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Conformational Study of Cyclic and Acyclic Phosphate Esters. CNDO/2 Calculations of Angle Strain and Torsional Strain

David G. Gorenstein,* [1] Debojyoti Kar, Bruce A. Luxon, and Robert K. Momii

Contribution from the Department of Chemistry, University of Illinois, Chicago Circle, Chicago, Illinois 60680. *Received July 18, 1975*

Abstract: Angle strain and torsional strain energies were calculated using the CNDO/2 LCAO-MO method for various geometries of dimethyl phosphate monoanion and compared with energies for 2′,3′-cyclic ribose phosphate and 3′,5′-cyclic ribose phosphate. While the calculations fail to identify the source of the strain energy in the 3′,5′-cyclic six-membered ring nucleotides, they do indicate that a significant portion of the high heat of hydrolysis of the five-membered ring, 2′,3′-cyclic nucleotides is associated with relief of torsional strain and that preferred torsional conformations of acyclic esters are strongly coupled to the RO-P-OR bond angles. This coupling of ester, O-P-O bond angles, and torsional angles is also demonstrated by CNDO calculations on various geometries of trimethyl phosphate. Eclipsing of one of the phosphate ester bonds reduces the bond angle between the esterified oxygen atoms by ca. 5° and eclipsing of both ester bonds further reduces the bond angle by another 5°. These predictions of the CNDO calculations are shown to be supported by x-ray crystallographic structures of cyclic and acyclic, monoanionic, and neutral phosphate esters.

One of the major unresolved questions regarding the manner in which 3′,5′-cyclic-adenosine monophosphate (cAMP) stimulates the activity of many different enzyme systems (glycogenolysis, lipolysis, protein synthesis, active transport, etc.)[2a] is its mode of binding to the enzyme cAMP protein kinase.[2b,3] The very large heat of hydrolysis of cAMP (−14 kcal/mol)[4,5] has prompted suggestions that cAMP covalently binds to the enzyme.[5,6] As pointed out by Westheimer and co-workers[7] this heat of hydrolysis quite inexplicably is not coupled with any demonstrable ring strain such as found in cyclic five-membered ring phosphate diesters; the O-P-O bond angle is similar to that of acyclic phosphates.[8,9]

A potential explanation for the anomalous behavior of the 3′,5′-cyclic nucleotides is that the strain energy is not associated with a decreased O-P-O bond angle but rather with a combination of factors such as ribose ring strain and phosphate diester torsional strain. In this paper we wish to present some semiempirical quantum mechanical calculations directed toward the elucidation of the importance of torsional strain and bond angle strain in both cyclic and acyclic phosphate esters. The acyclic phosphate diesters have aroused considerable theoretical interest because of the primary role the phosphate moiety plays in the structural definition of the polynucleic acids.[10,11]

Method of Calculation

Dimethyl phosphate monoanion and trimethyl phosphate, modeled on the basis of x-ray crystallographic structures of acyclic alkyl phosphates (see references in Tables II and III), were chosen for the quantum mechanical calculations on the acyclic esters (Figures 1 and 2). For the cyclic monoanions, molecular parameters from the crystal structures of 2′,3′-cyclic-cytidine monophosphate (cCMP)[12], 3′,5′-cyclic-uridine monophosphate (cUMP),[8] and 3′,5′-cyclic-guanosine monophosphate (cGMP)[13] have been used. However, we have retained only the ribose and phosphate ester portion of the structure, substituting a hydroxyl group for the nucleotide base.

The semiempirical SCF LCAO-MO calculations employed the CNDO option in the CNINDO/2 program of Pople and Segal.[14] Only the valence basis orbitals were considered and for phosphorus, 3d orbitals were included. The major structural parameters that we have varied in the dimethyl phosphate model are the RO-P-OR bond angle, θ, and the two dihedral angles ω and ω' defined in Figure 1. The angle θ' in Figure 1 is determined by the assumed C_{2v} symmetry of the phosphate tetrahedron, the fixed O-P-O bond angle, and the variable bond angle θ. Dihedral angles are defined by clockwise rotation about the phosphate ester bond, RO-POR (see ref 11 for convention). For the trimethyl phosphate molecule only bond angle, θ, and dihedral angle, ω, were varied (Figure 2). A torsional potential for a phosphate diester with a fixed 105° O-P-O bond angle was obtained by computing the energy of the dimethyl phosphate molecule using different torsional angles taken at 30° intervals (Table I and Figure 3). The symmetry of the problem has limited greatly the number of separate structures required to define this map (note the reflection planes along the diagonals).

