to the importance of solvation effects in the biochemical activity of 3′,5′-cyclic nucleotides.

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Theoretical Calculations on the Geometric Destabilization of 3′,5′- and 2′,3′-Cyclic Nucleotides[1]

Frederick J. Marsh,[2a] Paul Weiner,[2a] John E. Douglas,[2a]
Peter A. Kollman,*[2a,d] George L. Kenyon,*[2a,b,e] and John A. Gerlt*[2c,f]

Contribution from the Department of Pharmaceutical Chemistry, School of Pharmacy,
Department of Biochemistry and Biophysics, School of Medicine,
University of California, San Francisco, California 94143, and the Department of Chemistry,
Yale University, New Haven, Connecticut 06520. *Received February 5, 1979*

Abstract: We present molecular mechanics and ab initio quantum mechanical calculations on some cyclic nucleotides and analogues with the goal of accounting for the unusually exothermic heats of hydrolysis of the six membered ring containing phosphodiester, 3′,5′-cyclic adenosine monophosphate (AMP), and of the five membered ring containing phosphodiester, 2′,3′-cyclic AMP. We attribute the greater exothermicity for hydrolysis of 3′,5′-cyclic AMP and the corresponding simple model compounds relative to that for trimethylene phosphate (8 kcal/mol) to the following effects: (1) the trans ring fusion in 3′,5′-cyclic AMP, which leads to 4-5 kcal/mol of strain energy in this molecule, and (2) the presence in the 3′,5′-cyclic nucleotide of an unfavorable O-C-C-O interaction which is relieved upon phosphate ring cleavage. This second interaction, which is solvent dependent, can account for about 1-2 kcal/mol of the difference in hydrolysis energy. (3) This leaves approximately 1-2 kcal/mol unaccounted for, and this may arise from differential solvation energies of the reactant and product. Our calculations overestimate the exothermicity of hydrolysis of the five membered ring containing phosphodiester, 2′,3′-cyclic AMP, but find, in agreement with experiment, that its exothermicity is much closer to the simple model phosphodiester, ethylene phosphate, than found in the corresponding six membered ring phosphodiesters.

Introduction

One of our laboratories has recently obtained thermochemical evidence that the unusually exothermic enthalpy of hydrolysis of 3′,5′-cyclic adenosine monophosphate (AMP) is determined by the trans-fused trimethylene phosphate-tetrahydrofuran portion of the structure, i.e., the substituents on C1′ (the heterocyclic base) and on C2′ (a hydroxyl group) are not required to produce the 8 kcal/mol more exothermic enthalpy of hydrolysis which cyclic AMP (and analogues which have this structure) display relative to trimethylene phosphate.[3] However, the enthalpy of hydrolysis of a structural analogue having trans-fused trimethylene phosphate-cyclopentane rings was about 3 kcal/mol less exothermic than those with tetrahydrofuran rings, suggesting that both the trans ring fusion and some uncharacterized effect resulting from the tetrahydrofuran oxygen were responsible for the total heat effect. Presumably, the trans ring fusion effect is caused by geometric distortion (strain), but the origin of the effect associated with the oxygen atom was not clear. Perhaps this latter effect could also be due to geometric distortion, since C-O bonds are about 0.08 Å shorter than C-C bonds and this shorter bond length could introduce additional strain.

In this paper we describe the results of molecular mechanics and ab initio calculations which support the notion of considerable strain being associated with the trans ring fusion in the 3′,5′-cyclic AMP, but which suggest that *inter*molecular considerations (solvation) are necessary to provide the complete explanation for the enthalpy of hydrolysis of 3′,5′-cyclic AMP. Results on calculations of hydrolysis energies on 2′,3′-cyclic nucleotides are also presented for comparison purposes. In the following paper,[4] experimental data from one of our laboratories are presented which implicate differential solvation of the reactants and products of the hydrolysis reaction as the source of the unaccounted for enthalpy of hydrolysis in the 3′,5′-cyclic AMP case.

