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Conformational Properties of 5-Alkoxy and 5-Alkyl Substituted Trimethylene Phosphates in Solution

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Abstract: The results of thermochemical experiments and ab initio and molecular mechanics calculations on the enthalpies of hydrolysis of structural analogues of cyclic AMP suggested that 5 kcal/mol of the 8 kcal/mol more exothermic enthalpy of hydrolysis of cyclic AMP relative to trimethylene phosphate can be explained by geometric strain resulting from the trans fusion of the trimethylene phosphate and ribofuranoside rings. The remaining 3 kcal/mol of excess enthalpy of hydrolysis could not be accounted for by strain. In this paper we present the results of NMR studies on the solution conformations of trimethylene phosphate (2-hydroxy-2-oxo-1,3,2-dioxaphosphorinane) substituted at the 5 position with alkyl and alkoxy groups. The conformational energies of the alkyl groups are essentially independent of solvent, with values ranging from 0.5 to 0.8 kcal/mol being found for the *equatorial* preferences of methyl, ethyl, isopropyl, and *tert*-butyl. However, with alkoxy groups, the conformational energies were found to be solvent dependent, with the values for 5-methoxy ranging from 1.0 kcal/mol favoring the *axial* position in D_2O to 0.2 kcal/mol favoring the *equatorial* position in acetone-d_6. These results can be explained by assuming that polar solvents preferentially solvate the most polar conformation of a conformationally flexible solute. Since the 5-alkoxy substituent of the trimethylene phosphate ring in cyclic AMP is constrained to be in an equatorial position by the trans fusion of the trimethylene phosphate–ribofuranoside ring system, solvation effects appear to be important in the observed thermodynamic instability of cyclic AMP in water. A biochemical role for this solvation effect is proposed.

In a previous paper in this issue,[2] we reported the results of calorimetric experiments which indicated that the more exothermic enthalpies of hydrolysis of 3′,5′-cyclic nucleotides relative to trimethylene phosphate could be understood on the basis of intramolecular geometric distortion, which amounted to about 5 kcal/mol of energetic destabilization, and on the basis of an unexpected and unexplained effect which appeared to be caused by the presence of the endocyclic oxygen atom of the ribofuranoside ring and was responsible for about 3 kcal/mol of enthalpic exothermicity. The results of ab initio and molecular mechanics calculations described in the preceding paper[3] indicate that the effect of the oxygen atom is not due to introduction of additional geometric distortion.

In this paper, we describe the results of ^1H NMR studies by which we examined the solution conformational behavior of trimethylene phosphates substituted at the 5 position[4] with either alkoxy or alkyl substituents. Our approach was to measure vicinal coupling constants and relate the observed values to the conformational equilibria experienced by the cyclic esters. We have determined that the 5 position is not sterically demanding, since the conformational energies of alkyl substituents with increasing steric requirements (methyl, ethyl, isopropyl, and *tert*-butyl) were no larger than 0.8 kcal/mol favoring the equatorial conformer. However, the conformational energies of alkoxy substituents at the 5 position depend not only on their steric requirements, *but, more significantly, on the polarity of the solvent.* These 5-alkoxy substituted cyclic phosphates might be considered to be simple models of 3′,5′-cyclic nucleotides, and their behavior, therefore, suggests an explanation for the enthalpic effects caused by the ribofuranoside oxygen atom.

Experimental Section

Melting points were measured in open capillaries with a Hoover melting point apparatus and are corrected. ^{31}P NMR spectra were obtained at 32 MHz and ambient temperature with a Varian CFT-20 spectrometer. Phosphorus chemical shifts are expressed relative to an external capillary containing 85% H_3PO_4; upfield chemical shifts are expressed as *negative* numbers. High-resolution ^1H NMR spectra were obtained at 30 °C and at 270 MHZ using the Bruker spectrometer of the Southern New England High Resolution NMR Facility at Yale University; this instrument is equipped for simultaneous proton and phosphorus decoupling experiments. Elemental analyses were performed by Galbraith Laboratories, Knoxville, Tenn. Deuterated solvents were purchased from Stohler Isotopes.

General Synthetic Procedures. The syntheses of the phenyl esters of cyclic phosphates were accomplished by phosphorylation of a diol with phenyl dichlorophosphate in dry pyridine. The usual workup was evaporation of the solvent, partitioning of the residue between dilute aqueous hydrochloric acid and chloroform, and extraction of the chloroform solution with additional acid, water, and finally 7.5%

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aqueous sodium bicarbonate solution. The dried chloroform layer ($MgSO_4$) was evaporated to yield the phenyl triester (a mixture of two isomers).

The cyclic phosphate diesters were routinely obtained from the phenyl esters either by hydrogenolysis of an ethanolic solution of the triester in a Parr apparatus using Adam's catalyst or by base-catalyzed hydrolysis.

