To the Graduate School:

I am submitting herewith a dissertation written by Charles Leslie Barnes entitled "Crystallographic Studies of Nucleic Acid Components." I recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Biochemistry.

Stuart W. Hawkinson

Stuart W. Hawkinson, Major Professor

We have read this dissertation
and recommend its acceptance:

Accepted for the Council:

Vice Chancellor
Graduate Studies and Research

CRYSTALLOGRAPHIC STUDIES OF NUCLEIC ACID COMPONENTS

A Dissertation

Presented for the

Doctor of Philosophy

Degree

The University of Tennessee, Knoxville

Charles Leslie Barnes

March 1980

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ABSTRACT

The purpose of this study was to determine the three dimensional structures of several nucleic acid components by X-ray crystallographic techniques in order to examine the conformational details of these important metabolites. The study was conducted in two parts, the first part consisting of the determination of the structures of one natural and one synthetic nucleotide, and of one synthetic nucleoside. The second part of the study involved the synthesis of four modified pyrimidine bases believed to be involved in the mutations caused by hydroxylamine and bisulfite, and the determination of the crystal structures of these compounds. All data were collected on automated diffractometers using the θ-2θ scan technique. All structures were solved using heavy-atom or direct methods.

Crystals of adenosine-5'-methylphosphonate hemihydrate are triclinic, space group P1, with $\underline{a}$ = 10.510(2), $\underline{b}$ = 9.419(2), $\underline{c}$ = 7.803(2)Å, and α = 76.77(1), β = 97.24(2) and γ = 95.99(1)°. Full-matrix least-squares refinement resulted in a final $\underline{R}$ factor of 0.029 for the 3339 significant data. The two independent molecules in the asymmetric unit are very similar in conformation, with unusual ribose conformations.

Crystals of disodium guanosine-5'-phosphate heptahydrate are orthorhombic, space group $P2_12_12_1$, with $\underline{a}$ = 22.267(2), $\underline{b}$ = 21.360(2) and $\underline{c}$ = 9.035(1)Å. Block-diagonal least-squares refinement resulted in a final $\underline{R}$ Factor of 0.059. The aromatic bases of the nucleotide molecules are not stacked, but are packed in a "herringbone" pattern primarily stabilized by coordination of N(7) of the bases to a sodium ion.

Crystals of 4-thiopseudouridine are monoclinic, space group $P2_1$, with $\underline{a}$ = 15.434(2), $\underline{b}$ = 7.4381(5), c = 14.818(2)Å and β = 110.45(1)°. The structure was solved by direct methods, but the determination was hampered by a high degree of pseudosymmetry in the crystal. The correct structure was chosen by refinement to a final $\underline{R}$ value of 0.043 for the 3972 significant data.

Crystals of sodium 5,6-dihydrouracil-6-sulfonate monohydrate are monoclinic, space group $P2_1/c$, with $\underline{a}$ = 5.668(1), $\underline{b}$ = 11.026(2), $\underline{c}$ = 13.261(2)Å and β = 94.62(2)°. The compound was obtained as a by-product of the reaction of semicarbazide and bisulfite with cytosine. Full-matrix least-squares refinement resulted in a final $\underline{R}$ value of 0.032.

Crystals of sodium cytosine-5-methylene sulfonate trihydrate are monoclinic, space group $P2_1/c$, with $\underline{a}$ = 5.235(1), $\underline{b}$ = 22.843(5), $\underline{c}$ = 9.140(2)Å and β = 90.93(1)°. This compound is the product of the reaction of bisulfite with 5-hydroxymethylcytosine. Full-matrix least-squares refinement resulted in a final $\underline{R}$ value of 0.038.

Crystals of sodium N^4-hydroxy-5,6-dihydrocytosine-6-sulfonate monohydrate are monoclinic, space group $P2_1/c$, with $\underline{a}$ = 12.977(2), $\underline{b}$ = 8.384(2), $\underline{c}$ = 8.552(2)Å and β = 106.89(1)°. Crystals of sodium 1-methyl-N^4-hydroxy-5,6-dihydrocytosine-6-sulfonate tetrahydrate are monoclinic, space group $P2_1/c$, with $\underline{a}$ = 6.491(1), $\underline{b}$ = 11.129(2), $\underline{c}$ = 17.883(3)Å and β = 93.50(1)°. In both structures, the N(4) hydroxyl group is located *syn* to the ring N(3) atom. This conformation would prevent such modified cytosine residues in a polynucleotide from participating in Watson-Crick base pairing.

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CHAPTER I

INTRODUCTION

Nucleic acids, and the subunits of nucleic acids, are of central importance to biological processes. One tool which may be used to study the properties of these important metabolites is X-ray crystallography. With the exception of the transfer ribose nucleic acids, polynucleotides do not lend themselves to examination by single crystal X-ray study. However, it is possible to obtain suitable crystalline specimens of nucleotides, nucleosides, and the purine and pyrimidine bases, which aside from their roles as components of polynucleotides, are involved in numerous biochemical pathways. Analysis of the crystalline structures of these molecules, and of analogs or derivatives, can provide information about how they perform their biological functions.

