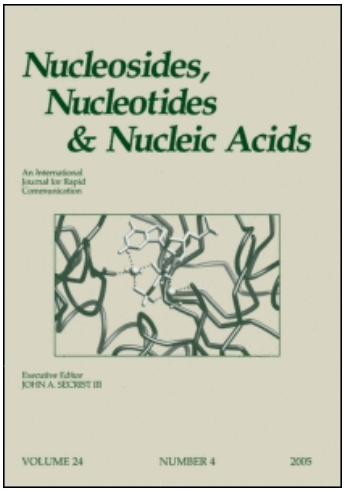

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CYTIDINE CYCLIC (3′, 5′) MONOPHOSPHATE: CYCLIC NUCLEOTIDE WITH A NON-CHARACTERISTIC RIBOSE RING CONFORMATION

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ABSTRACT: Cytidine 3′,-5′-cyclic phosphate (cCMP) occurs in nature and has growth stimulatory activity on L-1210 cells. The initiation of cell growth by cCMP, under conditions where cAMP, cGMP and cUMP delay the onset of proliferation suggests that cCMP may play a regulatory role in the cell metabolism. It has been reported that in 3′,5′-cyclic nucleotides, the phosphate ring fused to the furanose ring restricts the conformation of the furanose ring to the twist form C(3′) endo C(4′) exo ($^3T_4$), in contrast to the C(2′)endo C(3') exo ($^2T_3$) and C(3') endo C(2') exo ($^3T_2$) twist forms normally found in nucleotides and nucleosides. We have carried out an accurate crystal structure of cCMP and found that the furanose ring in cCMP has the C(3′) endo C(2′) exo conformation ($^3T_2$), with a pseudo rotation amplitude (P) of 44° and phase angle τ_m of 12°. cCMP is in low anti conformation (X_{CN} = 15.4°) and O(5′) has the fixed g⁻ conformation. The phosphate ring is constrained to the chair conformation, as in other cyclic nucleotides. The two exocyclic P-O bond distances are short (1.489, 1.476Å) and the ring angle at N(3) is large (125.2°) suggesting that the molecule in the solid state is a zwitterion with a plus charge on N(3). The crystals are hydrated and highly unstable. The three water molecules are highly disordered in ten locations. The crystals of cCMP $3H_2O$ are hexagonal, a = 16.294(3), b = c = 11.099(4)Å, space group $P6_1$, final R value is 0.067 for 1620 reflections ≥3σ.

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INTRODUCTION: The pyrimidine nucleotide cyclic 3′,5′ cytidine monophosphate (cCMP) was first identified in mouse L-1210 leukemia cells by Bloch[1]. Later he described its presence in normal and regenerating rat liver and in the urine of patients with acute myelocytic leukemia. The initiation of cell growth by cCMP, under conditions where cAMP, cGMP and cUMP delay the onset of proliferation suggests that cCMP may play a regulatory role in the cell metabolism[2]. It has been reported in the past [3-5] that in 3′,5′-cyclic nucleotides, the phosphate ring fused to the furanose ring restricts the conformation of the furanose ring to the twist form C(3′) endo C(4′) exo ($^3T_4$) in contrast to the twist forms C(3′) endo C(2′) exo ($^3T_2$) and C(2′) endo C(3′) exo ($^2T_3$) found in nucleosides and nucleotides. We have examined the crystal structure of cCMP and find that cCMP has a conformation that is quite different from that observed for the other common 3', 5′-cyclic nucleotides. This paper describes the results of our crystallographic studies on cCMP[6].

