Ab Initio Quantum Mechanical Calculations of the Magnetic Shielding Tensor of Phosphorus-31 of the Phosphate Group

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Abstract: The ^{31}P magnetic shielding tensor is calculated by an ab initio CHF method for the gauche-gauche and gauche-trans conformations of the dimethyl phosphate anion taken as a model of the phosphate group of nucleic acids and of the polar head of phospholipids. The results show that the geometry is more important than the conformation for the value of the chemical shift of ^{31}P. The calculated orientation of the principal values of the shielding tensor is found to agree with the experimental determinations. The role of the conformation, of hydration, and of the state of ionization on the value of the ^{31}P chemical shift and on the anisotropy of the tensor is discussed in relation with available experimental data.

Introduction

^{31}P nuclear magnetic resonance appears to be a very powerful tool for the study of the backbone of nucleic acids and of the polar head of phospholipids, in solution. Experimental studies on nucleoside phosphates, polynucleotides, and nucleic acids in solution have shown that the ^{31}P magnetic screening constant is influenced by the chemical structure of the substituents bound to the ester oxygens,[1-4] by the ionization state of the phosphate group,[2,3,5-15] by the complexation of metal ions,[11,16-21] and by intermolecular interactions.[9,10,13,14,18,21-23] Similar results have been obtained for the phosphate group of phospholipids.[12,24] However, measurements made on di- and polynucleotides[25,28] as well as on nucleic acids[8,29] as a function of temperature have shown that a very important factor in the determination of ^{31}P chemical shift is the conformation of the phosphate group, namely, the value of the rotation angles (ω and ω') about the P–O ester bonds. The role of the variations of ω and ω' on the value of the phosphorus screening constant is also observed in the study of the complexation of di-[26,30] and polynucleotides[6,28] with intercalating drugs. This dependence of ^{31}P chemical shift is of particular interest since the determination of the value of ω and ω' in solution from spin-spin coupling constants ($^3J_{\mathrm{OPOC}}$ in this case), as that of most of the other rotational angles which determine the conformation of these molecules, has not been possible up to now.

It would thus be of great interest to be able to deduce, from ^{31}P NMR experiments, for example, the population of the gauche-gauche conformer ($\omega = \omega' \simeq \pm 60°$) of the phosphate groups of the polynucleotide studied. Such an estimation is possible only if the chemical shift difference between the pure gauche-gauche (gg) and pure gauche-trans (gt) conformations is known. The chemical shift of ^{31}P of a pure gg phosphate group can be taken to be equal to the value measured in 3′,5′ cyclic mononucleotides,[3,31] but there is no equivalent stable rigid compound with a gt phosphate group. So the chemical shift variation ($\Delta\delta$) of ^{31}P due to the gg → gt conformational change can be evaluated only from theoretical calculations. Gorenstein and Kar[31] have used the empirical correlation which supposes a direct relation between the electron density of an atom and the magnetic shielding constant of the nucleus of this atom to make an estimation of the differences between the chemical shift of the phosphorus atom for the two conformations gg and gt of a phosphate group. If this type of correlation has been empirically found to be in qualitative agreement with experiment,[31-36] there are a number of examples to illustrate the limitations of such rules.[36-38] It appears therefore that the computation of the ^{31}P magnetic screening constant for the gg and gt conformations of a phosphate group in order to have an estimation of the chemical shift variation between the two conformers as reliable as possible could be useful for the interpretation of experimental data. The development of ab initio methods for the calculation of magnetic shielding constants[39-42] makes such studies possible nowadays for molecules of medium size. In addition to the magnetic shielding constant, which is the quantity measured in solution, ab initio calculations of the magnetic shielding tensor determine also the principal values of the tensor and their orientation with respect to the molecular framework. One or both of these two sets of quantities have been determined for the phosphate group from measurements on crystals and/or powders of some nucleotides,[43,44] polynucleotides,[44] and phospholipids.[45-47] The comparison of the experimental and theoretical results for the anisotropy of the magnetic screening tensor of ^{31}P ($\Delta\sigma$) and for the determination of its principal axes will provide an estimation of the numerical reliability of our results.

In addition the experimental studies have shown that the anisotropy of the ^{31}P shielding tensor is sensitive to the hydration of the phosphate group[45,47] and is several tens of parts per million smaller in magnitude in monoester than in diester phosphates,[44,46,47] in nucleic acid constituents as well as in phospholipids. The calculation of these two types of effect may indicate the factors which are responsible for the measured variations.