CHAPTER II

STRUCTURE OF ADENOSINE-5'-METHYLPHOSPHONATE HEMIHYDRATE

Introduction

Structure determinations of nucleotide analogs in general are useful for the information they yield about the parent compounds. Phosphonate analogs of nucleoside phosphates have been used to study the mechanism of action of phosphatases and phosphate kinases, leading to interest in this group of nucleotide analogs (Myers, Nakamura and Danielzadeh, 1965).

Cell Data

Crystals of adenosine-5'-methylphosphonate hemihydrate ($C_{11}H_{16}O_6N_5P \cdot 1/2H_2O$) are triclinic, space group P1, with $\underline{a}$ = 10.510(2), $\underline{b}$ = 9.419(2), $\underline{c}$ = 7.803(2)Å, α = 76.77(1), β = 97.24(2), γ = 95.99(1)°. Calculated and observed densities are 1.582 and 1.576 g cm^{-3}, respectively, for Z=2.

Experimental

A sample of adenosine-5'-methylphosphonate was supplied by Dr. Bimal C. Pal, Biology Division, Oak Ridge National Laboratory, and was recrystallized from aqueous/ethanol solution in a thermal gradient. The crystallization apparatus was similar to one recently described (Watkin, 1972). A specimen 0.35x0.35x0.22 mm was selected for the diffraction study. The space group and approximate cell dimensions were determined from precession and Weissenberg photographs. More accurate cell dimensions

were obtained with the use of the observed setting angles for 10 Mo $K\alpha_1$ reflections, in the range $39<2\theta<43^\circ$, measured with an Oak Ridge computer-controlled diffractometer (Busing, Ellison, Levy, King and Roseberry, 1968). The density of the crystals was measured by flotation in mixtures of chloroform and carbon tetrachloride. Intensity data were collected on the diffractometer with Nb-filtered Mo $K\bar{\alpha}$ radiation using the θ-2θ scan technique. Of 3444 reflections with $2\theta<55^\circ$, 3339 were considered observed ($|F_o|<3\sigma(F_o)$). Each structure factor was assigned a variance $\sigma^2(F)$ based on counting statistics plus a term $(0.03F)^2$, empirically derived during refinement. No absorption correction was applied (μ=0.22 mm^{-1}). No significant changes in two standard reflections were observed during the course of data collection.

The structure was solved from the sharpened Patterson function only after considerable difficulty and with significant assistance from the fast rotation function of R.A. Crowther (ROTRAN, B.M. Craven, 1975). The phosphorus positions were readily determined from the Patterson map, but provided insufficient phasing information to produce a Fourier synthesis significantly different from the Patterson map. It was later found that the positions of the phosphonate oxygen atoms were obscured by extensive vector overlap. The orientation of the adenine rings was found using ROTRAN, with 9-methyladenine (Kistenmacher and Rossi, 1977) as a model search fragment. The self-rotation function confirmed the relative orientations of the adenine rings, which, with packing considerations and assumptions concerning reasonable hydrogen bonding, allowed interpretation of the Patterson map. Successive Fourier syntheses, using $(2|F_o|-|F_c|)\exp(i\phi_c)$ coefficients, completed determination of the

structure. The 47 non-hydrogen atoms in the unit cell were refined by full-matrix least-squares methods. During the later stages of anisotropic refinement, the 34 hydrogen atoms were placed from difference Fourier synthesis alternating with least-squares refinement of non-hydrogen atom parameters, minimizing $\Sigma w(|F_o|-|F_c|)^2$ with $w=1/\sigma^2(F_o)$. The final values of R, the weighted R $\{[\Sigma w(F_o-F_c)^2/\Sigma wF_o^2]^{1/2}\}$, and σ, the goodness of fit $\{[\Sigma w(F_o-F_c)^2/(n-p)]^{1/2}$, where n=3339 reflections and p=423 variables$\}$, were 0.029, 0.043 and 1.16, respectively. The average shift on the final cycle of refinement was 20% of the estimated standard deviation. All refinements were performed with the XRAY system (Stewart, Kruger, Ammon, Dickinson and Hall, 1972). The final atomic parameters are given in Table A-1. The anisotropic thermal parameters for the non-hydrogen atoms are given in Table B-1.

Discussion

A drawing of the molecules, including bond distances and angles, is shown in Figure 1. The two independent molecules are nearly identical in conformation. Both molecules are zwitterions, with N(1) protonated and the methylphosphonate group ionized. The P-C distances [1.779(3), 1.789(3) Å] are consistent with the P-C distance of 1.78 Å found in pyridoxal-5'-methylphosphonate (Cole, Lachmann & Korytnyk, 1972). Other distances and angles are consistent with those determined for other nucleotide structures. Torsion angles descriptive of the molecular conformation are given in Table 1. In both molecules, the ribose ring is <u>anti</u> relative to the base. The phosphonate ester oxygen, O(5'), is placed <u>g^+</u> relative to the sugar ring, while the phosphorous atom is <u>trans</u> to C(4').

