Structural Chemistry of Cyclic Nucleotides. IV. Crystal and Molecular Structure of Tetramethylene Phosphoric Acid[1]

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Abstract: Cyclic nucleoside phosphates are biologically active compounds with high heats of hydrolysis. Comparable thermodynamic and structural data are available for five- and six-membered cyclic alkyl and nucleoside phosphates, but no seven-membered cyclic phosphate structures have been reported. Tetramethylene phosphoric acid ($C_4H_9PO_4$) forms orthorhombic crystals of space group $P2_12_12_1$ with $a = 8.101$ (2), $b = 6.213$ (3), $c = 13.321$ (2) Å and four molecules per cell. X-Ray diffraction data to $2\theta = 50°$ were collected using Mo Kα radiation. The structure was solved by Patterson methods and refined by least-squares to an R value of 2.4% for the 639 data. The thermally corrected C–C bond distances average 1.506 Å, 0.02 Å less than found in cyclohexane. The seven-membered ring is in a twist-chair conformation with the P and an ester oxygen on one side of the best four-atom plane and a methylene carbon on the other. Comparison of the enthalpies of hydrolysis and structures of five-, six-, and seven-membered cyclic phosphates suggests that tetramethylene phosphate is no more strained than trimethylene phosphate, and that the high heats of hydrolysis of cyclic 3′,5′-nucleotides are mainly due to the high energy ribofuranosyl conformation required to make the 3′,5′-diester linkage.

The thermodynamic, kinetic, and structural aspects of the hydrolysis of cyclic nucleoside phosphates have been studied extensively in recent years.[2-5] Hydrolysis data for cyclic alkyl phosphates are also available,[6,7] and joint consideration of the alkyl phosphate and the nucleotide data leads to some useful and general conclusions. Cyclic 3′,5′-AMP[8] has a large free energy[2] and enthalpy[2-4] of hydrolysis, both values being about 2 kcal/mol more exothermic than the values for the hydrolysis of ATP to ADP and inorganic phosphate under the same conditions.[9] Other cyclic 3′,5′ nucleotides have similar enthalpies of hydrolysis.[4] Five-membered cyclic phosphate diesters such as ethylene phosphate ($(CH_2)_2PO_4^-$) and cyclic 2′,3′-nucleoside phosphates have lower heats of hydrolysis, but the values are still about 5 kcal/mol more exothermic than those for the noncyclic phosphate diesters.[4,6] In addition, the five-membered cyclic phosphates have hydrolysis rates 10^4–10^7 above those for alkyl esters or for hydrolysis of six- and seven-membered phosphodiester rings such as cyclic 3′,5′-nucleoside phosphates, $(CH_2)_3PO_4^-$ and $(CH_2)_4PO_4^-$.[5,10-14] Hydrolysis of phosphate esters probably occurs via trigonal bipyramidal penta-coordinate phosphorus intermediates,[10,15-17] with the ester ring bridging an axial and an equatorial position in cyclic phosphates.[5] The enhanced hydrolysis rates and large enthalpies of hydrolysis of five-membered cyclic phosphates relative to noncyclic phosphate esters have been attributed to strain in the phosphodiester ring[10,15] which leads to a lower activation energy for the hydrolysis and a larger free energy change for the reaction in the case of cyclic esters. This analysis does not account for the thermodynamic data on the hydrolysis of six- and seven-membered phosphate rings. For the six-membered rings, the enthalpies of hydrolysis of cyclic 3′,5′-nucleoside phosphates are about 8 kcal/mol more exothermic than the enthalpy of hydrolysis of $(CH_2)_3PO_4^-$.[4,6,7] The seven-membered ring compound, $(CH_2)_4PO_4^-$, has a heat of hydrolysis about 4 kcal/mol below that of the five-membered cyclic phosphates,[7] even though cyclopentane and cycloheptane seem to be equally strained.[18,19] In order to determine whether conformational features in the cyclic phosphate rings could explain these observations, I undertook a detailed structural study of $(CH_2)_4PO_4H$, the results of which I report here.

