from the pyridine moiety imparting organization and rigidity to the ring both in the uncomplexed and complexed forms. Combining these results with the unusual stability effects recorded for ligand **3**, we conclude that it is participation of the pyridine group in complexation which is responsible for the behavior of ligand **2** rather than conformational factors.

The replacement of an oxygen atom in **6** by sulfur produces **4** which shows no heat of reaction with Na^+, K^+, or Ba^{2+}. The large size of the sulfur may force the sulfur atom out of the ring, as in the case of sulfur-substituted 18-crown-6.[8] The observation that **4** complexes alkali metal ions even more poorly than 1,10-dithia-18-crown-6, **8**, suggests that the steric consideration mentioned, namely orientation of the sulfur atom away from the ring, if present in **4**, does not enhance carbonyl participation in complexation. If this conclusion is extended to the case of the pyridine-containing ligand, it lends even greater support to the idea that the pyridine nitrogen, not the ester carbonyls, is responsible for its unusual behavior.

The log K, ΔH, and $T\Delta S$ values were obtained by a calorimetric titration procedure which has been described previously.[9] Ligand **2** was standardized by calorimetric titration with K^+ and found to be 98.2% pure. It was not possible to standardize **4** by this direct procedure since the log K value for formation of the Ag^+ complex was not sufficiently high to produce an end point. Variation of a ligand purity factor in the curve fitting computer routines used for data analysis indicated that **4** is greater than 98% pure.

To prepare **2**, 2,6-pyridinedicarboxylic acid chloride (20.4 g, 0.1 mol) and 19.4 g (0.1 mol) of tetraethylene glycol, both in 200 mL of a 50:50 mixture of tetrahydrofuran and benzene, were slowly added while stirred to 1 L of benzene at 50 °C. The resulting reaction mixture was stirred for 48 h. After cooling, the mixture was filtered and the solvents were removed under vacuum. The resulting light viscous oil was repeatedly extracted with hot hexane to yield 25.4 g (77%) of a white solid: mp 83.5-85 °C; IR 1730 cm^{-1} (C=O); NMR δ 3.85 (s, 8 H, OCH_2CH_2O), 3.95 (m, 4 H, $COOCH_2CH_2O$), 4.63 (m, 4 H, $COOCH_2$), and 8.0-8.5 (m, 3 H, aromatic H). Anal. Calcd for $C_{15}H_{19}NO_7$: C, 55.38; H, 5.88, N, 4.31; mol wt, 325.32. Found: C, 55.46; H, 5.96; N, 4.47; mol wt, 329.

Compound **4** was prepared by slowly dripping 19.0 g (0.102 mol) thiodiglycolyl dichloride and 19.7 g (0.102 mol) tetraethylene glycol, each diluted to 150 mL with benzene, from separate addition funnels into 1 L of rapidly stirring benzene at 50 °C. After the mixture was stirred for 68 h at 50 °C, the benzene was removed under reduced pressure and the crude product was distilled: 6.29 g (20%); bp 181 °C (0.4 mm); mp 41-43 °C. The product was recrystallized from chloroform-ether yielding long white needles, mp 43.5-44.5 °C. Compound **4** exhibited the following spectra: IR 1740 cm^{-1} (C=O); NMR δ 3.54 (s, 4 H, $COCH_2S$), 3.71 (s, 8 H, OCH_2CH_2O), 3.77 (m, 4 H, $COOCH_2CH_2O$), 4.36 (m, 4 H, $COOCH_2$). Anal. Calcd for $C_{12}H_{20}O_7S$: C, 46.74; H, 6.54; S, 10.40; mol wt, 308.35. Found: C, 46.95; H, 6.62; S, 10.48; mol wt, 306.

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^{31}P Chemical Shift Anisotropy in Solid Nucleic Acids

Sir:

So far, no report seems to have appeared on direct measurements of ^{31}P NMR chemical shift tensors of nucleic acids in solid state, although some efforts have been made in solutions.[1-3] In the present study, principal values of ^{31}P chemical shift tensors of nucleoside monophosphates (both in salt form and in free acid form), a cyclic mononucleotide, homopolyribonucleotides, tRNA, and DNA (and DL-α-dipalmitoyllecithin for comparison) were determined in their powder-line states by a combined use of cross-polarization technique and proton-dipolar decoupling.[4]

The experiments were done at 290 K on a home-built spectrometer[5] operating at 24.112 MHz for ^{31}P and 59.526 MHz for ^1H. The Hartman-Hahn condition was satisfied with rotating fields of 50 G for ^{31}P and 20 G for ^1H. In order to shorten the recycle time, the proton magnetization was brought back to the direction of a static field by a 90° pulse immediately after decoupling.