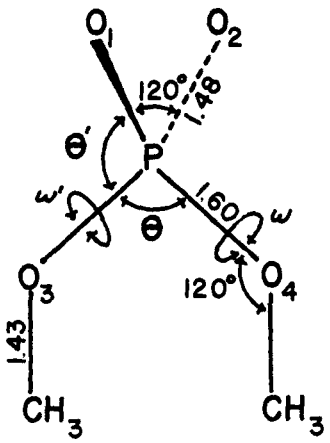

Figure 1. Structure of dimethyl phosphate monoanion. $\omega = \omega' = 0°$ represents the cis, eclipsed conformation. $\angle O_1PO_3 = \angle O_1PO_4 = \angle O_2PO_4 = \theta'$.

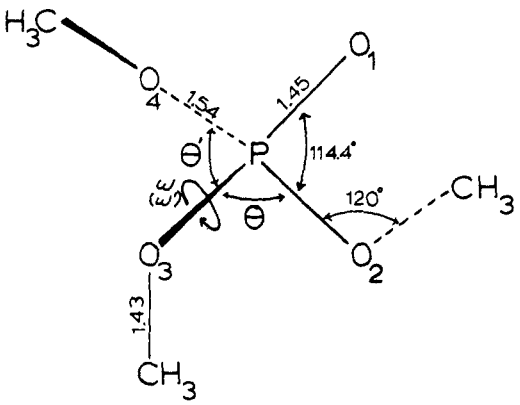

Figure 2. Structure of the trimethyl phosphate model used in the CNDO calculations. Bond angle θ and torsional angle ω (defined by CO_3PO_2) were varied. Bond angles O_2PO_1, O_4PO_1, and O_3PO_1 were kept fixed at 114.4°. Bond angle O_2PO_4 was fixed (104.0°) and torsional angles CO_4PO_2 and CO_2PO_4 were kept constant at +60°.

Results and Discussion

The isoenergy contours of Figure 3 are in general quite comparable to those obtained from a semiempirical (PCILO) calculation[15] and an ab initio calculation[16] employing a minimal Slater-type orbital basis set without 3d orbitals using similar dimethyl phosphate monoanion structures. However, whereas we find a local energy minimum for the $\omega = \omega' = 180°$ (trans, trans) conformation which is 4 kcal/mol above the minimum energy conformation ($\omega = \omega' = +64°$; + gauche, + gauche), Newton[16] finds that the t, t conformation represents an energy maximum, 7 kcal/mol above the low energy, +g, +g conformation. The PCILO[15] results are in general agreement with those of the ab initio calculation.

Empirical, force-field calculations and other semiempirical quantum mechanical calculations[17] have indicated that a local energy minimum exists for the t, t conformation, with the g, g conformation being the lowest energy. X-ray crystallographic studies show that all acyclic phosphate diesters (Table II) have either (+g, +g), (−g, −g), or (g, t) conformations which we calculate are at most only 2 kcal/mol above the minimum. As pointed out by Newton,[16] the low energies for the g, g conformations, the high energy calculated for the t, t conformation, and the absence of any experimentally observed t, t conformation are understandable in terms of the "gauche" and "anomeric" effects.[19,20] Apparently gauche-type interactions between adjacent electron pairs or bonding pairs and polar bonds (P-O) are most favored, providing an explanation for the results obtained.

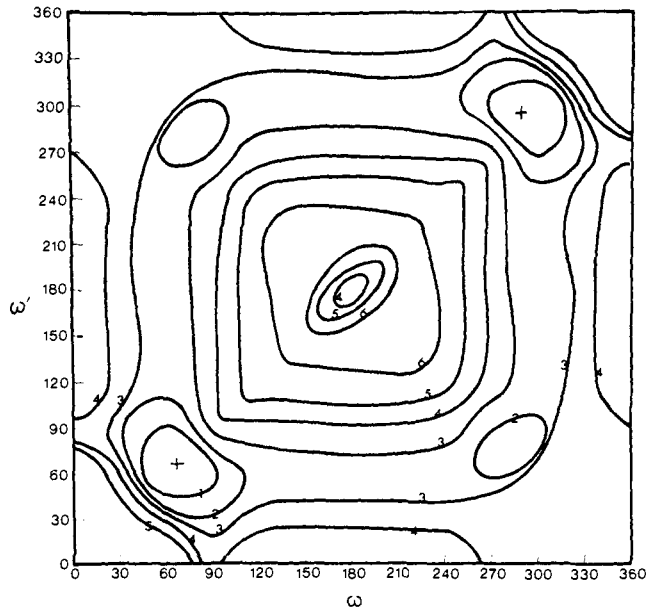

Figure 3. Torsional strain energy contour map of dimethyl phosphate monoanion. Dihedral angles ω, ω' are defined in Figure 1. Isoenergy contours are in kcal/mol over the lowest energy conformation ($\omega = \omega' = 64°$).

