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^{31}Phosphorus NMR Chemical Shielding Tensors of L-O-Serine Phosphate and 3'-Cytidine Monophosphate

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ABSTRACT

^{31}P nuclear magnetic resonance chemical shielding tensors have been measured from single crystals of L-O-serine phosphate and 3'-cytidine monophosphate. The principal elements of the shielding tensors are -48, -2, and 51 ppm for serine phosphate and -68, -13, and 64 ppm for 3'-cytidine monophosphate, relative to 85% H_3PO_4. In both cases four orientations of the shielding tensor on the molecule are possible; in both instances one orientation correlates well with the P-O bond directions. This orientation of the shielding tensor places the most downfield component of the tensor in the plane containing the two longest P-O bonds and the most upfield component of the shielding tensor in the plane containing the two shortest P-O bonds. A similar orientation was reported for the ^{31}P shielding tensor of phosphorylethanolamine (Kohler and Klein, Biochemistry, 15, 967 (1976)) and a comparison is made between the three molecules.

INTRODUCTION

The recently-developed high resolution multiple pulse and cross-polarization nuclear magnetic resonance (NMR) techniques have made it possible to resolve chemical shifts in solids, and therefore to accurately determine chemical shielding tensors.[1,2] Shielding tensors are intrinsically interesting in that they reflect the distribution of electronic orbitals around the nucleus. Several theoretical approaches have been used with varying degrees of success to predict shielding tensors.[3,4] On a less theoretical level, shielding tensors serve as a monitor of the chemical environment of the nucleus, and are useful in the analysis of anisotropic motion.[5-11] If the pertinent shielding tensor information is available, NMR spectra of anisotropic systems may be interpreted in terms of the modes of motion experienced by the molecule.

With a view toward the importance of phosphates in biological systems, especially nucleic acids and the phospholipid components of membranes, we have undertaken a study of the chemical shielding tensors of relevant organophosphates. This work reports the ^{31}P chemical shielding tensors of two such compounds, L-O-serine phosphate ($H_3N^+CH(COOH)CH_2OPO_2^-OH$) and 3'-cytidine monophosphate (3'-CMP), and compares them to the previously-determined ^{31}P shielding tensor of phosphorylethanolamine ($H_3N^+CH_2CH_2OPO_2^-OH$).[12]

EXPERIMENTAL TECHNIQUES AND SAMPLES

Techniques. The spectrometer used for the ^{31}P NMR experiments is a home-built double resonance instrument described in detail elsewhere.[12,13] The spectrometer operates at 24.3 MHz for ^{31}P detection, 60 MHz for ^{1}H decoupling, and is equipped with a 9.21 MHz ^{2}H field-frequency lock.

The ^{31}P Fourier transform spectra were taken using the cross-polarization nuclear enhancement technique, as described previously.[2,12] This technique simultaneously removes the ^{1}H dipolar broadening and enhances the ^{31}P signal by a factor of approximately two. The single contact version of this experiment was used, with a cross-polarization time of 1 msec and an ^{1}H decoupling field of approximately 8.6 gauss in the rotating reference frame. Except for the 3'-CMP single crystal experiments, quadrature phase detection was employed.

The shielding tensors for each of the two molecules were determined from plots of resonance frequency as a function of rotation angle for three different orientations of the single crystal samples. The methods of sample orientation and data analysis were identical with those previously described.[13] The values of the principal elements of the shielding tensors obtained from powder spectra were determined by computer simulation and comparison with the observed spectra.

Samples. Single crystals of serine phosphate were grown at room temperature by slow evaporation from an aqueous solution originally containing 5 g serine phosphate in 175 ml distilled water. The crystals were harvested after 4 months. A crystal measuring 5 x 4 x 3 mm was used for the NMR experiments. The Laue precession method was used to measure the unit cell dimensions, which were a = 7.70, b = 9.97, c = 9.02 Å in agreement with the literature values of a = 7.737, b = 10.167, and c = 9.136 Å.[14]

3'-CMP crystals were grown by slow evaporation from aqueous solution at room temperature. The solution originally contained 1.0 g 3'-CMP in 50 ml distilled water; a seed crystal

was suspended in the solution to enhance crystallization. After 18 days the tabular crystals were harvested. The crystal used in the NMR experiments measured approximately 1.4 x 3 x 0.5 mm. The measured unit cell dimensions were a = 8.75, b = 21.4, c = 6.82 Å, in agreement with the values of a = 8.80, b = 21.7, c = 6.85 Å reported by Alver and Furberg.[15]

