Stereochemistry of Nucleic Acids and Their Constituents. XXVII.[1] The Crystal Structure of 5′-Methyleneadenosine 3′,5′-Cyclic Monophosphonate Monohydrate, a Biologically Active Analog of the Secondary Hormonal Messenger Cyclic Adenosine 3′,5′-Monophosphate. Conformational "Rigidity" of the Furanose Ring in Cyclic Nucleotides

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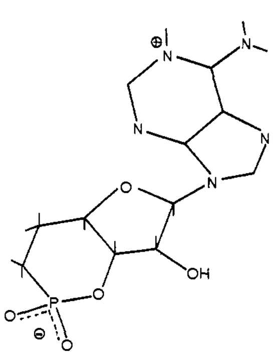
Abstract: The crystal structure of the isosteric phosphonate analog of cyclic AMP has been determined using three-dimensional data measured on a diffractometer. The crystals belong to the orthorhombic system, space group $P2_12_12_1$. The unit cell constants are: $a = 10.755$ (2), $b = 8.869$ (2), $c = 15.198$ (2) Å, $Z = 4$, $D_{\text{obsd}} = 1.578$ g cm^{-3}, $D_{\text{calcd}} = 1.579$ g cm^{-3}. The structure was solved by the use of the Patterson method and the application of the tangent formula. Refinement by the method of full-matrix least squares with anisotropic temperature factors for nonhydrogen atoms and isotropic temperature factors for the hydrogen atoms gave a final R of 0.04 for the 1199 observed reflections. The base displays the syn conformation with respect to rotation about the glycosyl bond, χ being $-125.8°$. The constraints of the cyclization freeze the ribose into the conformation, $^3T_4$(C(3′)-*endo*-C(4′)-*exo*), which appears to be characteristic of the cyclic nucleotides. For a similar reason the bond distances and bond angles in the ribose ring show marked differences from those of the nucleotides themselves. The phosphonate ring is in the chair conformation and flattened at the phosphorus end. The molecule is a zwitterion with the base site N(1) protonated by a phosphonate hydrogen. The nucleotides are packed in a head-to-tail fashion and are involved in an intricate scheme of hydrogen bonding both to themselves and the water of crystallization. A comparison of the conformation of the analog is made with the available data on cyclic AMP and cyclic UMP.

Cyclic adenosine 3′,5′-monophosphate (cyclic AMP) was discovered in 1960 by Sutherland[2,3] who demonstrated that it acted as a link or "messenger" between a large number of different hormones and the control and regulatory mechanisms of the human body. Cyclic AMP dependent protein kinases occur in a wide variety of tissues throughout the animal kingdom. In recent years a number of structurally related analogs of cyclic AMP have been synthesized and tested for their biological activity.[4,5] Perhaps the most notable of these are the N(6)-substituted derivatives which have been found to be more specific in their action than the natural metabolite.[5] The 5′-methylene cyclic phosphonate analog of cyclic AMP, **1**, is known to cause varying degrees of activation, with up to 50% of the maximal velocity of the natural compound, of the different cyclic AMP dependent protein kinases.[6-8] The present crystal-structure analysis of the 5′-methylene analog was undertaken to compare its molecular conformation with that of the natural metabolite, a preliminary report of which appeared in 1968,[9] but as yet the detailed crystal structure has not been reported. It was also hoped that the molecular conformation and the intermolecular interactions in the crystal would provide some relevant information regarding its biological function. A brief paper on the structure of the analog has been reported.[10] The only other crystal structure that is available on a cyclic nucleotide is uridine 3′,5′-monophosphate (cyclic UMP).[11] Since the complete crystal structure of this compound is available, the ribophosphate ring system is compared with the ribophosphonate system of the analog.



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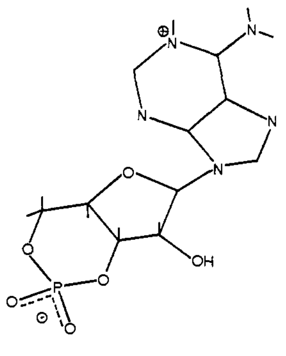

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Table I. Positional and Thermal Parameters of Atoms in 5′-Methylene Analog of Cyclic AMP[a]