Table I. Dimethyl Phosphate Energies

CH_3O-P-OCH_3 bond angle θ, deg	Dihedral angles ω, ω', deg	Total energy, hartrees	Relative energy, kcal/mol
105	60, 60	−99.1814	0.19
105	64, 64	−99.1817	0
105	60, 180	−99.1774	2.70
105	180, 180	−99.1763	3.39
105	0, 180	−99.1746	4.45
105	60, −60	−99.1577[a]	
95	60, 60	−99.1776	2.57
95	60, 180	−99.1800	1.07
95	180, 180	−99.1791	1.63
95	0, 180	−99.1752	4.07

[a] Steric interaction of the methyl groups is responsible for this high energy.

Bond Angle Coupling to Torsional Angles

Significantly this ordering of the conformational stabilities holds only for dimethyl phosphate geometries with an O-P-O bond angle of 105°. In molecules with the 95° O-P-O bond angle, the *t, t conformation appears to be slightly more stable than the g, g (the lowest energy conformation is in fact g, t for this structure)*. Since the gauche effect results from a balancing of large attractive and repulsive forces,[19] it is conceivable that such a reversal of conformational energies can occur.

These "anomalous" inverted energies for the 95° ester reveal a very important principle which has hitherto been ignored or only suggested[15] in previous empirical, semiempirical, and ab initio calculations on phosphate ester torsional barriers. In Table II we have compiled nearly all of the x-ray crystal structures of monoanionic phosphate esters for which accurate O-P-O bond angles and torsional angles are either reported or can be calculated from the crystal data. As can be seen from this compilation, esters with a g, g conformation have RO-P-OR bond angles around 103-106°. In contrast, esters with a g, t conformation have O-P-O bond angles around 97-101°. This "coupling" of bond and torsional angles can be most clearly seen in a comparison of the crystal structures of three different hydrogen bonding, amino complexes of bis(*p*-nitrophenyl)

Table II. X-Ray Crystallographic Structural Data On Monoanionic Phosphate Diesters and Monohydrogen Monoesters

	Compd	RO-P-OR, deg	Dihedral angles ω, ω', deg
1.	β-Adenosine-2′-uridine 5′-phosphate[a]	102.8	−128, −47
2.	L-α-Glycerophosphoryl choline I[b]	104.0	−71, −59
3.	L-α-Glycerophosphoryl choline II[b]	102.2	64, 66
4.	Vitamin B_{12} (air dried)[c]	102	−60, 157
5.	Vitamin B_{12} (coenzyme)[d]	100	−70, 173
6.	Vitamin B_{12} (wet)[e]	101	−61, 174
7.	Bis(*p*-nitrophenyl) phosphate, benzocaine complex[f]	97.8	167.1, −66.7
8.	Bis(*p*-nitrophenyl) phosphate, phenacaine complex[g]	97.2	179.6, 53.5
9.	Bis(*p*-nitrophenyl) phosphate, procaine complex[h]	103.3	77.4, 82.3
10.	Guanosine 5′-monophosphate[i]	104.9	80.3, 75
11.	Pyridoxal phosphate oxime[j]	107.1	71.7
12.	$H_2PO_4^-$ (ephedrine phosphate)[k]	102.6	169.3, 97.7
13.	3′-Cytidine monophosphate[l] (orthorhombic)	101.5	170.7, −75.3
14.	3′-Cytidine monophosphate (monoclinic)[m]	104.3	68.0, 61.0
15.	L-Serine phosphate[n]	101.5	−161.4, −97
16.	3′-Adenosine monophosphate[o]	105.5	−64.7, −70.7
17.	2-Aminoethanol phosphate[p]	106.2	−63, 113.3
18.	Pyridoxamine 5′-phosphate[q]	105.9	62, g
19.	5′-Deoxycytidine monophosphate[r]	105.5	69.9, g
		106.1	68.3, g
20.	Dimethyl phosphate[s]	104.8	57.5, 52.4
21.	Uridine-3′, 5′-adenosine monophosphate (1)[t]	100.3 (100.6)	−87.8, 162.7
22.	Uridine-3′, 5′-adenosine monophosphate (2)[u]	102.3 (104.6)	83.6, 84.0
23.	Guanosine-3′,5′-cytidine monophosphate[v]	104.3	−76, −69
24.	Silver diethyl phosphate[w]	102.4	68.0, 125.0
25.	Putrescinium diethyl phosphate[x]	105.9	77.1, 71.8
26.	Propylguanidium diethyl phosphate[y]	107.3	−62.9, −67.0
27.	Arginine diethyl phosphate[z]	105.0	−58.3, −66.0
28.	Magnesium diethyl phosphate[aa]	108.2	77.6, 87.4
29.	Barium diethyl phosphate[bb]	103.5	(−72, −68) 72, 68
30.	Adenosylyl-3′,5′-uridine (1)[cc]	105.0	−71, −66
31.	Adenosylyl-3′,5′-uridine (2)[cc]	103.7	−64, −75
32.	Phosphoenol pyruvate[dd]	101.1	−29.5, 159.5

