Structural Chemistry of Cyclic Nucleotides. II. Crystal and Molecular Structure of Sodium β-Cytidine 2′,3′-Cyclic Phosphate[1]

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Abstract: Pyrimidine 2′,3′-cyclic phosphates are substrates for the enzyme ribonuclease, and are formed in the enzymatic hydrolysis of ribonucleic acid. The hydrated sodium salt of β-cytidine 2′,3′-cyclic phosphate forms monoclinic crystals of space group $P2_1$ with $a = 6.737$, $b = 11.007$, and $c = 19.54$ Å, $\beta = 95.0°$, and two nucleotides in the asymmetric unit. X-Ray diffraction data to $2\theta = 125°$ were collected with a diffractometer using Cu Kα radiation. The structure was solved by Patterson and Fourier synthesis methods in conjunction with the tangent formula, and was refined using block-diagonal least-squares methods. The final R value was 4.7% for the 1964 reflections used in the refinement and 5.2% for all 2442 data. Both of the cytosine bases are in the syn conformation about the β-glycosidic bond, and seem to be locked in this unusual conformation. The bicyclic ribose–phosphodiester ring system holds the sugar with the ribose ring carbons nearly in one plane and the oxygens puckered. The H atoms on C(2′) and C(3′) are thus eclipsed, with dihedral angles of 1 and 4°. The conformations about the exocyclic CH_2OH bond are gauche-gauche and gauche-trans for the two anions. The nucleotides pack into base-stacked antiparallel-stranded ribbons, held together with intra- and inter-ribbon hydrogen bonds and coordination and hydrogen bonding through the hydrophilic sodium ion–water molecule region.

Cyclic 2′,3′-phosphate esters of cytidine and uridine are intermediates in the ribonuclease-catalyzed hydrolysis of ribonucleic acid, and are themselves substrates for ribonuclease. The molecular structures of these esters are thus relevant to the proposal and evaluations of precise mechanisms for ribonuclease catalysis. Saenger and Eckstein[2] have reported the structure of cyclic uridine 2′,3′-*O*,*O*-phosphorothioate, but this is the first detailed study of a cytidine cyclic ester. As was noted in the preliminary report on this work[3] the structure found for the cytidine 2′,3′-cyclic phosphate, prepared using dicyclohexylcarbodiimide,[4] is unusual and quite different from that of the cyclic uridine phosphorothioate prepared by a different means.[5] The cytosine bases are in the syn conformation about the glycosidic bond and they seem to be locked in this conformation. The bicyclic ribose–phosphodiester ring system imparts unusual rigidity to the ribose, with O(1′) the only atom which easily puckers. One of the two nucleotides in the crystal has O(1′) 0.48 Å out of the sugar plane, and the other ribose is virtually planar. These conformations are very unusual and leave H(2′) and H(3′) eclipsed. Saenger and Eckstein[2] observed a similar rigidity in the ribose ring of the uridine compound, with O(1′) 0.29 Å out of the sugar ring. The rates of hydrolysis of uridine and cytidine 2′,3′-cyclic phosphates differ, the cytidine ester being hydrolyzed to cytidine 3′-phosphate at twice the rate of the uridine ester in the presence of ribonuclease.[6] The rate difference must be explained in terms of structure and the chemical nature of the bases in the context of the mechanism of ribonuclease catalysis; this study is a contribution toward that end.

Experimental Section

Crystals of the sodium salt of cytidine 2′,3′-cyclic phosphate (Schwarz BioResearch, Lot No. 6802) were prepared by diffusing ethanol into an aqueous solution of the salt over a period of several weeks. The crystal data are given in Table I. A crystal with approximate dimensions of 0.2 × 0.1 × 0.15 mm was used to collect the intensity data to $2\theta = 125°$ using a G.E. XRD-5 diffractometer and nickel-filtered copper X-rays. The method of data collection has been described previously.[7] The error in conversion of the stationary crystal–stationary counter data (for which $2\theta < 90°$) to integrated intensities using the method of Alexander and Smith[8] was less than 5%. Data for which $90° \leq 2\theta \leq 125°$ were scanned over 3° at a rate of 2°/min, with 10- or 20-sec backgrounds collected at each end of the scan. Variation in the intensities of the six reference peaks monitored throughout the data collection was less than 5% provided the indoor relative humidity was 10% or greater. At lower humidities reversible shifts in intensities were noted. Insufficient data were collected at low humidites to explain the variations structurally. The final observational data consisted of 1964 nonzero intensities and 478 data which were too weak to measure (less than twice background).