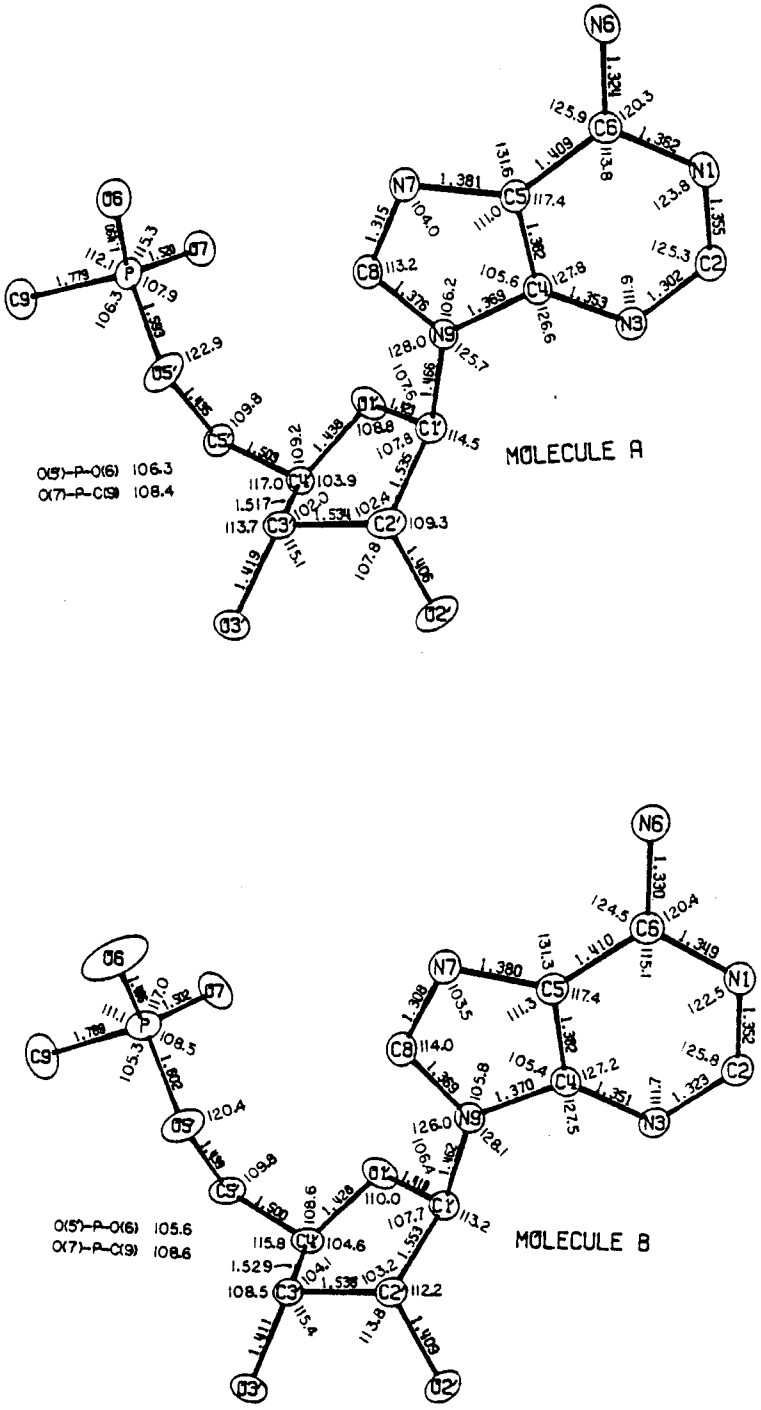


Figure 1. Bond Distances (Å) and Angles (°) for Adenosine-5'-Methylphosphonate Molecules A and B.

TABLE 1. TORSION ANGLES AND RIBOSE PSEUDO-ROTATION PARAMETERS FOR MOLECULES A AND B OF ADENOSINE-5'-METHYLPHOSPHONATE

		A	B
χ	O(1')-C(1')-N(9)-C(8)	56.5 (3)	52.9 (3)
τ_0	C(4')-O(1')-C(1')-C(2')	-11.0 (2)	- 17.3 (2)
τ_1	O(1')-C(1')-C(2')-C(3')	-13.8 (2)	- 3.6 (2)
τ_2	C(1')-C(2')-C(3')-C(4')	31.6 (2)	21.2 (2)
τ_3	C(2')-C(3')-C(4')-O(1')	-39.1 (2)	- 31.9 (2)
τ_4	C(3')-C(4')-O(1')-C(1')	31.7 (2)	31.0 (2)
ϕ	P-O(5')-C(5')-C(4')	136.1 (2)	143.1 (2)
ψ	O(5')-C(5')-C(4')-C(3')	50.1 (3)	51.5 (3)
ψ'	C(5')-C(4')-C(3')-O(3')	75.9 (3)	85.1 (2)
Ribose pseudo-rotation parameters			
P	Phase angle from $^{3}_{2}$ T	34.5	49.1
τ_m	Magnitude of pucker	39.2	33.1
	Conformational descriptor	$^{3}_{4}$T	$_{4}$T^{3}