Atom	X	Y	Z	β_{11} or B^b	β_{22}	β_{33}	β_{12}	β_{13}	β_{23}
N(1)	1660 (3)	5353 (4)	3641 (2)	55 (3)	136 (4)	33 (1)	4 (4)	3 (2)	−8 (2)
C(2)	2808 (4)	5326 (6)	4012 (3)	66 (4)	123 (7)	27 (2)	−11 (5)	3 (2)	−8 (3)
N(3)	3826 (3)	4868 (4)	3618 (2)	59 (3)	116 (6)	25 (1)	−4 (4)	3 (2)	−8 (2)
C(4)	3598 (4)	4419 (5)	2774 (3)	52 (3)	84 (6)	25 (2)	0 (4)	3 (2)	−8 (2)
C (5)	2490 (4)	4467 (5)	2331 (3)	53 (3)	115 (8)	23 (2)	−11 (4)	3 (2)	−8 (3)
C(6)	1433 (4)	4950 (5)	2796 (3)	59 (4)	125 (8)	33 (2)	−10 (4)	3 (2)	−8 (3)
N(7)	2613 (3)	3956 (5)	1474 (2)	50 (3)	149 (6)	29 (2)	−10 (4)	3 (2)	−8 (3)
C(8)	3784 (4)	3594 (5)	1417 (3)	62 (4)	117 (8)	29 (2)	−9 (4)	3 (2)	−8 (3)
N(9)	4443 (3)	3846 (4)	2184 (2)	44 (3)	98 (5)	25 (2)	1 (3)	3 (2)	−8 (2)
N(6)	307 (3)	5023 (6)	2455 (3)	43 (5)	24 (9)	42 (2)	7 (5)	3 (2)	−8 (4)
C(1′)	5759 (4)	3534 (5)	2294 (3)	54 (4)	108 (6)	29 (2)	13 (4)	3 (2)	−8 (3)
O(1′)	5899 (3)	2580 (3)	3046 (2)	83 (3)	95 (4)	42 (1)	3 (3)	3 (2)	−8 (2)
C(2′)	6604 (3)	4928 (5)	2414 (2)	47 (3)	132 (6)	27 (2)	−2 (4)	3 (2)	−8 (3)
O(2′)	7712 (3)	4774 (6)	1941 (2)	49 (3)	329 (9)	27 (1)	−23 (4)	3 (2)	−8 (3)
C(3′)	6913 (3)	4785 (4)	3385 (2)	47 (3)	88 (5)	29 (2)	5 (3)	3 (2)	−8 (3)
O(3′)	8054 (2)	5493 (4)	3631 (2)	55 (2)	135 (5)	21 (1)	−10 (3)	3 (1)	−8 (2)
C(4′)	6994 (4)	3098 (5)	3498 (3)	69 (4)	100 (6)	35 (2)	13 (4)	3 (2)	−8 (3)
C(5′)	7060 (5)	2647 (5)	4458 (3)	136 (6)	102 (6)	46 (2)	−11 (5)	3 (3)	−8 (3)
C(6′)	8277 (5)	3288 (5)	4825 (3)	120 (5)	132 (7)	43 (2)	10 (5)	3 (3)	−8 (3)
P	8428 (1)	5299 (1)	4659 (1)	49 (1)	125 (2)	20 (1)	−3 (1)	3 (1)	−8 (1)
O(6)	9751 (3)	5837 (4)	4705 (2)	53 (3)	239 (6)	30 (1)	−21 (4)	3 (2)	−8 (3)
O(7)	7516 (3)	6087 (4)	5239 (2)	72 (3)	162 (5)	27 (1)	−4 (3)	3 (2)	−8 (2)
O(W)	5019 (3)	5800 (6)	5658 (2)	87 (4)	303 (9)	43 (2)	28 (5)	3 (2)	−8 (3)
H(1)	102 (4)	564 (5)	391 (3)	5 (1)					
H(2)	286 (3)	563 (4)	459 (2)	2 (1)					
H(8)	431 (3)	320 (4)	86 (2)	2 (1)					
H(61)	−34 (5)	528 (6)	277 (3)	6 (1)					
H(62)	15 (6)	484 (6)	190 (3)	6 (1)					
H(1′)	599 (3)	298 (4)	174 (2)	2 (1)					
H(2′)	615 (3)	589 (4)	229 (2)	2 (1)					
H(02′)	762 (5)	434 (7)	138 (3)	7 (1)					
H(3′)	618 (4)	520 (5)	373 (3)	4 (1)					
H(4′)	776 (3)	273 (4)	317 (2)	3 (1)					
H(5′1)	640 (4)	304 (6)	479 (3)	6 (1)					
H(5′2)	706 (3)	149 (4)	456 (2)	2 (1)					
H(5′′1)	840 (5)	308 (6)	540 (3)	6 (1)					
H(5′′2)	904 (5)	284 (7)	447 (4)	7 (2)					

[a] Positional parameters of heavy atoms $\times 10^4$. Positional parameters of hydrogen atoms are $\times 10^3$. Anisotropic thermal parameters are $\times 10^4$. Anisotropic temperature factor is of the form $\exp[-(\beta_{11}hh + \ldots + 2\beta_{12}hk + \ldots)]$. Standard deviations refer to the least significant digits. [b] Denotes isotropic temperature factors of the hydrogen atoms.

Experimental Section

Suitable crystals of 1 for the X-ray work were supplied by Dr. J. G. Moffatt and Dr. G. H. Jones, Institute of Molecular Biology, Syntex Research, Palo Alto, Calif. The crystals grew as flat plates, and one measuring approximately 0.3 × 0.3 × 0.1 mm³ was chosen for the X-ray work, and mounted about the b axis. Preliminary photographic investigation revealed that the crystals belonged to the orthorhombic system with systematic extinctions occurring for the odd order reflections, along each axis, thus consistent with the space group $P2_12_12_1$. The unit cell dimensions were determined by a least-squares refinement of the angles ϕ, χ, and 2θ of 12 reflections recorded at medium 2θ angles. They are a = 10.775 (2), b = 8.869 (2), and c = 15.198 (2) Å. The experimental crystal density of 1.578 g cm^{-3} measured using the flotation technique in a mixture of carbon tetrachloride and benzene is in agreement with the calculated density of 1.579 g cm^{-3} assuming four formula units of the monohydrate, $C_{11}H_{14}N_5O_5P \cdot H_2O$, in the unit cell.

Intensity data were collected on a Picker FACS-I diffractometer using the θ–2θ scan technique with a scan speed of 2°/min. A total of 1372 independent reflections were scanned to a maximum 2θ of 128°; of these, 1199 reflections were greater than 1.5 times their standard deviations and were used in the structural analysis after correcting for the usual Lorentz and polarization effects.