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phosphate (footnotes *f*–*h* in Table II). In the benzocaine and phenacaine complexes with the phosphate diester monoanion the O-P-O bond angle is 97.2–97.8° and the torsional conformation is g, t. In the procaine complex the O-P-O angle is 103.3° and the conformation is g, g.

The CNDO calculations of Table I, which hinted that such coupling existed, were extended by computing a bond angle–torsional angle energy surface. As indicated in Table II, at least one of the torsional angles in acyclic esters is always approximately gauche. Therefore, we have computed the energy of a dimethyl phosphate with one gauche (ω = 60°) and a second variable torsional angle and a variable RO-P-OR bond angle (θ). The torsional angle was varied between 30 and 330° at 30° intervals and the bond angle was varied between 95 and 110° in 2–3° intervals. The bond angle–torsional angle contour map created from these calculations is shown in Figure 4.

As expected, the g, g conformation is most stable and the "best" RO-P-OR bond angle for this conformation is 103.4°. An ester in a g, t conformation minimizes its energy by distorting the O-P-O bond to 97.5°. A g, t ester with O-P-O bond angle of 103.4° is ca. 1 kcal/mol higher energy than the ester with a g, t conformation and bond angle of 97.5°. In contrast a g, g ester with bond angle of 97.5 is 1–2 kcal/mol higher energy than the normal, g, g, 103.4° ester.

Although CNDO calculations may give a very good torsional barriers as supported by ab initio calculations, they may often as not be quite inaccurate. In the present case, however, additional support for the coupling phenomenon calculated here is provided by a study of the x-ray structures of phosphate esters. Thus, included in Figure 4 are the bond and torsional angles of various esters listed in Table II (only the torsional angle differing most from the +g conformation is plotted and any ester with a −g conformation is converted to the conformation of its mirror image for the purpose of plotting Figure 4). Although some scatter is found, the correlation is found to be quite good. For the 19 esters possessing the g, g (or −g, −g) conformations, the average RO-P-OR angle is 104.8 ± 1.6° while for the nine esters possessing the g, t (or −g, t) conformation the average bond angle is 100.2 ± 1.6°. Clearly, they separate into two well-defined groups (as is also verified by statistically analyzing the separability of the sets by the F test at the 5% confidence level). It is likely that crystal packing considerations in the solid state largely determine the torsional angles and that the O-P-O bond angles then "readjust" to minimize the energy of the molecule.

Perahia et al.[15] have suggested that the geometry of the phosphate group defines the torsional conformation in phosphate diesters. However, in their study they kept the RO-P-OR bond angle (θ) constant and varied the O-P-OR bond angles (θ'). As demonstrated by the observed variation in the diester bond angle, this procedure ignores one of the most important components of the phosphate geometry distortion associated with torsional angle changes. Our bond angle variations (both θ and θ') more nearly approximate the experimental distortions, although variations in all of the θ' angles must really also be taken into account to fully characterize this bond angle–torsional angle coupling phenomenon.

These distortional effects, of course, have long been recognized in complete geometry optimization calculations.