Methods of Procedure

For the quantum-mechanical calculations, we employed an ab initio SCF approach using the program GAUSSIAN 70[5] and an STO-3G basis set.[6] The molecular mechanics calculations

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used the QCFF/PI[7] program developed by Warshel and Levitt. This program contains parameters for only some of the atoms in the molecules we considered, so we had to add parameters for phosphorus, sp^3 oxygen, and the partially negative oxygens of the phosphate anion grouping. We either chose these parameters from the literature[8] or based them on the properties of known, simple model compounds. We are aware of the perils of using a hybrid "force field", i.e., some parameters from one source, some from another, because the parameters are often very interdependent. Thus, changing or adding parameters to a set is likely to change the optimum value for remaining parameters. We attempted to avoid these perils by judicious comparison of calculated structures and relative energies for model compounds with those found in X-ray crystal structures and ab initio calculations. When we were satisfied that the parameters gave reasonable agreement with the model cases, we proceeded to study the cyclic phosphates. The parameters we used that were not in the Warshel–Levitt program are listed in Tables V–VIII. Unless otherwise specified, we completely optimized the molecular geometry until the energies changed less than 0.01 kcal/mol and until the gradients were smaller than 0.1 kcal/mol.

Model Calculations

We compared the structures of sugar rings found by X-ray crystallography (Table I) with those found in the molecular mechanics calculations. The bond-length and bond-angle agreement was reasonable, and a comparison of the sugar puckering parameters is also given in Table I. In general, the magnitude of the sugar pucker from a least-squares plane (q) was somewhat underestimated by the force-field calculations, but the agreement between the calculations and experiment for the sense of the puckering (ϕ) was quite good. The results of the calculations on 5′-AMP with and without the base are interesting because the calculations without the base (in parentheses) give a very different pseudorotational parameter than those with the base, with the latter in very good agreement with experiment. For 3′-AMP, the calculations with and without base gave very similar values of ϕ (~80°).

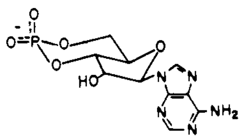
Next, we turned to torsional parameters for the phosphate group. We desired these to reproduce both the well-known tendency of ROPO groups to adopt a gauche rather than a trans conformation and the fact that this gauche tendency increased as θ (OPO) increased. The set of parameters $V_2 = -0.75$ and $V_3 = +0.75$ does this in a qualitatively satisfactory way for the dimethyl phosphate anion (**7**). It gives a ϕ_1, ϕ_2 map that is very similar to that obtained by Newton[21] and does indeed lead to a greater tendency for g,g as compared to g,t isomers at θ(OPO) = 105° (ΔE = 1.06 kcal/mol) than at θ(OPO) = 95° (ΔE = 0.93 kcal/mol).[22] These parameters do not lead to so large a conformational sensitivity to θ(OPO) as found either experimentally or by quantum-mechanical calculations.[22] However, the OPO angle in 3′,5′-cyclic AMP, 3′-AMP, and 5′-AMP is near to 102° where a g,g–g,t energy difference of about 1 kcal/mol is reasonable.

Finally, we turn to consideration of the torsional parameter for OCCO. It is well known that 1,2-difluoroethane prefers a gauche to a trans conformation with the preference very much more pronounced in polar solvents,[23] and this can be modeled with a V_2 torsional potential, which favors either 90 or 270° FCCF dihedral angles over either 0 or 180° angles. There are some indications in the literature that a similar tendency exists for OCCO linkages,[24] although there is no definitive information for the appropriate size of the V_2 potential to use to model this effect. Gerlt et al. have studied the axial–equatorial ratio of the OCH_3 group in **8** (shown axial) in a variety of solvents and have found this ratio to be quite solvent dependent.[4] They showed that in nonpolar solvents the gauche tendency for OCCO linkages was similar to that found for OCCC linkages. In aqueous solution, however, there was a significant increase in the preference of the OCCO linkage to be gauche. We can calculate the energy difference for **8** between OCH_3 axial (two gauche OCCO groups) and equatorial (two trans OCCO groups) for a variety of V_2(OCCO) values. The results we obtain are $V_2 = 0$, ΔE(axial–equatorial) = −0.2 kcal/mol; $V_2 = -2$, ΔE(axial–equatorial) = −3.6 kcal/mol; $V_2 = -4$, ΔE(axial–equatorial) = −7.0 kcal/mol. In view of the finding of Gerlt et al.[4] that **8** is more stable in the axial conformation than the equatorial in aqueous solution by 1.8 kcal/mol, an appropriate V_2 to represent this gauche tendency is $V_2 = -1$ kcal/mol.