Table I. Crystal Data

Name, hydrated sodium salt of β-cytidine 2′,3′-cyclic phosphate
Crystal system, monoclinic
a, 6.737 ± 0.006 Å
b, 11.007 ± 0.010 Å
c, 19.54 ± 0.017 Å
β, 95.0 ± 0.1°
λ, 1.5418 Å (Cu Kα)
Systematic absences, $0k0$ for $k = 2n + 1$
Space group, $P2_1$
D_{obsd}, 1.66 g cm^{-3}
D_{calcd}, 1.67 g cm^{-3}
Z, four, with eight water molecules per cell

All calculations were done on the IBM 7094/7040 system at the University of Chicago using programs cited in earlier publica-

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[1] Previous paper in this series: C. L. Coulter, *Acta Crystallogr., Sect. B*, **25**, 2055 (1969).

[2] W. Saenger and F. Eckstein, *J. Amer. Chem. Soc.*, **92**, 4712 (1970).

[3] C. L. Coulter and M. L. Greaves, *Science*, **169**, 1097 (1970).

[4] W. Szer and D. Shugar, *Biochem. Prep.*, **10**, 139 (1963).

[5] F. Eckstein and H. Gindl, *Chem. Ber.*, **101**, 1670 (1968).

[6] H.-J. Wieker and H. Witzel, *Eur. J. Biochem.*, **1**, 251 (1967).

[7] S. W. Hawkinson and C. L. Coulter, *Acta Crystallogr., Sect. B*, **27**, 34 (1971).

[8] L. E. Alexander and G. S. Smith, *Acta Crystallogr.*, **15**, 983 (1962).

Table II. Positional Parameters ($\times 10^4$) and Their Estimated Standard Deviations ($\times 10^4$)

Atom	Anion A			Anion B		
	x/a	*y/b*	*z/c*	*x/a*	*y/b*	*z/c*
P	−2329 (3)	3834 (2)	5428 (1)	5113 (3)	3.6 (2)	775 (1)
O(6)	−4149 (7)	3499 (5)	5755 (3)	3703 (7)	−154 (5)	1317 (2)
O(7)	−865 (7)	4626 (5)	5811 (3)	6846 (7)	794 (5)	938 (3)
O(2′)	−2931 (7)	4379 (5)	4670 (3)	3960 (7)	361 (5)	47.8 (2)
O(3′)	−1261 (7)	2628 (5)	5172 (2)	5829 (8)	−1320 (5)	538 (2)
C(1′)	−89.4 (10)	4771 (7)	3993 (3)	6272 (9)	546 (5)	−855 (3)
C(2′)	−1704 (10)	3876 (8)	4156 (3)	4755 (10)	−278 (8)	−524 (3)
C(3′)	−513 (11)	2806 (7)	4497 (3)	6054 (11)	−1343 (7)	−196 (3)
C(4′)	1646 (11)	3222 (7)	4566 (3)	8175 (10)	−1108 (8)	−353 (3)
O(1′)	1724 (7)	4133 (5)	4036 (2)	8184 (7)	40.8 (5)	−730 (2)
C(5′)	3146 (11)	2252 (7)	4459 (4)	9016 (12)	−2123 (8)	−787 (4)
O(5′)	2789 (9)	1672 (6)	3820 (3)	718 (8)	−1715 (5)	−1104 (3)
N(1)	−393 (8)	5351 (5)	3309 (3)	5797 (8)	723 (5)	−1596 (3)
C(2)	−534 (9)	4615 (7)	2713 (3)	5583 (9)	−294 (6)	−2033 (3)
N(3)	−691 (8)	5153 (6)	2099 (3)	5460 (8)	−112 (5)	−2718 (3)
C(4)	−732 (10)	6359 (7)	2048 (4)	5512 (10)	1008 (7)	−2970 (3)
C(5)	−673 (11)	7136 (7)	2647 (4)	5630 (10)	2060 (6)	−2534 (4)
C(6)	−481 (10)	6579 (7)	3254 (3)	5835 (10)	1867 (7)	−1857 (3)
O(2)	−542 (7)	3495 (4)	2771 (2)	5509 (7)	−1315 (4)	−1785 (2)
N(4)	−841 (10)	6863 (6)	1417 (3)	5405 (9)	1143 (6)	−3663 (2)
Na(1)	7436 (4)	2254 (3)	1888 (1)			
Na(2)	2741 (5)	2571 (3)	2760 (2)			
$H_2O(1)$	5165 (8)	1114 (5)	2501 (2)			
$H_2O(2)$	108 (10)	924 (7)	2527 (4)			
$H_2O(3)$	55.5 (8)	4368 (6)	8548 (3)			
$H_2O(4)$	2977 (10)	4682 (8)	6437 (3)			