With ab initio computational methods, as the one that we shall utilize, it is not possible to treat a phosphate group in a dinucleoside monophosphate or in a phospholipid because of the great size of these molecules. As in previous ab initio studies which have been carried out on the phosphate group the computations are actually performed for the dimethyl phosphate, monomethyl phosphate, and $PO_4H_2^-$ anions,[48,55] taken as model compounds of the biological phosphate groups.

Computational Details

The calculations have been carried out by a nonempirical method utilizing a Gaussian basis set. The elements of the magnetic shielding tensors are calculated by the coupled Hartree–Fock (CHF) procedure[39] developed by Ditchfield[40] for gauge invariant atomic orbitals (GIAO), which presents the great advantage of leading to values of the elements of the tensors which are independent of the system of axis chosen for the molecular geometry, even for basis sets of very limited size.

The SCF molecular eigenvalues and eigenvectors which are used as input for the CHF computation are obtained from an extended version[56] of the program GAUSS 70.[57,58] As in our

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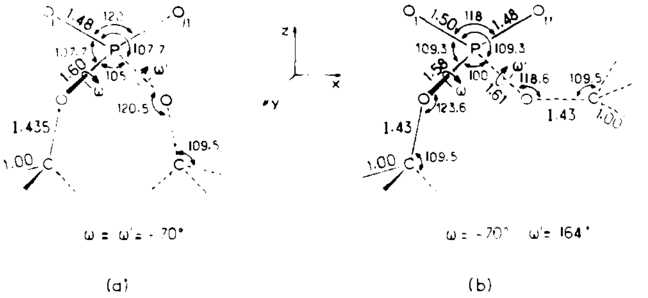

Figure 1. Geometries used for input. O_IPO_{II} defines the $x0z$ plane with $0z$ bisecting the O_IPO_{II} angle.

previous paper[38] the partitioning of the screening constant into a diamagnetic and a paramagnetic part is in the present work different from the one proposed by Ditchfield.[40] In the tabulated results the diamagnetic term corresponds to

$$\begin{aligned}\sigma_{\alpha\beta}{}^{d^{31}P} = \sum_{i\,\mathrm{occ}} \sum_{sq} c^{0\star}{}_{is} c^{0}{}_{iq} \frac{1}{2c^2} [\langle \chi_s | \mathbf{r}_q \wedge \mathbf{r}_P \delta_{\alpha\beta} \\ - (\mathbf{r}_q)_\alpha \cdot (\mathbf{r}_P)_\beta r_P{}^{-3} | \chi_q \rangle \\ + \langle \chi_s | \mathbf{r}_q \wedge \mathbf{r}_P \delta_{\beta\alpha} - (\mathbf{r}_q)_\beta \cdot (\mathbf{r}_P)_\alpha \cdot r_P{}^{-3} | \chi_q \rangle \\ - \langle ((\mathbf{R}_s - \mathbf{R}_q) \wedge \mathbf{r}_s)_\alpha \chi_s | (\mathbf{r}_P \wedge \nabla)_\beta \cdot r_P{}^{-3} | \chi_q \rangle \\ - \langle ((\mathbf{R}_s - \mathbf{R}_q) \wedge \mathbf{r}_s)_\beta \chi_s | (\mathbf{r}_P \wedge \nabla)_\alpha \cdot r_P{}^{-3} | \chi_q \rangle] \\ = \sigma_{\beta\alpha}{}^{d^{31}P}\end{aligned}$$

and the paramagnetic one is equal to

$$\begin{aligned}\sigma_{\alpha\beta}{}^{p^{31}P} = \sum_{i\,\mathrm{occ}} \sum_{s} \sum_{q} \\ - \left[\frac{1}{2c^2} c^{0\star}{}_{is} c^{0}{}_{iq} ((\mathbf{R}_s \wedge \mathbf{R}_q)_\alpha \langle \chi_s | (\mathbf{r}_P \wedge \nabla)_\beta \cdot r_P{}^{-3} | \chi_q \rangle \right. \\ + (\mathbf{R}_s \wedge \mathbf{R}_q)_\beta \langle \chi_s | (\mathbf{r}_P \wedge \nabla)_\alpha \cdot r_P{}^{-3} | \chi_q \rangle) \\ + \frac{1}{4c} ((c^{0\star}{}_{is} c^{H_\alpha}{}_{iq} + c^{H_\alpha\star}{}_{is} c^{0}{}_{iq}) \langle \chi_s | (\mathbf{r}_P \wedge \nabla)_\beta \cdot r_P{}^{-3} | \chi_q \rangle \\ + (c^{0\star}{}_{is} c^{H_\beta}{}_{iq} + c^{H_\beta\star}{}_{is} c^{0}{}_{iq}) \\ \left. \times \langle \chi_s | (\mathbf{r}_P \wedge \nabla)_\alpha \cdot r_P{}^{-3} | \chi_q \rangle) \right] = \sigma_{\beta\alpha}{}^{p^{31}P}\end{aligned}$$