Structural Analysis. The observed reflections were converted into the normalized structure amplitudes, $|E|$'s, and a sharpened three-dimensional Patterson synthesis was calculated with $(|E|^2 - 1)$ as coefficients. The phosphorus coordinates were obtained from the Harker sections. The phases of 228 reflections with $|E| > 1.2$ from the structure factor calculation using the phosphorus coordinates were refined by the application of the tangent formula.[12] The refinement converged after five cycles and the R value ($R = \Sigma||E_o| - |E_c||/(|E_o|)$) for the 228 reflections was 0.20. The E map generated using these reflections and the refined phases revealed the entire structure including the water molecule.

The coordinates of the atoms picked out from the E map were subjected at first to two rounds of refinement by full-matrix least squares with isotropic temperature factors, and then followed by one cycle of refinement with anisotropic temperature factors. The R factor for the 1199 observed reflections dropped to 0.093. From a difference electron density map computed at this point, 14 of the 16 hydrogen atoms were picked. Further anisotropic least-squares refinement and difference density maps proved to be of no help in determining the remaining two hydrogen atoms, which belonged to the water. The R factor at the conclusion of the refinement was 0.04 for the observed reflections. At this point the ratios of the shifts to the estimated standard deviations in all of the parameters were less than one.

The weighting scheme used in the refinement was $w = 1$ for $F_{\mathrm{obsd}} \leq 10.5$, and $w = 1/(0.03F_{\mathrm{obsd}} + 0.234)$ for $F_{\mathrm{obsd}} > 10.5$. This weighting scheme was derived from the plot of $|\Delta F|$ *vs.* $|F_{\mathrm{obsd}}|$, where $|\Delta F| = |F_{\mathrm{obsd}} - F_{\mathrm{calcd}}|$. The reflections 002, 020, and 112 were given zero weight because they appeared to be suffering from secondary extinction. The atomic form factors that were used are those of Cromer and Waber[13] for the nonhydrogen atoms, and that of Stewart, *et al.*,[14] for hydrogen atoms.

Results

The atomic positional and thermal parameters obtained from the least-squares refinement are given in Table I. The average standard deviations for the

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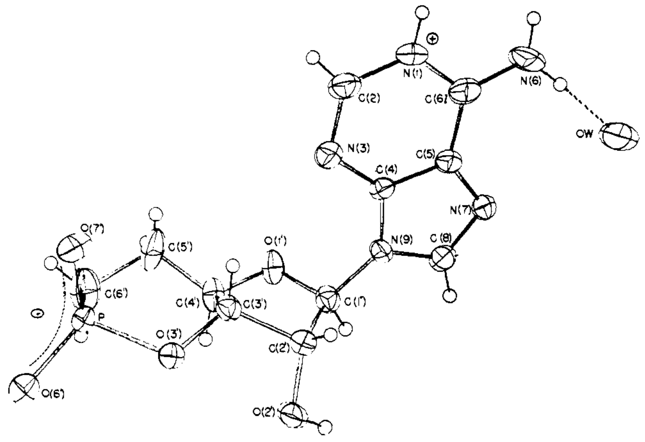

Figure 1. A drawing showing the thermal ellipsoids of the non-hydrogen atoms and atom numbering. Note the relatively large anisotropic vibration of the ribophosphonate atoms O(1′), C(4′), C(5′), and C(6′) (see also text).

various atoms are: P = 0.001, O = 0.003, N = 0.003, C = 0.004, and H = 0.04 Å. A table of structure amplitudes will appear in the microfilm edition of this volume of the journal.[15] The thermal ellipsoids for the atoms are represented in Figure 1. The covalent bond distances and angles involving hydrogen atoms are within the usual range found in X-ray determinations and are not given.

Discussion

The molecule occurs as a zwitterion, N(1) of the adenine base being protonated by an adjacent phosphonate proton. Similar zwitterion structures have been found for all the other known adenosine nucleotides, *viz.*, 3′-AMP,[16] 5′-AMP,[17] 3′-methylene analog of 3′-AMP,[1] U3′p5′A,[18] and A2′p5′U.[19]

Bond Distances and Bond Angles. Base. The bond distances and bond angles are given in Figure 2. The average estimated standard deviation in the bond lengths is 0.005 Å and in angles is 0.3°. The bond distances and bond angles in the adenine cation are similar to the values found in other N(1)-protonated adenine deviatives,[16] and have been discussed in detail elsewhere.[20,21] The exocyclic angle C(8)–N(9)–C(1′) is smaller than the angle C(4)–N(9)–C(1′). This seems to be the case in structures exhibiting the syn conformation, while the opposite is true for the anti conformation.[21]

Ribose. The bond distances and bond angles in the furanose ring (Table II) are significantly different from the values found in the common nucleotides,[20] but are generally in good agreement with the values found in cyclic UMP.[11] Although the corresponding values are not yet available for cyclic AMP, they are expected to be similar to the above values. The largest C–C bond in the cyclic nucleotide is C(1′)–C(2′), while the shortest is C(3′)–C(4′). The former is greater than the average ribose C–C bond (1.525 Å)[20-22] by about 0.02 Å and the latter is smaller by about 0.015 Å. The C(2′)–C(3′) bond is intermediate in value to the above bond distances. The ring C–O bond distances, C(1′)–O(1′) = 1.430 Å, and C(4′)–O(1′) = 1.441 Å, are equal within the experimental errors. This is in contrast to the observation that has been made in the nucleotides themselves where the C(1′)–O(1′) bond (1.409 Å) is significantly shortened in comparison with the C(4′)–O(1′) bond (1.450 Å).[22] The differences in the bond