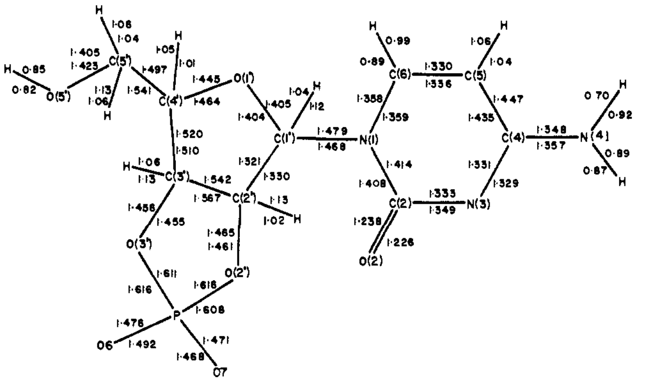

Figure 1. Bond distances (in ångströms) for the cytidine 2′,3′-cyclic phosphate anions; upper figures are for anion A and lower figures for anion B (Table II).

tions.[1,7,9] Scattering factors for phosphorus, nitrogen, and valence carbon were taken from the International Tables[10] and the values of Stewart, *et al.*,[11] were used for hydrogen.

Solution and Refinement. The phosphate groups, located using a sharpened Patterson synthesis, were used to get initial phase angles.[3] These phases were then refined using the tangent formula[9,12] and a Fourier map based upon refined phases and normalized amplitudes revealed the main features of the structure. As noted earlier,[3] isotropic least-squares refinement reduced R to 8.5%, where $R = \Sigma||F_o| - |F_c||/\Sigma|F_o|$ with $|F_o|$ the observed and $|F_c|$ the calculated structure factor. Peaks appeared in a difference Fourier map at heights of abous 0.5 e Å^{-3} in appropriate positions for the ribose and base hydrogen atoms. The hydrogens on water molecules were not found; the density near these oxygens was generally low and diffuse. Several cycles of block-diagonal least-squares refinement using anisotropic thermal parameters were required before the weighted sum of the squares of the deviations, $\Sigma w \cdot (|F_o| - |F_c|)^2$, leveled off. Half-shifts were used in this refinement, and the hydrogen atom positions were also refined. An isotropic B of 6 Å^2 was assigned to the hydrogens. The weight, w, was of the form $\sigma_h = a + b|F_o|_h$ where σ_h is $[1/(w_h)^{1/2}]$ and h (= h,k,l) refers to an individual observed reflection. The final values of a and b, derived from graphs of $\langle\Sigma(|F_o| - |F_c|)^2\rangle^{1/2}$ *vs.* $\langle|F_o|\rangle$, were 0.66 and 0.002 which correspond closely to unit weights.

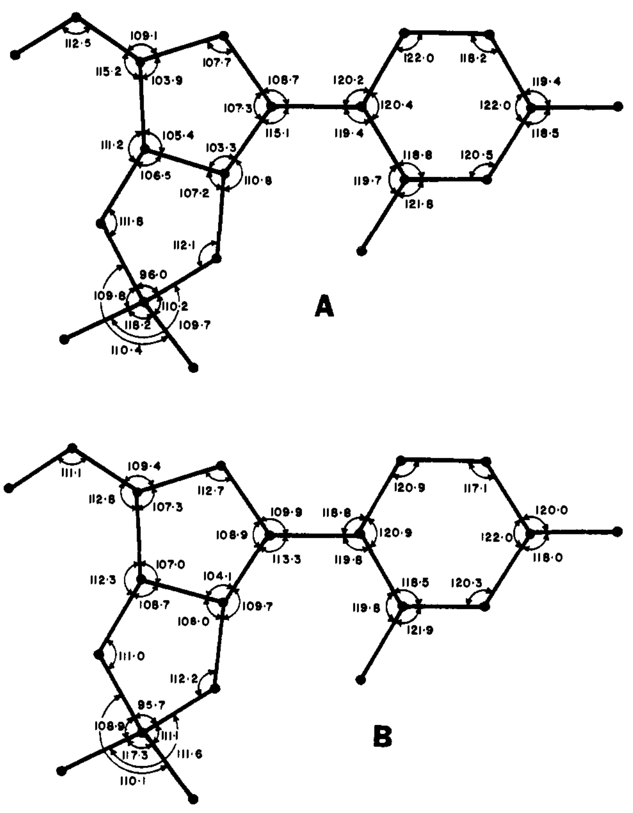

Figure 2. Bond angles (degrees). The bond angles involving H are not shown, but average 122° for those expected to be 120 and 109° for those expected to be tetrahedral, with an estimated standard deviation of 5°.

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(9) C. L. Coulter, *ibid.*, 27, 1730 (1971).

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(11) R. F. Stewart, E. R. Davidson, and W. T. Simpson, *J. Chem. Phys.*, 42, 3175 (1965).

(12) J. Karle and H. Hauptman, *Acta Crystallogr.*, 9, 635 (1956).