RESULTS

Serine Phosphate. A ^{31}P NMR spectrum taken of a powder of serine phosphate appears in Figure 1. The spectrum has the shape characteristically observed for a non-axial shielding tensor, and the values measured for the three principal elements of the tensor are given in Table I. Figure 1 also shows a spectrum taken of a single crystal of serine phosphate oriented with its b^* axis perpendicular to the magnetic field. The orthorhombic symmetry (space group $P2_12_12_1$) and presence of one serine phosphate molecule per asymmetric unit lead to a prediction that a NMR spectrum of an arbitrarily oriented crystal would have four lines; the reduction to two lines in this case is the consequence of the alignment of the crystal along a symmetry axis.

Rotation plots were generated from single crystal spectra taken as a function of sample orientation in the magnetic field. In each case the crystal was rotated about a symmetry axis, producing spectra with two well-resolved lines and obviating the problems associated with the resolution of four resonances. Analysis of the rotation data produced four possible shielding tensors, having the same principal values and related spatially by the symmetry operations of the unit cell of the crystal. The principal values are listed in Table I, and the possible orien-

tations of the tensors on the molecule are summarized in Table II. It is not possible on the basis of the NMR experiment alone to determine which is the proper tensor orientation, however for reasons to be presented in the discussion, it is judged that the orientation shown in Figure 2 (Orientation 1 of Table II) is the proper choice.

TABLE II

FIG. 2

3'-Cytidine Monophosphate. Spectra of powder and single crystalline samples of 3'-CMP are in general similar to those of serine phosphate. Again, the shielding tensor is non-axial; the principal values measured from a powder spectrum are contained in Table I. As was the case for serine phosphate, 3'-CMP crystals are orthorhombic (space group $P2_12_12$) and contain one molecule per asymmetric unit.[15] Therefore four symmetry-related shielding tensors were obtained from the analysis of the single crystal data. The principal elements of the tensors are in Table I, and the possible orientations of the tensors on the phosphate molecule are given in Table II. Figure 3 diagrams the most probable orientation of the tensor (Orientation 1 in Table II), as explained in the discussion.

FIG. 3

DISCUSSION

The problem of matching the symmetry-related shielding tensors with the symmetry-related molecules in the unit cell of the crystal cannot be solved directly by NMR experiments,[4] and recourse must be made to other information. In the case of the monoclinic phosphorylethanolamine (space group $P2_1/c$), there were only two possible tensor assignments, one that showed an obvious correlation with the bond directions, and one that did not.[12] The orientation which correlated with the bond

directions was therefore assumed to be the proper choice. For convenience, this orientation is repeated here in Figure 4, and both possible tensor orientations are listed in Table II.

FIG. 4

Serine phosphate and phosphorylethanolamine are analogous compounds having the structure $ROPO_2OH$. One would therefore expect that these compounds should have similar orientations of their ^{31}P chemical shielding tensors. As Figures 2 and 4 and the data in Table II indicate, orientation 1 of the serine phosphate shielding tensor is similar to the phosphorylethanolamine shielding tensor orientation assumed to be correct (Orientation 1 in Table II). Therefore the orientation diagrammed in Figure 2 is assumed correct in conformity with the phosphorylethanolamine results.

The question of the 3'-CMP ^{31}P chemical shielding tensor orientation is not quite as readily answered because the $ROPO(OH)_2$ structure of 3'-CMP is not strictly analogous to the $ROPO_2OH$ structures of serine phosphate and phosphorylethanolamine. However if one consistently chooses a molecular reference frame such that the Z axis is perpendicular to the two shortest P-O bonds and the X axis is their bisector, then it is immediately obvious that an orientation similar to orientation 1 for the phosphorylethanolamine shielding tensor may be found in all cases (Orientations 1 in Table II). Therefore, in keeping with the phosphorylethanolamine and serine phosphate results, the orientation shown in Figure 3 is assumed to be the proper orientation of the ^{31}P shielding tensor in 3'-CMP.