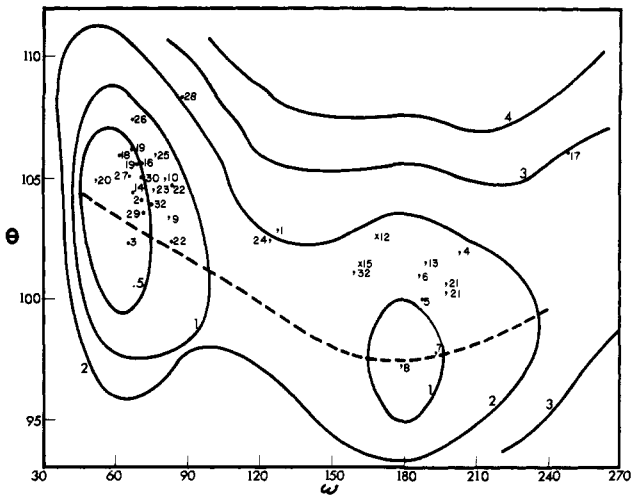

Figure 4. Bond angle (θ)-torsional angle (ω) contour map of dimethyl phosphate monoanion. The other dihedral angle was fixed at +60°. Isoenergy contours are in kcal/mol over the lowest energy geometry ($\omega = \omega' \sim 60°$, $\theta \sim 103.4°$). Points (●) and numbers refer to ester geometries in Table II. Other dihedral angles not plotted are ~+g (●) or ac (×).

Thus, the torsional geometry of hydrogen peroxide was not correctly predicted by even extended basis set, ab initio calculations until a complete geometry optimization involving the bond angle, bond distances, and torsional angle was considered.[21]

Coupling of Bond Angle–Torsional Angles in Neutral Esters

As a further demonstration of this coupling phenomenon in phosphate esters we have calculated a bond angle-torsional angle map for the neutral triester, trimethyl phosphate (Figure 5). Two of the methyl groups have been fixed in a gauche (+60°) conformation relative to each other while only the third (ω in Figure 2) has been varied. Only bond angle θ was varied with an automatic, geometrically required variation occurring in bond angle θ'. Note that ω', the dihedral angle defined by atoms CO_3PO_4, is trans when ω is gauche. It thus is impossible in the same triester molecule to have only gauche interactions between the three methyl groups. At least one set of dihedral angles will be g, t. By varying θ and ω we also create another complementary set of bond and torsional angles, θ' and ω' (Figure 2) which have also been used in defining the contours shown in Figure 5. The bond angle-torsional angle energy contour map was created in a similar fashion as described for dimethyl phosphate. The energy of the gauche, gauche (ω = 60°) structure is minimized at a RO-P-OR bond angle of 108°. The gauche, trans conformation has a minimum energy when the bond angle θ is ca. 103°. Finally, a trans, trans geometry is predicted by these calculations to have a bond angle of 98-99°.

Perhaps more important than the semiempirical calculations, these bond angle distortions are easily seen in actual structures of neutral phosphate esters. Thus, the x-ray structural data shown in Table III for neutral esters are incorporated into the bond angle-torsional angle maps of Figure 5. The second torsional angle that is not plotted in Figure 5 was assigned the synclinal (gauche; 30° < ω < 90°) or antiperiplanar (trans; 150° < ω < 210°) label based upon the convention of Prelog and Klyne.[22] In several instances the torsional angle was best characterized as anticlinal,[22] ac (90° < ω < 150°). Neutral esters with g, g; g, −g; or −g, −g torsional conformations have average O-P-O bond angles of 108.2 ± 1.43° and esters with ±g, t conformations have average bond angles of 103.2 ± 1.2°. The sin-