Six-membered cyclic phosphate diesters take up a chair or twist-chair conformation with O(2), C(3), C(5), and O(6) in the best plane and P and C(4) puckered in opposite directions. Trans fusion of this six-membered ring with a five-membered ribofuranoside ring as in 3′,5′-cyclic nucleotides causes very little change in the phosphate ring geometry. In five-membered phosphate rings, the above four atoms are also nearly coplanar with the P usually puckered. In the seven-membered ring of $(CH_2)_4PO_4H$, this constraint is relaxed, and the ring has a twist-chair conformation with O(2), C(3), C(5), and C(6) nearly coplanar, C(4) puckered one way, and the P atom and O(7) puckered the other direction out of the plane. The distances and angles within the phosphate ring are similar to those observed for cyclic six-membered alkyl and bicyclic esters and do not suggest as much strain as is evident in the angles for the five-membered cyclic phosphate rings. Thus phosphate ring strain alone does not explain the differences in the enthalpies of hydrolysis of $(CH_2)_3PO_4^-$ and cyclic 3′,5′-nucleotides. Consideration of the structural and thermodynamic data together suggests that strain involving the sugar ring of cyclic 3′,5′-nucleotides is a major factor, and these "high energy phosphate" compounds may be so because of the fairly rigid, high energy ribofuranosyl conformation required to make the 3′,5′-diester linkage.

Experimental Section

Tetramethylene phosphoric acid was prepared by Dr. John Gerlt,[7] using the dicyclohexylcarbodiimide method of Khorana et al.[20] $(CH_2)_4PO_4H$ crystallizes from benzene as colorless plates; the crystal data are given in Table I. Initial attempts to collect X-ray diffraction data were unsuccessful, as the crystals sublimed in the air. A crystal of dimensions 0.30 × 0.14 × 0.73 mm was coated with a drop of oil and used to collect intensity data to $2\theta = 50°$ using monochromated molybdenum radiation and a Picker FACS-I diffractometer. The cell dimensions in Table I were derived by least-squares fit to the $\pm 2\theta$ values for 13 reflections. Three quadrants of data were collected using $\theta - 2\theta$ scans (2°/min) with 20-sec background measurements at each end of the scan. Three reference peaks were monitored every 100 reflections; these intensities decreased about 6% during data collection, presumably because of crystal sublimation, and the data were rescaled to allow for this decrease. The intensities were corrected for Lorentz and polarization effects and the symmetry-related reflections were averaged. The average deviation in intensity from the mean of equivalent reflections was below 2%. The final observational data consisted of 639 intensities above 2σ, where σ was the stan-

Table I. Crystal Data for Tetramethylene Phosphoric Acid

Crystal system: orthorhombic
Space group: $P2_12_12_1$
$a = 8.101 \pm 0.002$ Å
$b = 6.213 \pm 0.003$ Å
$c = 13.321 \pm 0.002$ Å
$D_{\text{obsd}} = 1.506$ g/cm^3; $D_{\text{calcd}} = 1.507$ g/cm^3
$Z = 4$; Absorption coefficient: $\mu = 0.036$ cm^{-1}
$\lambda = 0.71069$ Å (Mo Kα)

Table II. Positional and Thermal Parameters (× 10^4) and Their Estimated Standard Deviations (× 10^4)[a]

Atom	x/a	y/b	z/c	B_{11}	B_{22}	B_{33}	B_{12}	B_{13}	B_{23}
P	297.4 (0.8)	1507 (1)	680.0 (0.5)	102 (1)	257 (2)	44.9 (0.3)	27 (1)	−8.3 (0.5)	−9 (1)
O(8)	−1445 (2)	1793 (5)	962 (2)	126 (3)	498 (9)	64 (1)	84 (5)	13 (2)	13 (3)
O(9)	715 (3)	2706 (5)	−283 (2)	121 (4)	479 (9)	59 (1)	35 (4)	−9 (2)	36 (3)
O(2)	642 (2)	−943 (3)	571 (2)	122 (3)	267 (6)	86 (2)	−10 (3)	−23 (2)	−39 (2)
O(7)	1557 (3)	2385 (3)	1463 (1)	204 (4)	213 (5)	56 (1)	5 (4)	−36 (2)	−7 (2)
C(3)	2332 (4)	−1786 (6)	506 (2)	153 (5)	252 (8)	80 (2)	49 (5)	−3 (3)	−36 (4)
C(4)	2993 (4)	−2253 (5)	1535 (3)	164 (5)	271 (8)	83 (2)	50 (6)	3 (3)	36 (4)
C(5)	3389 (4)	−319 (6)	2169 (2)	141 (5)	382 (10)	56 (2)	−24 (6)	−22 (2)	52 (4)
C(6)	1971 (5)	1182 (6)	2372 (2)	212 (6)	328 (10)	48 (2)	−12 (7)	−21 (2)	0 (3)