Calculation of the pseudorotation parameters (Altona and Sundaralingam, 1972) for the ribose rings reveals the unusual ${}^{3}_{4}T$ and ${}_{4}T^{3}$ conformations. The conformations of riboses A and B are intermediate between 3'-endo and 4'-exo, with B very close to 4'-exo, an extended conformation with the C(4')-C(5') bond pseudo-equatorial.

The molecules are packed in A-B pairs stabilized by base stacking and base to phosphonate oxygen hydrogen bonds (Figure 2). Within the pair the stacking distance is about 3.5Å, the closest contact distances being C(5)a---C(5)b, 3.507(4)Å, C(5)---N(7)b, 3.507(3)Å, C(6)a---C(8)b, 3.433(4)Å and C(8)a---C(6)b, 3.453(4)Å. The stacked bases are oriented so that N(1) and N(6) are near the ionized methylphosphonate group of the paired molecule. N(1)a-H---O(7)b, N(1)b-H---O(7)a, and N(6)b-H---O(6)a hydrogen bonds are present (Table 2), but N(6)a is more distant from O(6)b than from O(7)b (3.312(4)Å versus 3.101(3)Å, so that only a weak interaction with O(7)b is likely.

Adjacent A-B pairs are held together by stacking forces [stacking distances about 3.3Å; N(1)a---C(2)b, 3.458(3)Å, C(2)a---C(2)b, 3.221(4)Å, C(2)a---N(3)b, 3.360(4)Å, N(3)a---C(2)b, 3.384(4)Å, closest contacts] and an intricate network of hydrogen bonds, including interactions at position N(7) of both adenine rings. The water molecule participates as a donor in hydrogen bonds to N(3)b and O(6)a. Both C(8)-H groups are involved in close contacts: C(8)a---O(w), 3.134(3)Å; C(8)b---O(2')a, 2.916(3)Å.

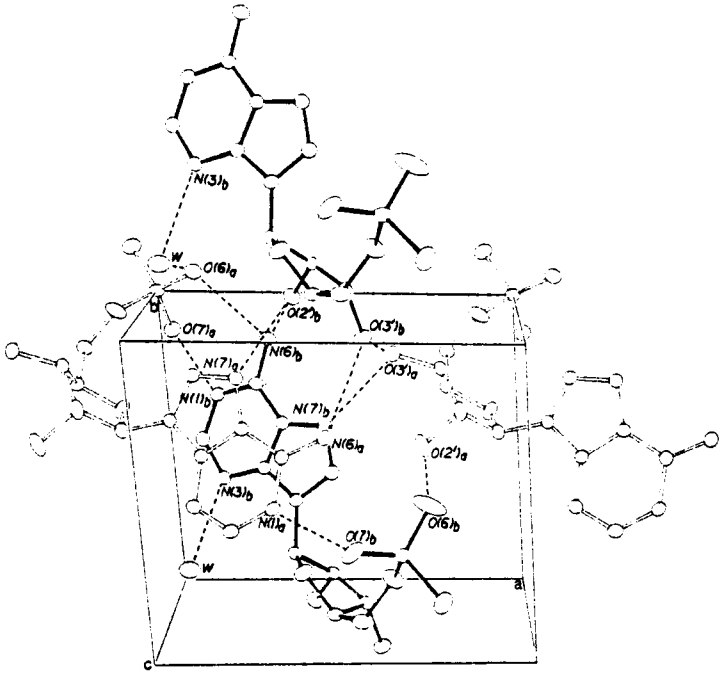


Figure 2. Packing Interactions for Adenosine-5'-Methylphosphonate. Molecule A is drawn with hollow bonds. Molecule B is drawn with solid bonds. The dashed lines represent hydrogen bonds.