where $\mathbf{r}_s$ and $\mathbf{r}_P$ are the position vectors of the electron with respect to the nucleus of the atom carrying orbital χ_s and to the phosphorus atom, respectively, $\mathbf{R}_s$ and $\mathbf{R}_q$ are the position vectors of the nuclei of the atoms carrying the orbitals χ_s and χ_q, $c^0{}_{is}$ and $c^0{}_{iq}$ are the zeroth-order coefficients of orbitals χ_s and χ_q in the eigenvectors obtained from the SCF MO run, and $c^{H_\alpha}{}_{is}$ are the first-order perturbed coefficients given by the CHF procedure for the direction α of the external magnetic field.

The principal values of the magnetic shielding tensor and their orientation with respect to the molecular framework are then obtained from the diagonalization of the 3 × 3 tensor calculated with a chosen system of axis.

The basis set utilized in the present work is minimal. The orbital exponents and contraction coefficients of O, C, and H atoms come from Clementi's work[59] while those of the phosphorus atom have been determined by Roos and Siegbahn.[60] This basis set, supplemented by six d Gaussian functions on the phosphorus atom, has already been used in our laboratory[51,52] for the study of the binding of alkali and alkaline earth cations to the dimethyl phosphate anion and has given results which are much more satisfactory than those obtained from the STONG type of minimal basis set.[51,52,61] Concerning the calculation of the magnetic shielding tensor itself Ditchfield[40] has shown that the STONG basis gives unsatisfactory results and has retained the split 4-31G basis set for his calculations.[62] In previous works[38,63] we have found that the basis just described and the 4-31G one give values of the shielding constants which are in qualitative agreement and which compare favorably with the measured values of the corresponding chemical shifts. The main advantage of the basis set utilized in this work over the 4-31G one is that for DMP⁻ it introduces 142 Gaussian functions instead of 180 in the computation of the two-electron integrals between the GIAO, computation of which is the bottleneck of the methods of calculation of the magnetic screening tensor using this type of atomic orbitals.

Results and Discussion

^{31}P Magnetic Shielding Constant in the Gauche–Gauche and Gauche–Trans Conformers of DMP⁻. Since the experimental studies on the dependence of the chemical shift of the phosphorus atom of the phosphate group upon the value of ω and ω' rotation angles[65] about the -O-P- bonds have been carried out on di- and polynucleotides[6,25,26,28,64] or nucleic acids[8] we have chosen the geometrical parameters of the dimethyl phosphate anion (DMP⁻) from X-ray data on dinucleoside monophosphates. There exist, for a number of these compounds, precise and refined crystallographic studies from which it is possible to deduce reasonable representative values for bond lengths and bond angles of the phosphate group in this class of molecule.

For the gauche-gauche conformation of DMP⁻ we have taken the geometry reported in Figure 1a; the reported bond lengths and bond angles are symmetrized and rounded off values of the corresponding quantities in GpC studied by Rosenberg et al.[66] and which appear to be typical for a dinucleoside monophosphate with a g^-g^- phosphate group. We have taken a totally symmetrical geometry in order to have identical gt conformers by varying ω or ω'.

With this input the calculated value of the magnetic shielding constant of ^{31}P is 644.54 ppm (Table I). For the gt conformer of DMP⁻ we have run two calculations, one called A in Table I for which the only difference from the previous computation is the value of the rotation angle ω', which is now taken equal to 164°, and one called B in Table I with the geometry reported in Figure 1b which is built from the molecule with the gt phosphate group observed in the crystal of UpA hemihydrate.[67]