EXPERIMENTAL SECTION: Transparent hexagonal plate-like crystals of cCMP were obtained by slow evaporation of a solution of cCMP in aqueous methanol. The unit cell dimensions were obtained by a least-squares refinement of a dozen reflections ($28° \; 2\theta \geq 8°$) on a CAD-4 diffractometer. These values are given in Table 1 along with other pertinent crystallographic data. The crystals were very unstable and had to be sealed in a glass capillary tube. Three-dimensional intensity data were collected on a CAD-4 diffractometer by the $\omega/2\theta$ scan method. A total of 2043 reflections were measured ($2\theta \leq 154°$) using CuKα radiation. The scan widths were calculated using the relation ($A + B \tan \theta$) where A and B had values of 0.70° and 0.15° respectively, aperture widths were determined using the equation ($3.0 + 1.2 \tan \theta$) mm. The maximum time spent on any reflection measurement was 100 seconds and the background count time was half the scan time. A faster scan was used for strong reflections. The intensities were monitored by measuring three reflections after every hour of x-ray exposure and the variation in the intensities of these three was less than 3% during the complete data collection. The orientation matrix was checked every 100 reflections. Out of the 2043 reflections measured, 1620 had their intensities $\geq 3\sigma$ (I). Lorentz and polarization corrections were applied to all reflections. The intensities of three reflections at $\chi \approx 90°$ were measured for all values of ϕ from 0° to 360° and the resultant transmission as a function of

Table 1. Crystallographic Data for cCMP

Stoichiometry	$C_9H_{12}N_3O_7P \cdot 3H_2O$
a	16.294 (3) Å
b	16.294(3)
c	11.099(4)
volume	2552.1 Å^3
space group	$P6_1$
μ, cm^{-1}	22.2
ρ (obsd), g.cm^{-3}	1.42 (by flotation in bromoform / benzene)
ρ (calc.), g.cm^{-3}	1.40
dimensions of crystal, mm	0.11 x 0.09 x 0.04
no. of reflections ($2\theta_{max} = 150°$ for Cukα)	2043 ($1620 \geq 3\sigma$)
$R = \sum \lvert \lvert F_o \rvert - \lvert F_c \rvert \rvert / \sum \lvert F_o \rvert$	0.069
temp = 22 ± 3 °C	
λ (CuKα) = 1.54051Å	

Q was applied to all the reflections. The maximum and minimum transmissions were 0.86 and 0.99 with an average of 0.94.

SOLUTION AND REFINEMENT OF THE STRUCTURE: The structure was solved by a straightforward application of the direct methods [7]. The structure determination package (SDP) of Enraf-Nonius[8] for the PDP 11/34 computer was used for the solution of the structure. At the end of the isotropic refinement, the R factor was 0.159. Further refinement was continued with anisotropic thermal parameters and the R fell to 0.122. A difference Fourier map computed at this stage and the

Table 2. Final Positional Parameters and their Standard Deviations

ATOM	X	Y	Z	B(A2)	Occupancy Factor
P	0.23169(9)	0.4339(1)	0.1390	3.85(3)	
O5′	0.2770(3)	0.5434(3)	0.1060(5)	4.6(1)	
O2P	0.2342(3)	0.4265(3)	0.2698(4)	4.9(1)	
O3′	0.1205(3)	0.3879(3)	0.1032(4)	3.92(9)	
O1P	0.2742(3)	0.3892(3)	0.0636(5)	5.0(1)	
C5′	0.2582(5)	0.5758(5)	-0.0073(7)	4.8(2)	
O4′	-0.1130(3)	0.5451(3)	-0.1175(4)	4.1(1)	
O2	-0.1204(3)	0.3419(3)	-0.2857(4)	4.4(1)	
N1	0.0398(3)	0.4317(3)	-0.2698(4)	3.5(1)	
N3	-0.0312(3)	0.3528(4)	-0.4478(5)	3.8(1)	
N4	0.0544(4)	0.3663(4)	-0.6194(6)	4.8(1)	
C1′	0.0278(4)	0.4622(4)	-0.1489(6)	3.6(1)	
C4′	0.1509(4)	0.5252(4)	-0.0123(5)	3.6(1)	
C3′	0.1086(4)	0.4207(4)	-0.0124(5)	3.3(1)	
C2′	0.0063(4)	0.3881(4)	-0.0506(6)	3.6(1)	
O2′	-0.0448(3)	0.4042(3)	0.0406(5)	4.7(1)	
C2	-0.0437(4)	0.3728(4)	-0.3305(6)	3.6(1)	
C4	0.0524(4)	0.3869(4)	-0.5045(6)	4.1(1)	
C5	0.1352(4)	0.4424(5)	-0.4367(7)	4.4(2)	
C6	0.1255(4)	0.4641(5)	-0.3244(6)	4.1(1)	
OW1 †	0.420(2)	0.780(2)	0.071(3)	6.0(5)*	0.22
OW2 †	0.778(2)	0.937(2)	0.228(3)	8.4(8)*	0.21
OW3 †	0.917(2)	0.129(2)	0.129(3)	14(1)*	0.40