[a] Temperature factor = $\exp(-(B_{11}h^2 + B_{22}k^2 + B_{33}l^2 + 2B_{12}hk + 2B_{13}hl + 2B_{23}kl))$ with (h, k, l) crystal indices.

Table III. Positional (× 10^3) and Isotropic Thermal Parameters for the Hydrogen Atoms[a]

Atom	x/a	y/b	z/c	B, Å^2
H3A	221	−307	7	6.3
H3B	300	−72	7	4.9
H4A	221	−323	191	5.0
H4B	402	−316	144	6.5
H5A	371	−76	281	5.0
H5B	443	60	183	6.3
H6A	85	29	254	4.8
H6B	230	231	280	6.1
H9	152	284	−41	6.3

[a] Each hydrogen is given the number of the atom to which it is bound. Estimated standard deviations are 0.05 Å for the positional entries and 1.0 Å^2 for the B.

dard deviation derived from counting statistics. No corrections were applied for absorption or extinction. All calculations were done on the IBM 370/168 system at the University of Chicago, using programs cited in earlier publications.[21,22] Scattering factors were taken from the international tables.[23]

Solution and Refinement

The structure was solved by inspection of a sharpened Patterson synthesis. The phosphorus position was deduced from peaks in the Harker sections, and the four oxygens and one methylene carbon located by consideration of inter- and intramolecular phosphorus to light atom vectors. A Fourier synthesis using phase angles based upon the positions of these six atoms contained peaks for the other three carbon atoms. The structure was refined by isotropic least-squares methods, and a difference Fourier synthesis was calculated. This map contained peaks of height 0.3-0.6 e/Å^3, in appropriate positions for the hydrogens which were then included in the calculations. For the anisotropic least-squares refinement, the weighting system used was $\sigma_h = 0.5 + 0.021|F_o|$ where σ_h is $[1/(w_h)^{1/2}]$ and h $(= h, k, l)$ refers to an individual reflection. The constants were derived from a plot of $\langle \Sigma|F_o| - |F_c|)^2\rangle^{1/2}$ vs. $\langle|F_o|\rangle$. The final refinement included positional and isotropic thermal parameters for the hydrogen atoms. After convergence, the R value for the 639 data was 2.4%, where $R = \Sigma||F_o| - |F_c||/\Sigma|F_o|$, and the weighted R was 2.9%. The final shifts in parameters were less than 0.3 of the estimated error in the parameter. The goodness of fit (1.0 for optimum weighting) was 0.86.

Results

The positional and thermal parameters for the nine nonhydrogen atoms in the asymmetric unit are given in Table II. Table III contains the hydrogen atom parameters. The estimated standard deviations of the nonhydrogen atom positions are 0.004 Å, and for the hydrogen atoms, 0.05 Å. The bond lengths not involving hydrogen should thus be accurate to ±0.01 Å or better and the bond angles, ±0.3°.