The theoretical values of σ^{31P} for these two geometries of the gt conformer of DMP⁻ are 643.03 and 637.98 ppm, respectively (Table I). The calculated downfield shift associated with the gg → gt conformational change is in qualitative agreement with the experimental data and the empirical calculations of Gorenstein and Kar.[31] But our results indicate that the molecular geometry is far more important for the value of σ^{31P} than the value of the torsional angles about the PO ester bonds since the $\Delta\delta$ between the gg and gt conformer is -1.5 if bond lengths and bond angles have identical values for the two conformers, while it is -6.5 when bond lengths and angles have values which are measured for gauche-gauche and gauche-trans phosphate groups in crystals. Similarly Gorenstein and Kar[31] have found that the variation of σ^{31P} of DMP⁻ with ω and ω' does not present the same value when the different conformers have identical geometry and when they do not. But the large difference (4 ppm) between the calculated values of σ^{31P} for the two geometries of the gt conformer raises the problem of the choice of the theoretical value of $\Delta\delta$ which is actually representative of the phenomenon occurring in the conditions of NMR experiments.

The examination of the results of X-ray crystallographic studies shows that dinucleotide monophosphates in which the phosphate group is gg[66-70] have bond lengths and bond angles between the atoms of this group which differ in a rather systematic manner from those observed in dinucleoside mono-

Table I. Calculated Principal Values (ppm) and Corresponding Direction Cosines of the Principal Axes of the ^{31}P Magnetic Shielding Tensor of Phosphates

	σ^a	$\sigma^{d\,b}$	$\sigma^{p\,c}$	σ_{11}	x	y	z	σ_{22}	x	y	z	σ_{33}	x	y	z	$\Delta\sigma^d$
DMP^-gg	644.54	984.74	−340.21	569	0.0254	0.9997	0.	604	0.	0.	1.	761	0.9997	−0.0254	0.	174
$DMP^-gt(A)$	643.03	984.61	−341.58	571	0.0185	0.9995	−0.0265	602	0.0180	0.0206	0.9995	759	0.9997	−0.0190	−0.0175	172
$DMP^-gt(B)$	637.98	984.53	−346.54	577	0.0214	0.9632	0.2681	584	0.0095	−0.2683	0.9633	753	0.9997	−0.0180	−0.0149	172
$PO_4H_2^-gg$	647.47	984.24	−336.77	577	0.0224	0.9998	0.	609	0.	0.	1.	756	0.9998	−0.0224	0.	163
$PO_4H_2^--H_2O$	651.34	983.93	−332.59	586	0.0261	0.9996	0.0111	618	0.0912	−0.0134	0.9957	751	0.9955	−0.0250	−0.0914	149
MMP^-gg^e	646.08	984.49	−338.41	573	0.0237	0.9995	−0.0961	607	0.0139	0.0958	0.9953	758	0.9996	−0.0249	−0.0116	168
$MMP^{2-\,f}(A)$	626.20	986.91	−360.71	599	0.0590	0.8280	0.5576	638	−0.0793	−0.5530	0.8294	641	0.9951	−0.0931	0.0331	-40^g
$MMP^{2-\,f}(B)$	622.35	986.77	−364.41	594	0.0259	0.7901	0.6124	633	−0.0395	−0.6114	0.7904	639	0.9989	−0.0446	0.0153	-42^g

[a] $\sigma = (\sigma_{11} + \sigma_{22} + \sigma_{33})/3$. [b] Diamagnetic part of σ. [c] Paramagnetic part of σ. [d] $\Delta\sigma = \sigma_{33} - (\sigma_{11} + \sigma_{22})/2$. [e] Monomethyl phosphate monoanion. [f] Monomethyl phosphate dianion. [g] Here $\Delta\sigma = \sigma_{11} - (\sigma_{22} + \sigma_{33})/2$.

phosphates with a gt phosphate group.[67,71] In addition quantum-mechanical calculations of the molecular energy of the gg and gt conformations of DMP^-[49,53-55,72] have shown that the stability of the gt conformer can become larger than the stability of the gg one if variations of the geometrical characteristics of the molecule occur upon the gg → gt conformational change. In particular these works put forward the role of the OPO angle between the ester bonds on the respective stability of the gg and gt conformers, a decrease from about 105° to about 100° of this bond angle stabilizing the gt conformation with respect to the gg one in agreement with the measured values in crystals.

From these results it seems that the conformational equilibrium DMP^- gg $\leftrightarrows$ DMP^- gt(B) is more probable than DMP^-gg $\leftrightarrows$ DMP^-gt(A); if this assumption is correct our calculations predict a −6.5 ppm downfield shift for the phosphorus atom when the diester phosphate group changes from a gg to a gt conformation.