Table 2 Continued

OW4 †	0.775(3)	0.801(3)	0.100(5)	7(1)*	0.33
OW5 †	0.238(3)	0.148(3)	0.579(5)	18(1)*	0.37
OW6 †	0.088(8)	0.878(8)	0.20(1)	8(3)*	0.36
OW7 †	0.968(3)	0.138(3)	0.174(5)	20(1)*	0.31
OW8 †	0.855(3)	0.818(3)	0.159(6)	28(2)*	0.40
OW9 †	-0.001(4)	0.850(4)	0.104(7)	44(3)*	0.40
H1N4	0.113(5)	0.414(5)	-0.672(9)	7(2)*	
H2N4	-0.024(6)	0.302(6)	-0.67(1)	7(2)*	
HC3′	0.142(5)	0.395(6)	-0.070(9)	6(2)*	
HN3	-0.070(3)	0.320(3)	-0.477(6)	2(1)*	
HC4′	0.130(4)	0.535(4)	0.037(6)	4(1)*	
HC2′	-0.028(4)	0.311(4)	-0.088(6)	3(1)*	
HC1′	-0.032(5)	0.469(4)	-0.159(7)	5(2)*	
HO2′	-0.081(5)	0.359(5)	0.076(8)	7(2)*	
HC6	0.187(5)	0.508(5)	-0.261(7)	5(2)*	
HC5	0.199(5)	0.469(5)	-0.466(9)	6(2)*	
H1C5′	0.284(6)	0.489(6)	-0.081(8)	8(2)*	
H2C5′	0.231(6)	0.590(6)	-0.013(9)	10(3)*	

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† Disordered water molecules. Starred atoms were refined isotropically. Isotropic equivalent thermal parameter defined as: (4/3) [a^2 B(1,1) +b^2 B(2,2) + c^2 B(3,3) + ab (cosγ) B(1,2) + ac (cosβ)B (1,3) + bc (cos α) B(2,3).

positions of most of the hydrogen atoms were obtained (except those of water molecules). Refinement was continued using isotropic thermal parameters for the hydrogen atoms and anisotropic thermal parameters for the non-hydrogen atoms. The R value fell to 0.092. A difference Fourier map computed at this stage gave the positions of the water oxygens. All the water molecules were disordered. Their occupancies were determined using their relative peak heights from the difference Fourier map and the occupancies were refined. A total of three water molecules were present for each cCMP molecule. The three water molecules were highly disordered into nine locations. The final R value was 0.069 for the 1620 observed reflections with $I \geq 3\sigma$. The quantity minimized was $\Sigma\, \omega(|Fo| - (1/k)|Fc|)^2$ where the weight $\omega = 4|Fo|^2/\sigma(|Fo|^2)^2$ and $\sigma^2(|Fo|)^2 = [\sigma^2(I) + p^2I^2]/LP$, where p is an "ignorance factor" to reduce the weight of intense reflections (p = 0.05). The symbol $\sigma(I)$ is the standard deviation of the intensity I based on counting statistics and k is the scale factor. The atomic scattering factors and anomalous dispersion factors were taken from International Tables [9]. For the hydrogen atoms, the scattering factors given by Stewart, Davidson and Simpson[10] were used.

RESULTS AND DISCUSSION: The final positional and isotropic thermal parameters for all the atoms are given in Table 2. Fig. 1 gives the conformation of cCMP as observed in the crystal as well as the atom numbering scheme followed in the paper. The bond lengths and angles for non-hydrogen atoms are given in Table 3. Bond lengths involving the hydrogen atoms are in the usual range for an x-ray determination.