Table I summarizes the chemical shift parameters relative

Table I. Principal Values of the Chemical Shift Tensor for ^{31}P in Some Nucleic Acids[a]

Ester	Nucleic acid	σ_{11}	σ_{22}	σ_{33}	$\bar{\sigma}$[b]	σ_{soln}[c]
Monoester (salt)	5′-AMP(Na_2)	−78	29	29	−7	−4.1[d]
	5′-GMP(Na_2)	−63	25	25	−4	−4.1[d]
	5′-UMP(Na_2)	−67	27	27	−4	−4.0[d]
	3′-UMP(Na_2)	−66	27	27	−4	−4.1[d]
Monoester (free acid)	5′AMP	−77	−8	83	−1	−0.3[e]
	3′-AMP	−71	−11	90	3	
	3′-CMP	−67	−11	72	−2	
	5′-dCMP	−77	−1	87	3	
Diester	poly A(K)	−89	−24	110	−1	1.0[f]
	poly G(K)	−83	−24	112	2	1.1[f]
	poly C(K)	−92	−25	114	−1	1.2[f]
	poly U(K)	−85	−24	112	1	0.7[f]
	$tRNA^{phe}_{yeast}$	−86	−30	107	−3	0∼2[g]
	DNA(salmon)(Na)	−83	−23	110	2	∼1[h]
	3′,5′-cAMP	−88	−38	124	−1	1.6[e]
	DPL[i]	−103	−35	131	−2	

[a] All values are in parts per million relative to 85% H_3PO_4, taking upfield as positive. Principal values are determined by differentiation of powder spectra and believed to be accurate to ±5 ppm. [b] $\bar{\sigma} = (\sigma_{11} + \sigma_{22} + \sigma_{33})/3$. [c] Chemical shift values in aqueous solutions. [d] Values at pH 8.0.[13] [e] Values at pH 3.0.[13] [f] Reference 14. [g] Main peak only.[1] [h] Reference 15. [i] DL-α-dipalmitoyllecithin (DPL). The principal values of DPL were already reported by other authors. Our results are in good agreement with those reported by Griffin,[16] and a little different from those by Kohler and Klein.[17]