Above we have used a phenomenological V_2 term to represent the gauche OCCO effect; it is worth commenting on the origin of this effect. van der Waals and electrostatic repulsions would lead one to expect 1,2-difluoroethane to be trans; the fact that this molecule is observed to be gauche,[23] even in the gas phase, can be best rationalized by the fact that the stabilizing C–H bond–CF antibond interactions are maximal when these bonds are trans and minimal when they are cis.[25] In the related molecules which have oxygens on two adjacent carbons, the gauche effect is less well established in vacuo,[24] perhaps because the van der Waals and electrostatic repulsions are relatively larger than the gauche effect. In polar solvents, however, the preferential solvation of the gauche conformer, due to its larger dipole moment, shifts the equilibrium in both FCCF and OCCO compounds toward the gauche conformer. Another way to look at this solvent effect is that any solvent (relative to the gas phase) will damp out the larger electrostatic O···O or F···F repulsion in gauche (relative to trans) conformers and preferentially stabilize the gauche structure.

Results

Hydrolysis of Six-Membered Phosphodiester Rings. We first focused our attention on the comparison of 3′,5′-cyclic AMP (**1**) and trimethylene phosphate (**2**). In view of the experimental fact that the presence of the nucleic acid base had little effect on the hydrolysis energy of the cyclic nucleotide, we first studied the hydrolysis of model compounds **5** and **9** and trimethylene phosphate (**2**).



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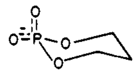

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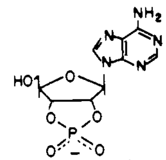

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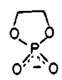

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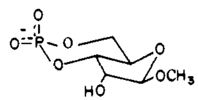

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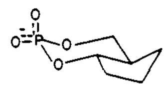

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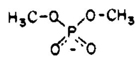

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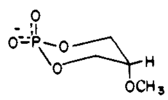

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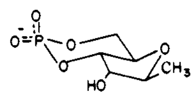

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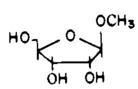

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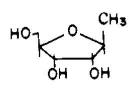

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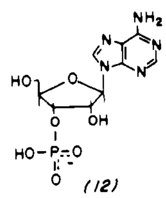

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Our initial working hypothesis was that there was extra strain in both rings of **5** and **9** not seen in either **10** or **11**, which serve as models for the monocyclic systems.

Since we had an X-ray crystal structure for the trans-fused rings (3′,5′-cyclic AMP)[17] (**1**) and the "unstrained" models

Table I. Comparison of Sugar Puckering Parameters Determined from X-ray Analyses and by Force-Field Calculations

sugar ring from	X-ray q[a]	X-ray ϕ[a]	calcd q[a]	calcd ϕ[a]
methyl α-lyxofuranoside[b]	0.426	256.8	0.313	246.9
adenosine[c]	0.361	60.6	0.298	58.0
7-ethyl 3β-D-ribofuranosylimidazo[2,1-*i*]purine[d]	0.395	212.6	0.281	216.5
3′,5′-cyclic AMP[e]	0.514	287.0	0.394	268.8
2′,3′-cyclic CMP[f]	0.020	304.1	0.098	293.8
3′-AMP[g]	0.364	75.2	0.271	82.3
5′-AMP[h]	0.433	65.9	0.297 (0.271)	73 (135.9)

[a] Sugar puckering parameters as described by Cremer and Pople (ref 13). Our q and ϕ correspond to their q_2 and ϕ_2. [b] Reference 14. [c] Reference 15. [d] Reference 16. [e] Reference 17. [f] Reference 18. [g] Reference 19. [h] Reference 20; values in parentheses are calculations not including the base.

Table II. Total Energies (E_t) of ab Initio Calculations on Phosphate and Sugar Rings

molecule	E_t, au
trimethylene phosphate, geometry from 3′,5′-cyclic AMP[a]	−747.71026
trimethylene phosphate, geometry from X-ray structure[b]	−747.71822
sugar ring (**9**) from 3′,5′-cyclic AMP X-ray structure[c]	−526.78412
sugar ring (**9**) from 3′-AMP X-ray structure[d]	−526.78294
sugar ring (**9**) from 5′-AMP X-ray structure[e]	−526.78210

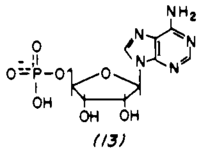
[a] Reference 17. [b] Reference 26. [c] Reference 17. [d] Reference 9. [e] Reference 10.