TABLE 2. HYDROGEN BONDING DISTANCES AND ANGLES IN THE STRUCTURE OF ADENOSINE-5'-METHYLPHOSPHONATE

Donor (D)	Acceptor (A)	In Molecule at	Distance (Å) D-A	Distance (Å) H-A	D-H-A Angle (°)
N(1)a	O(7)b	1+x,y,z	2.618(3)	1.93	166
N(1)b	O(7)a	-1+x,y,z	2.542(3)	1.85	174
N(6)a	O(3')b	1+x,1+y,z	2.870(3)	1.92	170
N(6)b	O(6)a	-1+x,y,z	2.843(3)	2.22	162
N(6)b	O(2')b	x,1+y,z	2.902(3)	2.21	136
O(2')a	O(6)b	x,y,z	2.601(3)	1.88	173
O(2')b	N(7)a	-1+x,-1+y,z	2.783(3)	2.03	172
O(3')a	N(7)b	x,y,z	2.857(3)	2.06	169
O(3')b	O(3')a	x,-1+y,z	2.750(3)	2.06	161
O(w)	N(3)b	x,y,z	2.912(3)	2.27	142
O(w)	O(6)a	-1+x,-1+y,z	2.837(4)	1.94	173
C(8)a	O(w)	1+x,1+y,z	3.134(3)	2.28	157
C(8)b	O(2')a	x,y,z	2.916(3)	2.33	126

CHAPTER III

STRUCTURE OF DISODIUM GUANOSINE-5'-PHOSPHATE HEPTAHYDRATE

Introduction

Guanosine-5'-phosphate (GMP) is one of the four common nucleotides of ribonucleic acids and an important metabolite. Disodium GMP crystallizes as the heptahydrate, space group $P2_12_12_1$, with Z=8. Another crystal form of this nucleotide was reported earlier which displayed a hair-like habit, the crystals being too fine for single crystal work (Zimmerman, 1976). Fiber diffraction studies of bundles of these crystals indicated a stacked arrangement of the GMP monomers, held together in a tetrameric hydrogen bonded array. A stacked helical arrangement of the monomers of the mono-sodium salt of GMP is believed to be responsible for the unique viscous gels observed when aqueous solutions of the disodium salt are acidified (Sasisekharan, Zimmerman and Davies, 1975). In addition, base stacking has been observed in a majority of the crystal structures of the ribose nucleosides and ribose and deoxyribose mononucleotides of guanine and the related base hypoxanthine reported to date (Nagashima, Wakabayashi, Matzuzaki and Iitaki, 1974; Young, Tollin and Wilson, 1974; Murayama, Nagashima and Shimizu, 1969; Thewalt, Bugg and Marsh, 1971; Munns and Tollin, 1970). We have found that in the crystals of disodium GMP heptahydrate the bases are not stacked but are instead arranged in a "herringbone" pattern primarily stabilized by coordination of N(7) of the bases to a sodium ion.

Cell Data

Crystals of disodium GMP heptahydrate ($C_{10}H_{12}O_8N_5PNa_2 \cdot 7H_2O$) are orthorhombic, space group $P2_12_12_1$, with a = 22.267(2), b = 21.360(2), c = 9.035(1)Å. Calculated and observed densities are 1.65 and 1.63 g cm^{-3}, respectively, for Z=8.

Experimental

Disodium GMP obtained from Sigma Chemical Company was crystallized from an aqueous/ethanol solution in a thermal gradient. The crystallization device was similar to one recently described (Watkin, 1972). A specimen 0.05x0.1x0.3 mm was selected for diffraction study. The space group and approximate cell dimensions were determined from precession and Weissenberg photographs. More accurate cell dimensions were obtained by least-squares refinement with the use of the observed setting angles for 12 Cu $K\alpha_1$ reflections in the range $100 < 2\theta < 116^\circ$, measured with an Oak Ridge computer-controlled diffractometer (Busing, Ellison, Levy, King and Roseberry, 1968). The density of the crystals was measured by flotation in mixtures of chloroform and dibromomethane. Intensity data were collected on the diffractometer with Ni-filtered Cu $K\bar{\alpha}$ radiation using the θ-2θ scan technique. Of 4220 reflections with $2\theta < 132^\circ$, 3931 were considered observed ($F_o > 3\sigma(F_o)$). Each intensity was assigned a variance $\sigma^2(I)$ based on counting statistics plus a term $(0.04I)^2$, empirically derived during refinement. No absorption correction was applied. No significant changes in two standard reflections were observed during the course of data collection.

The structure was solved using the direct methods program MULTAN (Germain, Main and Woolfson, 1971) and completed from difference Fourier syntheses. The 66 non-hydrogen atoms in the asymmetric unit were refined by block-diagonal least-squares methods. Following initial isotropic refinement, those atoms with unusually high temperature factors were allowed to refine anisotropically. Forty-four of the total of 52 hydrogen atoms were included in the model during the final stages of refinement. Those hydrogen atoms whose positions could be calculated with confidence were placed at calculated positions which were updated after each round of refinement. The remainder of the hydrogen atoms were located from difference Fourier syntheses, except those of waters 11, 12, 13 and 14. All hydrogen atoms were arbitrarily assigned temperature factors approximately equivalent to those of the atoms to which they were bonded. Structure factors calculated with the hydrogen atoms alone were applied as partial contribution during refinement of the non-hydrogen atoms. The quantity minimized during refinement was $\Sigma w(|F_o|-|F_c|)^2$ with $w=1/\sigma^2(F_o)$. The final values of R, the weighted R $\{[\Sigma w(F_o-F_c)^2/\Sigma wF_o^2]^{1/2}\}$, and σ, the goodness of fit $\{[\Sigma w(F_o-F_c)^2/(n-p)]^{1/2}$, where n=3927 reflections and p=396 variables$\}$, were 0.056, 0.075 and 1.76, respectively. Four reflections were excluded from the final refinement because their weighted structure factor differences were greater than six times the standard deviation of an observation of unit weight. The average shift on the last cycle of refinement was 25% of the estimated standard deviation. All refinements were performed with the XRAY system (Stewart, Kruger, Ammon, Dickinson and Hall, 1972). The final atomic parameters are given

in Table A-2. The final anisotropic thermal parameters for those atoms refined anisotropically are given in Table B-2.