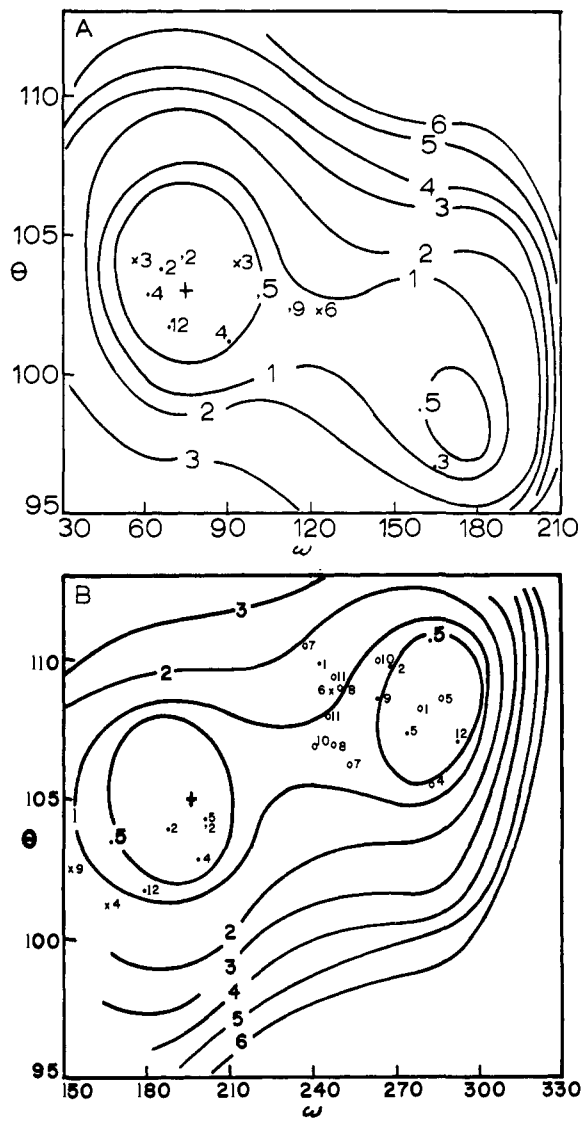

Figure 5. Bond angle (θ)-torsional angle (ω) contour map of trimethyl phosphate. In A the unplotted dihedral angle is trans. In B the unplotted dihedral angle is −gauche. Isoenergy contours are in kcal/mol over the lowest energy conformation (+). Data points and numbers refer to ester geometries given in Table III. In A, the second dihedral angle not plotted is trans (●) or anticlinal (×). In B, the second dihedral angle not plotted is −gauche (●), +gauche (○), or anticlinal (×).

gle example of a neutral ester with a set of dihedral angles of t, t description agrees very nicely with the CNDO predicted bond angle (the observed bond angle for triphenyl phosphate is 96.6° and the predicted value is 98°). Our proposed correlation of torsional angles and bond angles thus accurately accounts for the large distortions from C_{3v} symmetry of the phosphate oxygen tetrahedron in the triphenyl phosphate crystal structure. The (57°, −125°) and (−93°, 134°) sets of torsional angles are associated with bond angles of 104° (as predicted) while the t, t (163°, 161°) set of torsional angles couples with the significantly smaller bond angle of 96.6°. Note also that this coupling is found in both acyclic *as well as cyclic neutral esters* (only exocyclic angles are plotted in Figure 5). In fact, the small, endocyclic O-P-O bond angles in five-membered ring esters (98.0-98.5°, esters No. 6-11 in Table III) conform as well with our proposal that any eclipsing of the ester bonds as found in either trans or cis conformations results in a decrease in the O-P-O bond angle. The small endocyclic bond angle in both neutral *and* monoanionic esters is thus likely a reflection of their eclipsed torsional geometry. That eclipsing re-

Table III. X-Ray Crystallographic Structural Data on Neutral Esters

	Compd	RO-P-OR′ bond θ, deg	Dihedral angles ω, ω′, deg
	Acyclic		
1.	Bis(*p*-chlorophenyl) hydrogen phosphate[a]	108.1	82,82
		109.7	−34.1, 118.8
2.	Dibenzyl hydrogen phosphate[b]	103.8	−66.6,−172.0
		104.2	73.2, −159.2
		109.6	44.2, 90.2
3.	Triphenyl phosphate[c]	96.6	163, 161
		104	−125,57
		104	134, −93
4.	Tris(*p*-nitrophenyl) Phosphate[d]	101.1	−165.1, 90.3
		102.9	−161.0, −60.6
		105.4	45.0, 88.1
5.	*myo*-Inositol-2-phosphate[e] (dihydrogen)	104.3	−158.8, −39.8
		107.3	59.2, 86.2
		108.5	−53.1, +74.4
	Cyclic		
6.	Methyl pinacol phosphate[f]	98.4	−12.5, −12.2
		102.2	−123.9, −143.4
		108.9	94.0, 113.3
7.	Methyl ethylene phosphate[g]	98.1	11,2
		106.1	−108,47
		110.4	124, −58
8.	Methyl acetoine diol cyclophosphate[h]	98.5	0, −1
		106.8	−113,53
		108.9	110, −51
9.	*trans*-Methyl hydrobenzoin phosphate (a)[i]	98.2	22.5, 1.3
		108.6	−83.6, −95.4
		102.4	112.5, 161.4
10.	*trans*-Methyl hydrobenzoin phosphate (B)	98.0	−14.5, −5.8
		109.9	96.6, −51.9
		106.8	−119.5, 53.4
11.	Catechol cyclic phosphate[j]	98.4	1.0, −0.9
		108.1	114.7, −34.5
		109.3	−113.5, 71.7
12.	Phenyl trimethylene phosphate[h]	106.6	43, −42
		101.7	179.6, −69.2
		107.0	68.0, 66.5