Phenyl Esters of 5-Methoxytrimethylenephosphoric Acid. 2-Methoxy-1,3-propanediol[5] was phosphorylated with phenyl dichlorophosphate to yield a mixture of two phenyl triesters: isomer a, δ_{31P} −17.55 ppm ($CDCl_3$), and isomer b, δ_{31P} −15.00 ppm ($CDCl_3$), in a ratio of 64:36, as assessed by integration of the ^{31}P NMR spectrum. The isomeric phenyl esters were separated by fractional crystallization from ligroin–ethanol to yield isomer a, uncontaminated by isomer b, and isomer b, which had an isomeric purity of 95%, as judged by ^{31}P NMR. Neither of the isomers was subjected to elemental analysis, since analytically pure 5-methoxytrimethylenephosphoric acid could be obtained in high yield by hydrogenolysis of a mixture of the isomers. In addition, both ^{31}P and ^1H NMR spectra were in accord with their proposed structures.

Phenyl Esters of 5-Methyltrimethylenephosphoric Acid. 2-Methyl-1,3-propanediol[6] was phosphorylated with phenyl dichlorophosphate to yield a mixture of two phenyl triesters: isomer a, δ_{31P} −16.26 ppm ($CDCl_3$), and isomer b, δ_{31P} −16.13 ppm ($CDCl_3$), in a ratio of 55:45, as assessed by integration of the ^{31}P NMR spectrum. The isomeric phenyl esters were also separated by fractional crystallization from ligroin–ethanol to yield isomer a, uncontaminated by isomer b, and isomer b, which had an isotopic purity of 90%, as judged by ^{31}P NMR. Again, neither isomer was subjected to elemental analysis, since pure 5-methyltrimethylenephosphoric acid was obtained in high yield by hydrogenolysis of a mixture of the two isomers. Both the ^{31}P and ^1H NMR spectra of these compounds were in accord with their proposed structures.

5-Methoxytrimethylenephosphoric Acid. Hydrogenolysis of a mixture of the phenyl esters of this diester afforded a residue which was crystallized from dioxane, mp 101.5–104.0 °C (lit. mp 101.5–104.0 °C[2]).

5-Ethoxytrimethylenephosphoric Acid. 2-Ethoxy-1,3-propanediol[7] was phosphorylated to yield a mixture of phenyl esters which was hydrogenolyzed to give the acid which crystallized from chloroform as colorless prisms, mp 108.5–109.5 °C. The structure of this compound was established by X-ray crystallography.[8]

Cyclohexylammonium 5-Isopropoxytrimethylene Phosphate. 2-Isopropoxy-1,3-propanediol[7] was phosphorylated and the resulting mixture of triesters was hydrolyzed in 2 N NaOH dissolved in a dioxane–water mixture (1:3). The reaction mixture was neutralized to pH 5 with Amberlite IR-120 (H^+) and the phenol removed by extraction with ether. The sodium salt was absorbed to DEAE-Sephadex A-25 (HCO_3^-) and eluted with a gradient of 0–0.2 M trimethylammonium bicarbonate, pH 8.5. The fractions containing the diester product were concentrated in vacuo, and the triethylammonium salt was converted to the cyclohexylammonium salt by percolation through a column of IR-120 (cyclohexylammonium). The cyclohexylammonium salt was crystallized from ethanol–diethyl ether. A correct elemental analysis could not be obtained for this compound, although it appeared pure by both ^{31}P and ^1H NMR.

Cyclohexylammonium 5-*tert*-Butoxytrimethylene Phosphate. This salt was prepared from 2-*tert*-butoxy-1,3-propanediol[7] according to a procedure analogous to that used for the isopropoxy cyclic ester. The salt was crystallized from ethanol–diethyl ether to yield colorless material, mp 182–190 °C dec. Anal. Calcd for $C_{13}H_{28}NO_5P$: C, 50.48; H, 9.12; N, 4.53; P, 10.01. Found: C, 50.35; H, 9.23; N, 4.63; P, 10.19.

5-Methyltrimethylenephosphoric Acid. Hydrogenolysis of a mixture of the phenyl esters of this diester afforded a residue which was crystallized from ethyl acetate, mp 112.5–114 °C (lit. mp 112.5–114 °C[2]).

5-Ethyltrimethylenephosphoric Acid. The acid was prepared by phosphorylation of 2-ethyl-1,3-propanediol[6] with phenyl dichlorophosphate and hydrogenolysis of the resulting mixture of phenyl esters. Crystallization from ligroin gave analytically pure crystals, mp 68–69 °C. Anal. Calcd for $C_5H_{11}O_4P$: C, 36.15; H, 6.68; P, 18.65. Found: C, 36.19; H, 6.64; P, 18.48.

5-Isopropyltrimethylenephosphoric Acid. The acid was prepared as described for the 5-ethyl cyclic phosphate, but using 2-isopropyl-1,3-propanediol.[6] The acid was crystallized from ligroin, mp 87–88 °C. Anal. Calcd for $C_7H_{15}O_4P$: C, 43.30; H, 7.79; P, 15.95. Found: C, 43.15; H, 7.46; P, 15.83.