In all three cases the most downfield component of the

shielding tensor is approximately in the plane of the two longest P-O bonds (low electron density bonds) and the most upfield component of the shielding tensor is approximately in the plane of the two shortest P-O bonds (high electron density bonds). Recently this was also found to be the orientation for the shielding tensor of a phosphodiester, barium diethyl phosphate,[17] as had been previously predicted.[12] Attempts to calculate the ^{31}P chemical shielding tensors of organophosphates have not been satisfactory,[18] yet the collection of empirical data from molecules of the structures $ROPO_2OH$, $ROPO(OH)_2$, and $(RO)_2PO_2$ seems to indicate that as a first-order approximation the shielding tensors of the organophosphates are aligned with the planes containing the P-O bonds. The deviations from this alignment and the variations of tensor orientation seen from molecule to molecule indicate that the ^{31}P shielding tensors are sensitive to more subtle environmental effects as well as to the P-O bond distribution, and in fact similar sensitivities have been reported for ^{13}C [19-21] and ^{19}F [7,22] chemical shielding tensors.

ACKNOWLEDGEMENTS

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REFERENCES

1. J.S. Waugh, L.M. Huber, and U. Haeberlen, Phys. Rev. Let., 22, 180-181 (1968).

2. A. Pines, M.G. Gibby, and J.S. Waugh, J. Chem. Phys., 59 569-590 (1973).

3. U. Haeberlen, "High Resolution NMR in Solids: Selective Averaging," Academic Press, New York, N.Y., 1976, p 162.

4. M. Mehring, "High Resolution NMR Spectroscopy in Solids," Springer Verlag, New York, N.Y., 1976, ch 5.

5. S.J. Kohler and M.P. Klein, Biochemistry, 16, 519-526 (1977).

6. M. Mehring, R.G. Griffin, and J.S. Waugh, J. Chem. Phys., 55, 746-755 (1971).

7. R.G. Griffin, J.D. Ellett, Jr., M. Mehring, J. G. Bullitt, and J.S. Waugh, J. Chem. Phys., 57, 2147-2155 (1972).

8. A. Pines, M.G. Gibby, and J.S. Waugh, Chem. Phys. Lett., 15, 373-376 (1972).

9. H. W. Spiess, Chem. Phys., 6, 217-225 (1974).

10. H.W. Spiess, R. Grosescu, and U. Haeberlen, Chem. Phys., 6, 226-234 (1974).

11. J. Seelig and H-U. Gally, Biochemistry, 15, 5199-5208 (1976).

12. S.J. Kohler and M.P. Klein, Biochemistry, 15, 967-971 (1976).

13. S.J. Kohler, J.D. Ellett, Jr., and M.P. Klein, J. Chem. Phys., 64, 4451-4458 (1976).

14. M. Sundaralingam and E.F. Putkey, Acta Cryst., B26, 790-800 (1970).

15. E. Alver and S. Furberg, Acta Chemica Scand., 13, 910-924 (1959).

16. J. Kraut, Acta Cryst., 14, 1146-1152 (1961).

17. R.G. Griffin, to be published.

18. S.J. Kohler, unpublished results.

19. R.G. Griffin, A. Pines, S. Pausak, and J.S. Waugh, J. Chem. Phys., 63, 1267-1271 (1975).

20. R.G. Griffin and D.J. Ruben, J. Chem. Phys., 63, 1272-1275 (1975).

21. A. Pines, J.J. Chang, and R.G. Griffin, J. Chem. Phys., 61, 1021-1030 (1974).

22. R.G. Griffin, H-N. Yeung, M.D. LaPrade, and J.S. Waugh, J. Chem. Phys., 59, 777-783 (1973).

Figure Captions

Figure 1. 24.3 MHz NMR spectra of L-O-serine phosphate. (a) Spectrum of a powder of serine phosphate obtained from a cross-polarization experiment with ^1H decoupling. 420 passes were accumulated using a cross-polarization time of 1 msec. The scale is in ppm relative to 85% H_3PO_4 and assumes resonances at lower field strengths have negative chemical shifts. (b) Spectrum from a cross-polarization experiment with ^1H decoupling of a single crystal of serine phosphate oriented with its b* axis perpendicular to the magnetic field. 400 passes were accumulated. The better signal to noise ratio of the powder spectrum was due to the larger sample size used.