Table III. Thermal Parameters (×10⁴) and Their Estimated Standard Deviations (×10⁴)[a]

Atom[b]	B_{11}	B_{22}	B_{33}	B_{12}	B_{13}	B_{23}
P, A	163.7 (4)	53.0 (1.6)	14.8 (0.5)	9.6 (2.4)	10.6 (1.1)	5.6 (0.8)
P, B	159.8 (4)	53.5 (1.6)	13.3 (0.4)	4.7 (2.3)	16.1 (1.1)	0.0 (0.8)
O(6), A	196 (13)	97 (6)	22 (2)	28 (8)	23 (4)	11 (3)
O(6), B	221 (13)	84 (6)	18 (1)	4 (7)	30 (3)	1 (2)
O(7), A	229 (14)	88 (6)	28 (2)	10 (8)	2 (4)	−6 (3)
O(7), B	203 (13)	84 (6)	24 (2)	−10 (8)	20 (4)	−11 (3)
O(2′), A	173 (12)	83 (5)	19 (1)	26 (7)	14 (3)	20 (2)
O(2′), B	163 (12)	90 (6)	18 (1)	50 (7)	20 (3)	1 (2)
O(3′), A	223 (13)	64 (5)	19 (1)	23 (7)	32 (4)	7 (2)
O(3′), B	298 (15)	56 (5)	17 (1)	13 (7)	24 (4)	7 (2)
C(1′), A	163 (17)	73 (8)	15 (2)	1 (10)	4 (4)	2 (3)
C(1′), B	128 (15)	63 (7)	14 (2)	−5 (8)	13 (4)	−1 (3)
C(2′), A	168 (16)	72 (7)	17 (2)	−16 (10)	−12 (4)	4 (3)
C(2′), B	177 (17)	79 (8)	14 (2)	−4 (10)	18 (4)	−5 (3)
C(3′), A	189 (18)	61 (7)	16 (2)	−4 (9)	18 (5)	3 (3)
C(3′), B	244 (20)	62 (7)	15 (2)	−17 (10)	36 (5)	1 (3)
C(4′), A	226 (20)	73 (8)	12 (2)	−8 (10)	2 (5)	7 (3)
C(4′), B	185 (17)	75 (7)	17 (2)	11 (10)	13 (5)	2 (3)
O(1′), A	166 (12)	84 (6)	20 (1)	−18 (7)	0 (3)	18 (2)
O(1′), B	152 (11)	70 (5)	20 (1)	−1 (7)	12 (3)	4 (2)
C(5′), A	187 (19)	48 (7)	25 (2)	−12 (9)	9 (5)	10 (3)
C(5′), B	208 (19)	85 (8)	22 (2)	32 (11)	18 (5)	2 (4)
O(5′), A	302 (16)	88 (6)	20 (2)	−4 (8)	26 (4)	6 (2)
O(5′), B	222 (14)	81 (6)	29 (2)	50 (8)	37 (4)	3 (3)
N(1), A	152 (13)	58 (6)	11 (1)	−9 (7)	6 (3)	4 (2)
N(1), B	184 (14)	48 (5)	13 (1)	0 (7)	16 (4)	2 (2)
C(2), A	118 (15)	59 (7)	16 (2)	−13 (8)	1 (4)	5 (2)
C(2), B	99 (14)	57 (6)	16 (2)	6 (8)	10 (4)	1 (3)
N(3), A	163 (13)	56 (5)	11 (1)	−1 (7)	11 (3)	7 (2)
N(3), B	171 (14)	48 (5)	14 (1)	−15 (7)	13 (3)	−1 (2)
C(4), A	135 (16)	69 (7)	19 (2)	1 (9)	12 (5)	10 (3)
C(4), B	119 (15)	70 (7)	17 (2)	6 (9)	7 (4)	9 (3)
C(5), A	187 (19)	48 (7)	25 (2)	−12 (9)	9 (5)	10 (3)
C(5), B	179 (18)	40 (6)	20 (2)	0 (8)	5 (5)	5 (3)
C(6), A	152 (17)	66 (7)	15 (2)	−5 (9)	4 (4)	1 (3)
C(6), B	155 (18)	52 (6)	20 (2)	1 (9)	10 (5)	−3 (3)
O(2), A	224 (13)	47 (4)	18 (1)	−2 (6)	9 (3)	0 (2)
O(2), B	244 (13)	44 (4)	16 (1)	−2 (7)	13 (3)	1 (2)
N(4), A	298 (20)	72 (7)	20 (2)	13 (9)	17 (5)	17 (3)
N(4), B	219 (16)	70 (6)	16 (2)	1 (9)	9 (4)	0 (3)
Na(1)	191.1 (7)	69.1 (3)	22.4 (0.9)	−21.9 (4)	19.7 (2)	−1.7 (1.3)
Na(2)	244.5 (8)	80.8 (3)	24.3 (0.9)	30 (4)	23.1 (2)	10.5 (1.4)
H_2O (1)	246 (14)	84 (5)	19 (1)	30 (8)	23 (3)	8 (2)
H_2O(2)	359 (20)	124 (8)	42 (2)	14 (12)	9 (6)	−9 (4)
H_2O(3)	245 (16)	89 (6)	34 (2)	20 (8)	10 (4)	−6 (3)
H_2O(4)	296 (19)	200 (11)	34 (2)	−2 (12)	10 (5)	−44 (4)

[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. [b] The anion (A or B) in the asymmetric unit is indicated next to the atom symbol.