5-*tert*-Butyltrimethylenephosphoric Acid. The acid was prepared as described for the 5-ethyl analogue, but using 2-*tert*-butyl-1,3-propanediol.[9] The acid was crystallized from ligroin, mp 123–128 °C. Anal. Calcd for $C_7H_{15}O_4P$: C, 43.30; H, 7.79; P, 15.95. Found: C, 43.15; H, 7.46; P, 15.83.

For ^1H NMR studies of the 5-substituted cyclic esters, tetrabutylammonium or tetramethylammonium salts were prepared by percolation of the acid through an Amberlite IR-50 (tetraalkylammonium) column.

Coupling-Constant Determinations. For non-first-order spectra, the vicinal proton–proton and phosphorus–proton coupling constants were obtained by an iterative least-squares procedure using the program LACX, a copy of which, modified for use on an IBM 370, was kindly provided by Professor K. B. Wiberg of this department. Spectra were simulated on a PDP 11/45 using the program NMRSIM, which was kindly provided by Professor J. W. Faller of this department. For first-order spectra, the coupling constants were obtained directly from the spectra.

Conformational Free Energies. Conformational free energies for the substituted trimethylene phosphates were calculated from the equilibrium mole fraction populations of the two possible chair conformers, that with the substituent axial, X_{ax}, and that with the substituent equatorial, X_{eq}, according to the equation

$$\Delta G = -RT \ln (X_{ax}/X_{eq}) \quad (1)$$

so a negative conformational free energy corresponds to an equilibrium in which the axial conformer is the more populated.

The quantities X_{ax} and X_{eq} were obtained from the vicinal proton–proton (J_{HCCH}) and phosphorus–proton (J_{POCH}) coupling constants observed for an equilibrating system, J_{obsd}, according to the general equations

$$J_{obsd} = J_{ax}X_{ax} + J_{eq}X_{eq} \quad (2)$$

and

$$X_{ax} + X_{eq} = 1 \quad (3)$$

where J_{ax} and J_{eq} refer to the coupling constants for nonequilibrating axial or equatorial conformers, respectively. The separated cis and trans phenyl esters of 5-methoxytrimethylenephosphoric acid and of 5-methyltrimethylenephosphoric acid were used as models of the nonequilibrating conformers.

Since the values obtained for J_{POCH} for the neutral triesters might not be valid for anionic diesters, we calculated separately values for the conformational free energies for J_{HCCH} and for J_{POCH}. We found that both methods yield similar values, but we have reported free-energy values based on only the values for J_{HCCH} since those for J_{POCH} could be influenced by structural effects, e.g., changes in bond angle at the oxygen atom.

Results and Discussion

Assignment of the Conformations of the Phenyl Triesters. The 270-MHz ^1H NMR spectra of isomer a of the phenyl ester of 5-methoxytrimethylenephosphoric acid dissolved in Me_2SO-d_6 is shown in Figure 1 and that of isomer b of the same cyclic triester dissolved in CS_2 is shown in Figure 2; the coupling constants derived from non-first-order analyses of these spectra and proton and/or phosphorus decoupled spectra are listed in Table I. The spectrum of isomer a is that of a deceptively simple A_2B_2MX system, in which fewer lines than the number expected are observed; this is caused by fortuitous values for the chemical-shift difference between the A and B protons and their coupling constants to the phosphorus nucleus. On the basis of the coupling constants for these isomeric materials and Majoral et al.'s finding that 2-phenoxy substituents of 2-oxo-1,3,2-dioxaphosphorinane ring systems prefer an axial orientation in all solvents[10] (although the preference is more pronounced in polar solvents), we have assigned isomer a as the trans ester and isomer b as the cis ester. The coupling-constant evidence for this assignment is the equal and small values of J_{HCCH} observed in isomer a, this implying two gauche arrangements between the pairs of nonequivalent protons on

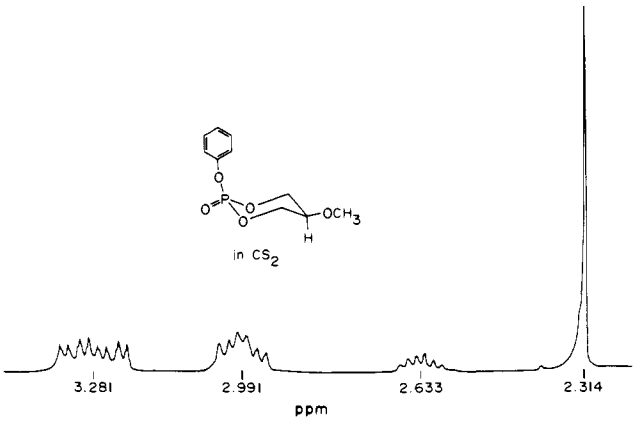

Figure 1. 270-MHz ^{1}H NMR spectrum of isomer a of the phenyl ester of 5-methoxytrimethylene phosphate dissolved in Me_2SO-d_6.