Figure 2. Chemical shielding tensor of L-O-serine phosphate. The most probable orientation of the serine phosphate shielding tensor (Orientation 1 of Table II) is shown in three orthogonal projections of the phosphate region of the molecule. The shielding tensor is shown as an ellipsoid with the most downfield component of the shielding tensor represented by the shortest ellipsoid axis. The numbering of the atoms corresponds to that used in the x-ray crystallographic work.[14]

Figure 3. Chemical shielding tensor of 3'-CMP. The most probable orientation of the 3'-CMP shielding tensor

(Orientation 1 in Table II) is shown in three orthogonal projections of the phosphate region of the molecule. The shielding tensor is shown as an ellipsoid with the shortest axis representing the most downfield element of the shielding tensor. The numbering of the atoms corresponds to that used in the x-ray crystallographic study.[15]

Figure 4. Chemical shielding tensor of phosphorylethanolamine.[12] The most probable orientation of the phosphorylethanolamine shielding tensor (Orientation 1 of Table II) is shown in three orthogonal projections of the phosphate region of the molecule. The shielding tensor is represented as an ellipsoid with the shortest ellipsoid axis corresponding to the most downfield element of the shielding tensor. The numbering of the atoms corresponds to that used in the x-ray crystallographic study.[16]

Table I. Values of the principal elements of the chemical shielding tensors.[a]

	Powder[b]			Single Crystal[c]		
	σ_{11}	σ_{22}	σ_{33}	σ_{11}	σ_{22}	σ_{33}
Serine phosphate	-53	-4	59	-48	-2	51
3'-CMP	-63	-9	71	-68	-13	64
Phosphoryl-ethanolamine[d]	-63	-8	65	-67	-13	69

[a]All values are in ppm relative to 85% H_3PO_4, using the convention that resonances at lower field strengths have negative chemical shifts.

[b]The powder values were determined by matching computer-simulated spectra with the empirical spectra; uncertainties are estimated to be approximately ± 5 ppm.

[c]These are the simple numerical averages of the values from the individual symmetry-related tensors. The standard deviations were less than 4 ppm for the serine phosphate values and less than 6 ppm for the 3'-CMP values.

[d]Data from reference 12.

Table II. Direction cosines of the principal axes of the tensors relative to a molecular reference frame[a]

		Orientation 1			Orientation 2			Orientation 3			Orientation 4		
		x	y	z	x	y	z	x	y	z	x	y	z
Serine Phosphate	σ_{11}	-.1533	.0706	.9857	-.9553	.2720	-.1161	.8262	.4535	-.3341	.2821	-.7958	-.5358
	σ_{22}	.9652	-.2032	.1647	.0856	-.1217	-.9889	-.2053	.7947	.5712	-.8456	-.4701	.2529
	σ_{33}	.2119	.9766	-.0370	-.2831	-.9546	.0930	.5246	-.4034	.7497	-.4532	.3817	-.8056
3'-CMP	σ_{11}	-.1246	-.0368	.9915	-.9619	-.1936	-.1929	.6793	-.6012	-.4209	.4068	.8317	-.3780
	σ_{22}	.9760	.1752	.1291	.1824	.0706	-.9807	-.4386	-.7924	.4240	-.7191	.5467	.4290
	σ_{33}	-.1785	.9838	.0141	.2035	-.9785	-.0325	-.5884	-.1034	-.8019	.5634	.0973	.8204
Phosphoryl-ethanol-amine	σ_{11}	.0038	-.0746	.9972	-.9384	.3446	-.0235						
	σ_{22}	.9872	.1593	.0081	-.1270	-.4074	-.9044						
	σ_{33}	-.1595	.9844	.0743	-.3212	-.8457	.4261						

[a]In each case, the axis system was chosen such that the Z axis is the normal to the molecular plane containing the two shortest P-O bonds, and the X axis is their bisector. The direction cosines were calculated from the average values of the Euler angles of the equivalent tensor orientations for each molecule. These equivalent orientations were obtained by matching the symmetry-related shielding tensors with the symmetry-related molecules in the unit cell of the crystal. The standard deviations of the Euler angles were 4° or less for serine phosphate and phosphoryl-ethanolamine and 9° or less for 3'-CMP.