The final R value was 4.67% for the observed data and 5.2% when the unobserved data were included at the average calculated value.

Results and Discussion[13]

Tables II and III contain the positional and thermal parameters for the 46 nonhydrogen atoms in the asymmetric unit. The estimated standard deviations of the C, N, or O positions are 0.007 Å, well above the maximum final least-squares shift (0.002 Å). The bond lengths should thus be accurate to ±0.01 Å and the bond angles to ±0.4°; these figures are supported by comparison of the independent distances within the two nucleotides. Table IV lists hydrogen atom positional parameters.

Table IV. Positional Parameters (×10³) for Hydrogen Atoms[a]

	Anion A			Anion B		
Atom	x/a	y/b	z/c	x/a	y/b	z/c
H(C-1′)	20	550	432	629	142	−56
H(C-2′)	−264	356	368	351	−49	−84
H(C-3′)	−69	198	422	521	−216	−41
H(C-4′)	209	365	503	918	−89	4.5
H-1(C-5′)	316	161	486	935	−294	−54
H-2(C-5′)	468	267	447	804	−248	−119
H(O-5′)	245	93	386	99	−99	−113
H(C-5)	−71	809	256	544	296	−270
H(C-6)	−54	696	371	603	254	−161
H-1(N-4)	−63	642	118	555	186	−390
H-2(N-4)	−47	764	144	520	54	−395

[a] Estimated standard deviations are 0.07 Å for all entries.

Bond distances and bond angles for the cytidine 2′,3′-cyclic phosphate anions are given in Figures 1 and 2. The bond distances are close to those expected[14,15]

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(13) Tables of the structure factors 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 Street, N.W., Washington, D. C. 20036, by referring to code number JACS-73-570. Remit check or money order for $3.00 for photocopy or $2.00 for microfiche.

(14) V. Sasisekharan, A. Lakshiminarayanan, and G. N. Ramachandran in "Conformation of Biopolymers," Vol. 2, G. N. Ramachandran, Ed., Academic Press, New York, N. Y., 1967, pp 641–654.

(15) J. Donohue, *Arch. Biochem. Biophys.*, **128**, 591 (1968).

except for the rather long C(4′)–C(5′) distance in anion B; the expected value is 1.514 Å.[14] The C(5′)–O(5′) bonds are differently oriented in the two nucleotides, but such conformational features rarely affect bond lengths. Averaged bond distances involving hydrogen atoms are as follows: C(sp^3)–H, 1.09 Å; C(sp^2)–H, 1.00 Å; N–H and O–H, 0.84 Å, all with estimated standard deviations of 0.07 Å.

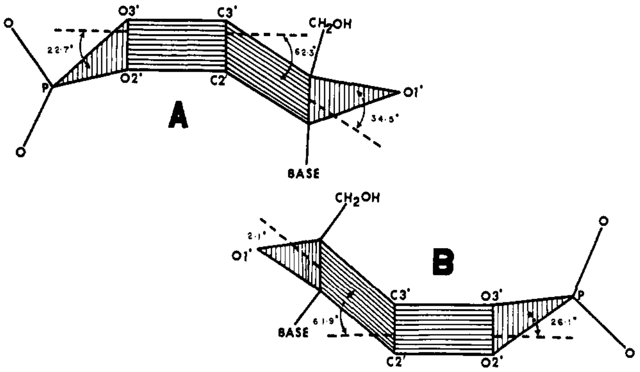

Figure 3. Schematic representation of the bicyclic system.

Table V. Some Dihedral Angles for Cytidine 2′,3′-Cyclic Phosphate[a]

Bond	Anion A, deg	Anion B, deg
C(4′)–O(1′)–C(1′)–C(2′)	−33.2	1.9
O(1′)–C(1′)–C(2′)–C(3′)	16.1	−0.7
C(1′)–C(2′)–C(3′)–C(4′)	5.5	−0.7
C(2′)–C(3′)–C(4′)–O(1′)	−24.4	1.8
C(3′)–C(4′)–O(1′)–C(1′)	36.0	−2.3
O(3′)–P–O(2′)–C(2′)	−21.2	−25.7
P–O(2′)–C(2′)–C(3′)	10.7	15.2
P–O(3′)–C(3′)–C(2′)	−21.6	−21.7
O(2′)–C(2′)–C(3′)–O(3′)	6.6	4.0
C(3′)–O(3′)–P–O(2′)	25.6	28.2
O(1′)–C(1′)–N(1)–C(6)	−117.1	−105.5
O(1′)–C(4′)–C(5′)–O(5′)[b]	60.2	42.4
C(3′)–C(4′)–C(5′)–O(5′)[b]	56.1	161.7
H(2′)–C(2′)–C(3′)–H(3′)[b]	3.6	0.5
H(1′)–C(1′)–C(2′)–H(2′)[b]	139.6	111.3
H(3′)–C(3′)–C(4′)–H(4′)[b]	144.1	133.2
H(4′)–C(4′)–C(5′)–H-1(5′)[b]	61.1	61.0
H(4′)–C(4′)–C(5′)–H-2(5′)[b]	56.8	174.5