Final bond distances and bond angles for tetramethylene phosphoric acid are given in Figures 1 and 2. The C–C bond distances average 1.502 Å vs. 1.528 Å for cyclohexane[24] and 1.52 Å for $(CH_2)_3PO_4H$,[25] a significant difference. Thermal effects can give short X-ray bond lengths and thermally corrected bond distances based upon a riding atom model are given in Table IV. The bond distances remain symmetric, with the C–C average, 1.506 Å, still below the

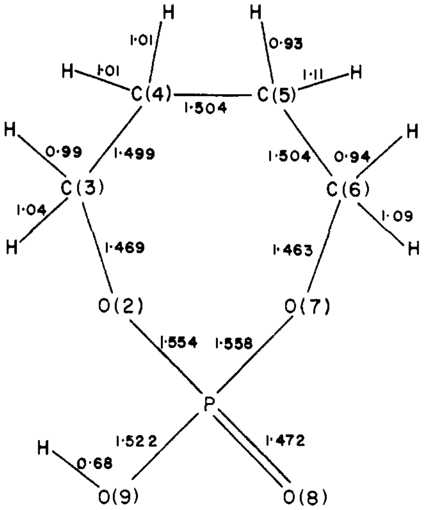

Figure 1. Bond distances (in Å) for tetramethylene phosphoric acid.

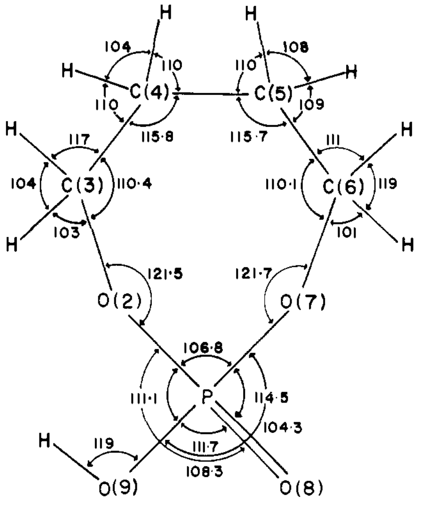

Figure 2. Bond angles (deg). Not all angles involving hydrogen are shown, but the others are similar.

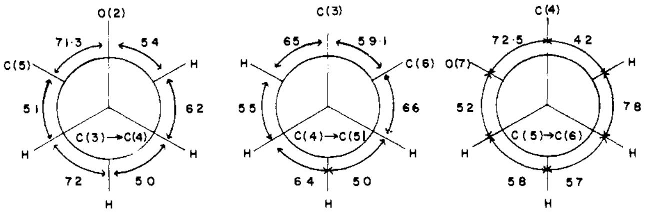

Figure 3. Newman projections down the C–C bonds. Estimated error of dihedral angles involving hydrogens are ±4°.

Table IV. Thermally Corrected Bond Distances[a]

Bond	Bond length, Å
P–O(2)	1.568
P–O(7)	1.568
P–O(8)	1.496
P–O(9)	1.541
O(2)–C(3)	1.470
O(7)–C(6)	1.468
C(3)–C(4)	1.505
C(4)–C(5)	1.508
C(5)–C(6)	1.505

[a] The second atom is assumed to ride on the first in all cases.

cyclohexane value. For cyclic hydrocarbons, bond lengths and strain energies decrease with increasing ring size to cyclohexane and then increase again.[19] This effect, and the internal agreement of chemically equivalent bond distances, suggests that the 1.506-Å value is correct. The bond angles (Figure 2) are also symmetric and slightly above the trigonal and tetrahedral values as is commonly found for ring systems. Table V gives the best least-squares plane data with O(2), C(3), C(5), and C(6) in a plane. The P and O(7) atoms pucker to one side of this plane and C(4) to the other to give a twist-chair conformation. The twist is shown by the O(2)–C(3) and C(6)–C(5) bonds which cross the best plane in opposite directions. The hydrogen atoms on adjacent carbons are gauche or trans in all cases; the dihedral angles are shown in Figure 3. The C(3)–C(4)–C(5)–C(6) angle is the only nonhydrogen contact below 60°. The packing is shown in Figure 4 and involves a hydrogen bond (2.491 Å) between O(9) and O(8) on an adjacent molecule. The hydrophilic and hydrophobic regions of the crystals are well separated, and there are no unusually close nonbonded contacts in the crystal structure.

Table V. Best Weighted Least-Squares Plane[a]

Atom	Distance from plane, Å
O(2)*	−0.027
C(3)*	0.094
C(5)*	−0.081
C(6)*	0.093
P	0.973
O(7)	1.196
C(4)	−0.671

[a] Atoms marked with an asterisk were used to define the plane. Equation of the plane, $2.832x + 4.793y - 7.083z = -0.6480$ Å, where x, y, and z are from Table II.