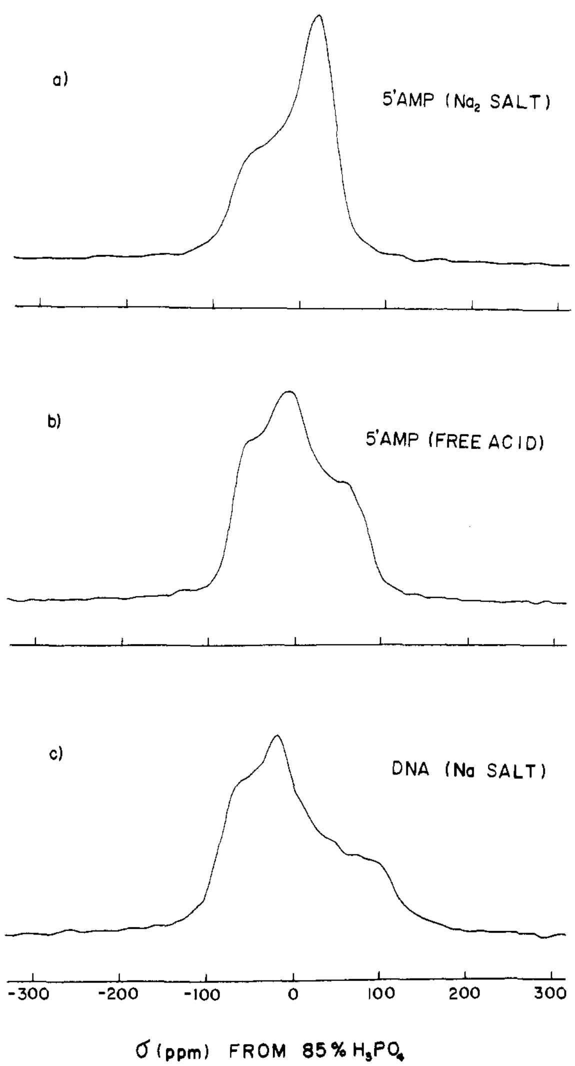

Figure 1. Typical ^{31}P NMR spectra in powder-line nucleic acids: (a) adenosine 5′-monophosphate (disodium salt); (b) adenosine 5′-monophosphate (free acid); (c) deoxyribonucleic acid (from salmon, sodium salt). These spectra were taken at 290 K by a combined use of cross-polarization technique and proton-dipolar decoupling. The static magnetic field increases to the right.

to 85% H_3PO_4 for various nucleic acids studied. It is to be noted that one third of the trace of the chemical shift tensor in solids ($\bar{\sigma}$) coincides with the isotropic value of the chemical shift in solutions (σ_{soln}) within the experimental error. However, while the variations both in $\bar{\sigma}$ and in σ_{soln} are small for different types of nucleic acids, the principal values of the chemical shift tensor, σ_{11}, σ_{22}, and σ_{33}, vary widely. It may be emphasized, therefore, that the anisotropic part of the ^{31}P chemical shift tensor is significantly more sensitive to the chemical or conformational environment of the phosphate group than its isotropic counterpart.

The chemical shift tensors of mononucleotides are axially symmetric in sodium salts but clearly nonaxial in free acid forms. Typical spectra for the two cases are shown in Figures 1a and 1b, respectively, with 5′-AMP as an example. All polynucleotides (including tRNA and DNA) exhibit nonaxial patterns with considerably larger anisotropy. An example from DNA is shown in Figure 1c. All the chemical shift tensors of the polynucleotides studied are almost identical within the experimental error, irrespective of the base and the ribose species and of whether they are single or double stranded. 3′,5′-cyclic AMP and DL-α-dipalmitoyllecithin which have phosphodiester bonds like polynucleotides, exhibit resonance patterns similar to those of polynucleotides, but the anisotropy is slightly larger.

From these results, we conclude the following. The ^{31}P chemical shift tensor of a nucleic acid is primarily determined by whether the phosphate group exists in a monoester form or in a diester form and when in the monoester form, by whether it exists in a free acid form or in a salt form. Namely, it is primarily sensitive to esterification, protonation, and ionization of the phosphate group.

The effect of esterification and protonation on the ^{31}P chemical shift tensor in phosphorylethanolamine which is a phosphate monoester was empirically discussed by Kohler and Klein.[6] They found that the principal axes of the chemical shift tensor correlate with the ground-state electronic distribution around the phosphorus atom, with the most downfield tensor component along the direction of lowest electron density. They used this correlation to understand qualitatively powder spectra of several organophosphate mono- and diesters.

The powder spectra of the salt forms of nucleic acid monoesters can be discussed similarly. The electron density of all the P-O bonds except a P-O(R) bond would become about the same by ionization of the P-O(H) bond, leaving the P-O(R) bond with the lowest electron density. This would result in axially symmetric patterns in the powder spectra with the peak in the upfield and the symmetry axis lying along the P-O(R) bond. The anticipation that the P-O(R) bond has the lowest electron density is actually supported by the fact that, in the molecular structure of monosodium 5′-IMP in crystal, the P-O(R) bond is the longest (1.62 Å) whereas the three P-O bonds are equally short (1.53 Å).[7] In fact, the observed powder spectra of the sodium salts of monoesters were axially symmetric with the peak in the upfield (Figure 1a). Of course, the directions of the principal axes can not be determined until single-crystal data are available, but the symmetry axis is considered to be nearly parallel to the P-O(R) bond from the above argument.