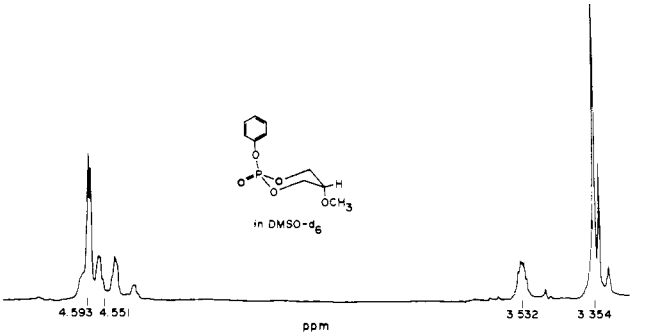

Figure 2. 270-MHz ^{1}H NMR spectrum of isomer b of the phenyl ester of 5-methoxytrimethylene phosphate dissolved in CS_2.

C_4 and C_6. The spectrum for isomer b is not deceptively simple, and the protons on C_4 and C_6 are characterized by two unequal values of J_{HCCH} which are consistent with one gauche and one trans arrangement of protons.[11]

By a similar line of reasoning, the cis and trans esters of 5-methyltrimethylenephosphoric acid were assigned to the b and a isomers, respectively. Neither of these spectra was deceptively simple, and the coupling constants derived from first-order analysis of spectra obtained for these isomers dissolved in Me_2SO-d_6 are listed in Table II.

Conformational Energies of the Diesters. A 270-MHz spectrum of the tetrabutylammonium salt of 5-methoxytrimethylene phosphate dissolved in D_2O is reproduced in Figure 3; this is also a deceptively simple non-first-order spectrum. The vicinal proton–proton and phosphorus–proton coupling constants are compared with those of the two phenyl esters in Table I. The values we have found for the diester salt are essentially the same as those found for the trans phenyl ester, with a small and perhaps significant difference being found for the values of J_{HCCH} for the salt and trans ester.[12] The relatively large uncertainties in the values of J_{POCH} for the trans ester and the salt are the result of their spectra being deceptively simple. Using the values for J_{HCCH} in Table I and the equations in the Experimental Section, the conformational free energy for the 5-methoxy substituent in the tetrabutylammonium salt of the diester is 1.0 kcal/mol favoring *axial* disposition; the values for J_{POCH} do not reveal any equilibration. The reason for this discrepancy presumably is that for values of J_{POCH} the phenyl esters may not be perfect models for the diester conformers. Moreover, when the conformational equilibrium strongly favors either conformer, evaluation of conformational free energies from either J_{HCCH} or J_{POCH} may not be extremely precise, but this approach is adequate to describe the effects we have found and is the only one possible for rapidly equilibrating diester anions discovered in polar solvents. The accuracy of our free energies is also limited by the ±0.2-Hz resolution of the Fourier transform spectra and the error in the analyses of deceptively simple non-first-order spectra.

In Table I we have also listed the coupling constants found for the tetrabutylammonium salt of 5-methoxytrimethylene phosphate in acetonitrile-d_3. From the values for J_{HCCH} and an analysis analogous to that used for the spectrum of this salt in D_2O, we can calculate that in acetonitrile the conformational free energy is 0.6 kcal/mol favoring the *axial* conformer and that in acetone the conformational free energy is 0.2 kcal/mol favoring the *equatorial* conformer (Table III). Thus, the conformational free energy of this cyclic phosphate is solvent dependent.

In contrast to this behavior, the conformational free energy

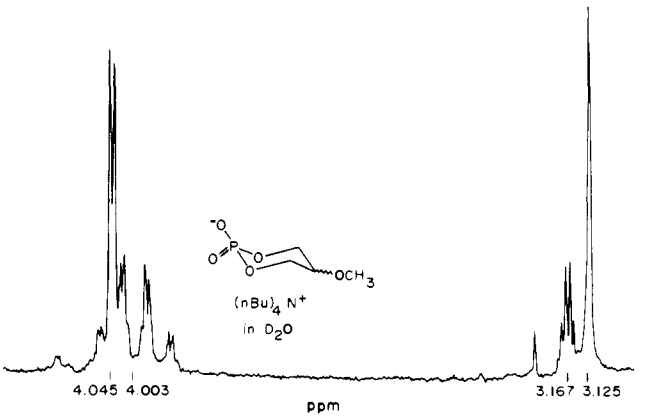

Figure 3. 270-MHz ^{1}H NMR spectrum of the tetrabutylammonium salt of 5-methoxytrimethylene phosphate dissolved in D_2O.

of 5-methyltrimethylene phosphate is much less solvent dependent. In Table II we also compare the coupling constants for the tetrabutylammonium salt of 5-methyltrimethylene phosphate dissolved in D_2O and acetonitrile-d_3 with its trans and cis phenyl esters. No coupling-constant information can be obtained in acetone-d_6, since the chemical shifts of the methylene protons are identical. The average value for the conformational free energy of the 5-methyl group is 0.5 kcal/mol favoring the *equatorial* position.