Discussion

Phosphate bond angles for several five-, six-, and seven-membered cyclic phosphates are given in Table VI. The table also lists the angle changes required to give symmetric trigonal bipyramidal geometry in the hydrolysis intermediate; the tetrahedral phosphate values are 57 and 33°. No phosphates are in fact tetrahedral,[26] and the transition-state symmetry probably need not be exact, but the table does show the five-membered cyclic phosphates to be closer to the symmetric five-coordinate geometry than any of the six- or seven-membered ring diesters, mainly because of the small O(3)–P–O(4) angle. The angles for cyclic 3′,5′-nucleotides are similar to those observed in $(CH_2)_3PO_4H$ and $(CH_2)_4PO_4H$, with the differences most likely due to packing and the protonation of the phosphate. The P–O–C(3′) angles in cyclic 3′,5′-nucleotides have values[27,28] of 111–114°, significantly below the expected 120° value or the P–O–C(5′) angles (118–122°), as noted by Gerlt et al.[7] The P–O–C angles in $(CH_2)_3PO_4H$ are[25] 116.9 and 120.8°; in

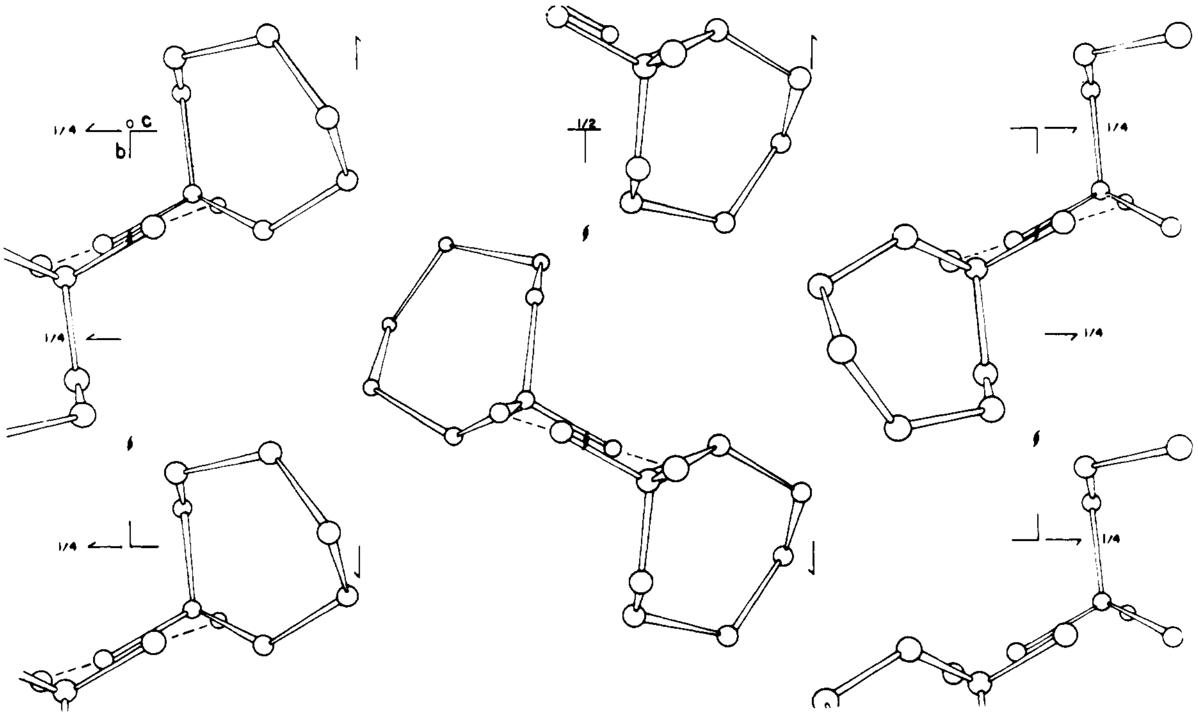

Figure 4. The crystal structure viewed down *a*. Hydrogen atoms are not shown. The hydrogen bond between O(9) and O(8) appears as a dashed line; the O(9) to O(8) distance is 2.491 Å, and the P–O(9)···O(8) angle is 124.9°.