The principal values of the chemical shift tensors of the mononucleotides (free acid form) are very similar to the values for phosphorylethanolamine,[6] so that only one oxygen of the phosphate group seems to be protonated as in phosphorylethanolamine. This is in fact true in the x-ray analysis of the free acid form of 5′-AMP.[8,9] The powder spectra of the diesters (3′,5′-cyclic AMP and all of the polynucleotides) are again similar to those of mononucleotides in free acid forms, but the anisotropies of the former are larger than those of the latter, indicating that the perturbation of esterification to the electron density would be qualitatively similar to that of protonation but would be larger in magnitude.[6]

Finally, we note that, even within the salt forms of monoesters, the free acid forms of monoesters, or the diesters of nucleic acids, the variation in the magnitude of anisotropy is beyond the experimental error in certain cases; for example, among the diesters, cyclic AMP and DL-α-dipalmitoyllecithin show definitely large anisotropies than polynucleotides. This observation suggests that the ^{31}P chemical shift tensor can reflect conformational variation in the phosphorus moiety, i.e., in the O-P-O bond angle and/or the rotational angle (ω,ω') about the P-O bonds. Such a possibility is well expected from theoretical calculations[10,11] and experimental observations[12] of the dependence of σ_{soln} on conformations of nucleic acid phosphodiesters in solutions.

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Prolongation of the Lifetime of the ^2E State of Tris(2,2′-bipyridine)chromium(III) Ion by Anions in Aqueous Solution[1]

Sir:

Compared to the emission lifetimes of ^2E states of other octahedral N-bonded Cr(III) complexes in aqueous solution ($\tau < 2$ μs),[2,3] the ^2E state[4] of $Cr(bpy)_3^{3+}$ is remarkably long-lived ($\tau = 63$ μs at 22 °C in N_2-purged solutions).[5] The $Cr(bpy)_3^{3+}$ ^2E state is readily quenched by O_2 and by a wide variety of electron-transfer agents (including N_3^- and I^-)[5-8] resulting in a diminution in the intensity of the $^2E \rightarrow {}^4A$ phosphorescence bands at 695 and 727 nm and a concomitant decrease in the ^2E lifetime. In neutral and basic aqueous solution, $Cr(bpy)_3^{3+}$ is photochemically active, undergoing ligand loss to form $Cr(bpy)_2(OH)_2^+$ and free bpy. The ^2E state is formed with a quantum yield of ~1 and is believed to be the direct precursor of the photoaquation intermediates.[9]

We have been examining the effect of changing the solution medium (solvent, salts) on the ^2E state of $Cr(bpy)_3^{3+}$ by monitoring the excited-state absorption spectrum (flash photolysis), lifetime (decay of the ^2E absorption), and phosphorescence spectrum (spectrofluorimetry). Substitution of D_2O for H_2O effects no change in the ^2E lifetime. Similarly, in nonaqueous solvents (CH_3OH, CH_3CN, DMF, ethylene glycol), the profiles of excited-state emission, excitation, and absorption spectra, as well as the excited-state lifetime, remain unchanged. However, in aqueous solution in the presence of high salt concentrations (>1 M), the lifetime is *increased* while all the other excited-state parameters remain unchanged. This general effect has been observed for NaCl, $NaClO_4$, $NaHSO_4$, and KNO_3; the same effect is observed when $HClO_4$ is used in place of $NaClO_4$. We have examined this phenomenon in detail for ClO_4^- in order to establish the effect of anion on the radiative, nonradiative, and reactive (photochemical) pathways.

In dilute aqueous solution, the quantum yield of phosphorescence is very small ($\Phi_{rad} < 10^{-3}$)[5] indicating that k_{rad}, the radiative rate constant, is small compared to the sum of the nonradiative and reactive rate constants, $k_{nr} + k_{rx}$. In concentrated ClO_4^- solutions, k_{rad} is still negligible.[10] The effect of ClO_4^- on Φ_{rx}, k_{rx}, k_{nr}, and τ is shown in Table I. Perchlorate ion decreases both the nonradiative and reactive rate constants but has a greater effect on the latter. This is in marked contrast to the behavior of the ^2E state of $Cr(en)_3^{3+}$ which is unaffected[12] by the presence of 5.2 M $MgCl_2$ in aqueous solution at 25 °C.