Since all the experimental values of $\Delta\delta^{31P}$ with increasing temperature are, for di- or polynucleotides, smaller than −1.5 ppm,[75] the calculated value of −6.5 ppm seems too large and the difference of −1.5 ppm between DMP^-gg and DMP^-gt(A) would appear more satisfactory at first sight; but a careful examination of the available experimental data in relation with the results of our calculations for DMP^-gt(B) shows that they are not unreasonable.

From the tabulation of experimental values of ^{31}P chemical shifts of a variety of organic phosphate esters Gorenstein[4] has clearly demonstrated that for molecules in which the -O-P-O- bond angle is about 105° the resonance of the phosphorus atom is shifted several parts per million upfield with respect to the shift of this nucleus in molecules in which this angle is about 100°. Our results for A and B geometries of the gt conformer of DMP^- are on that matter in complete agreement with experiment. In addition the NMR experimental procedure for the measurement of the variation of the chemical shift of ^{31}P with the gg → gt conformational change starts with dinucleoside monophosphates (we shall limit ourselves to this class of molecule in this part of the discussion) in solution at low temperature (4 °C); in these experimental conditions the ^1H NMR experimental studies on these compounds have shown[76-81] that an equilibrium exists between stacked conformations with a g^-g^- or g^+g^+ phosphate group and skewed conformations with a g^+t phosphate as well as extended ones. With increasing temperature the equilibrium is modified in favor of the unstacked conformations. It is possible to deduce from the ^1H NMR experiments an evaluation of the percentage of stacked conformation present in the solution[76,82] for the dinucleoside monophosphate under investigation. Lee and Tinoco[81] have determined this percentage for some fo the dinucleoside monophosphate studied by Gorenstein et al.[25] for temperatures quite similar to those used in the ^{31}P study. Thus, it is meaningful to compare for the compounds concerned the measured values of $\Delta\delta^{31P}$ with increasing temperature with those which are calculated for the variation of the percentage of stacked conformations determined by Lee and Tinoco[82] with the assumption that $\Delta\delta^{31P}$ between gg and gt conformations of the phosphate group is equal to the theoretical differences

$$\sigma^{31P}(\mathrm{DMPgg}) - \sigma^{31P}(\mathrm{DMPgt}) = \sigma_{gg} - \sigma_{gt}$$

obtained for the two geometries A and B that we have utilized for the gt conformer. The results are reported in Table II together with the value of what we call $(\sigma_{gg} - \sigma_{gt})_{exp}$, that is, the value of the chemical shift difference between the gg and gt conformations which is obtained from the measured value of $\Delta\delta^{31P}$ and the variation of the percentage of stacked conformations with increasing temperature. The results reported show that the experimental values are larger than the calculated ones if we consider that DMP^-gt has geometry A but smaller than those that we obtain when we retain geometry B for the gt conformation.

Table II. Measured and Calculated Values of $\Delta\delta^{31P}$ Due to Temperature Increase (ppm)

	$\Delta\delta^{31P}$				
	exptl[a]	calcd (A)[b]	calcd (B)[b]	Δ % stacked[b,c]	$(\sigma_{gg} - \sigma_{gt})_{exptl}$
ApA	−1.220	−0.413	−1.788	27.5	−4.4
ApU	−1.191	−0.548	−2.373	36.5	−3.1
CpC	−1.237	−0.518	−2.243	34.5	−3.6

[a] Relative to D_2O lock and for the temperature range 5-82 °C. [b] For the temperature range 4-75 °C. [c] From Lee and Tinoco[81] average values for pN and Np units.

Since the Gaussian basis set that we have used in the present computation of σ^{31P} is very limited the numerical value of the calculated quantity $\sigma_{gg} - \sigma_{gt}$ is most probably not very accurate, but, from the comparisons that we have made between the experimental data and our results, the following conclusions appear concerning the chemical shift's variation that ^{31}P undergoes during a gg → gt conformation transition.

(1) The geometry of the conformers seems to be the principal factor in the determination of δ^{31P} as it is for the energy difference between the two conformers.[54,74]

(2) The value of $\Delta\delta^{31P}$ is most probably in the range −3.5 to −6.5 ppm.