Table VI. Phosphate Bond Angles (deg) for Cyclic Diesters[a]

Bond	$(CH_2)_4PO_4H$ (7)	$(CH_2)_3PO_4H$ (6)	3′,5′-UMP[b] (6)		3′,5′-GMP (6)	$MeAcPO_4$ (5)	2′,3′-CMP[b] (5)	
O(3)–P–O(4)	106.8	104.6	102.7	103.5	104.0	97.2	96.0	95.8
O(1)–P–O(2)	111.7	116.7	119.2	119.7	116.9	115.3	118.2	117.2
O(2)–P–O(3)	111.2	110.1	109.8	109.2	106.2	104.2	110.4	110.2
O(1)–P–O(3)	108.3	109.5	107.2	107.9	108.7	115.2	109.8	108.8
O(2)–P–O(4)	104.3	110.2	109.2	109.4	108.8	106.0	110.5	111.1
O(1)–P–O(4)	114.5	105.1	107.4	106.0	111.3	116.8	109.7	111.5
Angle changes for intermediate[c]	56 28	49 23	50 25	50 25	49 23	46 23	46 21	45 21
Reference		(25)	(27)		(28)	(30)	(21)	

[a] The ring size is given next to each entry; the phosphate oxygens are numbered O(1)–O(4), with O(3) and O(4) the ester oxygens, and O(2) also substituted in $(CH_2)_4PO_4H$ and the cyclic acyl phosphate.[30] The acid hydrogen was not located in $(CH_2)_3PO_4H$ but presumed[25] to be on O(1). [b] Two independent molecules are in the crystal structure. [c] The upper figure is for deviations from 90° and the lower figure from 120° for a symmetric trigonal bipyramid with a bridging ring. The values for a tetrahedron would be 57 and 33°.

Table VII. Dihedral Angles (deg) Involving Phosphorus for Cyclic Phosphate Diesters[a]

Bond	$(CH_2)_4PO_4H$ (7)	$(CH_2)_3PO_4H$ (6)	3′,5′-UMP[b] (6)		3′,5′-GMP (6)	2′,3′-CMP[b] (5)	
C(4)–O(4)–P–O(3)	−40.8[c]	46.6	50.2	49.3	44.3	21.3	25.7
C(4)–O(4)–P–O(2)	−158.6[c]	164.9	163.2	161.4	159.0	134.8	138.6
C(4)–O(4)–P–O(1)	79.0[c]	−68.7	−65.9	−67.1	−74.4	−93.2	−88.5
O(1)–P–O(3)–C(1)	−166.8	−167.1	−167.2	−164.1	−157.2	−139.1	−143.3
O(4)–P–O(3)–C(1)	−43.0	−48.8	−54.4	−50.7	−44.4	−25.6	−28.2
O(2)–P–O(3)–C(1)	70.1	63.4	62.1	65.6	72.5	88.9	86.8
Reference		(25)	(27)		(28)	(21)	

[a] The ring size is given next to each entry; positive angles correspond to clockwise rotation of the far atom about the central bond. [b] Two independent molecules are in the crystal structure. [c] For $(CH_2)_4PO_4H$, the ring extends to the left of the C(4)–O(4)–P plane when looking down the O(4)–P bond while, for the other entries, the rings extend to the right of this plane; thus these signs differ.