[a] Positive angles correspond to clockwise rotation of the far atoms about the central bond. [b] These angles are absolute values.

Table VI. Least-Squares Planes of Ribose, Phosphate, and Cytosine Residues

Atoms in the plane	Displacements, Å	Displacements of other atoms		Coeff[a]
C(1′), A	−0.022	O(1′)	−0.475	$a = -1.230$
C(2′), A	0.032	C(5′)	−0.814	$b = 4.798$
C(3′), A	−0.033	N(1)	−0.901	$c = 17.467$
C(4′), A	0.022	O(2′)	1.323	$d = -9.297$
		O(3′)	1.152	
		P	2.311	
C(1′), B	0.003	O(1′)	0.029	$a = 0.559$
C(2′), B	−0.004	C(5′)	−1.246	$b = 5.674$
C(3′), B	0.004	N(1)	−1.143	$c = 16.459$
C(4′), B	−0.003	O(2′)	1.255	$d = 0.750$
		O(3′)	1.212	
		P	2.311	
C(2′), A	−0.022	P	0.475	$a = 5.399$
C(3′), A	0.023	C(1′)	1.297	$b = 6.451$
O(2′), A	0.015	C(4′)	1.305	$c = 0.943$
O(3′), A	−0.015			$d = -2.361$
C(2′), B	−0.037	P	0.417	$a = 4.769$
C(3′), B	0.037	C(1′)	1.221	$b = 6.572$
O(2′), B	0.024	C(4′)	1.382	$c = 6.127$
O(3′), B	−0.023			$d = -4.318$
N(1), A	0.013	O(2)	−0.062	$a = 6.733$
C(2), A	−0.012	N(4)	0.058	$b = 0.318$
N(3), A	−0.003	C(1′)	0.090	$c = -1.584$
C(4), A	0.016			$d = 0.631$
C(5), A	−0.015			
C(6), A	0.001			
N(1), B	0.007	O(2)	−0.051	$a = 6.728$
C(2), B	−0.015	N(4)	0.049	$b = -0.521$
N(3), B	0.002	C(1′)	0.218	$c = -1.589$
C(4), B	0.018			$d = -4.110$
C(5), B	−0.026			
C(6), B	0.014			

[a] The plane equations are in the form $ax + by + cz + d = 0$; (x,y,z) refer to atomic positions in fractional cell coordinates, as in Table II.

The bond angles within the ribose and phosphate portions of the nucleotides do differ from the expected values[14] reflecting the rather unusual rigidity imposed upon the sugar ring by the ester linkage. Torsion angles about the ribose and phosphate bonds are given in Table V. The five-membered ester rings of the two unique anions have similar conformations, with the phosphorus puckered toward the sugar ring in both cases. This is the opposite of the puckering observed for the uridine thioate ester,[2] but the sulfur in that case was on the sugar side which would make the conformation observed here sterically less favorable. Figure 3 illustrates this and the other geometrical aspects of the bicyclic ring system, and can be directly compared with Saenger and Eckstein's Figure 2.[2] The conformations about the C(4′)–C(5′) bond differ for the two cytidine nucleotides (Table V) with O(5′) gauche to both O(1′) and C(3′) for anion A and in a gauche–trans orientation in anion B. The gauche–gauche conformation is the most common,[16] with O(5′) over the sugar ring. When the pyrimidine base is in the syn conformation, as here, the base carbonyl oxygen, O(2), and O(5′) would be very close in the gauche–gauche conformation; the contact is relieved in this case by puckering O(1′) of the A-ribose endo (toward C(5′)), thus pivoting both the base and the C(4′)–C(5′) bond away (Table VI). The O(5′)–O(7) hydrogen bond between adajcent A nucleotides (Table VII) must account for this mode of adjustment. The least-squares plane data in Table VI show O(1′) 0.475 Å out of the plane (endo) of the other four sugar ring atoms in nucleotide A. The similar uridine nucleotide[2] had O(1′) of the ribose exo by 0.29 Å. The puckering of the furanose oxygen involves nonsymmetric torsional flexing about the C(1′)–C(2′) and C(3′)–C(4′) bonds (Table V and Figure 4); this also affects the orientations of C(2′), C(3′), O(2′), O(3′), H(2′), and H(3′) to a degree. Lapper, *et al.*,[17] suggested such flexing in solution to account for the nonsymmetric P–H and P–^{13}C nmr coupling constant for nucleoside 2′,3′-cyclic phosphates, and predicted a 3′-*endo*-ribose conformation at equilibrium for pyrimidine 2′,3′-cyclic nucleotides. The puckering of O(1′) is much greater than that of

