^{31}P CHEMICAL SHIFTS IN PHOSPHATE DIESTER MONOANIONS. BOND ANGLE AND TORSIONAL ANGLE EFFECTS.

David G. Gorenstein[†] and Debojyoti Kar

Department of Chemistry, University of Illinois, Chicago Circle
Chicago, Illinois 60680

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Summary

The ^{31}P chemical shifts of phosphate diester monoanions are correlated with the phosphorus total electron densities calculated by the CNDO/2 molecular orbital method. The large downfield chemical shift of cyclic five-membered ring phosphate diesters is associated with both a decrease in the O-P-O bond angle and an eclipsing of the phosphate ester bonds. The upfield shift of cyclic six-membered ring diesters is associated with a torsional angle effect only. A chemical shift-torsional angle contour map has been created and shown to explain ^{31}P chemical shifts in oligo- and polynucleic acids.

INTRODUCTION

We have recently described an empirical correlation between ^{31}P chemical shifts and O-P-O bond angles in phosphate esters.[1] In pursuing the theoretical foundation for this bond angle effect we have observed that large ^{31}P shifts are apparently associated with rotation about the phosphate ester bond as well. We wish to describe in this communication some semi-empirical quantum mechanical calculations which reveal the structural basis for the variation in the ^{31}P chemical shifts of phosphate diester monoanions. This particular class of phosphate esters has aroused considerable theoretical interest since they are represented by the polynucleic acids.[2,3]

Any successful theory of ^{31}P chemical shifts in phosphate esters must account for the large downfield shift of cyclic five-membered ring diesters and the upfield shift of six-membered ring diesters. Thus, the ^{31}P chemical shift of the five-membered ring diester, 2',3'-cyclic cytidine monophosphate (2',3'-cCMP) is -20.3ppm relative to 85% phosphoric acid and that of the

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[†] Fellow of the Alfred P. Sloan Foundation.

six-membered ring diester, 3',5'-cyclic adenosine monophosphate (3',5'-cAMP) is +2.6ppm.[1] The chemical shift of acyclic diester monoanions are typically 0 to 1ppm.[1]

In an extensive series of quantum-mechanical calculations, Letcher and Van Wazer[4] have concluded that ligand electronegativity dominates ^{31}P chemical shifts. Another theoretical study of ^{31}P shifts in phosphoryl compounds by Purdela[5] has provided evidence for an X-P-X bond angle correlation. We wish to demonstrate that in phosphate esters ^{31}P chemical shifts are influenced by changes in both O-P-O bond angles and phosphate torsional angles.

METHODS

The molecular parameters for the cyclic diester monoanions used in our calculations were obtained from the X-ray structures of 2',3'-cyclic cytidine monophosphates (cCMP)[6], 3',5'-cyclic uridine monophosphate (cUMP)[7a], and 3',5'-cyclic guanosine monophosphate (cGMP).[7b] However, we have retained only the ribose and phosphate ester portion of the structure, substituting a hydroxyl group for the nucleotide base. For the acyclic diesters a dimethyl phosphate in the lowest energy, gauche, gauche ($\omega = \omega' = 64°$) conformation[8] has been assumed. This conformation is 2-4 kcal/mol more stable than any other potential minimum conformation and is likely the predominant species in solution.[2,3] The self-consistent field, LCAO molecular orbital (MO) calculations employed the CNDO/2 option of the semi-empirical, quantum mechanical program CNINDO/2.[9] Only valence electrons were considered and for phosphorus, 3d orbitals were included in the basis set.

RESULTS AND DISCUSSION

Total phosphorus electron densities, P_{AA}, were calculated for the four diester monoanions using the CNDO/2 program, and are reported in Table I. Unfortunately, we have only these four different structure to provide the basis for our correlation of electron densities and ^{31}P chemical shifts which may be related by eq 1:

$$\delta_{calc}\ (\mathrm{ppm}) = 1703.1\ P_{AA} - 7882.9 \qquad (1)$$

In using calculated electron densities we are not claiming that these shifts are dominated by a diamagnetic term.[10] As pointed out by Van Wazer[4] and others[11] the diamagnetic screening contribution should be small in comparison to a paramagnetic term. However, electron density changes can

Table I.