Table VIII. Least-Squares Plane Data for Cyclic Phosphate Diesters[a]

Compd	Deviation from best plane (A)						Ref
	O(3)	C(3)	O(4)	C(4)	P	C	
$(CH_2)_3PO_4H$	−0.0005	0.0005	0.0005	−0.0005	−0.62	0.67	25
3′,5′-UMP(A)	0.004	−0.004	−0.004	0.004	0.72	−0.72	27
3′,5′-UMP(B)	−0.02	0.02	0.02	−0.02	0.69	−0.71	27
3′,5′-GMP	0.02	−0.02	−0.02	0.02	−0.61	0.73	28
Thio-2′,3′-UMP	−0.004	0.007	0.004	−0.006	0.21		29
2′,3′-CMP(A)	−0.02	0.037	0.02	−0.037	0.42		21
2′,3′-CMP(B)	−0.015	0.023	0.015	−0.022	0.475		21

[a] Oxygen numbering as in Table VI, with C(3) bound to O(3) and C(4) to O(4). The first four atoms were used to define the planes in all cases, and the P and C atoms complete the six-membered rings.

noncyclic nucleotides, they range from 115 to 122°. Projected views down the ester bonds for the six-membered rings are given in Figure 5, and Table VII lists the torsion angles for seven- and five-membered diester rings as well. Again $(CH_2)_4PO_4H$ and $(CH_2)_3PO_4H$ have values similar to those for 3′,5′-UMP[27] and 3′,5′-GMP,[28] while the 2′3′-CMP[21] values are quite different. Table VIII contains least-squares plane data for these rings, based upon published coordinates;[21,25,27-30] the best plane for $(CH_2)_4PO_4H$ was given in Table V. The conformations of the six-membered rings are all twist chair, with similar P and C puckering. The five-membered rings can either have the P puckered as in 2′,3′-CMP, or be planar as in the five-membered cyclic acyl phosphate reported by Smith et al.[30] In the seven-membered ring, this constraint is relaxed, and only one phosphate oxygen is in the best plane (Table V). Geometrically then, both alkyl and bicyclic five-membered phosphodiester rings seem to be significantly strained. The six-membered phosphate rings of 3′,5′-nucleotides differ slightly from the $(CH_2)_3PO_4H$ and $(CH_2)_4PO_4H$ rings, but the structural features suggesting strain are poorly defined and clearly involve the sugar as well as the phosphate rings.

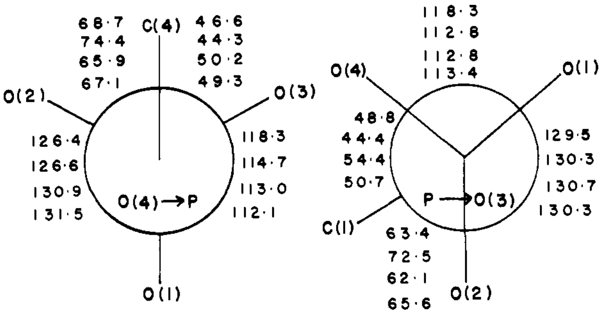

Figure 5. Newman projections down the phosphate ester bonds for six-membered diester rings. The values (in order) are for $(CH_2)_3PO_4H$, 3′,5′-GMP, and the two 3′,5′-UMP anions. See Table VII for values for other ring sizes.

A summary of the relevant enthalpy data for phosphate hydrolysis, taken from Sturtevant et al.[6] and Gerlt et al.[7] is given in Table IX. The enhanced rates and heats of hydrolysis of five-membered cyclic phosphates relative to alkyl phosphates seem largely to be due to phosphate ring strain. 2′,3′-AMP has an enthalpy change 2.5 kcal/mol more exothermic than $(CH_2)_2PO_4^-$, and 2′3′-CMP 1.2 kcal/mol more exothermic, so there is some sugar effect, but all three

Table IX. Enthalpies of Hydrolysis of Phosphate Diesters[a]

Diester	$-\Delta H_{\text{obsd}}$,[b] kcal/mol
Diethyl phosphate	2.50
$(CH_2)_2PO_4^-$	6.92
$(CH_2)_3PO_4^-$	3.82
$(CH_2)_4PO_4^-$	2.62
3',5'-AMP	11.49[c]
2',3'-AMP	9.4 (2' bond, ref 4)
2',3'-CMP	8.1 (2' bond, ref 4)
Methyl β-D-ribofuranoside 3,5-Cyclic phosphate	11.74
Methyl α-D-glucopyranoside 4,6-Cyclic phosphate	6.87