[trimethylene phosphate[26] (**2**) and the sugar ring of 3′- (**12**)[19] or 5′-AMP (**13**)[20]], we first carried out separate ab initio calculations on the phosphodiester rings of **1** and **2** and on the sugar rings of **1**, **12**, and **13**, always keeping the geometries as found in the X-ray crystal structures.[27] Extensive geometry optimization would have been too expensive. The results (Table II) were interesting and suggested that most if not all of the strain existed in the phosphodiester ring of cyclic AMP (0.007 96 au = $\sim$5 kcal/mol), with the sugar ring of 3′,5′-cyclic AMP calculated to be *more stable* than that of either 3′- (**12**) or 5′-AMP (**13**) by 0.7–1.3 kcal/mol.

Because we were concerned that our incomplete geometry optimization might prejudice the results, we turned to complete geometry optimization of **2**, **5**, and **9** and their hydrolysis products **14–18** using the molecular mechanics program (Table III). It was gratifying to find that the reaction energies calculated in this way

$$\text{reactant} + \text{H}_2\text{O} \rightarrow \text{product}$$

were qualitatively consistent with our ab initio strain estimates; i.e., reactions (2)–(4) were calculated to be more exothermic than reaction (5) by 4–6 kcal/mol, respectively.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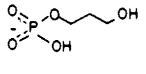
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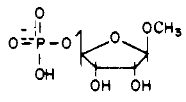
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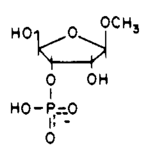
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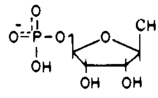
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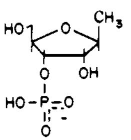
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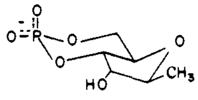
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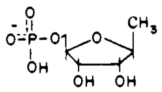
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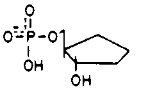
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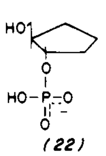
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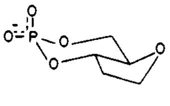
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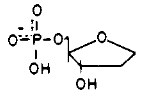
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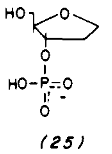
The above calculations were carried out without a V_2(OCCO) term included in the calculation; the inclusion of such a term [V_2(OCCO) = −1 kcal/mol] increases the exothermicity of reactions (1) and (2) by about 1 kcal/mol (see Table III).

Experimentally, there is very little effect on the exothermicities of reaction (2) by adding the nucleic acid base, so we decided to test this using a very crude force field for the base for reaction (1). We used the original Warshel-Levitt parameters, which treat all sp^2 carbons, nitrogens, and oxygens in a similar fashion. This very crude force field leads to an exothermicity for reaction (1) including the base which is about 2 kcal/mol more than found without the base (Table III).

In the molecular mechanics calculations described up to this stage, we did not include any electrostatic or dipolar effects. We did this because we wanted to mimic the molecular properties in aqueous solution, where all the hydrogen bonding and highly charged atoms would be heavily solvated. Recalculating the ΔH values of reactions (1), (4), and (5) using partial charges from the ab initio calculations[28] led to exothermicities of $\sim$37, 20, and 29 kcal/mol, respectively (Table III). It is clear that the energies calculated with the partial charges are not so appropriate for analyzing reaction energies in aqueous solution as those without these charges; it also appears that any lack of complete solvation for reactants and products of reactions (1)–(4) is comparable to that found in reaction (5).

We have also studied a number of analogues of **5** and **9** (Table III). Reaction (6) suggests that replacing the sugar -O- with $-CH_2-$ has little effect on the intrinsic phosphodiester strain [compare with reaction (2)], but we would not expect any V_2(OCCO) contribution here, so this molecule should have a less exothermic hydrolysis energy than reaction (5). Calculations on reactions (7)–(10) suggest that removing the 1′-CH_3 and 2′-OH as well as replacing the sugar -O- by $-CH_2-$ has little effect on the hydrolysis energy [compared with reactions (1) and (2)]. However, calculations on the hydrolysis of two trans-fused six-membered rings [reactions (11) and (12)] suggest an exothermicity not substantially greater than that for hydrolysis of trimethylene phosphate.