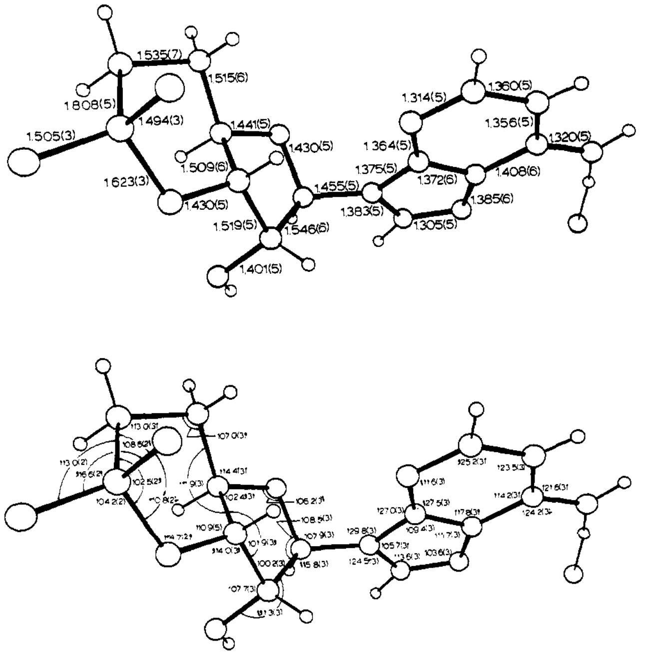

Figure 2. Bond distances and bond angles in the 5′-methylene analog of cyclic AMP.

Table II. A Comparison of the Bond Distances and Bond Angles in the Ribose of **1** and Cyclic UMP

	1	Cyclic UMP Molecule 1	Cyclic UMP Molecule 2
O(1′)–C(1′)	1.430 (5)	1.444 (8)	1.431 (8)
C(1′)–C(2′)	1.546 (6)	1.565	1.560
C(2′)–C(3′)	1.519 (5)	1.531	1.529
C(3′)–C(4′)	1.509 (6)	1.509	1.502
C(4′)–O(1′)	1.441 (5)	1.434	1.436
C(2′)–O(2′)	1.401 (5)	1.396	1.411
C(1′)–N	1.455 (5)	1.458	1.448
O(1′)–C(1′)–C(2′)	108.5	107.9	108.4
C(1′)–C(2′)–C(3′)	100.2	99.4	99.8
C(2′)–C(3′)–C(4′)	101.9	102.6	102.9
C(3′)–C(4′)–O(1′)	102.4	101.4	101.8
C(4′)–O(1′)–C(1′)	106.2	106.0	105.1
O(1′)–C(1′)–N	107.9	107.4	108.2
C(2′)–C(1′)–N	115.8	117.4	116.0
C(1′)–C(2′)–O(2′)	111.3	107.6	108.1
C(3′)–C(2′)–O(2′)	107.7	113.6	112.7
C(2′)–C(3′)–O(3′)	114.0	115.7	116.8
O(1′)–C(4′)–C(5′)	114.4	114.8	112.7
σ	0.3	0.7	0.7

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(15) A table of observed and calculated structure amplitudes will appear following these pages in the microfilm edition of this volume of the journal. Single copies may be obtained from the Business Operations Office, Books and Journals Division, American Chemical Society, 1155 Sixteenth St., N.W., Washington, D. C. 20036, by referring to code number JACS-72-5070. Remit check or money order for $3.00 for photocopy or $2.00 for microfiche.

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lengths and angles between the cyclic nucleotides and the nucleosides are clearly a manifestation of the torsional strain in the furanose ring imposed by the cyclization.

The bond angles in the furanose ring (Table II) are again markedly different from those of the nucleotides,[20,21] but are in good agreement with the values found in cyclic UMP.[11] The angles C(1′)-C(2′)-C(3′), C(2′)-C(3′)-C(4′), and C(3′)-C(4′)-O(1′) are 100.2, 101.9, and 102.4°, respectively, and are close to each other. They are, however, markedly different from the angles O(1′)-C(1′)-C(2′) = 108.5° and C(4′)-O(1′)-C(1′) = 106.2°. Among the five intraring angles those at C(1′), O(1′), and C(4′) show the greatest difference to the common nucleotides.[21] The smallest valence angle in the cyclic nucleotide involves C(2′) while the largest angle involves the adjacent C(1′). It must be mentioned that the bond distances, bond angles, and torsion angles are interrelated. Thus, changing any one of these would result in a change of the other.

Phosphonate. The present structure represents the only known case of a cyclic nucleoside phosphonate. The bond distances in the phosphonate ring that are significantly different from those of cyclic UMP are C-P = 1.808 ± 0.005 Å, and C(5′)-C(6′) = 1.537 ± 0.007 Å. The remaining bond distances are in agreement with cyclic UMP (Table III). The presence of the methylene group instead of the O(5′) atom produces a contraction in the valence angle C(5′)-C(6′)-P = 113.0°, in comparison with the angle C(5′)-O(5′)-P = 118.8°. Whereas the angles C(4′)-C(5′)-C(6′) and P-O(3′)-C(4′) show an expansion of about 2-3° from those of cyclic UMP (Table III), the smallest and largest angles in the phosphonate group are C(6′)-P-O(3′) and O(6)-P-O(7), respectively. A similar trend is observed in cyclic UMP.