Part of the difference of 3 ppm between the "experimental" and theoretical values of $\sigma_{gg} - \sigma_{gt}$ may originate from a nonoptimal choice of the molecular geometries since our input data come from crystallographic data while the experiments are dealing with solutions where the values of ω and ω' for the gg conformation seem to deviate[76-80] from the crystallographic average of ±70°, indicating a different molecular geometry, if the interdependence found theoretically for the two set of parameters is not an artifact. Moreover, we do not take into account a possible rearrangement of the solvent with the conformational change[83] which may play a role in σ^{31P} since, as we shall see later, hydrogen bonding of a solvent to the phosphate group does produce a chemical shift variation for ^{31}P.

Another possible contribution to the difference between experimental and theoretical values of $\sigma_{gg} - \sigma_{gt}$ is that the unstacking of the bases which is observed with increasing temperature can be obtained by the variation of rotational angles other than ω and ω'; in this case the variation of the percentage of stacked conformations given by ^1H NMR spectra is an upper limit of the variation of the percentage of gg conformation in the solution studied.

To end this part of the discussion we would like to remark that the comparison of the diamagnetic and paramagnetic parts of the magnetic shielding constant of ^{31}P in the three computations run for DMP^- shows that the paramagnetic component, which in ab initio methods has no direct relation with the electron population of the phosphorus atom, is the determining term for the calculated chemical shift variations.

Principal Values and Orientation of the Principal Axes of the Magnetic Shielding Tensor. The principal values of the ^{31}P magnetic shielding tensor of DMP^- reported in Table I show that there is one direction for which the magnetic shielding is larger by more than 100 ppm than for the two others in agreement with experimental measurements on polynucleotides and nucleic acids[44] as well as on diester phospholipids.[44-47] The calculated value of the anisotropy of the magnetic shielding given by

$$\Delta\sigma = \sigma_{33} - \tfrac{1}{2}(\sigma_{22} + \sigma_{11})$$

is 174 ppm for experimental values ranging from 150 to 200 ppm.[44,47,84] But it seems that our results underestimate the quantity $\sigma_{22} - \sigma_{11}$, which is theoretically 35 ppm while the range of measured values goes from 50 to 70 ppm.

The calculated direction cosines of the principal axes of the magnetic shielding tensor for DMP^-gg compare favorably with the measurements of the corresponding quantity for barium diethyl phosphate.[47] The principal axes are almost collinear with the axis of the *xyz* reference frame. In particular theory and experiment agree on the direction of each of the principal values; the smallest one is perpendicular to the O_IPO_{II} plane and coplanar with the PO ester bonds while the largest is in the O_IPO_{II} plane and perpendicular to the -O-P-O- one.

The 0° angle that we calculate between σ_{22} and the $0z$ axis instead of the nonzero measured value is due to the totally symmetric geometry that we have used as input. Since the experimental measurements of the anisotropy of the magnetic shielding as well as of the directions of the principal values of the shielding tensor are correctly reproduced for DMP^-gg by our calculations we can reasonably expect that the corresponding results that we obtain for the gt conformer of DMP^- are meaningful. If this assumption is correct it implies that gg and gt conformations have similar values of $\Delta\sigma^{31P}$ and of the direction cosines of the principal values of the tensor, as can be seen from the values reported in Table I. The comparison of the values obtained for the two geometries of the gt conformer of DMP^- shows that the molecular geometry has little influence on the orientation of the principal axes, but the one to one comparison of the principal values of the tensor puts into evidence a marked dependence of each individual principal value upon molecular geometry so that it is most probably totally fortuitous if $\Delta\sigma$ has the same value for the two geometries of the gt conformer. Unfortunately there is no experimental evidence to check these theoretical results, but it is possible, in order to test further their validity, to try to reproduce variations of $\Delta\sigma$ and of σ actually observed experimentally.