Table I. Effect of $NaClO_4$ on ^2E State of $Cr(bpy)_3^{3+}$

	No added salt	5 M $NaClO_4$
$\Phi_{rx}{}^a$	0.16	0.009
$\tau_{air}{}^b$	4.8×10^{-5} s	7.1×10^{-5} s
$k_{rx}{}^c$	$3.4 \times 10^3\ s^{-1}$	$1.2 \times 10^2\ s^{-1}$
$\tau_0{}^d$	6.3×10^{-5} s	2.9×10^{-4} s
$k_{nr}{}^e$	$1.3 \times 10^4\ s^{-1}$	$0.3 \times 10^4\ s^{-1}$

[a] Quantum yield of photochemical reaction (λ = 313 nm) at 22 °C measured in air-saturated solution at pH 9.6 relative to Φ_{rx} of 0.11 under the same conditions at 11 °C.[9] [b] Lifetime of ^2E state for conditions, given above, under which ϕ_{rx} is determined. [c] Rate constant for the reactive pathway $k_{rx} = \Phi_{rx}\tau_{air}^{-1}$, estimated uncertainty ±25%. [d] Lifetime in N_2-purged solution at 22 °C; $\tau_0^{-1} = k_{nr} + k_{rx} + k_{rad}$. [e] Rate constant for nonradiative pathway calculated from $k_{nr} = \tau_0^{-1} - k_{rx}$, estimated uncertainty ±30%.

The photochemical reaction of $Cr(bpy)_3^{3+}$ is believed to proceed through a seven-coordinate intermediate formed by the addition of a water molecule to the Cr(III) metal center.[9] By analogy to $Fe(phen)_3^{2+}$, $Cu(phen)_3^{2+}$,[13] and closely related Cr(II) complexes,[14] pockets undoubtedly exist between the bpy ligands large enough to accommodate small molecules such as H_2O; in the presence of 5 M ClO_4^-, where ion pairing would be extensive,[15] some of the interligand and solvation sphere water molecules would be expected to be replaced by ClO_4^-. In addition, in concentrated salt solutions a considerable amount of bulk solvent is bound up in solvation of the ions,[16] thereby lowering the activity of water and the rate of formation of the photochemical intermediate, k_{rx}.

The lack of a solvent isotope effect on k_{nr} is indicative of the lack of direct vibrational coupling between the metal-centered ^2E state of $Cr(bpy)_3^{3+}$ and the solvent; a similar lack of a D_2O isotope effect has been reported[17] for $Cr(CN)_6^{3-}$. However, k_{nr} may be affected by changes in solvent polarity owing to electronic dipole perturbation of the ^2E state.[18] Solvent-dependent lifetimes have been reported for other Cr(III) complexes;[16,17,19] in the case of $Cr(CN)_6^{3-}$, the ^2E lifetime has been shown to be a function of solvent polarity.[17] The lack of a solvent effect on the lifetime of the ^2E state of $Cr(bpy)_3^{3+}$ indicates that changes in solvent polarity do not cause sufficient perturbation to alter k_{nr} (within experimental uncertainty). In addition, the ^2E and ^4T states in $Cr(bpy)_3^{3+}$ are effectively isolated.[9] As a result, despite the Laporte and spin forbiddenness of the $^2E \rightarrow {}^4A$ transition in all Cr(III) complexes, the ^2E states of other Cr(III) complexes are substantially shorter lived than the ^2E state of $Cr(bpy)_3^{3+}$

Nonradiative decay of the ^2E state of $Cr(bpy)_3^{3+}$ occurs by transformation of electronic energy into vibrational energy; the ligand may act as both the perturbation (an oscillating dipole) and as the energy acceptor.[18] The anion effect on k_{nr} can be seen as arising from extensive ion pairing and placement of anions in interligand pockets, thereby decreasing both vibrational freedom of the ligands and the efficiency of energy transfer. This model suggests that increasing the rigidity of the bpy ligands should cause a decrease in the rate of energy transfer and a corresponding increase in the ^2E state lifetime. Such an increase in rigidity, accomplished by substituting phen for bpy, is reflected in the low energy metal-nitrogen vibrational modes which are at a higher frequency for tris(phen) complexes than for tris(bpy) complexes.[20] Thus, the ^2E state of $Cr(phen)_3^{3+}$[21] is expected to be longer lived than the corresponding state of $Cr(bpy)_3^{3+}$. Furthermore, the effect of anions on the ^2E state lifetime should be less pronounced for $Cr(phen)_3^{3+}$ than for $Cr(bpy)_3^{3+}$ because the more rigid phen ligands would be less susceptable to perturbation by the interligand anions.