Hydrolysis of Five-Membered Phosphodiester Rings. As a second major area of interest we have focused on heats of hydrolysis of 2′,3′-cyclic nucleotides and analogues. Our mo-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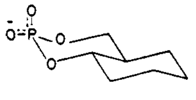
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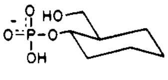
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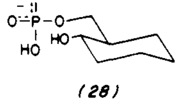
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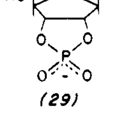
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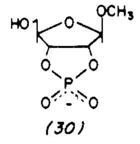
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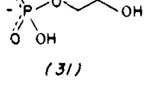

(31)

Table III. Energies of Hydrolytic Cleavage of Six-Membered Phosphodiester Rings (kcal/mol)

reaction no.[a]	reactant	product	ΔH(exp)[b]	ΔE(MM, no V_2, no charges)[c]	ΔE(MM, V_2)[d]	ΔE(MM, charges)[e]	ΔE(ab initio)[f]
(1)	**1**	**12**	−11.1	−8.9		−36.8	−19.6
(2)	**5**	**16**	−11.1	−6.7			
(3)	**9**	**18**		−7.7			−19.6
(4)	**9**	**17**		−5.8	−7.1	−20.0	
(5)	**2**	**14**	−3.0	−1.5		−29.3	−14.6
(6)	**19**	**20**		−5.6			
(7)	**6**	**21**	−7.6	−6.0		−26.8	
(8)	**6**	**22**		−8.0			
(9)	**23**	**24**	−10.1	−6.4	−9.0	−28.8	
(10)	**23**	**25**		−7.2			
(11)	**26**	**27**		−3.4			
(12)	**26**	**28**		−2.1			

[a] Reaction is reactant + $H_2O \rightarrow$ product. [b] References 3 and 4. [c] MM ≡ molecular mechanics calculations, neither V_2 (OCCO) nor charges included. [d] Molecular mechanics calculations, V_2 (OCCO) included. [e] Molecular mechanics calculations, partial charges included. [f] Ab initio calculations on phosphodiester rings described above and using energies of phosphopropanol and H_2O, calculated using standard, extended geometries (ref 19).

Table IV. Hydrolytic Energies of Cleavage of Five-Membered Phosphodiester Rings (kcal/mol)

reaction no.	reactant	product	ΔH(exp)[a]	ΔE(MM, no V_2, no charges)[b]	ΔE(MM, charges)[c]	ΔE(ab initio)[d]
(13)	**3**	**12**	−9.4	−10.2	−29.8	−13.1 (−10.3)
(14)	**29**	**18**		−10.3	−26.2	
(15)	**30**	**16**		−9.8		
(16)	**4**	**31**	−6.4	−12.5	−39.1	(−7.8)

[a] References 3 and 4. [b] Molecular mechanics calculations, no charges. [c] Molecular mechanics calculations, charges included. [d] Ab initio calculations on five-membered phosphodiester ring using X-ray or (in parentheses) molecular mechanics optimized geometries. A standard extended geometry was used for **12**.

Table V. Bond Length Parameters: $E_{\text{stretch}} = K_s(R - R_0)^2 + D_0$

bond	K_s	R_0	D_0	ref
OC	179.5	1.43	80.4	8
OP	166.6	1.61	80.0	8
$^{\delta-}$OP	393.1	1.48	80.0	8
OH	150.0	0.96	111.7	9

Table VI. Bond Angle Parameters: $E_{\text{bend}} = K_b(\theta - \theta_0)^2$

bond angle	K_b	θ_0	ref
OCH	79.2	109.5	10
OCC	115.0	109.5	10
COH	45.0	107.3	10
COC	100.0	109.5	10
POC	140.0	120.5	10
$^{\delta-}$OPO	105.0	108.2	10
$^{\delta-}$OPO$^{\delta-}$	105.0	119.9	10
OPO	105.0	102.6	10
OCO	115.0	109.5	10
POH	45.0	107.3	10

Table VII. Dihedral Angles Parameters: $E_{\text{tor}} = V_1(1 + \cos\phi) + V_2(1 - \cos 2\phi) + V_3(1 + \cos 3\phi)$

torsional angle	V_1	V_2	V_3	ref
-OC-	0	0	0.605	11
OPOC	0	−0.75	+0.75	9
-CN-	0	0	−0.3	12