CYTOSINE BASE: The molecule exists as a zwitterion; N(3) of the cytosine ring is protonated. The average estimated standard deviations in a C-C, C-O, C-N bond length are 0.005Å and the average deviation in a bond angle is 0.4°. Because of protonation, the angle at N(3) is increased to 125.6°, similar to the values found in other protonated bases[11]. The bond distances and angles in the cytosine base are similar to the values found in other cytidine and cytosine structures[12]. In contrast to 3′, 5′-UMP[13] but similar to 2′-acteyluridine 3′, 5′-cyclophosphate benzyl triester[14], the external angle C(1′)-N(1)-C(6) is larger than C(1′) -N(1)-C(2) by 8°. The angle C(5)-C(4)-N(4) is 3° larger than N(3)-C(4)-N(4).

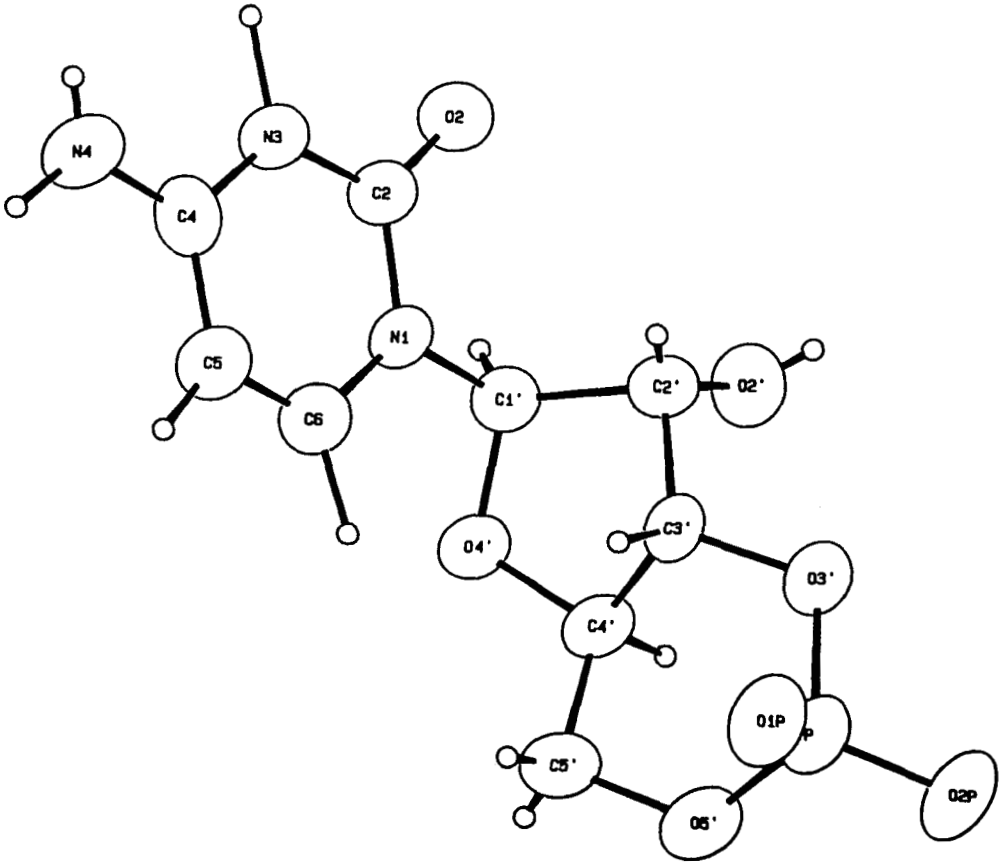

Fig.1. An ORTEP diagram of Cytidine cyclic (3',5') monophosphate showing the conformational details and the atomic numbering scheme.

RIBOSE RING: The bond distances C(1′)-O(4′) and C(4′)-O(4′) are 1.413(5) and 1.431 (5)Å respectively. All the other 3′,5′ cyclic nucleotides whose structures have been determined cUMP[13-14], cAMP[15-16] and cGMP[17] do not show an anomeric effect as these bond distances are equal in these structures. The C(1′)-O(4′)-C(4′) bond angle in cCMP is 108.3°, nearly a tetrahedral value, whereas in the other cyclic analogs, this angle is 3 to 5° less than the expected tetrahedral value. The ribose has the C(3′) *endo*C(2′) *exo* conformation ($^2T_3$). This differs from the $^3T_4 \rightleftharpoons {}_4T^3$ conformation that has been suggested to be the characteristic conformation for 3′,5′ cyclic nucleotides and has been found in cUMP[13-14], cAMP[15-16] and cGMP[17]. A few years later, in