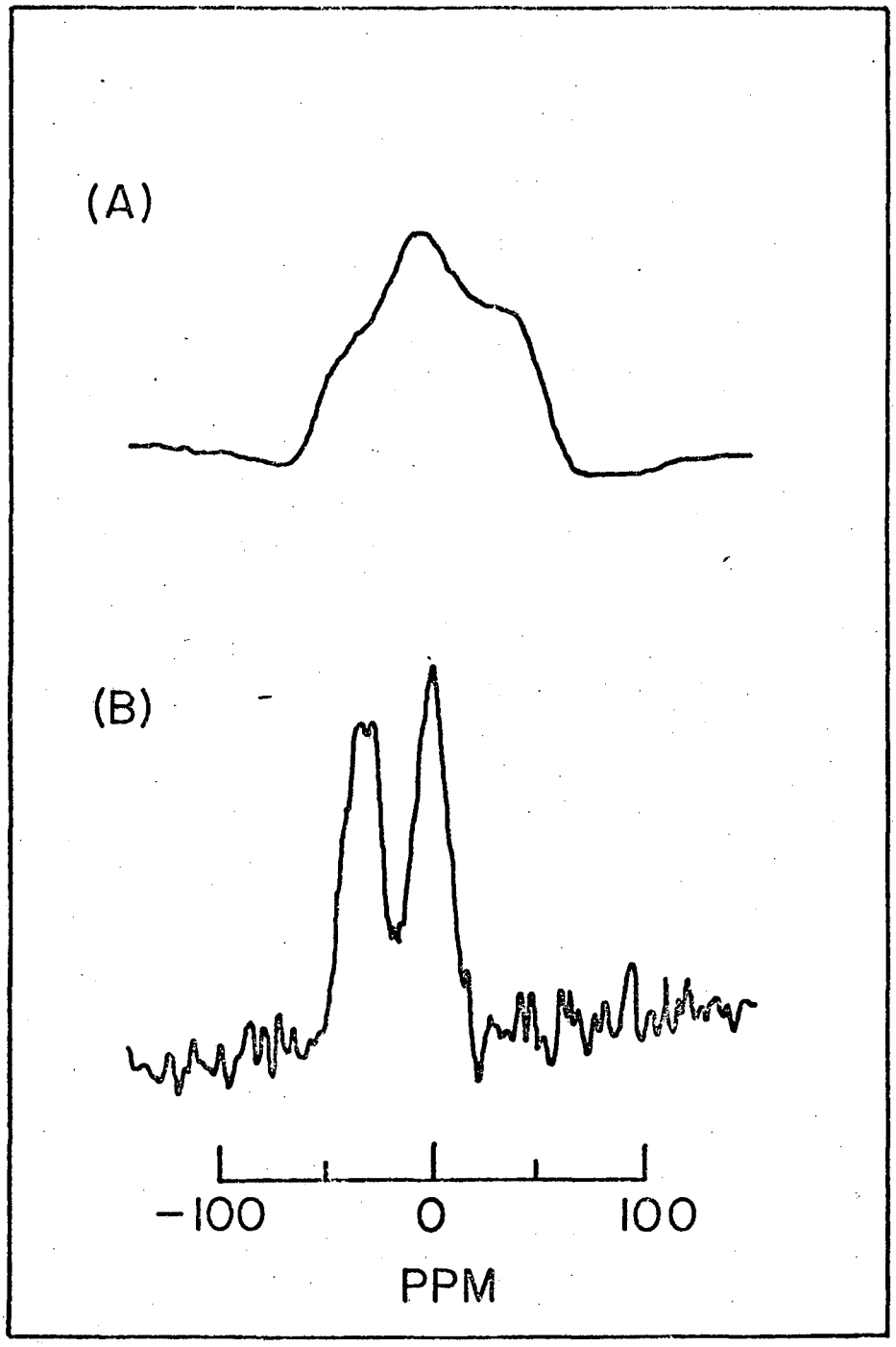

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FIGURE 1
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