A solvent-dependent behavior similar to that found for the 5-methoxy group is also observed in the spectra of the tetrabutylammonium salts of 5-ethoxy-, 5-isopropoxy-, and 5-*tert*-butoxytrimethylene phosphates (Table III); the conformational energies for 5-ethyl, 5-isopropyl, and 5-*tert*-butyl groups were observed to be solvent independent. We find that the conformational free energies of the alkyl groups favor the equatorial conformers, with only a slight increase being observed as the size of the substituent increases. Bentrude has documented a value of 1.0 kcal/mol favoring the equatorial conformation for *tert*-butyl groups in related 1,3,2-dioxaphosphorinane ring systems.[13] The range of values we observe is much smaller than that associated with either the cyclohexane or 1,3-dioxane ring systems and is presumably the result of small diaxial interactions and the flattened nature of the 1,3,2-dioxaphosphorinane ring system. Qualitatively, the behavior we observe for the alkoxy series is a minimal steric effect added to a more pronounced solvent-dependent effect. There is little diminution or enhancement of the solvent-dependent effect by increasing the steric requirement or hydrophobicity of the alkoxy substituent.

Solvent Dependence of Conformational Energies. What is the explanation for the solvent dependency of the conformational energies of alkoxy groups when compared to alkyl groups of similar size? A simple qualitative explanation for the observed conformational behavior of alkoxy groups relative to

Table I. Coupling Constants of Phenyl Esters and Tetrabutylammonium Salt of 5-Methoxytrimethylene Phosphate[a]

	J_{POCH}		J_{HCCH}	
	axial H	equatorial H	axial H	equatorial H
trans ester in Me_2SO-d_6	2.2 ± 1.0	22.0 ± 1.0	1.5 ± 0.2	1.5 ± 0.2
cis ester in CS_2	4.7 ± 0.2	20.3 ± 0.2	9.2 ± 0.2	4.5 ± 0.2
salt in D_2O	1.2 ± 0.4	22.2 ± 0.4	2.2 ± 0.2	2.2 ± 0.2
salt in acetonitrile-d_3	7.1 ± 0.2	15.6 ± 0.2	2.2 ± 0.2	3.6 ± 0.2
salt in acetone-d_6	10.6 ± 0.2	12.5 ± 0.2	5.4 ± 0.2	3.2 ± 0.2

[a] In hertz. Axial and equatorial assignments were made by designating the proton with the smaller J_{POCH} as the axial proton, i.e., the one spending most of its time in a gauche relationship to the phosphorus atom.

Table II. Coupling Constants of Phenyl Esters and Tetrabutylammonium Salt of 5-Methyltrimethylene Phosphate[a]

	J_{POCH}		J_{HCCH}	
	axial H	equatorial H	axial H	equatorial H
trans ester in Me_2SO-d_6	2.7 ± 0.2	20.6 ± 0.2	2.6 ± 0.2	3.1 ± 0.2
cis ester in Me_2SO-d_6	<1.0	22.7 ± 0.2	11.4 ± 0.2	4.5 ± 0.2
salt in D_2O	7.9 ± 0.2	15.7 ± 0.2	9.0 ± 0.2	4.1 ± 0.2
salt in acetonitrile-d_3	6.1 ± 0.2	17.3 ± 0.2	9.7 ± 0.2	4.5 ± 0.2

[a] In hertz. Axial and equatorial assignments were made by designating the proton with the smaller J_{POCH} as the axial proton, i.e., the one spending most of its time in a gauche relationship to the phosphorus atom.

Table III. Solvent Dependence of Conformational Energies of 5 Substituents[a]

	5-methoxy	5-ethoxy	5-isopropoxy	5-*tert*-butoxy
D_2O	−1.0	−1.1	−0.9	−0.2
acetonitrile-d_3	−0.6	−0.2	−0.3	+0.7
acetone-d_6	+0.2	+0.3	+0.3	+2.0
	5-methyl	5-ethyl	5-isopropyl	5-*tert*-butyl
D_2O	+0.6	+0.4	+0.6	+0.7
acetonitrile-d_3	+0.5	+0.2	+0.6	
acetone-d_6		+0.5	+0.7	+0.8

[a] In kcal/mol.

alkyl groups is that the axial conformer of an alkoxy substituted cyclic phosphate is more polar (possesses a larger dipole moment) than the equatorial conformer, and polar solvents should preferentially solvate the more polar conformation of a solute which has conformational flexibility. In less polar solvents, the predominant effect is the steric effect, and the conformational equilibria observed for the alkoxy substituents in acetone-d_6 are similar to those observed for the alkyl substituents; we have found in the cases of the salts of the 5-ethoxy- and 5-ethyltrimethylene phosphates that a further decrease in solvent polarity by using tetrahydrofuran-d_8 has negligible effect on the conformational free energies. That hydrogen bonding is *not* an important factor in the axial preference of the alkoxy groups is demonstrated by the fact that the conformational equilibria are the same in methanol-d_4 as in acetonitrile-d_3, two solvents of similar dielectric constant (32.6 vs. 36.2, respectively). We have also determined that the identity of the tetraalkylammonium cation is not important in these equilibria, since the conformational energies found for the 5-ethyl and 5-ethoxy substituents were identical in all solvents for the tetramethylammonium and tetrabutylammonium cations (used throughout this study to increase the solubility of the salts). The axial preference of polar substituents is not what one might have anticipated, since in the absence of intermolecular forces and the "gauche effect" [14] an electronegative substituent might have been expected to assume an equatorial position, owing to the repulsion of the net dipole of the phosphate ester ring and that of the substituent. The "gauche effect" does not appear to be significant for alkoxy substituents, since in relatively nonpolar solvents there is little difference between the conformational energies of alkoxy and alkyl substituents with similar steric requirements.