Compound	O-P-O Angle	Dihedral Angles ω,ω'(°)	[12] P_{AA}	Chemical Shifts[a]	
				δ_{obs}	δ^{b}_{calc}
2',3'-cCMP[c]	95.7	25.6,-21.2	4.6166	-20.3	-20.3
2',3'-cCMP[c,d]	96.0	28.2,-25.7	4.6214	---	-12.2
3',5'-cUMP[c]	102.7	50.1,-54.2	4.6308	2.6	3.8
3',5'-cUMP[c]	103.1	48.8,-50.4	4.6293	2.6	1.3
3',5'-cGMP[c]	103.9	44.2,-44.3	4.6303	2.6	3.0
Dimethyl Phosphate	105	64,64	4.6292	0.5	1.1
	105	60,60	4.6282		-0.6
	105	60,180	4.6227		-10.0
	105	180,180	4.6127		-27.0
	105	0,180	4.6162		-21.0
	105	60,-60	4.6299		2.3
Dimethyl Phosphate	95	60,60	4.6245		-6.9
	95	60,180	4.6240		-7.8
	95	180,180	4.6198		-14.9
	95	0,180	4.6164		-20.7

a. Chemical shifts in ppm from 85% H_3PO_4 (see ref. 1).
b. Chemical shifts calculated from eq 1.
c. Only the ribose phosphate portion of the molecule was considered.
d. This second molecule found in the asymmetric unit is highly strained (ca. 10 kcal/mol) with an unusual, planar ribose conformation. It would not be likely that this structure and the electron densities accurately reflected those in solution.

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influence the paramagnetic shielding term as well by altering the effective radius of the p and d orbitals.[12,13] Chemical shifts of other non-hydrogen nuclei[12] have similarly been shown to correlate satisfactorily with calculated electron densities.

While additional structural data on different phosphate diester monoanions is certainly desired to better establish the relationship we have proposed, it is significant that structural modifications on the dimethyl phosphate molecule appear to accurately reflect the chemical shift trends observed for the two cyclic diesters. Electron densities and calculated chemical shifts for several different conformations and bond angles for dimethyl phosphate are listed in Table I. Rather perversely, the data in this table do not support on first consideration our original empirical correlation of chemical shifts and O-P-O bond angles. Thus we note that a reduction of the diester O-P-O bond angle in dimethyl phosphate from 105° to 95° (keeping a g,g conformation) results in only a 6-7 ppm downfield shift with decreasing bond angles. (Note that the t,t conformation shows a 12ppm upfield shift in going from the 105° to 95° ester!) It is difficult to assess the importance of torsional angle changes in these shifts since steric interactions of the methyl groups preclude a MO calculation in the eclipsed, cis conformation of dimethyl phosphate, which would be analogous to the geometry of the five-membered ring diester. Possibly, other allowed eclipsed conformations (cis, trans and trans,trans) might be expected to show similar electron density trends. However, we have recently noted[3] that O-P-O bond angle distortions are coupled to changes in torsional angles. (Eclipsing of even one phosphate ester bond results in a reduction of 5-7° in the RO-P-OR' bond angle.[3]) Thus, any attempt to separate the chemical shift changes into a mix of bond angle and torsional angle effects will prove to be quite meaningless. Note though that the calculated chemical shifts of dimethyl phosphates with O-P-O bond angles of either 95° or 105° in a cis, trans conformation are both nearly the same as for 2',3'-cCMP.

Perhaps the best way of understanding the origin of the downfield shift in five-membered cyclic esters is that the ring strain **implied** by the eclipsed conformation is partially relieved by a reduction of the O-P-O bond angle and this coupled bond and torsional angle effect is responsible for the low electron density on phosphorus (and hence the downfield shift).

Torsional effects alone serve to explain the 2-3ppm upfield shift of the six-membered ring cyclic phosphates. Thus, a dimethyl phosphate (105°) with a 60°,-60° conformation shows a chemical shift nearly the same as that of the cyclic ester.

Carrying out MO and chemical shift calculations on dimethyl phosphates possessing other torsional geometries, we have been able to create a "chemical shift" contour map shown in Figure 1. Because of the limited structural data available to us which was used in defining eq 1, the possible errors in such a map may be substantial. At the very least, however, it is quite likely that relative shifts may be reasonably obtained from this diagram. The utility of a chemical shift-torsional angle contour map is demonstrated by its applicability to the definition of the solution structure of oligo- and polynucleic acids. Although we have previously interpreted the ^{31}P chemical shifts reported by Patel[14] of an actinomycin D- double helical, hexadeoxyribonucleotide dimer as arising from a change in O-P-O bond angles, an alternative (and likely equivalent) explanation based upon torsional angle changes is also possible.