Table VIII. Nonbonded Constants: $E_{\text{nonbond}} = (-A/R^6) + Be^{-CR}$

atom pair	A	B	C	ref
OO	746.8	42 155.7	3.6	9
OH	119.3	5 433.9	3.6	9
CO	746.8	49 028.4	3.6	9
PO	2400.6	256 772.0	3.6	9
PC	2400.6	305 366.8	3.6	9
PH	383.1	31 929.9	3.6	9

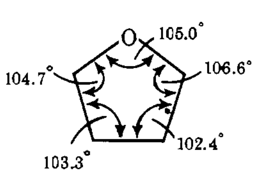
lecular mechanics calculations (Table IV) are less successful here and suggest (incorrectly) that not only are the five-membered phosphodiester rings [reactions (3) and (5)] more strained than the six-membered rings, but that ethylene phosphate has a larger negative heat of hydrolysis than 2′,3′-cyclic AMP. However, in this case, the ab initio calculations (at the molecular mechanics optimized geometries) give a good representation of the relative energies of reactions (1) and (13) and also suggest (correctly) a smaller heat of hydrolysis of these rings than six-membered phosphodiester rings. This latter result is consistent with experiment for the cyclic AMP case, but not for trimethylene phosphate.

An obvious question at this point is: why do the molecular mechanics calculations find a larger heat of hydrolysis for ethylene phosphate, where one is breaking one ring, than in the case of 2′,3′-cyclic AMP, where one is also breaking one ring, but in this way presumably relieving some strain in the remaining ring? The reason appears to be the "*strain*" in the product 3′-AMP (or 2′-AMP) when the bond is broken. If we force phosphoethanol [product of reaction (16)] to adopt a geometry like the phosphoethanol skeleton of 3′-AMP, the energy of reaction (13) becomes −8.1 to −9.4 kcal/mol instead of −12.5 kcal/mol, depending on the exact choice of geometry. Thus, we understand why the molecular mechanics calculations find the energy of reaction (16) more exothermic than those of reactions (13–15), but this is not the order found experimentally. This could come from either the fact that our force field overestimates the strain in 2′- or 3′-AMP or that there are subtle solvation effects that change the relative hydrolysis energies.

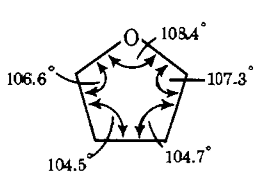
Discussion

It is of interest to compare the nature of the "strain energy" in five- and six-membered phosphodiester rings. To do this, we consider the molecular mechanics energy components. The main difference between the hydrolysis of trimethylene phosphate [reaction (5)] and 3′,5′-cyclic AMP [reactions (1–3)] comes from the internal angle (θ) strain, which decreases by 5 kcal/mol on hydrolysis of **1** and is almost unchanged on hydrolysis of **2.** The torsional (ϕ) strain actually increases by 2 kcal/mol in reactions (1-3), but this is compensated for by an increase in nonbonded attractions (3 kcal/mol). In reaction (5) all the component changes are very small.

Although the ab initio calculations on the model phosphate and sugar rings suggest that most of the strain is localized in the phosphate ring, the X-ray structures for 3′,5′-cyclic GMP,[29] trimethylene phosphate,[26] and 5′-GMP[30] and the calculated structures for compounds **23, 24,** and **2** suggest strain in both rings. (We focus on 3′,5′-cyclic GMP here rather than 3′,5′-cyclic AMP[17] because its X-ray structure has been more accurately determined.) The structural parameters for the phosphate ring in the trans-fused rings (**23**) or 3′,5′-cyclic GMP are very similar to those in trimethylene phosphate (**2**) (calculated or X-ray) with the exception of one POC angle, which is 113.6° in 3′,5′-cyclic GMP and 116.9° in trimethylene phosphate. Since the ideal value of this angle is 120.5°,[10] this would account for about 1 kcal/mol of strain energy in the trans-fused rings. In the sugar ring, the internal angles show a much larger deviation from tetrahedral in 3′,5′-cyclic GMP (or **23**) than in either 5′-GMP or **24.** The angles calculated for the sugar rings in **23** and **24** are shown below. Similar changes are found in comparing 3′,5′-cyclic GMP and 5′-GMP. Using the parameters of our force field, these changes in ring angles can account for 2-3 kcal/mol of the hydrolysis energy. The remainder of the ~5 kcal/mol "extra" internal angle strain appears to come from angles involving the hydrogens.