[a] M. Calleri and J. C. Speakman, *Acta Crystallogr.*, **17**, 1097 (1964). [b] J. D. Dunitz and J. S. Rollett, *ibid.*, **9**, 327 (1956). [c] G. W. Svetich and C. N. Caughlan, *ibid.*, **19**, 645 (1965). [d] M. Hague and C. N. Caughlan, *ibid.*, **26**, 1528 (1970). [e] C. S. Yoo, G. Blank, J. Pletcher, and M. Sax, *ibid.*, **30**, 1983 (1974). [f] M. G. Newton, J. R. Cox, Jr., and J. A. Bertrand, *J. Am. Chem. Soc.*, **88**, 1503 (1966). [g] T. A. Steitz and W. N. Lipscomb, *J. Am. Chem. Soc.*, **87**, 2488 (1965); X. Chiu and W. N. Lipscomb, *ibid.*, **91**, 4150 (1969). [h] D. Swank, C. N. Laughlan, F. Ramirez, O. P. Madan, and C. P. Smith, *ibid.*, **89**, 6503 (1967). [i] M. G. Newton and B. S. Campbell, *ibid.*, **96**, 7790 (1974). [j] F. P. Boer, *Acta. Crystallogr., Sect. B*, **28**, 1201. [k] H. J. Geise, *Recl. Trav. Chim. Pays-Bas*, **86**, 362 (1969).

duces the O-P-O bond angle apparently is true for the polyphosphates as well. Thus, in Table IV the structures of various tripolyphosphates and pyrophosphates support this point. In the adenosine monohydrogen triphosphate structure, the smallest RO-P-OR′ bond angle (on the α phosphate) is associated with the eclipsed torsional conformation (−ac, t).

This demonstration of a direct coupling between torsional angles and bond angles simplifies and makes enormously more useful our recent empirical correlation[23] of O-P-O bond angles and ^{31}P chemical shifts in phosphate esters and another, seemingly contradictory, theoretical correlation[24] of torsional geometries and ^{31}P chemical shifts. It now appears that one need not attempt any separation of bond and torsional angle contributions to ^{31}P chemical shifts since the bond and torsional angles are intimately interrelated.

It is also significant that correlations between phosphate tetrahedra O-P-O bond angles and P-O bond distances

Table IV. X-Ray Crystallographic Structural Data on Tripoly- and Pyrophosphates

	Compd	Phosphate	ROPOR bond angles, deg	Dihedral angles, deg
1.	Adenosine monohydrogen triphosphate[a]	α	97	64, 164
		β	96	232, 84
		γ	94	235, 180
2.	$Na_5P_3O_{10}$ (I)[b]	β	94	165, 165
3.	$Na_5P_3O_{10}$ (II)[c]	β	98	−169, −169
4.	$Na_2KP_3O_{10}$[d]	β	99.4	−52.2, 49.6
5.	$K_4P_3ONH_2$[e]	β	101.5	160, −39
6.	Thiamine pyrophosphate[f]	α	100.8	−78.1, 139.0
7.	$NaH_2P_2O_7$[g]		104.5	102.4

[a] O. Kennard, N. W. Isaacs, W. D. S. Motherwell, J. C. Coppola, D. L. Wampler, A. C. Larson, and D. G. Watson. *Proc. R. Soc. London, Ser. A*, **325**, 401 (1971). Dihedral angles references in D. Perahia, B. Pullman, A. Saran, *Biochem. Biophys. Res. Commun.*, **47**, 284 (1972). [b] D. E. C. Corbridge, *Acta Crystallogr.*, **13**, 263 (1960). [c] D. R. Davies and D. E. C. Corbridge, *ibid.*, **11**, 315 (1958). [d] P. I. Turdjman, A. Durif, and C. Cavero-Ghersi, *Acta Crystallogr., Sect. B*, **30**, 2701 (1974). [e] W. Hilmer, *ibid.*, **19**, 362 (1965). [f] J. Pletcher and M. Sax, *J. Am. Chem. Soc.*, **94**, 3998 (1972). [g] R. L. Collin and M. Willis, *Acta Crystallogr., Sect. B*, **27**, 291 (1971).

have recently been described.[25] There is little doubt that torsional angles, bond angles, and bond lengths are coupled together and that to obtain really accurate torsional barriers, complete geometry optimization is required. The present accuracy of semiempirical and even ab initio calculations probably limits any more accurate assessment of torsional barriers. Differences in torsional potentials calculated by different groups[15-17] are as likely a result of different energy calculation methods as different choices for model geometries (note Newton and Perahia et al. assume a diester O-P-O bond angle about 3° smaller than our dimethyl phosphate model).

Finally, the dependence of O-P-O angles on torsional angles indicates that it is wrong to simply average RO-P-OR bond angles from esters in different conformations. Thus, in x-ray structural studies of polynucleic acids at medium atomic resolution, O-P-O bond angles of 100-102° are often assumed in the models.[26] The conformations of these helical or double helical esters are normally g, g and therefore an angle closer to 105° should in fact be assumed.

