)	579 (72)	585 (60)	535 (39)	551 (67)
PO^*_2 wag	488 (29)	613 (108)	612 (108)	539 (38)	606 (96)	475 (17)	571 (48)
PO^*_2 twist	314 (0)	400 (6)	401 (8)	473 (16)	435 (10)	324 (1)	455 (14)
PO_2 sciss	391 (2)	391 (3)	388 (2)	403 (5)	392 (0)	382 (1)	404 (3)
CCO bend	531 (12)	310 (2)	310 (2)	282 (4)	332 (9)	572 (49)	280 (4)
CCC bend	413 (9)	453 (10)	452 (8)	328 (3)	301 (5)	418 (10)	333 (6)
COP bend (M)[a]	258 (7)	256 (6)	255 (5)	247 (2)	254 (4)	263 (6)	248 (2)
COP bend (P)[b]	221 (7)	163 (0)	156 (1)	263 (6)	130 (1)	242 (5)	282 (5)
CCCO tors	124 (0)	175 (2)	181 (1)	94 (1)	171 (1)	131 (0)	92 (1)
COPO tors (M)[a]	90 (2)	91 (1)	96 (2)	100 (0)	93 (2)	94 (6)	99 (2)
COPO tors (P)[b]	56[c] (5)	64 (4)	62 (3)	46 (2)	57 (2)	68[c] (0)	31 (3)
CCOP tors	45[c] (2)	27 (2)	31 (1)	70 (5)	39 (1)	33[c] (3)	68 (1)

[a] Methyl side of the molecule. [b] Propyl side of the molecule. [c] Assignments for COPO and CCOP torsions may be interchanged.

Table XI. Diagnostic Frequencies (in cm^{-1}), Range, and Average IR Intensities (km/mol) for Determining the Conformations of Dialkyl Phosphate Groups, Based on HF/6-31G* Results for Methyl Propyl Phosphate

	E	**F**	**G**	**H**	**I**	**J**	**K**		
	α_1: $-ac$	ap	ap	$-ac$	ap	$+sc$	$+sc$		
mode	θ_1: $-sc$	$+sc$	$-sc$	ap	ap	$+sc$	ap	range	intensity[a]
C_2–C_3 stretch	1083	1090	1091	1107	1112	1082	1105	29	35
C_1–C_2 stretch	930	940	941	960	970	927	959	43	9
PO^*_2 rock	608	507	504	616	527	617	617	113	31
PO^*_2 wag	488	613	612	539	606	475	571	138	63
PO^*_2 twist	314	400	401	473	435	324	455	159	8
COP bend (P)[b]	221	163	156	263	130	242	282	152	4

[a] Averaged over all conformers. [b] Propyl side of the molecule.

Thus, published crystal structures of phospholipids indicate that *all* of the 21 (14) observed values for α_1 (α_4) are in the $\pm ac/ap$ region, suggesting that this preference of α_1 (or α_4) is enhanced by intermolecular forces in crystals. Extensive NMR studies[41,42] of different choline glycerophospholipids also agree with this conclusion.

In contrast to α_1, θ_1 shows little preference: all three likely ranges for θ_1 are found in the minimal energy structures. Each of the three lowest energy conformers have θ_1 values in the $\pm sc$ region, although *ap* is close in energy. This also parallels experiment. For example, out of the 24 observed values for θ_1 in crystal structures, 11 are in $\pm sc$, 12 in *ap*, and the remaining one is in syn-periplanar. It should be noted that the CCCO torsion θ_1 in the methyl propyl phosphate anion may not be a good model for the NCCO (α_5) torsion in phosphorylcholines or ethanolamines (I and III) which strongly prefer $\pm sc$ due to electrostatic attraction between the negatively charged $>PO_4^-$ and positively charged $-NR_3^+$ moieties of these phospholipids (all but one of the observed α_5 being in $\pm sc$, with the sole exception being *ac* but close to *sc*). Nevertheless, the corresponding torsion in the lowest energy conformers of the methyl propyl phosphate anion are also in $\pm sc$ as stated above, showing the same structural preference though to a much lesser extent.

In the literature, there are many NMR studies on the flexibility of the glycerol moiety of phospholipids. Most of them[43,44] conclude that the glycerol part is flexible and that there are two or three rotamers coexisting in the gel or aqueous phases. In contrast, a few NMR studies have concluded the glycerol moiety to be rigid,[45] and further interpretation of NMR results is difficult and may not be unique.[46] In this connection, we note that all seven computed energy minima are within 1.0 kcal/mol in energy, and in fact the two lowest energy conformers are virtually isoenergetic when Gibbs free energies at 298 K are considered (Table III). These results indicate that the glycerol moiety may, in fact, be flexible with a large number of conformations thermally accessible.

B. Vibrational Frequencies. The harmonic vibrational frequencies were determined for all seven energy minima using the HF/6-31G* approximation. Frequencies for some important normal modes are reported in Table X. For all seven structures, the P–O*, P–O, and C–O stretching frequencies[47] are similar to each other and to the corresponding ones of the *gg* conformers of the dimethyl phosphate anion, since all of them are *gg* about the phosphate group.[40]

Close examination of Table X shows that frequencies of the following vibrations depend markedly on the conformation: C_2–C_3 stretching, C_1–C_2 stretching, PO^*_2 rocking, wagging, twisting, COP bending (of the propyl side), and CCCO torsion. The six modes showing the greatest variation in frequency with conformer

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(47) The P–O asymmetric stretching frequency in conformer **I** is about 60 cm^{-1} higher than that in the others. This may be due to a lack of mixing of angular/torsional motions in the P–O stretching, since both α_1 and θ_1 are in the trans region.