A similar conformational preference of alkoxy groups (and other polar substituents) has been noted for substituents at C_5 in the 1,3-dioxane ring system.[15] Kaloustian[16] has described the solvent dependence of this system by assuming that the conformational energy difference between two conformers can be represented by

$$\Delta E_{conf} = \Delta E_{struct} + \Delta E_{solv} + \Delta E_{dipole} \quad (5)$$

and

$$\Delta E_{struct} = \Delta E_{bonds} + \Delta E_{angles} + \Delta E_{torsional} \quad (6)$$

ΔE_{struct} should be solvent independent, since the energy terms are those arising from intramolecular effects. However, ΔE_{solv}, the difference in solvation energies, and ΔE_{dipole}, the difference in dipolar interactions, should be highly solvent dependent. ΔE_{solv} should be zero in the gas phase but should increase with increasing solvent polarity; ΔE_{dipole} is at a maximum in the gas phase and should approach some minimum value in very polar solvents. Thus, as the solvent polarity increases, both of these energy terms should favor the more polar conformation. This qualitative explanation, therefore, is consistent with the solvent-dependent conformational equilibria we have observed.

Additional evidence for the unusual conformational preferences of the 5-alkoxy group has been obtained by an X-ray crystallographic study of 5-ethoxytrimethylenephosphoric acid.[8] Two molecules are present in the asymmetric unit of these crystals, and each molecule is found to be in a chair conformation which is similar to those observed for trimethylenephosphoric acid and its phenyl ester. Interestingly, the ethoxy groups of both molecules are found to be *axial* substituents of the rings. Examination of the crystal packing diagram of the crystal provides an explanation for why this molecule is found to be in its most polar conformation in the solid state. Dipolar interactions between adjacent molecules in the crystal provide a polar environment for each molecule, thereby encouraging the axial disposition of the polar substituent. We are aware of no other example of a crystal structure of a conformationally flexible ring system in which a single polar substituent is found in an axial orientation. Our NMR data reported in the present paper and the crystallographic study provide compelling evidence that the conformation of a flexible molecule should be expected to be responsive to the polarity of its environment and experience a significant solvent-dependent conformational equilibrium.

In the case of the 1,3-dioxanes, no alkoxy substituent had as distinct an axial preference of many of those we have

Table IV, Solvent Dependence of Coupling Constants of Phenyl Esters of 5-Methoxytrimethylene Phosphate[a]

	J_{POCH}			J_{HCCH}		
	axial H	equatorial H	sum	axial H	equatorial H	sum
			Trans Ester			
Me_2SO-d_6	2.2 ± 1.0	22.0 ± 1.0	24.2	1.5 ± 0.2	1.5 ± 0.2	3.0
acetonitrile-d_3	1.6 ± 0.4	22.4 ± 0.4	24.0	1.6 ± 0.2	1.5 ± 0.2	3.1
acetone-d_6	1.1 ± 0.2	23.0 ± 0.2	24.1	1.7 ± 0.2	1.7 ± 0.2	3.4
chloroform-d	1.0 ± 0.3	22.2 ± 0.3	23.2	1.9 ± 0.2	1.9 ± 0.2	3.8
benzene-d_6	1.3 ± 0.2	22.4 ± 0.2	23.7	1.9 ± 0.2	1.8 ± 0.2	3.7
			Cis Ester			
Me_2SO-d_6	8.7 ± 0.3	11.5 ± 0.3	20.2	6.9 ± 0.2	3.8 ± 0.2	10.6
acetonitrile-d_3	7.6 ± 0.2	16.0 ± 0.2	23.6	7.3 ± 0.2	4.1 ± 0.2	11.4
chloroform-d	6.7 ± 0.2	18.3 ± 0.2	25.0	8.1 ± 0.2	4.3 ± 0.2	12.4
CS_2	4.7 ± 0.2	20.3 ± 0.2	25.0	9.2 ± 0.2	4.5 ± 0.2	13.7

[a] In hertz. Axial and equatorial assignments were made by designating the proton with the smaller J_{POCH} as the axial proton, i.e., the one spending most of its time in a gauche relationship to the phosphorus atom.

found;[15] however, acetonitrile was the most polar solvent studied, since water would have led to hydrolysis of the ring system.