Table III. A Comparison of the Bond Distances and Angles in the Phosphonate Ring of **1** and Phosphate Ring of Cyclic UMP

		Cyclic UMP	
	1	Molecule 1	Molecule 2
C(3′)-C(4′)	1.509 (6)	1.509 (8)	1.502 (8)
C(4′)-C(5′)	1.515 (6)	1.500	1.489
C(5′)-C(6′)	1.535 (7)	1.468	1.464
C-C(5′)-P	1.808 (5)	1.611	1.611
P-O(3′)	1.623 (3)	1.609	1.619
O(3′)-C(3′)	1.430 (4)	1.443	1.421
P-O(6)	1.505 (3)	1.485	1.471
P-O(7)	1.494 (3)	1.476	1.483
C(3′)-C(4′)-C(5′)	111.9 (3)	111.8 (7)	111.1 (7)
C(4′)-C(5′)-C(6′)	107.0 (3)	104.0	105.4
C(5′)-C(6′)-P	113.0 (2)	118.3	119.2
C-C(5′)-P-O(3′)	102.5 (2)	102.7	103.5
P-O(3′)-C(4′)	114.7 (2)	112.7	111.3
O(3′)-C(3′)-C(4′)	110.9 (3)	111.0	112.1
O(3′)-P-O(6)	104.2 (2)	107.1	107.9
O(3′)-P-O(7)	110.8 (2)	109.8	109.2
C(6′)-P-O(6)	108.6 (2)	109.2	109.4
C(6′)-P-O(7)	113.0 (2)	107.4	106.0
O(6)-P-O(7)	116.6 (2)	119.2	119.7

The Molecular Conformation. Least-Squares Planes for Base. The least-squares planes for the base are shown in Table IV. The displacements of the ring atoms from the plane are not significant, but the substituent atom C(1′) shows a significant deviation (0.070 Å) from the plane and lies on the side of O(1′). The dihedral angle between the planes of the pyrimidine and imidazole rings is 2.1°.

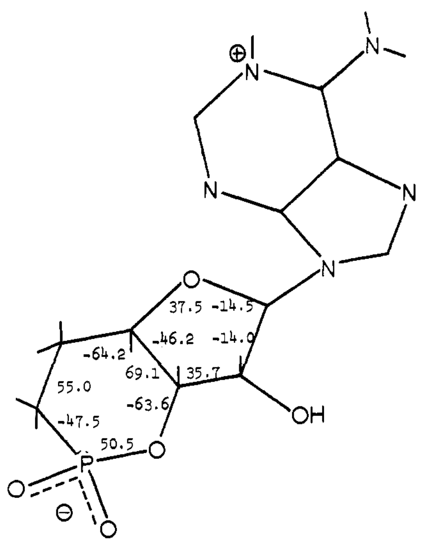

Figure 3. The intraring torsion angles for the ribose and phosphonate rings in 5′-methylene cyclic AMP.

Table IV. Deviations of Atoms from Least-Squares Planes through the Base[a]

Atom	Plane I, Å	Plane II, Å	Plane III, Å
N(1)	*−0.027*	*−0.013*	−0.075
C(2)	*0.010*	*0.010*	−0.009
N(3)	*0.022*	*0.006*	0.031
C(4)	*−0.001*	*−0.019*	*0.003*
C(5)	*0.022*	*0.016*	*−0.004*
C(6)	*−0.011*	*0.005*	−0.064
N(7)	*0.024*	0.011	*0.003*
C(8)	*−0.012*	−0.042	*−0.001*
N(9)	*−0.027*	−0.062	*−0.001*
N(6)	−0.021	0.004	−0.103
C(1′)	−0.070	−0.122	−0.009
O(1′)	−1.182	−1.236	−1.113
Rms	0.019	0.012	0.002

[a] Atoms used in the fitting of the planes are in italics.

Glycosyl Angle χ. This angle as defined originally by Donohue and Trueblood[23] describes the stereochemistry of the base with respect to the sugar. The definition of this angle used in this paper is that of Sundaralingam.[24] It is found that the cyclic nucleotide analog exhibits the syn conformation where χ = −125.7°. Crystals of the natural compound, cyclic AMP, have two indepent molecules in the asymmetric unit of structure.[9] One occurs in the syn conformation (χ = −102°), the other in the anti conformation (χ = 50°). Thus, the conformation of 50% of the molecules of the natural compound is similar to the analog. It appears that the syn conformation is preferable to the anti conformation in the cyclic nucleotides (purines). It is interesting that cyclic UMP[11] also crystallizes with two independent molecules in the same crystal, but both occur in the anti conformation χ = +77 and +58°.

Conformational "Rigidity" of the Ribose in Cyclic Nucleotides. The torsion angles about the furanose ring bonds are shown in Figure 3. The largest angle occurs about C(3′)-C(4′), the bond about which the

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(23) J. Donohue and K. N. Trueblood, *J. Mol. Biol.*, 2, 363 (1960).
(24) M. Sundaralingam, *Biopolymers*, 7, 821 (1969).

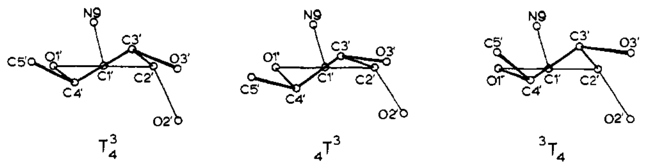

Figure 4. The puckering in cyclic nucleotides (a) symmetrical twist form ^3_4T (or T^3_4), (b) cyclic AMP and cyclic UMP $_4T^3$, and (c) present work $^3T_4$ (see also Table VI).

five-membered ring is fused to the phosphonate ring. Thus, C(3′) and C(4′) should show the most deviation and lie on opposite sides of the least-squares plane through the furanose ring atoms (see below). The similarity in the torsion angles C(2′)–C(3′) ≈ C(4′)–O(1′) and C(1′)–C(2′) ≈ C(1′)–O(1′) indicates that the furanose ring is close to the C_2 or symmetrical twist (T) conformation.