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Table VII. Possible Hydrogen Bonds and Angles

Atom A[a]	Atom B[a]	Symmetry code[b]	Distance, Å	Bond	Angle, deg
O(6), A	N(4), B	II	2.858	C(4)–N(4)–O(6)	119.9
				P–O(6)–N(4)	121.3
O(5′), B	N(3), A	III	2.828	C(5′)–O(5′)–N(3)	124.9
				C(4)–N(3)–O(5′)	132.5
				C(2)–N(3)–O(5′)	106.9
O(5′), A	O(7), A	IV	2.727	P–O(7)–O(5′)	108.1
				C(5′)–O(5′)·O(7)	100.8
$H_2O(1)$	O(6), B	I	2.806	P–O(6)–$H_2O(1)$	109.2
$H_2O(1)$	$H_2O(4)$	V	2.810	O(6)–$H_2O(1)$–$H_2O(4)$	115.6
$H_2O(2)$	O(2), A	I	2.910	C(2)–O(2)–$H_2O(2)$	162.1
$H_2O(2)$	$H_2O(3)$	IV	2.704	$H_2O(3)$–$H_2O(2)$–O(2)	138.3
$H_2O(3)$	O(6), B	IV	2.622	P–O(6)–$H_2O(3)$	140.8
$H_2O(3)$	O(7), B	V	2.731	P–O(7)–$H_2O(3)$	107.9
$H_2O(3)$	N(4), A	IV	2.808	C(4)–N(4)–$H_2O(3)$	112.8
$H_2O(4)$	O(7), A	I	2.765	P–O(7)·$H_2O(4)$	142.9
$H_2O(4)$	O(6), A	VI	2.771	P–O(6)–$H_2O(4)$	137.0
				O(7)–$H_2O(4)$–O(6)	116.3

[a] The anion (A or B) in the asymmetric unit is indicated next to the atom symbol [b] I, x, y, z; II, $1 + x, y, -1 + z$; III, $1 - x, -^1/_2 + y, -z$; IV, $-x, ^1/_2 + y, 1 - z$; V, $1 - x, -^1/_2 + y, 1 - z$; VI, $x, 1 + y, z$, where x, y, and z are from Table II.

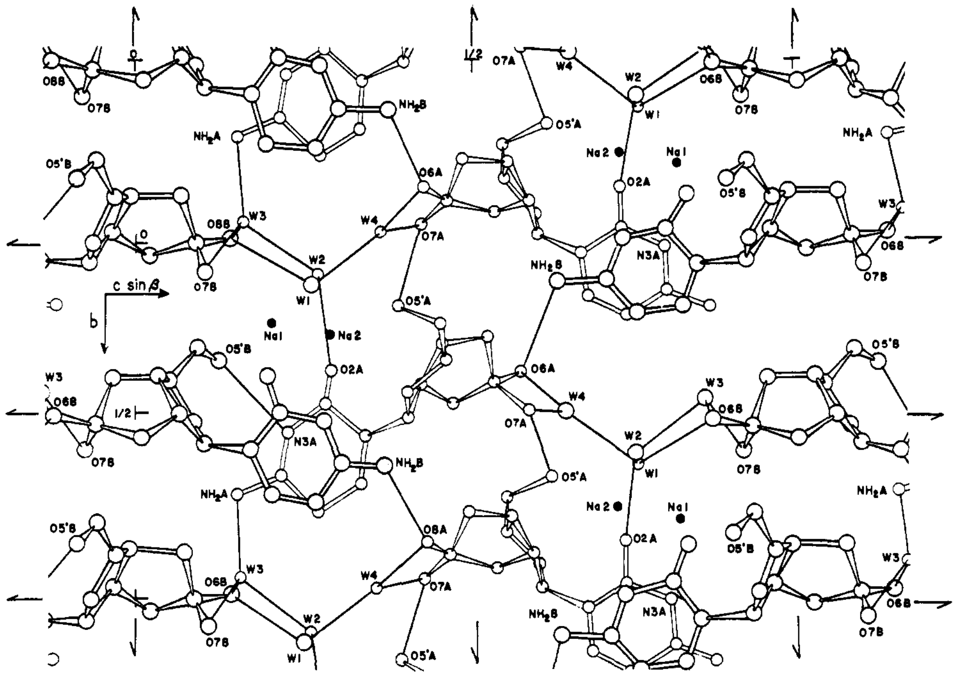

Figure 4. The crystal structure viewed down *a*. Water molecules are labeled W1 to W4 and the amino nitrogens as NH_2A and NH_2B. Hydrogen atoms are not shown, but hydrogen bonds appear as solid lines.