The magnitude of the solvent-dependent conformational effect can be estimated by comparing the conformational energies of an alkoxy group and an alkyl group with similar steric requirements, e.g., methoxy and ethyl; the difference in the conformational free energies for these groups is 1.5 kcal/mol. That the conformational energy of the alkoxy group in relatively nonpolar solvents is similar to that of the analogous alkyl group indicates that this 1.5 kcal/mol of energy is not primarily due to the "gauche effect", which by itself might be expected to be relatively solvent independent and which certainly should be observable in nonpolar solvents. In 1,2-difluoroethane, the polar C–F bonds are known to prefer the gauche orientations to a degree unexpected on the basis of electrostatics.[17] The enthalpy difference between the two rotamers is about zero in the gaseous state (a 2:1 ratio of gauche and trans rotamers) and about 0.9 kcal/mol in the liquid phase (a 9:1 ratio of gauche and trans rotamers); a study of the solvent dependence of this rotameric distribution indicates a substantial effect,[18] with increasing amounts of the gauche rotamer occurring with an increase in solvent polarity. Thus, in 1,2-difluoroethane the data indicate that the rotameric distribution is a composite of a significant gauche effect and a significant response of the polarity of the molecule to the polarity of the solvent. Molecules with vicinal C–O bonds do not appear to have as significant a gauche effect as that found for 1,2-difluoroethane.[19] Our laboratory is currently undertaking the syntheses of 5-fluoro-, 5-chloro-, and 5-bromotrimethylenephosphoric acids to determine the conformational preferences of these substituents and also to determine whether a gauche effect will be observed with the fluoro substituent.

Solvent-dependent conformational equilibria for the 5-alkoxy groups were also observed in the cis but not the trans phenyl ester of 5-methoxytrimethylenephosphoric acid. In Table IV we compare the values for J_{HCCH} and J_{POCH} for these isomeric compounds. The decrease in the sum of the values for J_{POCH} with increasing solvent polarity can be explained if the ring populates a twist-boat conformation, and result in increasing axial preference for both the 5-methoxy and 2-phenoxy groups.[20] This solvent-dependent effect accounts for our choice of solvents in obtaining the coupling constants for axial and equatorial conformers.

Relationship to 3′,5′-Cyclic Nucleotides. What is the relationship between these conformational preferences and the thermodynamic stability of 3′,5′-cyclic nucleotides? About 3 kcal/mol of the excess enthalpy of hydrolysis found for the cyclic esters derived from trans fusion of a trimethylene phosphate ring to a tetrahydrofuran ring and all of the excess enthalpy of hydrolysis of methyl α-D-glucopyranoside 4,6-cyclic phosphate (also about 3 kcal/mol) relative to that of trimethylene phosphate has been related by our calorimetric studies[2] and ab initio and molecular mechanics calculations[3] to the presence of the oxygen atom at the 5 position of the cyclic phosphate ring.[2] In all of these compounds, this alkoxy substituent is rigidly constrained to be an equatorial substituent of the cyclic phosphate ring. Our results reported in this paper indicate that this orientation is unfavorable, at least in polar solvents such as water, and we, therefore, propose that about one-half of the excess enthalpy of hydrolysis which is associated with the equatorial oxygen substituent is due to the preference of alkoxy groups to be axial substituents of six-membered cyclic phosphate rings, *leading to a destabilization of these compounds relative to trimethylene phosphate.*

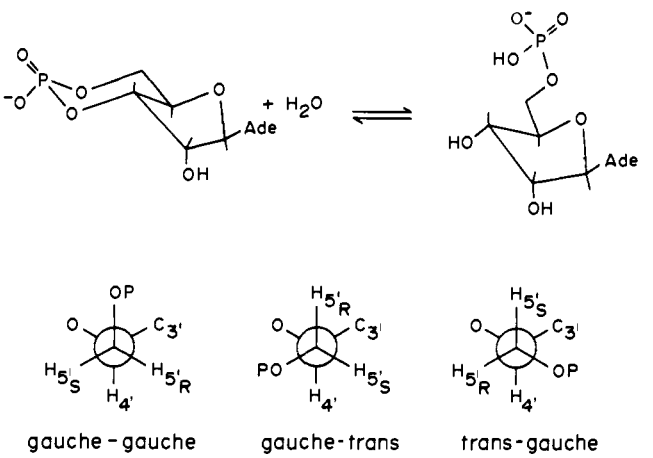

Figure 4. Rotameric geometries about the $C_{4'}$–$C_{5'}$ bond in cyclic AMP and 5′-AMP. In cyclic AMP, the trans-gauche geometry is imposed by the trans ring fusion, but in 5′-AMP all three rotameric geometries are, in principle, accessible.