Discussion

The two crystallographically independent GMP molecules in the asymmetric unit are quite similar and display no surprising conformational parameters (Figure 3). Both ribose rings are in the 2'-*endo* conformation and are in the *anti* conformation relative to the bases. The conformation about the exocyclic C(4')-C(5') sugar bond in both molecules is g^+. The relevant torsion angles and ribose conformational descriptors are given in Table 3.

Figure 4 is a stereo view of the packing, showing the alternating layers of solvent and nucleotide molecules. Despite the high degree of hydration in this crystal, there is no apparent disorder of the non-hydrogen solvent atoms. Table 4 is a list of the hydrogen bonds present in the structure. It should be noted that difficulty in placing some of the water hydrogen atoms results in ambiguities in the assignment of donor and acceptor status in the last several hydrogen bonds listed. The two GMP molecules, 14 water molecules and four sodium ions in the asymmetric unit form an intricate network of hydrogen and coordination bonds (Table 5). There are no base-base hydrogen bonds in the structure; such bonds are important in the description of the spontaneously formed helix of the gel of the monosodium salt (Sasisekharan, Zimmerman and Davies, 1975; Zimmerman, 1976). The atoms involved in base pairing in double stranded polynucleotides, N(1), N(2) and O(6), are in this case hydrogen bonded to a phosphate oxygen and waters, respectively. The

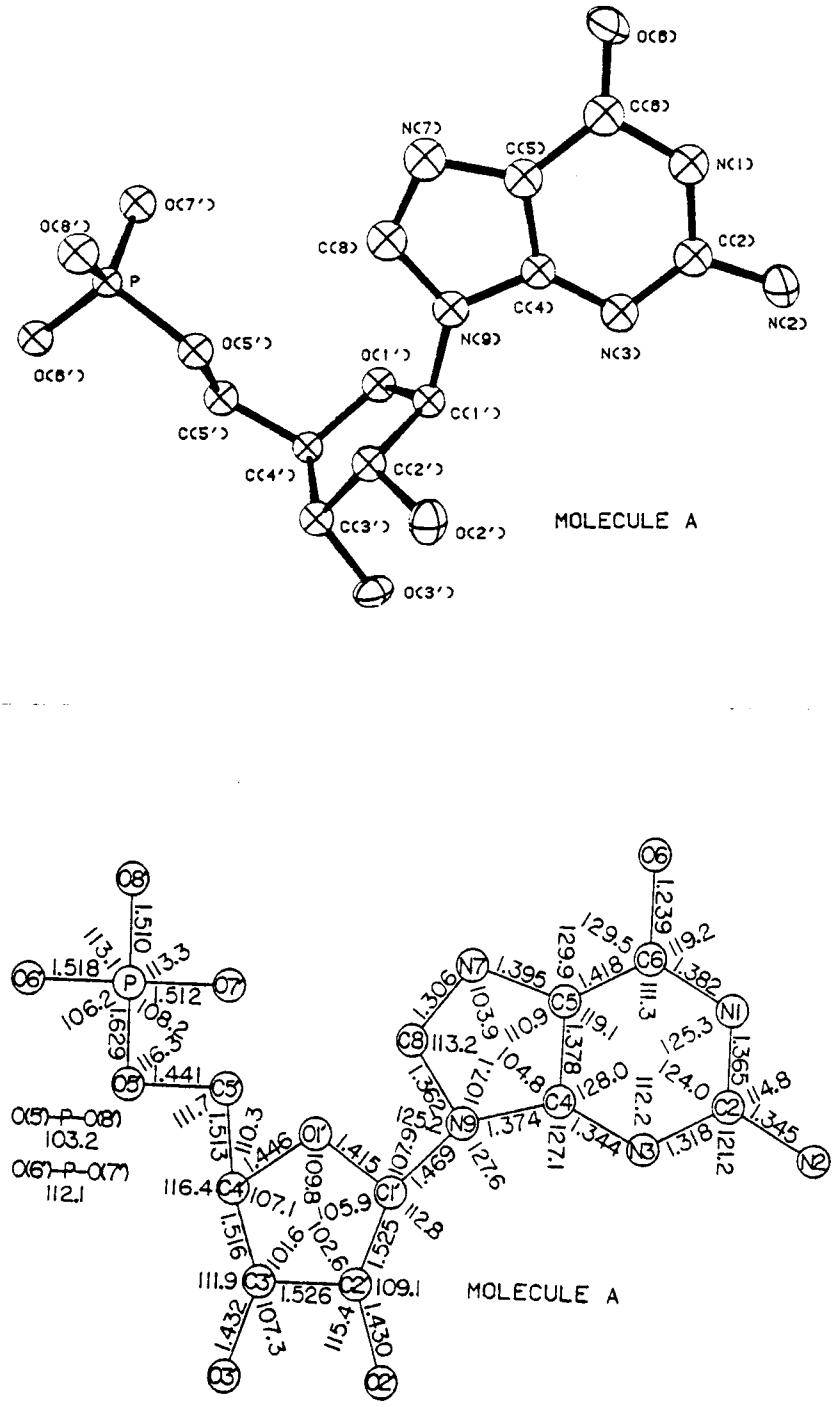
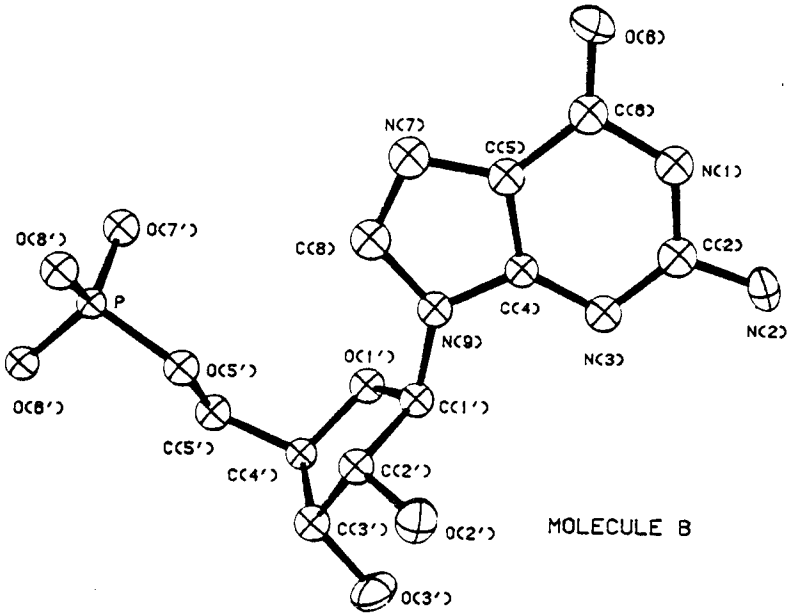