Herzfeld et al.[47] have observed that the binding of one molecule of water to a diester phospholipid produces a decrease of the measured anisotropy of the magnetic shielding tensor and an upfield shift of its trace (σ). There exist a number of theoretical studies[52,85,86] which have clearly established the most favorable (energywise) arrangement of a molecule of water hydrogen bonded to the phosphate group and which have shown that the hydration scheme is quite similar for the DMP^- and $PO_4H_2^-$ anions. Because of this similarity and because of the computer time required for the calculations of NMR chemical shift tensors we have for the study of the role of hydration on σ^{31P} limited our computations to $PO_4H_2^-$ with bond lengths and bond angles equal to those of the gg conformer of DMP^- and with the OH bonds taken equal to 1 Å. The results reported in Table I indicate that the replacement of the methyl groups by hydrogen atoms produces an increase of the shielding constant and a decrease of the anisotropy but leaves unchanged the cosine directions of the principal values. For the $PO_4H_2^-{-}H_2O$ complex with a water molecule located at the most favorable position determined by Pullman et al.[52] we obtain with respect to the preceding computation an increase of the shielding constant and a decrease of the anisotropy of the tensor in qualitative agreement with experiment. The comparison of the experimental and theoretical values show that the calculated $\Delta\delta^{31P}$ (3.5 ppm upfield) is larger than the measured ones (1.4-2.0 ppm upfield) while the variation of $\Delta\sigma$ due to hydration is calculated to be smaller than the measured value (1.4 and 3.7-4.0 ppm). The calculated direction cosines of the principal axes of the ^{31}P shielding tensor for $PO_4H_2^-$ and $PO_4H_2^-{-}H_2O$ (Table I) are almost identical, so that it seems that hydration does not influence the orientation of the principal axes of the tensor. This result should, however, be taken with caution; it might be due to the particular location of the molecule of water that we have retained (in the $x0z$ plane which contains two of the principal axes of the tensor).

The above results tend to show that there is an intrinsic effect of the hydration of the phosphate group on the ^{31}P magnetic shielding tensor and that the measured variations are most probably (at least for the first molecules of water) not due to any large modification of the conformation of the phosphate group.

Case of the Monoesters. The experimental data on the ^{31}P magnetic shielding tensor of monoester organophosphates exhibit systematic differences with the corresponding quantity in the diesters, in the case of nucleic acid constituents[44] or of phospholipids.[46,47] There is in particular a sensible decrease of the anisotropy of the tensor from 150-200 to 75-130 ppm, principally due to an important diminution of the value of σ_{33}. Moreover, in the case of mononucleotides the ^{31}P shielding tensor is axially symmetric in sodium salts and clearly nonaxial in the free acids as it is in all the phosphodiesters. But in spite of the measured differences between the principal values in mono- and diesters it appears from the work of Kohler and Klein[43,46] on one hand and of Griffin et al.[47] on the other that the direction of the principal axes is quite similar in the two types of molecules. This set of observations induced us to try to search for its interpretation.

If we take the gg conformer of DMP^- that we have studied previously and replace one of the methyl groups by a hydrogen atom (the procedure is similar to the one used in the case of $PO_4H_2^-$) we obtain a monomethyl phosphate anion. Since X-ray crystallographic studies show that the phosphate groups of the free acid form of mononucleotides[87-93] as well as of phosphorylethanolamine[94] exist as monoanions we can expect that the calculation of σ^{31P} for the molecule just described is representative, at least qualitatively.

The calculated values reported in Table I for monomethyl phosphate (MMP^-) show that the calculations do not reproduce the measured decrease of $\Delta\sigma$ since we obtain a lowering of 6 ppm for a measured value of about 50 ppm on the average.

Table III. Experimental Principal Values of the Magnetic Shielding Tensor of Adenosine Mono- and Polynucleotides with Respect to 85% H_3PO_4 (ppm) (from Terao et al.[44])

	σ_{11}	σ_{22}	σ_{33}	σ	$\Delta\sigma^a$
poly A(K)	−89	−24	110	−1	166.5
5′AMP	−77	−8	83	−1	125.5
5′AMP (Na_2)	−78	29	29	−7	−107

[a] See footnotes *d* and *g*, Table I.

But theory and experiment agree on the similarity of the direction of the principal axes in mono- and diester phosphates. This concordance between calculations and measurements shows that our results, although not satisfactory, are not unreasonable.

If now we remove the proton of the OH group of the monomethyl phosphate anion we obtain the dianion which can be taken as a first approximation of the phosphate group of sodium salts of the mononucleotides studied by Terao et al.[44] since in this case the phosphate is doubly ionized.

The calculated principal values for this dianion are reported in Table I (MMP^{2-}(A)) and clearly show that in this case the magnetic shielding tensor is axial in agreement with experimental data. In addition the measured downfield shift and the decrease of the anisotropy of the tensor (with respect to corresponding values in the monoanions) are reproduced by this calculation. Even the fact that σ_{11} is the least modified of the principal values (Table III) is reproduced by the theory. This qualitative agreement is not fortuitous: if the monomethyl phosphate dianion is built from the B geometry of the DMP^- gt by removing the trans methyl group the calculated values reported in Table I (MMP^{2-}(B)) show that the results differ only numerically from those obtained from MMP^{2-}(A).