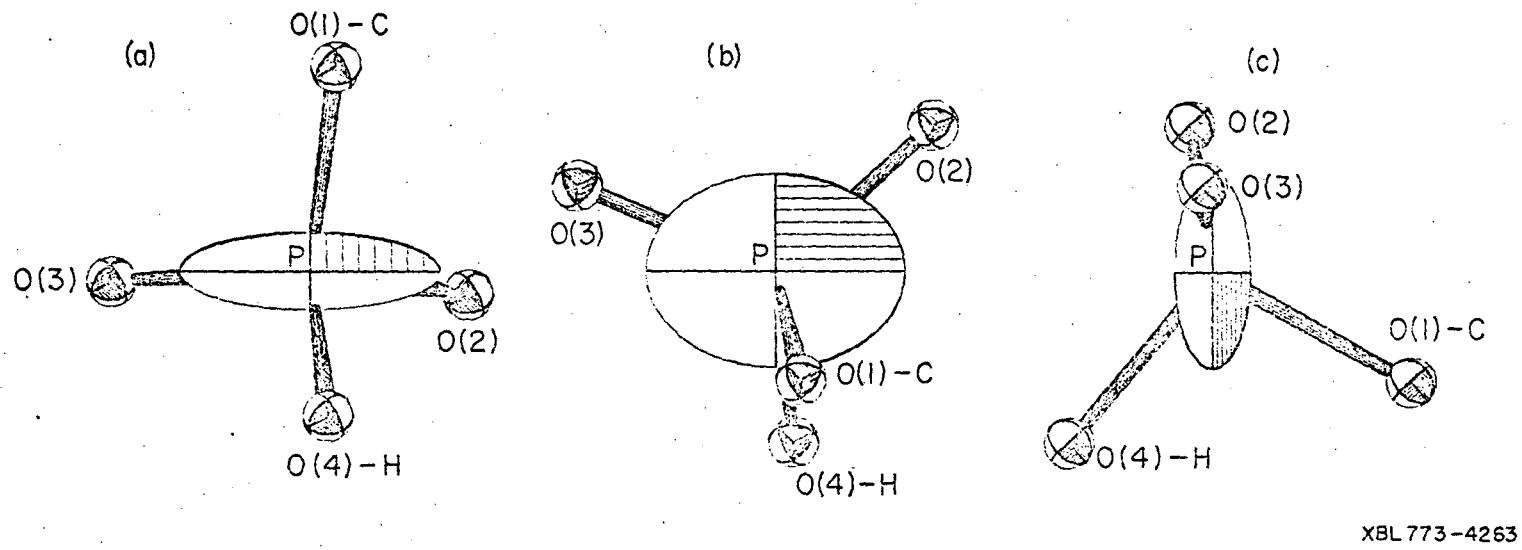

FIGURE 2
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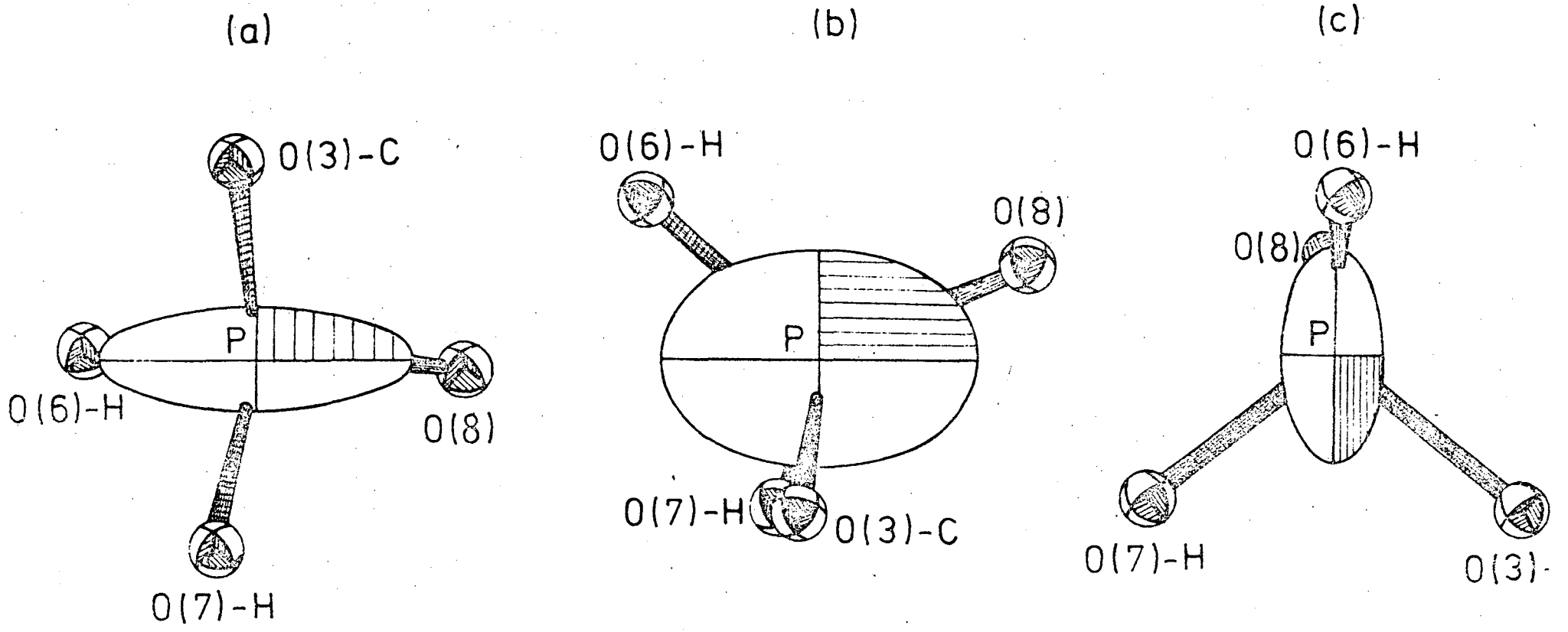