sugar ring of **23**



sugar ring of **24**

On the other hand, the strain energy of the five-membered rings has both a torsional component (ϕ) [−6 to −8 kcal/mol in reactions (13-16)] and internal angle (θ) component (−4 kcal/mol). Thus, the nature of the strain in the two cases is quite different; this gives us encouragement that by appropriate modification of torsional parameters one could reproduce the experimental fact that the hydrolysis of 2′,3′-cyclic AMP is less exothermic than that of 3′,5′-cyclic AMP. Previous CNDO/2 calculations[22] also found 2′,3′-cyclic nucleotides to be of higher energy than 3′,5′-cyclic nucleotides. Although our calculations give results in better agreement with experiment, neither set of calculations has satisfactorily reproduced the relative hydrolysis energies of five- and six-membered-ring cyclic nucleotides. Earlier calculations[31,32] were able to rationalize the relative kinetic instability of five-membered-ring cyclic phosphate esters.

We have carried out the above calculations both using partial charges (dielectric constant = 1) and neglecting them (dielectric constant = ∞). The correct situation is between these two extremes, although in aqueous solution one would expect the latter approximation to be more nearly correct. The inclusion of atomic partial charges in the calculations allows us to represent all levels of electrostatic interactions, including ion-ion, ion-dipole, and dipole-dipole. It is therefore not surprising that the calculations neglecting partial charges are in better qualitative agreement with ΔH(experimental) than those in which the partial charges are included. In a previous study[1] we showed that for hydrolysis reactions involving charge separations relative solvation energies were the single most important factor in modifying gas-phase hydrolysis energies. There is neither any *major* change in shape nor any separation of charge during the hydrolysis in the reactions studied in the present work, so simple solvation estimates[1] used previously are too crude to be of use here.

Although molecular mechanics techniques have been very usefully employed to study the structures and strain energies of nonpolar molecules in inert solvents, there have been many fewer applications to polar or ionic molecules.[31] The reason for this is clear from our study; structural properties are rather insensitive to the force field, but relative energies are very sensitive to partial charges. We have attempted to surmount this difficulty by using both molecular mechanics and ab initio techniques and by comparing relative hydrolysis energies of closely related compounds; however, we have been only partially successful in factoring out the many uncertainties in the calculations.

Conclusions

Both the molecular mechanics and ab initio results are consistent with the conclusion that there is 4-5 kcal/mol more strain in the sugar and phosphodiester rings of 3′,5′-cyclic AMP than found in the phosphodiester ring of trimethylene phosphate, most of which comes from internal angle (θ) effects. Experimental solution studies on the conformational energies of 5-methoxytrimethylene phosphate (**8**) suggest that 1 kcal/mol extra strain in 3′,5′-cyclic AMP comes from the trans $O_{1'}C_{4'}C_{5'}O_{5'}$ orientation in this molecule, which prefers a gauche conformation in the hydrolysis product. The remaining 3 kcal/mol of extra strain in 3′,5′-cyclic AMP relative to trimethylene phosphate is inferred to be due to the relative solvation energies of the reactant and product. The observation that the hydrolysis energy of **6** is less than that of **5** suggests that the furanose oxygen is playing a key role in the solvation energies; the role that this oxygen plays in solvation is discussed in the following paper.[4]

The calculations are able to reproduce the fact that fusing six-membered rings to the trimethylene phosphate ring [reactions (11) and (12)] causes significantly less strain than fusing five-membered rings to this phosphodiester ring.

The calculations are not able to reproduce the fact that ethylene phosphate and 2′,3′-cyclic AMP have less exothermic heats of hydrolysis than 3′,5′-AMP. Whether this is due to a poor representation of the torsional terms in the force field or to solvation effects remains to be seen. The calculations are, however, consistent with the fact that the hydrolysis energies are much closer to being equal for the 2′,3′-cyclic AMP and its "model" compound, ethylene phosphate, than for the corresponding six-membered ring phosphodiester species (3′,5′-cyclic AMP and trimethylene phosphate).

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

John A. Gerlt,*[1] Neal I. Gutterson, Reed E. Drews, and Jay A. Sokolow

Contribution from the Department of Chemistry, Yale University, New Haven, Connecticut 06520. *Received February 5, 1979*

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%