Table 3. Bond distances (in Å) and bond angles (°) in cCMP

Bond	Distance	Bond Angle	(°)
N(1) - C(2)	1.386(5)Å	N(1) - C(2) - N(3)	114.4(4)°
C(2) - N(2)	1.382(5)	N(1) - C(2) - O(2)	123.1(4)
C(2) - O(2)	1.197(5)	N(3) - C(2) - O(2)	122.5(4)
N(3) - C(4)	1.343(6)	C(2) - N(3) - C(4)	125.6(4)
C(4) - N(4)	1.324(6)	N(3) - C(4) - C(5)	117.6(4)
C(4) - C(5)	1.408(6)	N(3) - C(4) - N(4)	119.7(4)
C(5) - C(6)	1.326(6)	C(5) - C(4) - N(4)	122.7(4)
C(6) - N(1)	1.363(5)	C(4) - C(5) - C(6)	118.1(4)
N(1) - C(1′)	1.477(5)	C(5) - C(6) - N(1)	123.4(4)
C(1′) - C(2′)	1.532(6)	C(6) - N(1) - C(2)	120.8(3)
C(1′) - C(3′)	1.534(5)	C(6) - N(1) - C(1′)	123.8(3)
C(3′) - C(4′)	1.485(6)	C(2) - N(1) - C(1′)	115.3(3)
C(4′) - O(1′)	1.431(5)	N(1) - C(1′) - C(2′)	113.4(3)
O(1′) - C(1′)	1.413(5)	C(1′) - C(2′) - C(3′)	98.2(3)
C(2′) - O(2′)	1.417(5)	C(1′) - C(2′) - O(2′)	106.6(3)
C(3′) - O(3′)	1.440(4)	C(3′) - C(2′) - O(2′)	112.0(3)
O(3′) - P	1.626(3)	C(2′) - C(3′) - C(4′)	101.4(3)
P - O1P	1.488(3)	C(2′) - C(3′) - O(3′)	116.3(3)
P - O2P	1.463(3)	C(4′) - C(3′) - O(3′)	110.8(3)
P - O(5′)	1.593(4)	C(3′) - C(4′) - O(4′)	104.7(3)
O(5′) - C(5′)	1.455(6)	C(4′) - O(4′) - C(1′)	108.3(3)
C(5′) - C(4′)	1.516(7)	O(4′) - C(1′) - N(1)	108.3(3)

Table 3 Continued

C(3′) - O(3′) - P	117.2(1)
O(3′) - P - O1P	108.9(2)
O(3′) - P - O2P	105.9(2)
O1P - P - O2P	118.6(2)
O(3′) - P - O(5′)	104.3(2)
O(P1) - P - O(5′)	110.6(2)
O(P2) - P - O(5′)	107.6(2)
O(3′) - P - O(5′)	104.3(2)
P - O(5') - C(5′)	122.7(3)
O(5′) - C(5′) - C(4′)	103.3(4)
C(5′) - C(4′) - C(3′)	111.8(4)
C(5′) - C(4′) - O(4′)	114.8(4)

the crystal structure of cyclic Adenosine 3'-5'-monophosphate sodium salt carried out in our laboratory by Kartha and his co-workers [18], a similar conformation is found for one of the molecules of $_c$AMP . The torsion angles C(4′)-O(4′)-C(1′)-C(2′) and O(4′)-C(1′)-C(2′)-C(3′) for 3′,5′-cyclic nucleotides and their analogs range from -15 to -27° and from, 10 to 14° respectively; the corresponding values in cCMP are 5.2 and -29.4° respectively (Table 5). The other three torsion angles in the ribose rings C(1')- C(2')-C(3')- C(4'), C(2')-C(3')-C(4')-O(4') and C(3')-C(4')-O(4')-C(1') are 41.6, -41.1 and -22.7 ° respectively. The pseudo rotation parameters P and τ_m are 44 and 12° respectively. Thus the geometry and the pucker of the ribose ring in cCMP are quite different from those of other 3′,5′-cyclic nucleotides, but are similar to that of most nucleosides and nucleotides..