are listed in Table XI. For instance, the COP bending on the propyl side has a frequency of 221–282 cm^{-1} when α_1 is in the *ac/sc* region but only 130–163 cm^{-1} for α_1 in *ap*. However, from the experimental point of view, some of these vibrations (PO$^*_2$ twisting, COP bending of the propyl side, and CCCO torsion) may not be very useful in discriminating among differing conformers of this species since they are computed to have either low frequency and/or low IR intensity (less than 10 km/mol) and therefore would be difficult to identify. The calculated IR intensity for PO$^*_2$ rocking is relatively large (25–43 km/mol), but the observed PO$^*_2$ rocking absorption in calcium phosphatidylserines[39] is actually rather weak. The only vibration that may be useful in determining α_1 seems to be, again, the PO$^*_2$ wagging since its frequency is appreciably higher when α_1 is in *ap* (606–613 cm^{-1}) than in *ac/sc* (475–571 cm^{-1}) and its calculated IR intensities are relatively strong (about 100 km/mol), particularly when α_1 is in the *ap* region. But there may be a problem in using the PO$^*_2$ wagging frequency to determine α_1 since configurations about the P–O bonds (i.e., α_2 and α_3) also affect the PO$^*_2$ wagging frequency as discussed above. Specifically, a PO$^*_2$ wagging frequency at about 610 cm^{-1} can be interpreted as either an indication of α_1 being anti-periplanar or an indication of a *gt* or *tt* (α_2, α_3) configuration about the P–O bonds (see Tables IV and V). Thus, the observed peak at 664 cm^{-1} for calcium phosphatidylserines[39] may also be understood as resulting from a structure with a *gg* configuration about the P–O bonds and an anti-periplanar configuration about one or both of the two C–OPO$_3^-$ bonds (i.e., α_1 and/or α_4). This explanation would imply that the structures for the lithium phosphatidylserines have an α_1 angle in the *sc/ac* region (anti-clinal preferred to syn-clinal as discussed above) since no absorption is observed around 665 cm^{-1} as would show up if α_1 were anti-periplanar.[39]

For determining the θ_1 angle (noting that in none of the seven structures is θ_1 in $\pm$*ac*, and therefore we need to determine only whether it is *sc* or *ap*), the C$_2$–C$_3$ stretching should be the most useful according to our results. Thus, structures with θ_1 being in the $\pm$*sc* region have significantly lower frequencies (1082–1091 cm^{-1}) for this vibration than those in the *ap* configuration (1105–1112 cm^{-1}). This is consistent with experimental results[48] that the C–N stretching frequency in phosphatidylcholines is lower for the OCCN torsion (α_5) being gauche than trans. This fact has been employed in many experimental studies[49] to determine α_5. It should be noted that the C$_1$–C$_2$ stretching frequency also depends on θ_1 (927–941 cm^{-1} for $\pm$*sc* and 959–970 cm^{-1} for *ap*), but its IR intensities are relatively low (computed to be below 13 km/mol).

Conclusions

We have found that diffuse functions are not very important in predicting the geometries, relative energies, and vibrational frequencies of the dimethyl phosphate and methyl propyl phosphate anions. The HF/6-31G* results, as are summarized below, show that most of the critical structural features of phospholipids observed in crystals, in the gel and liquid crystal phases, and in solution are reproduced by ab initio quantum mechanical calculations of these two model species.

(1) The preferred structure of the dimethyl phosphate anion has the gauche-gauche conformation, with the gauche-trans and the trans-trans conformers being about 1.2 and 2.8 kcal/mol, respectively, higher in energy. (The differences increase to 1.5 and 3.7 kcal/mol at the MP2/6-31+G*//HF/6-31+G* level of approximation.) This agrees with crystallographic studies that indicate most of nucleic acids and phospholipids are in this conformation. However, the barrier for going from gauche-gauche to gauche-trans is only about 1.7 kcal/mol, indicating that the gauche-trans conformer may be present in solution.

(2) There is strong and unusual coupling between the OPO and O*PO* bond angles and the COPO torsional angles of the dimethyl phosphate anion: both of the OPO, O*PO* bond angles decrease as the two COPO torsions go from gauche-gauche to gauche-trans to trans-trans. The trans-trans structure has only C_2 symmetry instead of C_{2v} as one might expect, probably due to repulsion between lone pairs on the esterified oxygens.

(3) The CCOP torsional angle in the methyl propyl phosphate anion prefers to be anti-periplanar or trans with the gauche conformations being less stable. On the other hand, the CCCO torsion only marginally favors gauche. Both observations are in good agreement with experiments on phospholipids. All seven conformers computed for this molecule are within 1.0 kcal/mol in energy, indicating that the glycerol moiety in phospholipids may be flexible.

(4) Although the PO$^*_2$ wagging frequency in IR spectra may be able to uniquely determine the conformation of the dimethyl phosphate anion, that is not the case for the methyl propyl phosphate anion or phospholipids, since its frequency depends on the torsions about the P–O as well as the O–C bonds. Thus, the IR spectrum for the calcium phosphatidylserines[38] may be alternatively interpreted as resulting from a gauche-gauche conformation about the phosphate and an anti-periplanar conformation about one or both of the two C–OPO$_3^-$ bonds, instead of from a gauche-trans or trans-trans configuration about the phosphate.

Finally, we emphasize that the two molecules studied here are among the simplest model compounds for phospholipids. Employing the methyl propyl phosphate anion to model glycerol phosphate (or phosphatidylcholine) neglects effects arising from acyl substitutions (or changing from a methyl terminal group to trimethylammonium). Nevertheless, it is very encouraging that such simplified models still seem able to reveal many of the important features in phospholipid structures. Further ab initio studies on more realistic model compounds for glycerol and head groups and on other parts of lipid molecules are underway to generate a reliable and detailed force field for such systems.

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