Figure 3. Bond Distances and Angles for Disodium Guanosine-5'-Phosphate Molecules A and B.



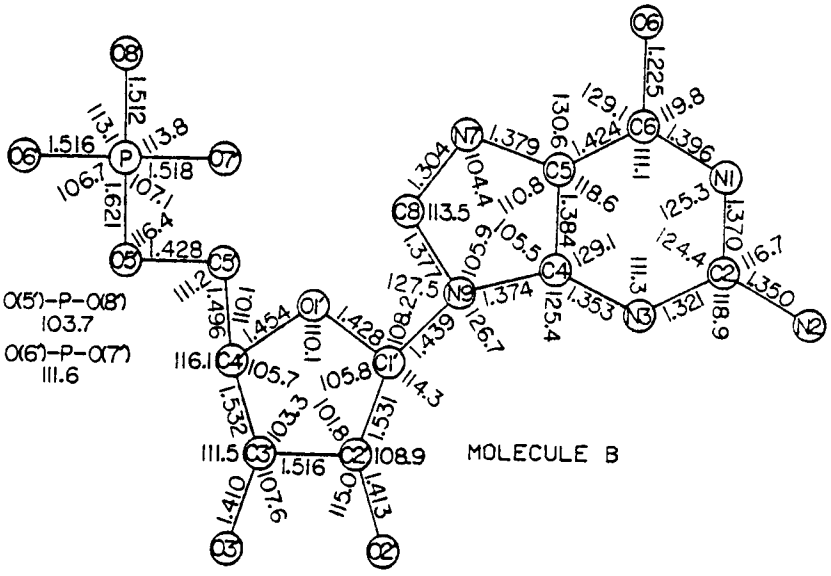


Figure 3. (Continued)