It is common to describe the deviations of the atoms from the least-squares plane as endo or exo depending on whether they are on the same side or opposite side, respectively, of C(5′). This popular practice, though apparently suitable for the common nucleotides, does not give a comparable description for the conformation of the furanose ring in cyclic 3′,5′ nucleotides; in the cyclic nucleotides the marked puckering of C(4′) brings C(5′) into the equatorial plane of the ring. Thus, in order to compare its puckering with the common nucleotides, the deviations are described here relative to the glycosyl nitrogen N(9). That is, endo and exo mean the deviation is on the same side or opposite side, respectively, of N(9) (Figure 4).

Some least-squares planes for the ribose are given in Table V. C(3′) and C(4′) show the greatest deviations from the best (plane II) and next best (plane III) four-atom planes. Therefore, the ribose is in the twist (T) conformation $^3T_4$ (C(3′)-*endo*-C(4′)-*exo*)[22-25] (Figure 4). The deviations of C(3′) and C(4′) from the plane O(1′)–C(1′)–C(2′) are also given (plane IV).

Table V. Deviations of Atoms from Least-Squares Planes through the Ribose[a]

Atom	Plane I, Å	Plane II, Å	Plane III, Å	Plane IV, Å
O(1′)	*−0.152*	*−0.082*	*0.056*	*0.000*
C(1′)	*−0.011*	*0.097*	*−0.083*	*0.000*
C(2′)	*0.161*	*−0.047*	*0.078*	*0.000*
C(3′)	*−0.264*	−0.638	*−0.051*	−0.363
C(4′)	*0.267*	*0.050*	0.629	0.347
C(5′)	−0.257	−0.567	0.404	−0.073
N(9)	−1.145	−0.872	−1.344	−1.118
O(2′)	1.508	1.212	1.368	1.287
O(3′)	0.287	−0.372	0.587	0.083
Rms	0.195	0.066	0.069	0.000

[a] The atoms used in fitting the planes are in italics.

The relevant torsion angles for the three known cyclic nucleotides are compared in Table VI. Although the details are not available in cyclic AMP,[9] it appears that the two independent molecules of cyclic AMP exhibit the $_4T^3$ conformation. A similar conformation is exhibited by the two different molecules of cyclic UMP. The subtle differences between the ribose conformation of the analog and cyclic AMP and cyclic UMP are also seen when the torsion angles are compared (Table VI) (see also Figure 4). The phosphonate ring of the analog probably is partly responsible for the above differences in the ribose conformation.

Both from the X-ray studies and considerations of molecular models it is seen that the ribose ring in the cyclic nucleotides has a restricted conformation (Figure 4). Although the natural cyclic AMP and UMP exist in the $_4T^3$ conformation, the present structure determination suggests that the conformation $^3T_4$ is probably also likely. Thus, the cyclic nucleotides can exhibit the small range of conformations $_4T^3 \leftrightarrow {}^3T_4$. In this connection it is interesting to note that the atoms O(1′), C(4′), C(5′), and C(6′) of the analog show rather marked thermal vibration approximately normal to the ribose ring (Figure 1). This perhaps suggests that the furanose ring executes a narrow range of conformations centered around $^3T_4$, which is the time and space averaged conformation obtained by the X-ray technique. The conformational distribution seen here as reflected by the thermal vibrations of the atoms apparently has not been seen in other X-ray studies of furanoside systems.

The torsion angles involving the hydrogen atoms of the ribose ring are also listed in Table VI. The moduli of these values (dihedral angles) are related to the coupling constants measured from proton magnetic resonance spectroscopy, and they can yield direct information on the conformation of the furanose ring in solution.

Conformation of the Phosphonate Ring. The phosphonate ring exhibits a distorted chair conformation (Table VI). It is more puckered about the C(3′)–C(4′) bond about which the ribose is fused to it, and flattened about the bonds involving the phosphorus atom. Clearly, the nonbonded interactions are responsible for the greater puckering about the C(3′)–C(4′) bond and the bonds adjacent to it in the phosphonate and ribose rings. The ribose ring is also found to be flattened at the opposite side of the bond C(3′)–C(4′). A detailed comparison of the torsion angles of the phosphonate and phosphate rings is given in Table VI. The torsion angles in the phosphate rings follow the same trend as in the phosphonate ring.

Molecule Packing and Hydrogen Bonding. The hydrogen bonding and crystal packing schemes are shown in Figure 5. The nucleotides are packed in a head-to-tail fashion with no overlap of the base rings. The closest contact between the bases involves the substituent N(6) atom.

In Table VII are listed the hydrogen bonding distances between heavy atoms and between hydrogen and heavy atoms as well as the hydrogen bonding angles. Some of these values are also indicated in Figure 5.

With the exception of the base site N(3) and possibly N(7) and the ribose ring O(1′), all donor and acceptor atoms participate in hydrogen bonding. N(3) is usually found to be involved in an intramolecular hydrogen bond O(5′)–H···N(3) in the nucleosides exhibiting the syn conformation.[21] This hydrogen bond restricts the conformation C(3′)–C(4′)–C(5′)–O(5′) to (+) gauche.[21] However, for the alternative trans or the (−) gauche (as in the cyclic nucleotides) conformations about the C(4′)–C(5′) bond, N(3) cannot participate in the intramolecular hydrogen bond. Also, the steric over-

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(25) M. Sundaralingam, *J. Amer. Chem. Soc.*, 93, 6644 (1971).