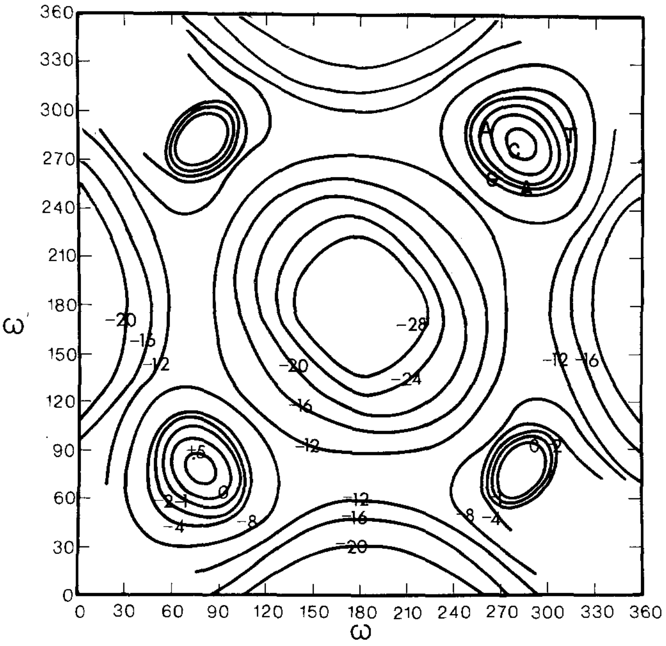

Figure 1: ^{31}P chemical shift-torsional angle contour map of dimethyl phosphate monoanion. "Isoshift" contours are in ppm from 85% H_3PO_4 as calculated by eq. 1. Letters, A, C, T, and G locate the conformations of the 3'-nucleotide residues deoxyadenosine, deoxycytidine, thymidine and deoxyguanosine respectively in the Jain and Sobell model of the d-(ApTpGpCpApT) duplex.

Jain and Sobell[15] have proposed a model for this intercalated complex involving partial unwinding of a specific section of the double helix. Included in the chemical shift-torsional angle contour map are the proposed dihedral angles in this model for the nucleic acid residues. Note that these are "unusual" conformations (normally the double helix torsional angles fall within a narrow, well-defined, -g,-g region[2,3]). The ^{31}P spectrum, showing individual signals at -2.2, -1.2, and -.6ppm and two large broad signals at +0.4 and +.7ppm is qualitatively consistent with our proposed shifts.

The ^{31}P spectrum of large polynucleotides also provides significant structural information. As shown in Figure 2, the broad envelop of ^{31}P signals between 0 and +1ppm for phenylalanine transfer polyribonucleic acid would be indicative of the large amount of double helical structure[16] in these molecules (i.e., -g,-g conformation). Most significantly we observe some fine structure in this spectrum and a few resonances downfield from the main peaks. These different signals must represent phosphate residues in various torsional conformations. More recently[17] (spectra not shown) we have observed a single

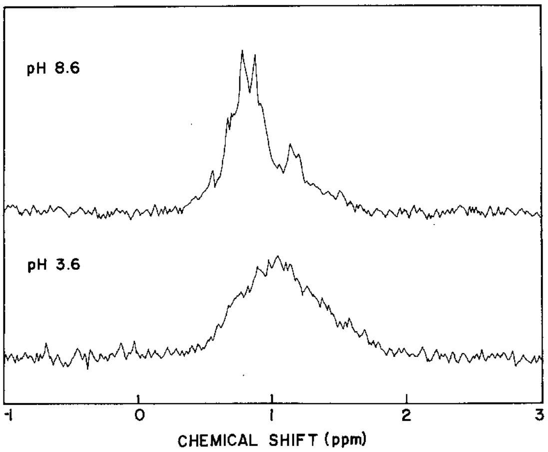

Figure 2: ^{31}P Fourier transform, (^{1}H decoupled) nmr spectra of phenylalanine transfer-RNA. 2x Dialyzed against H_2O. 2mM EDTA, .2 M NaCl, 20 mg/ml in H_2O. (upper) pH 8.6, 31,000 transients. (lower) pH 3.6, 16,000 transients. Spectrometer frequency 36.43 MHz.

phosphate signal 3-5 ppm downfield from the main envelop which titrates with pH and which must be the single 5'-terminal monoester phosphate. We also see ca. 6 individual peaks 20-21ppm downfield which we believe may represent the phosphates in the more open conformations presumably responsible for the hairpin loops in these complex, folded structures.[16] Gueron[18] has also reported fine structure in the ^{31}P spectra of t-RNA's (but only in the "expected" helical region).

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