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FIGURE 3

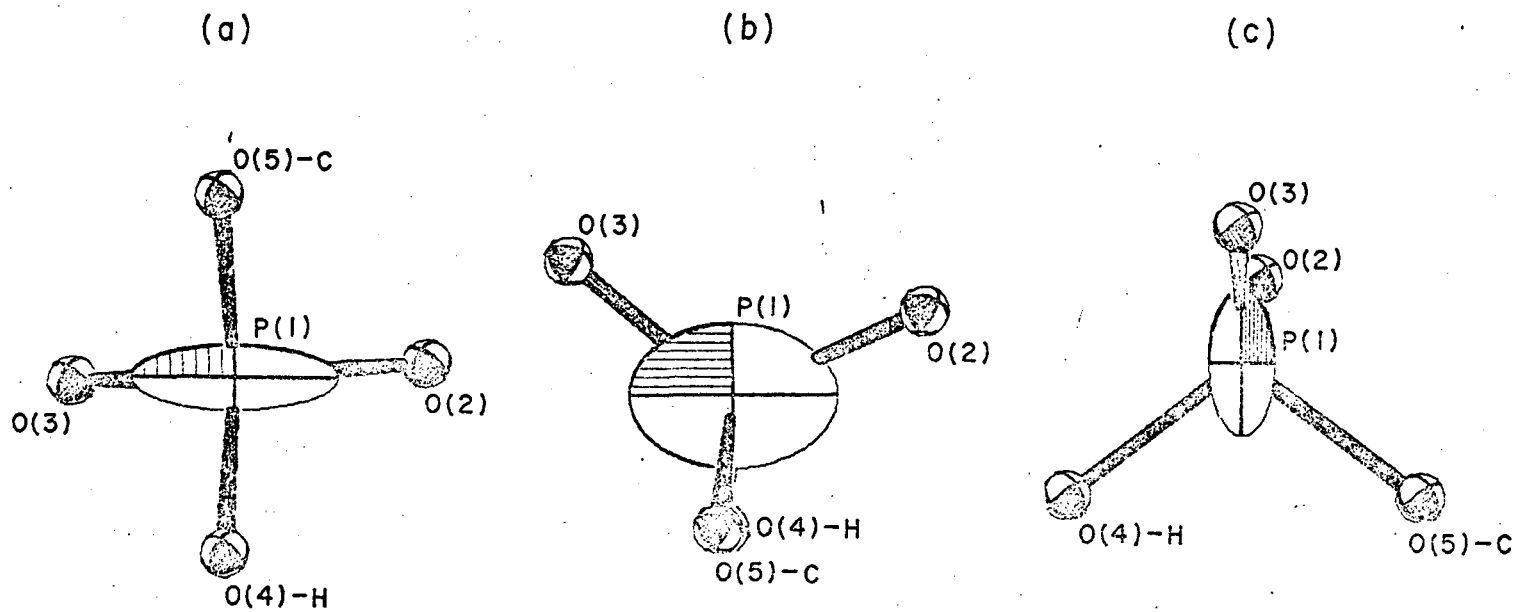

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FIGURE 4

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