It has been reported in the literature[19-22] that the transfusion of trimethylene and tetrahydrofuran rings causes geometric strain and this strain gives rise to the greater enthalpy of

hydrolysis for cyclic nucleotides as compared to that of "strain-free" diesters such as diethyl phosphate and trimethylene phosphate. The enthalpy of hydrolysis of cyclic 3'-5'- nucleotides are about -14Kcal/mol greater than those for 2',3'-nucleotides, whose values are approximately -8Kcal/mol. Semi-empirical quantum mechanical calculations[23] show that for the 2′,3′-cyclic nucleotides, a significant part of the heat of hydrolysis is associated with the relief of torsional and bond angle strain, which arises in 2′,3′-cyclic nucleotides because of the eclipsing of both ester bonds. However, these calculations do not identify the source of the strain energy present in 3′,5′-cyclic nucleotides, where the enthalpy of hydrolysis amounting to -14 kcal/mol is considerably different than the corresponding value of -8 kcal/mol for 2′,3′-cyclic nucleotides. This difference may be attributable to the fact that the bond shortening (anomeric) effect is not found (Table 4) in 3' ,5'-cyclic nucleotides but is observed in 2' ,3'-cyclic nucleotides[23-25] and in "strain free" nucleosides and nucleotides. Since the configuration at C(1′) for 3′,5′-cyclic nucleotides is the same as for other nucleosides and nucleotides, one would expect a bond-shortening effect to be present in 3′,5′-cyclic nucleotides as well. We suggest that the lack of this effect in 3′,5′-cyclic nucleotides is due to geometric strain in the furanose moiety as a result of 3′,5′-cyclization. This strain translates into a greater enthalphy of hydrolysis for 3′,5′-cyclic nucleotides than 2′,3′-cyclic nucleotides. The reason for the absence of the geometric strain in cCMP is not apparent, but measurement of the enthalpy of hydrolysis of this nucleotide will shed further light on this problem.

PHOSPHATE GROUP: The phosphate group is uncharged and the two bond distances P-O(P1) and P-O(P2) are 1.488(3) and 1.463(3)Å similar to the values found in other uncharged phosphate groups. The bond angles C(5′)-O(5′)-P and O(5′)-P-O(P1) of 122.7 and 110.6 ° are similar to the values found in cGMP and are about 3 ° greater than the values found in cUMP.

Table 4 gives the bond lengths and angles in the dioxophosphorinane ring in cCMP and compares them with the values found in other 3′,5′-cyclic nucleotides. The angle O(5′)-P-O(3′) is about 2 ° larger than the value found in cAMP but 2 ° smaller than the value found in AccUMP. The torsion angles (Table 5) show that this ring had a distorted chair conformation, as has been found

Table 4. Bond distances and angles in the phosphate six membered ring in 3′5′ cyclic nucleotides.

BOND	8,2′amino[16] $_c$AMP	AccUMP[14]	cAMP[15]	cCMP*	cUMP[13] I	cUMP[13] II	cGMP[17]	cAMP[4] Methyl Phos
P - O(3′)	1.614(2)	1.579(4)	1.57(1)	1.626(3)	1.609(8)	1.619(8)	1.610(3)	1.623(3)
O(3′) - C(3′)	1.435(3)	1.437(4)	1.42(2)	1.440(4)	1.443(8)	1.421(4)	1.429(4)	1.430(4)
C(3′) - C(4′)	1.507(4)	1.518(4)	1.51(2)	1.485(6)	1.509(8)	1.502(8)	1.501(5)	1.509(6)
C(4′) - C(5′)	1.516(5)	1.505(4)	1.48(3)	1.516(7)	1.489(8)	1.500(8)	1.495(5)	1.515(6)
C(5′) - O(5′)	1.461(4)	1.455(4)	1.45(2)	1.455(6)	1.464(8)	1.468(8)	1.462(4)	1.535(7)†
O(5′) - P	1.610(2)	1.568(4)	1.53(1)	1.593(4)	1.611(8)	1.611(8)	1.592(3)	1.808(5)‡
BOND ANGLE								
P-O(3′)-C(3′)	112.5(2)	111.0(2)	113(1)	117.2(1)	112.7(7)	111.3(7)	113.6(2)	114.7(2)
O(3′)-C(3′)-C(4′)	110.6(2)	110.4(2)	112(2)	110.8(3)	111.0(7)	112.1(7)	111.0(2)	110.9(3)
C(3′)-C(4′)-C(5′)	110.7(2)	111.1(2)	111(2)	111.8(4)	111.8(7)	111.1(7)	110.2(3)	111.9(3)
C(4′)-C(5′)-O(5′)	104.1(3)	105.1(2)	106(2)	103.3(4)	104.0(7)	105.4(7)	105.4(2)	107.0(3)**
C(5′)-O(5′)-P	117.8(2)	120.0(2)	122(1)	122.7(3)	118.3(7)	119.2(7)	121.9(2)	113..0(2)***
O(5′)-P-O(3′)	103.3(1)	106.3(2)	108.8(7)	104.3(2)	102.7(7)	103.3(7)	103.9(1)	102.5(2)