Cyclic Nucleotides

We may use the dimethyl phosphate torsional potential to assess the extent of torsional strain in the 2′,3′- and 3′,5′-cyclic-ribose phosphates. The heat of hydrolysis of cCMP is −8.1 kcal/mol[4] which is similar to the value found for the hydrolysis of the simple cyclic diester, ethylene phosphate (−6.8 kcal/mol).[7] The 4 to 5 kcal/mol observed strain energy in the five-membered cyclic phosphates has been generally assumed to be associated with bond angle strain[4,7,27] (O-P-O bond angle is constrained 6-7°). Our results indicate that a significant portion of this strain energy is associated with the torsional "gauche effect". Although steric interactions of the two methyl groups prevent a torsional strain calculation on a dimethyl phosphate conformation in the eclipsed, cis conformation as found in cCMP, it can be seen that the related eclipsed, trans, trans and cis, trans (ω = 0°, ω′ = 180°) conformations which should exhibit similar gauche interactions are 3-4 kcal/mol higher energy than the g, g conformation of the acyclic diester. Actually as pointed out earlier our demonstration of a coupling between O-P-O bond angles and torsional an-

Table V. Cyclic Phosphate Energies

Structure	RO-P-OR bond angle, deg	Dihedral angles ω, ω', deg	Energy, hartrees	Rel energy, kcal/mol
2′,3′-cCMP-(A)[a]	95.7	25.6, −21.2	−177.6790	12.2
2′,3′-cCMP-(B)[a]	96.0	28.2, −25.7	−177.6625	22.6
3′,5′-cUMP-(A)[a]	102.7	50.1, −54.2	−177.6947	2.4
3′,5′-cUMP-(B)[a]	103.5	48.8, −50.4	−177.6985	0
3′,5′-cGMP	103.9	44.2, −44.3	−177.6793	12.0

[a] Two molecules are found in the asymetric unit.

gles indicates that it is quite meaningless to attempt a separation of the strain energy in the five-membered cyclic phosphates into bond angle and torsional angle components. Again, the small O-P-O bond angle found in all five-membered cyclic phosphates is likely a reflection of geometry distortion resulting from the necessarily eclipsed conformation in these esters.

In Table V we have listed the CNDO/2 computed energies for the isomeric cyclic phosphates (limited to the ribose phosphate portion only). Significantly, we find that the six-membered cyclic esters are *more* stable than the five-membered cyclic ester. While the CNDO/2 method generally cannot provide very accurate energy differences for molecules of this size, the ordering indicated is quite likely reliable, especially since we are only comparing isomeric molecules. Perhaps as a more forceful argument than these direct energy calculations, the lower energy for the six-membered cyclic phosphate is consistent with the rather "normal" structure for this molecule (O-P-O bond angle of 103° and +g, −g conformation). Note that this conformation *maximizes* gauche interactions. Whatever the actual magnitude of this energy difference it is clear that our calculations indicate that the six-membered ring diester possesses no unusual strain energy compared to the five-membered ring diester. This theoretical calculation contrasts with the reported heats of hydrolysis of six-membered ring cyclic mononucleotide phosphates (−14 kcal/mol for cAMP[4,28]). The very high energy of hydrolysis for 3′,5′-cyclic nucleotides, which is larger even than the free energy of hydrolysis of ATP (−8.9 kcal/mol),[29] stands also in contrast to the "normal" heat of hydrolysis found for a simple six-membered ring cyclic phosphate, trimethylene phosphate. Thus, sodium ethylene phosphate releases 3-4 kcal/mol *more* heat upon hydrolysis than sodium trimethylene phosphate.[7] This result would be consistent with our energy calculations. The "high-energy" nature of the 3′,5′-cyclic nucleotides does not appear to be associated with either the phosphate or ribose ring structures and energy calculations on the nucleic acid base conformations[30] indicate that there are no unusual interactions here as well. In addition no unusual energies are found for the monoester products.[30] Whatever the source of the high energy for the 3′,5′-cyclic nucleotides, it unfortunately is not revealed by these calculations.

Note Added in Proof: An analogous coupling of O-C-O bond angles and torsional angles in dimethoxymethane has been demonstrated.[31] The same 4-5° reduction in O-X-O (X = C or P) bond angle is observed for each rotation about the O-X bond from a gauche to a trans conformation. The origin of this coupling is ascribed to bond-bond repulsion.

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