C(2′) or C(3′) for the crystalline nucleotides, and would probably remain so in solution. From nmr data, Smith, *et al.*,[18] predict dihedral angles of about 90° for P–O(3′)–C(3′)–C(4′) and 140° for P–O(2′)–C(2′)–C(1′) in uridine 2′,3′-cyclic phosphate. The values of these angles for anion A of cytidine 2′,3′-cyclic phosphate are 92.7 and 101.5°, corresponding to less distortion of the five-membered ester ring than predicted.[18] These dihedral angles are sensitive to the amount and direction of puckering of the P atom. The gauche–trans conformation observed about the C(4′)–C(5′) bond in anion B of the crystal gives no potentially close contacts, and the conformation is stabilized by a hydrogen bond to an adjacent pyrimidine nitrogen down the *a* axis (Table VII). The ribose in this case is virtually planar (±0.008 Å) as can be seen from the data in Tables V and VI with O(1′) slightly (0.029 Å) puckered exo as it was in the uridine case.[2] The P–O(3′)–C(3′)–C(4′) and P–O(2′)–C(2′)–C(1′) dihedral angles are 95.3 and 96.6°. These unusual sugar conformations are a result of the reduction of the degrees of freedom in the flexing of the five-membered ring because of the phosphodiester linkage. A very interesting feature brought about by this is that hydrogens H(2′) and H(3′) are nearly fully eclipsed in both anions, with H(2′)–C(2′)–C(3′)–H(3′) dihedral angles of 4 and 1°. The clearly limited range of variability of the H(2′)–H(3′) and H(3′)–H(4′) dihedral angles with changes in ribose conformation (Table V) may carry over to solution, and this then would permit correlation of the nmr spin–spin coupling constants

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(18) I. C. P. Smith, H. H. Mantsch, R. D. Lapper, R. Deslauriers, and T. Schleich in "Conformations of Biological Molecules and Polymers," E. Bergman and B. Pullman, Ed., Academic Press, New York, N. Y., in press.

for these protons with dihedral angle. Part III of this series reports such a study.[19]

A striking feature of this crystal structure is that the cytosine bases are both in the syn conformation about the β-glycosidic bond (Table V). Only two other pyrimidines, the β-nucleosides 4-thiouridine[20] and 6-methyluridine[21] have been observed in the solid state with this conformation, although the arrangement is more common with β-purine glycosides.[22] Meadows, *et al.*,[23] have suggested on the basis of an nmr study that cytidine 2′-phosphate is in the syn conformation when bound to ribonuclease. Models suggest that unless H(2′) can be pivoted away from the base—a difficult maneuver with the phosphodiester linkage—cyclic 2′,3′-CMP may be locked in the syn conformation as either O(2) or H(6) of the base would be too close to H(2′) for rotation to take place. Solution studies[19] seem to support this hypothesis, and the conformational feature could be important in considering mechanisms for ribonuclease catalysis. The H(2′)–O(2) distances are 2.39 and 2.60 Å in the nucleotides studied here.

The packing of the crystal structure is shown in Figure 4. The pyrimidine bases are aligned parallel to one another, roughly 3.3 Å apart, forming antiparallel stranded base-stacked ribbons, with hydrophilic channels of sodium ions and water molecules between the hydrophobic ribbons. The packing in 4-thiouridine hydrate[20] was similar; packing forces alone stabilized the syn conformation in that structure, and could be doing so here. Adjacent ribbons are linked with two direct hydrogen bonds, and there is one intramolecular hydrogen bond (O(5′), B, to N(3), A) within each ribbon. These links are reinforced with a number of water- and sodium-mediated linkages (Table VII and Figure 5). The sodium coordination is shown in Figure 5; both carbonyl oxygens of the bases are coordinated to the sodium ions. The coordination numbers for both sodium ions are 6, although one ligand for Na(1) (N(3), A) is rather far away. The other coordination distances are normal, but the angles among these ligands are rather irregular; the arrangement might best be described as a distorted trigonal prism. It is similar to the arrangement observed in adenosine triphosphate.[24] The hydrogen bond distances and angles in Table VII are also within the expected range, and there are no unusually close nonbonded contacts in the crystal structure.

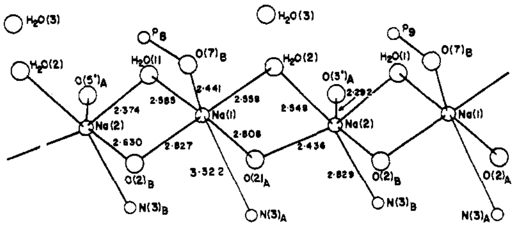

Figure 5. A view of the sodium ion coordination.

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