*This work †C(5′)-C(6′) bond ‡P-C(6′) bond **C(4′)-C(5′)-C(6′) ***C(5′)-C(6′)-P

The other angles show little variations from the values formed in other cyclic nucleotides.

in other cyclic nucleotides. As usual in cyclic nucleotides, the greatest puckering occurs about the C(4′)-C(3′) bond.

HYDROGEN BONDING AND PACKING: Table 6 gives the data for hydrogen bond distances and angles. Fig. 2 shows the molecules arranged in a helical manner, connected by N-H...O hydrogen bonds. Fig. 3 gives a stereoscopic view of the packing of the molecules in the hexagonal unit cell.

Table 5. Conformational angles in the phosphate six membered ring in 3′5′ cyclicnucleotides.

ANGLE	cAMP		8,2′ amino cAMP	AccUMP	cAMP	cCMP	cUMP		cGMP
	Mol. I	Mol. II					Mol. I	Mol. II	
C(3′)-C(4′)-C(5′)-O(5′)	-61.1	68.3	-62.9	-58.8	-59.3	-61.1	-61.4	-59.5	-64.2
C(4′)-C(5′)-O(5′)-P	57.2	56.6	60.5	54.1	50.9	53.7	60.5	56.5	55.0
C(5′)-O(5′)-P-O(3′)	-49.0	-49.9	-53.6	-49.3	-40.3	-46.4	-56.7	-50.5	-47.5
O(5′)-P-O(3′)-C(3′)	46.0	48.9	50.1	49.1	39.6	45.1	57.4	49.3	50.5
P-O(3′)-C(3′)-C(4′)	-58.1	-61.9	-60.1	-61.1	-54.1	-59.5	-66.7	-61.2	-63.6
O(3′)-C(3′)-C(4′)-C(5′)	66.6	68.3	66.8	67.4	65.9	70.2	69.1	67.1	69.1

Table 6. Hydrogen bond distances (in Å) and angles (°)

DONOR	HYDROGEN	ACCEPTOR	DISTANCES IN Å			ANGLE (°)	POSITION OF
D	H	A	D-H	H...A	D...A	D-H..A	ACCEPTOR ATOM
N4	H1N(4)	O2P	1.06	1.99	2.860	131.9	(x, y, 1 + z)
N(4)	H2N(4)	O1P	1.33	1.69	2.917	150.2	(y, x, 5/6 + z)
N(3)	HN(3)	O2P	0.68	2.02	2.683	167.9	(y, x, 5/6 + z)
O(2′)	HO(2′)	O1P	0.78	2.09	2.793	153.7	(y, x, z - 1/6)
C(6)	HC(6)	OW1	1.12	2.45	3.125	162.5	(1-y, x-y+1, 1/5+z)
C(5)	HC(5)	O(2)	0.92	2.39	3.208	152.8	(x-y , x , 1/6 + z)