Table VI. A Comparison of the Conformation of the 5′-Methylene Cyclic AMP Analog with Cyclic AMP[a]

	Cyclic AMP, analog	Cyclic AMP		Cyclic UMP	
		Molecule 1	Molecule 2	Molecule 1	Molecule 2
Glycosyl angle χ^b	−125.8°(syn)	50°(anti)	−102°(syn)	77°(anti)	58°(anti)
Ribose Conformation τ_0	−14.5			−19.3	−23.5
τ_1	−14.0			−10.4	−5.5
τ_2	35.7			34.6	30.8
τ_3	−46.2			−48.1	−46.7
τ_4	37.5			41.5	43.2
H(1′)-H(2′)	107			114	119
H(2′)-H(3′)	38			38	37
H(3′)-H(4′)	−177			−176	−174
	${}^3T_4{}^c$	${}_4T^3$	${}_4T^3$	${}_4T^3$	${}_4T^3$
Phosphonate and phosphate ring conformation					
P–O(3′)	50.5			57.4	49.3
O(3′)–C(3′)	−63.6			−66.7	−61.2
C(3′)–C(4′)	69.1			69.1	67.1
C(4′)–C(5′)	−64.2			−61.4	−59.5
C(5′)–C(6′) or C(5′)–O(5′)	55.0			60.5	56.5
C(6′)–P or O(5′)–P	−47.5			−56.7	−50.5

[a] Only the available preliminary data on cyclic AMP are given. [b] The glycosyl torsion angle as cited here is based on the definition in ref 24. [c] ${}^3T_4$ = C(3′)-*endo*–C(4′)-*exo*, ${}_4T^3$ = C(4′)-*exo*–C(3′)-*endo*. The ribose puckering in all these cyclic nucleotides may be also stated to be close to the usual C(3′)-*endo* puckering.[22,24,26] The ribose exhibits the twist (T) conformation rather than the alternative envelope (E) conformation. In the analog C(3′) is puckered more than C(4′), hence, the conformation is ${}^3T_4$, while in the natural compounds it is *vice versa;* consequently their conformations are ${}_4T^3$. The dlfference between ${}^3T_4$ and ${}_4T^3$ is only one of degree than of kind.

Table VII. Hydrogen Bond Distances and Angles[a]

	A···B, Å	H···B, Å	A–H···B, deg
N(1)–H(1)···O(6)	2.651	1.83	164
N(6)–H(61)···O(3′)	3.042	2.17	169
N(6)–H(62)···O(w)	2.850	1.98	172
O(2′)–H–O(2′)···O(7)	2.707	1.78	168
C(8)–H(8)···O(6)	3.085	2.20	138
O(7)···O(w)	2.776		
O(6)···O(w)	3.047		

[a] A and B designate the nonhydrogen atoms.

crowding by the ribose prevents it from being involved in even an intermolecular hydrogen bond.

Phosphate–Base Hydrogen Bonding. The shortest hydrogen bond (2.651 Å) is between O(6) (negatively charged) of the phosphonate and the base site N(1) (positively charged) which is protonated by a phosphonate proton. Even shorter hydrogen bonds between phosphate and base have been observed in other nucleotides.[26,27] As a general rule such short (strong) hydrogen bonds are observed between electronegative atoms that are positively or negatively charged. The $N(1)^+$–H···$O(6)^-$ hydrogen bonding has been observed in all of the adenine nucleotides and dinucleoside phosphates that have been determined to date (see also ref 1). The persistent observation of the above hydrogen bonding scheme in adenine and also cytidine nucleotides[26-28] suggests strongly that such hydrogen bonding may be important not only in nucleic acid–nucleic acid interactions, but also in intrastrand interactions that stabilize certain tertiary structures of transfer ribonucleic acids.[16]

The second anionic phosphonate oxygen O(7) is hydrogen bonded to the water of crystallization and the 2′ hydroxyl group of an adjacent ribose moiety. The ester O(3′) appears to be involved in a weak hydrogen bond, O(3′)···H–N(6) = 3.042 Å, to one of the amino hydrogen atoms. A similar hydrogen bonding is reported in cyclic AMP.[10] In general, however, the ester oxygen atoms show little tendency for hydrogen bonding.[24]

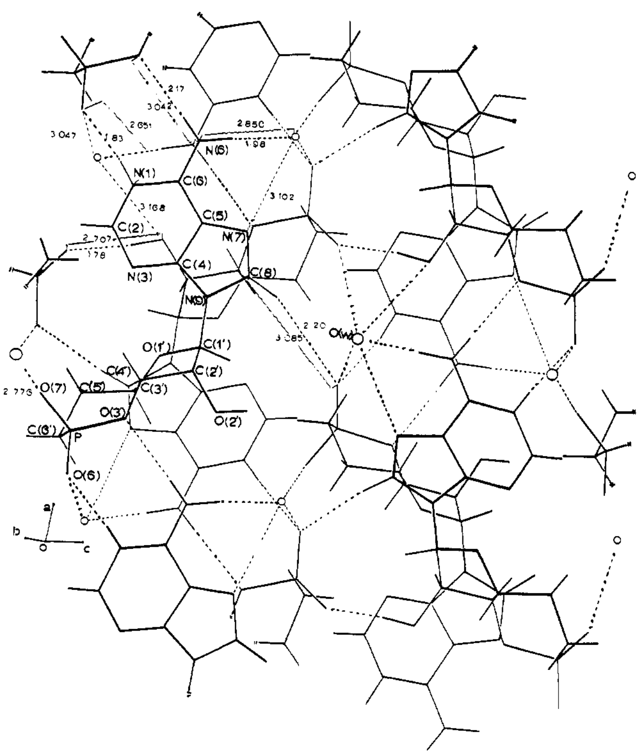

Figure 5. The molecular packing and hydrogen bonding scheme viewed approximately down the *a* axis.