We are well aware that the calculated numerical values of $\Delta\delta^{^{31}P}$ and of the variation of $\Delta\sigma$ between DMP^- or MMP^- and MMP^{2-} are much larger than the measured ones, but this is not unexpected if we recall that we have utilized for the dianion bond lengths and bond angles of dinucleoside monophosphates which are appreciably different from those observed in dianions of mononucleotides[95-99] and that the magnetic shielding tensor is very sensitive to the molecular geometry as shown in the case of DMP^-.

This set of results on monomethyl phosphate taken as a model of phosphate monoesters lead us to think that, since our calculations are able to reproduce satisfactorily the fundamental qualitative differences which are observed between the shielding tensors of the sodium salts of mononucleotides and the shielding tensors of their free acids without taking into account the molecular geometric rearrangement which accompanies the second ionization of the phosphate group, the observed differences between the ^{31}P shielding tensor of monoester and diester phosphates are principally due to differences in the molecular geometry and to the intermolecular hydrogen bonding which cannot exists between diesters. This conclusion is supported by the marked dependence of $\sigma^{^{31}P}$ upon these factors.

We would like to remark that the calculated $\Delta\delta$ between the mono- and dianions of monomethyl phosphate is due to the paramagnetic part of the screening constant and again has no relation with the electron population of the phosphorus atom.

Conclusion

The calculation of the magnetic shielding tensor of the phosphorus atom of dimethyl phosphate taken as a model of the phosphate group of nucleic acid constituents or of the polar head of phospholipids carried out in the present work shows that the molecular geometry is a factor more decisive than is the conformation of the phosphate group for the value of the ^{31}P magnetic screening constant. The calculated quantity $\sigma_{gg} - \sigma_{gt}$ with identical bond lengths and bond angles for gg and gt conformers of DMP^- is much too small when compared to the estimations of this quantity which can be obtained from the variation of ^1H and ^{31}P spectra of dinucleoside monophosphates with increasing temperature. The present calculations insofar as they are significant tend thus to confirm that the conformational change between gg and gt forms of a phosphate group is accompanied by a rearrangement of the molecular geometry of the part of the molecule concerned.

The comparison of the measurements of $\Delta\delta^{^{31}P}$ in dinucleoside monophosphates as a function of temperature with the calculated values of $\sigma^{^{31}P}$ for gg and gt conformations of DMP^-, taking into account the variation of the molecular geometry which seems among the most probable, induces us to propose a value of about 4–6 ppm downfield as the variation of ^{31}P chemical shift between a pure gg and a pure gt phosphate group.

The very limited size of the Gaussian basis set that we have utilized makes us cautious about the exact numerical values that we obtain from our computation. In spite of these limitations the present calculations are able to reproduce correctly the order of magnitude of the anisotropy of the magnetic shielding tensor as well as the orientation of each of its principal axes which are measured for diester phosphates. This agreement gives us confidence to predict that the observation of an axial tensor for ^{31}P of a phosphate group is indicative of a dianionic state for this group and that the differences which are observed between the shielding tensors of diester and monoester phosphates (powder or crystal) are principally due to differences in the molecular geometry and to the possible intermolecular hydrogen bonds.

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Chemistry of 2-Naphthyl Bis[bis(dimethylphosphino)ethane] Hydride Complexes of Iron, Ruthenium, and Osmium. 3. Cleavage of sp^2 C–H Bonds

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Abstract: Reactions of HMNp(dmpe)$_2$ complexes (Np = 2-naphthyl; dmpe = $Me_2PCH_2CH_2PMe_2$; M = Fe or Ru) to cleave various sp^2 C–H bonds, including those of aromatic hydrocarbons, are described. The new HMR(dmpe)$_2$ complexes are characterized spectroscopically. Studies of substituted benzenes show that product stability is favored by electron-withdrawing substituents and inhibited by electron donors. By a combination of NMR spectroscopy, specific deuterium labeling, and disubstitution, it has been possible to establish which isomeric products are formed. The overall study of HMNp(dmpe)$_2$ chemistry is summarized, and the factors favoring C–H bond cleavage by low-valent transition metals are discussed.

Introduction

The first reports of the activation of C–H bonds by soluble transition metal complexes appeared in 1963 and 1965. These reactions[1,2] (eq 1 and 2) provide examples of both *intra*- and *inter*molecular C–H bond cleavage at sp^2 carbon atoms (Np = 2-naphthyl; dmpe = $Me_2PCH_2CH_2PMe_2$). Subsequently,

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