[a] Enthalpies were measured at pH 7.3, 25°; values for monoanionic diesters hydrolyzing to give monoanionic products are 0.3–0.8 kcal/mol less exothermic. For detailed conditions and discussion, see Gerlt et al.[7] [b] The estimated errors are 0.45 kcal/mol or less. [c] The 5'-ester bond; the value for the 3' bond is 12.09 (ref 7).

cyclic five-membered diesters have hydrolysis heats well above that of diethyl phosphate. For six-membered rings, strain involving the ribose is more important. The nature of the purine or pyrimidine base in cyclic 3′,5′-nucleotides does not have much effect; the methyl-substituted ribofuranoside phosphate has the same enthalpy of hydrolysis (within error) as 3′,5′-AMP. Cyclic ribofuranoside phosphates have hydrolysis heats about 8 kcal/mol more negative than that of $(CH_2)_3PO_4^-$, so these sugar rings must be more strained than the ribofuranosyl rings in 2′,3′-nucleotides where the heat difference is 1–2.5 kcal/mol over $(CH_2)_2PO_4^-$. This strain seems to be partially relieved on linking the six-membered phosphate ring to a six-membered sugar, as the heat of hydrolysis of the cyclic glucosyl phosphate in Table IX is 5 kcal/mol less exothermic than that for the comparable ribofuranosyl phosphate. Taken together, the thermodynamic and structural data on six-membered cyclic phosphate rings suggest that cyclic 3′,5′-nucleotides have high heats of hydrolysis because of the fairly rigid high energy ribofuranosyl conformation required to make the 3′,5′-diester linkage. The phosphate ester rings in 3′,5′-nucleotides are similar geometrically to the phosphate rings in compounds with low heats of hydrolysis such as $(CH_2)_3PO_4^-$. These nucleotides are examples of compounds which have high heats of hydrolysis because of strain in the starting material which must be maintained in the transition state and thus does not lead to a greatly increased rate of hydrolysis. In addition, neither the enthalpy data nor the geometrical data suggest that $(CH_2)_4PO_4^-$ is significantly more strained than $(CH_2)_3PO_4^-$. The situation in cyclic hydrocarbons is different, and the common use of hydrocarbon potential functions to predict strain in phosphate rings leads to incorrect estimates of strain energy.[31]

In 1941, Lipmann[32] suggested that phosphate compounds with high free energies of hydrolysis could act as energy sources for biological systems. My results indicate that there are at least three different methods for storing energy in phosphate compounds, and I would expect selective hydrolytic enzyme systems to have developed to utilize each of these sources of energy to drive biochemical reactions. The sources, with examples of use, are: (1) storage in pyrophosphate bonds, as exemplified by ATP and the many kinase and phosphorylase enzyme systems with ATP as a substrate or product; (2) phosphate ring strain storage, found in the 2′,3′-nucleoside monophosphates involved in the ribonuclease-catalyzed hydrolysis of RNA; (3) ribose ring energy storage as in 3′,5′-AMP. The adenyl cyclase–phosphodiesterase enzyme systems involve 3′,5′-AMP directly, but no examples of coupled reaction systems using the available energy to drive otherwise unfavorable reactions are known. The thermodynamically favored hydrolysis may simply ensure that enzymatic hydrolysis is an effective mechanism for lowering the concentration of 3′,5′-AMP when regulatory activity is no longer required.[33]

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Supplementary Material Available. A listing of observed and calculated structure factors will appear following these pages in the microfilm edition of this volume of the journal. Photocopies of the supplementary material from this paper only or microfiche (105 × 148 mm, 24× reduction, negatives) containing all of the supplementary material for the papers in this issue may be obtained from the Journals Department, American Chemical Society, 1155 16th St., N.W. Washington, D.C. 20036. Remit check or money order for $4.00 for photocopy or $2.50 for microfiche, referring to code number JACS-75-4084.

References and Notes

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(8) Abbreviations used: 3',5'-AMP = cyclic adenosine 3',5'-monophosphate; 3',5'-GMP = cyclic guanosine 3',5'-monophosphate; 3',5'-UMP = cyclic uridine 3',5'-monophosphate; 2',3'-CMP = cyclic cytidine 2',3'-monophosphate.
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