Hydrogen Bonding to Water. The water of crystallization is trapped in a pocket surrounded by several nucleotides. There are a number of short contacts involving the water and the surrounding nucleotides (Figure 6). In view of the fact that the hydrogen atoms on the water were not determined, only a tentative hydrogen bonding scheme involving the water can be given. It is clear that the water accepts a hydrogen bond from N(6) and appears to donate a hydrogen bond

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(26) M. Sundaralingam and L. H. Jensen, *J. Mol. Biol.*, 13, 914 (1965).
(27) C. E. Bugg and R. E. Marsh, *ibid.*, 25, 67 (1967).
(28) M. A. Viswamitra, B. S. Reddy, G. Hung-Yin Lin, and M. Sundaralingam, *J. Amer. Chem. Soc.*, 93, 4565 (1971).

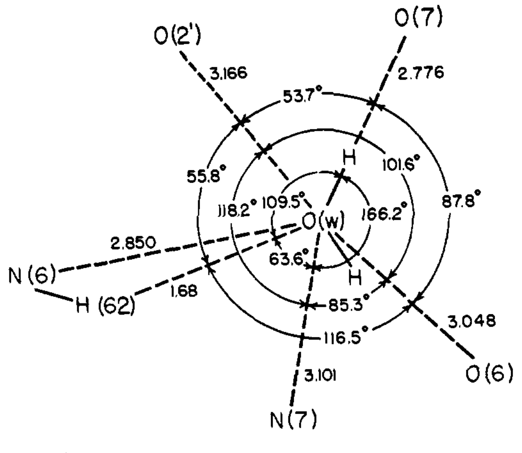

Figure 6. The close contacts and possible hydrogen bonds involving the water molecule.

to O(7) as judged by the hydrogen bond distances and angles (Figure 6 and Table VII). It also appears that the water donates its second proton to O(6) of a different phosphonate group. The remaining short contacts (Figure 6) are probably van der Waals interactions. The difficulty encountered in determining the water hydrogen atoms could in part be due to possible rotational disorder in the water molecule.

C–H···O Hydrogen Bond. In the purine derivatives the C(8)–H bond is relatively polarized, because the carbon atom is flanked by electronegative nitrogen atoms. Thus, this bond in the purine system presents an ideal case for involvement in a C–H···O hydrogen bonding. Indeed, many of the crystal structures containing the purine ring have been found to display C–H···O hydrogen bonding involving either the C(8) or C(2) carbon.[16,29] In the present structure the C–H···O hydrogen bond involves the phosphonate oxygen O(6). Therefore, O(6) participates in three hydrogen bonds. The tendency for the formation of the C(8)–H···O hydrogen bond seems to be even greater for the N(1) protonated adenine derivatives than the neutral adenine compounds.[21] It appears that the electronic perturbation of the adenine base is transmitted to the C(8)–H bond causing it to become more polarized. That this, in fact, is the case is supported by the easy substitution of H(8) by halide ions under acid conditions.

Conclusion

The molecular conformation of the 5′-methylene analog of cyclic AMP is close to the conformation found for one of the molecules of cyclic AMP, itself. It is not yet known whether it is the syn or anti conformation that has biological activity, but it is clear from available X-ray data[21,24] that both conformations are easily assumed by these compounds, *i.e.*, the glycosyl angle is the only angle about which the cyclic nucleotide can exhibit conformational freedom (anti and syn), since the ribophosphonate (or ribophosphate) group is frozen into the characteristic conformation $^{3}T_{4} \leftrightarrow {}_{4}T^{3}$. The intermolecular hydrogen bonding provides some insight into the mode of interaction of the cyclic nucleotides with enzymes. The 5′-methylene analog forms about 11 hydrogen bonds (Figure 7); three of these involve water molecules. The remaining hydrogen bonds involve the neighboring nucleotides. Each anionic phosphonate oxygen atom is hydrogen bonded to a water.

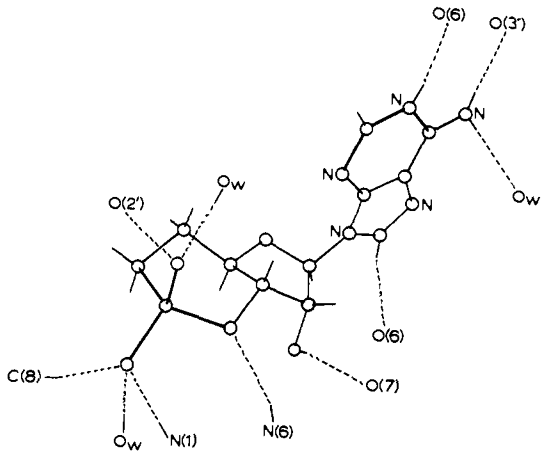

Figure 7. The hydrogen bonding environs of a single molecule of the 5′-methylene analog. Except for the base sites N(3) and possibly N(7) (see text) the remaining potential hydrogen bonding sites are involved in hydrogen bonding. The anionic oxygens of the phosphonate group participate in a total of five hydrogen bonds. It is envisaged that this substrate molecule will have at least part of this hydrogen bonding environment when it is bound to the "active site" of adenylyl cyclase, the enzyme that converts adenosine triphosphate to cyclic AMP. In other words, the three-dimensional arrangement of the donor and acceptor sites in the enzyme can be roughly obtained from the hydrogen bonding scheme.

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(29) D. J. Sutor, *J. Chem. Soc.*, 1105 (1963).