The remaining contribution to the enthalpies of hydrolysis of the cyclic esters trans fused to either a tetrahydrofuran or tetrahydropyran ring is not directly revealed by this study. But our observation that the polarity of the solvent is an important determinant in the solution structure of flexible molecules which can populate conformations of different polarity provides a suggestion as to the source of the remaining excess enthalpy. The hydrolysis products of these molecules can also undergo a solvent–solute interaction similar to that we have found for cyclic esters. Using 5′-AMP as an example, rotation about the $C_{4'}$–$C_{5'}$ bond from the trans-gauche geometry imposed by the cyclic phosphate ring to either the gauche-gauche or gauche-trans geometry permitted after hydrolysis will lead to a significant increase in the polarity of (at least a portion of) the hydrolysis product (Figure 4). An increase in the polarity of the solute should lead to enhanced solvation, and this would

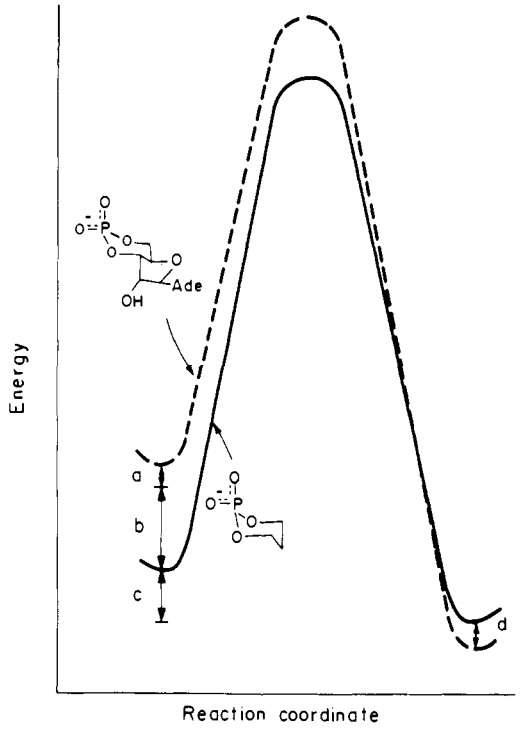

Figure 5. Energy diagram for the hydrolyses of cyclic AMP and trimethylene phosphate. The energy contribution designated by (a) represents the solvent-dependent destabilization of cyclic AMP relative to trimethylene phosphate, (b) represents the geometric destabilization in cyclic AMP caused by the trans ring fusion, (c) represents the enthalpy of hydrolysis of an unsubstituted six-membered ring, and (d) represents the solvent-dependent stabilization of 5′-AMP relative to 3-hydroxypropyl phosphate.

lead to *a stabilization of the hydrolysis product relative to that derived from trimethylene phosphate.* In experiments still in progress we have observed that the rotameric distribution about the exocyclic C–C bonds in analogues structurally related to 5′-AMP is significantly influenced by the polarity of the solvent with the polar rotamers being favored in water and the trans-gauche rotamer in less polar solvents (e.g., acetonitrile).[22]

In Figure 5 we present an energy diagram for the hydrolyses of cyclic AMP and trimethylene phosphate. The 8 kcal/mol difference in their enthalpies of hydrolysis is due to strain which amounts to 5 kcal/mol (b) and the solvent-dependent contributions just described which total about 3 kcal/mol (a and d).

Our conclusion that the thermodynamic instability of 3′,5′-cyclic nucleotides has a major component which is dependent on solvent may suggest a role for the "high-energy" nature of these molecules in biological systems. Utilization of this energy by opening of the cyclic phosphate ring when the cyclic nucleotides interact with protein kinases is apparently unimportant. However, the fact that the thermodynamic destabilization is solvent dependent may indicate that nature has chosen not to utilize the destabilization directly but rather indirectly by binding the molecule to a hydrophobic environment, e.g., a binding site on protein kinase. In binding small, polar molecules to hydrophobic sites, an enzyme would normally have to utilize a portion of the binding energy to cause desolvation of the small molecule, thereby decreasing the energy available for conformational changes, etc. However, with the cyclic nucleotides and their nonpolar arrangements of dipoles, the energy required for desolvation may be significantly less, leading to the observed tight binding and/or the conformational change which cyclic nucleotides induce in most protein kinases to cause dissociation of the regulatory and catalytic subunits (Figure 6). The importance of this polarity

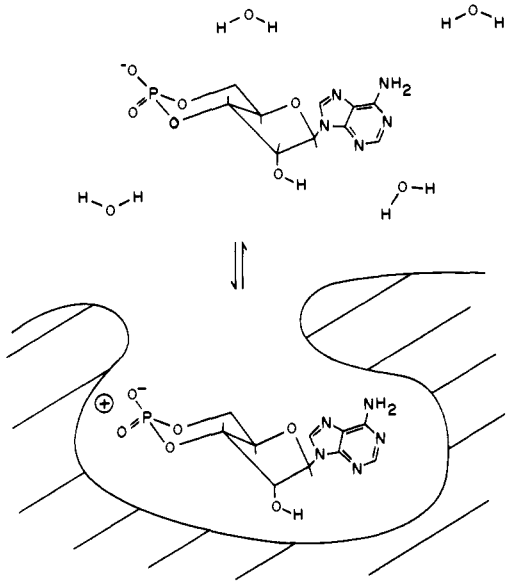

Figure 6. A conceptualized representation of the role of solvent–solute interactions in the biochemical activity of cyclic AMP. The low polarity of cyclic AMP (relative to acyclic nucleotides) results in poor solvation which increases the ability of cyclic AMP to be transferred from water to hydrophobic sites on protein molecules.

effect is currently being assessed in our laboratory in both chemical and enzymatic systems.

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