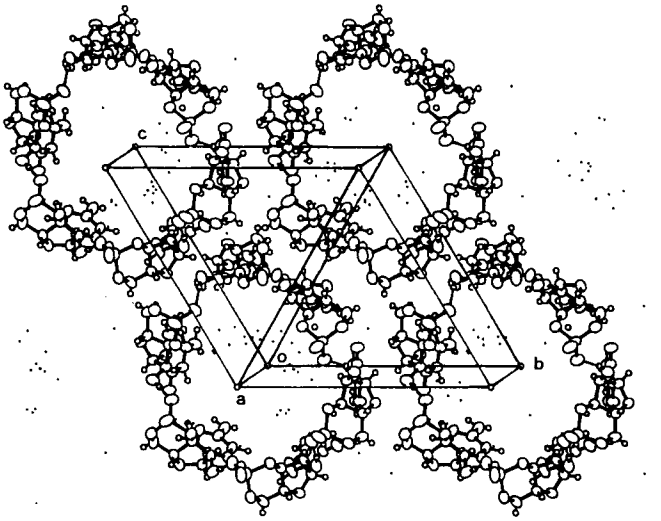

Fig. 2. An ORTEP diagram showing the helical arrangement of the molecules in the crystal lattice. The dots represent the various disordered water molecules, which form a channel running through the helices and stabilizing them by forming hydrogen bonds.

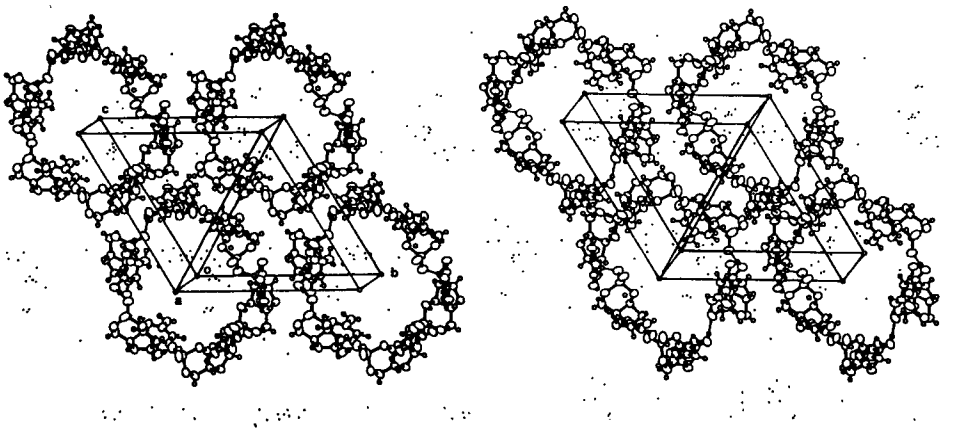

Fig. 3. A stereoscopic view of the packing of the molecules in the unit cell projected down the a-axis. The molecules related by the 6_1 screw form an helical arrangement stabilized by a N-H...O hydrogen bonds (shown by solid lines). The disordered water molecules are represented by dots and they form a channel running through the helices and stabilizing them by forming hydrogen bonds.

Molecules related by 6_1 screw axis form a helical arrangement stabilized by N-H...O hydrogen bond. The three water molecules are disordered into twelve different sites and these form a channel running through the helical arrangement of cCMP molecules.

CONCLUSION: Our analysis of the crystal structure of cytidine 3',5' cyclic phosphate shows that this compound has a non-characteristic ribose pucker, $^3T_2$, unlike the preferred $^3T_4 \leftrightarrows {}^4T_3$ conformation found in other cyclic nucleotides. This conformation is characterized by the presence of the anomeric effect in the furanose ring, a feature seen in cyclic 2',3' nucleotides but not in other 3',5' nucleotides. Our results suggest that the higher enthalpy of these cyclic nucleotides may be associated with the relief of the ring strain in the furanose, and not just the six- membered ring. The characteristic conformation of these cyclic nucleotides does not seem to depend on pH, since cGMP and its Na salt have the same basic conformation. This non-characteristic conformation observed for cCMP seems to be unique. It is tempting to hypothesize that the non- characteristic conformation and the other associated features in the furanose ring of cCMP might endow it with markedly different biological properties from those of cAMP, cGMP and cTMP. Further research is needed to assess this hypothesis.

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