TABLE 3. TORSION ANGLES AND RIBOSE PSEUDO-ROTATION PARAMETERS FOR MOLECULES A AND B OF DISODIUM GMP

		A	B
χ	O(1')-C(1')-N(9)-C(8)	55.5 (7)	57.4 (7)
τ_0	C(4')-O(1')-C(1')-C(2')	- 15.8 (5)	- 18.4 (5)
τ_1	O(1')-C(1')-C(2')-C(3')	32.0 (5)	33.5 (5)
τ_2	C(1')-C(2')-C(3')-C(4')	- 34.9 (5)	- 35.2 (5)
τ_3	C(2')-C(3')-C(4')-O(1')	26.6 (5)	25.4 (5)
τ_4	C(3')-C(4')-O(1')-C(1')	- 7.2 (5)	- 4.4 (5)
ϕ	P-O(5')-C(5')-C(4')	171.9 (3)	169.8 (3)
ψ	O(5')-C(5')-C(4')-C(3')	55.5 (6)	55.1 (6)
ψ'	C(5')-C(4')-C(3')-O(3')	148.4 (4)	147.8 (5)
Ribose pseudorotation parameters			
P	Phase angle from $^{3}_{2}T$	173.5	169.7
τ_m	Magnitude of pucker	35.2	35.7
	Conformational descriptor	$^{2}T_{3}$	$^{2}T_{3}$

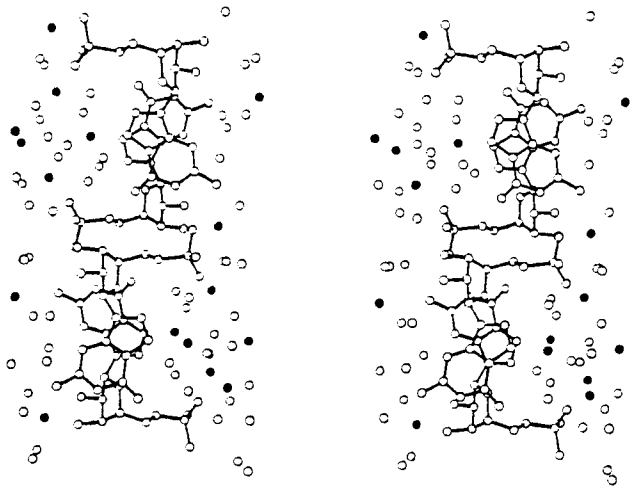


Figure 4. Stereo View of Packing Interactions in Disodium GMP Heptahydrate. The view is down the c axis. The sodium ions are shown as shaded ellipsoids.

TABLE 4. HYDROGEN-BONDING DISTANCES AND ANGLES IN THE STRUCTURE OF DISODIUM GMP[a]

Donor (D)	Acceptor (A)	Symmetry Code	Distance (Å) D-A	Distance (Å) H-A	D-H···A Angle (°)
N(1)a	O(6')a	10	2.702(6)	1.9	166
N(1)b	O(6')b	9	2.765(6)	1.9	170
N(2)a	W(11)	7	2.953(9)	2.1	167
N(2)b	W(13)	9	2.919(12)	2.2	148
O(3')a	O(8')b	1	2.713(6)	2.0	175
O(2')a	O(6')b	2	2.730(6)	1.8	167
O(3')b	O(8')a	3	2.690(6)	2.0	171
O(2')b	O(6')a	1	2.723(6)	1.9	158
W(1)	O(8')a	3	2.730(6)	2.0	169
W(1)	O(6)b	11	2.759(6)	1.9	177
W(2)	O(3')a	1	2.968(6)	2.3	156
W(2)	O(7')b	2	2.749(6)	1.8	153
W(3)	O(8')b	1	2.761(6)	1.9	160
W(3)	O(7')a	7	2.947(6)	2.0	155
W(4)	W(9)	9	2.849(7)	2.1	142
W(4)	O(7')b	1	2.811(6)	1.8	174
W(5)	O(8')a	7	2.806(6)	2.0	150
W(5)	O(6')b	1	2.752(6)	2.0	161
W(6)	O(7')a	1	2.703(6)	2.0	170
W(6)	O(3')a	5	3.066(6)	2.3	154
W(7)	O(6)a	8	2.718(6)	1.8	174
W(7)	O(8')b	1	2.733(6)	2.0	155
W(8)	W(14)	11	2.725(10)	1.9	163
W(8)	W(3)	5	2.814(7)	2.0	171
W(9)	O(7')a	1	2.711(6)	1.8	161
W(9)	W(4)	11	2.849(7)		
W(10)	O(7')b	6	3.025(6)	2.2	164
W(10)	W(4)	11	2.767(8)	2.0	160
W(11)	W(12)	8	2.740(12)		
W(11)	N(3)b	1	3.105(10)		
W(12)	O(2')a	1	2.782(9)		
W(12)	N(3)a	1	3.038(10)		
W(13)	W(11)	7	2.793(12)	1.9	167
W(14)	O(7')b	1	2.808(9)		
W(14)	O(1')b	1	2.979(9)		

[a]The donor atom belongs to the basic set listed in Table A-2 and the acceptor atom is related to this set by the symmetry operations of the space group and/or translations. The code used is as follows:

TABLE 4 (Continued)

1	x	y	z	7	-1/2+x	1/2-y	-z
2	x	y	1+z	8	1-x	1/2+y	1/2-z
3	x	y	-1+z	9	1-x	1/2+y	-1/2-z
4	1/2-x	1-y	1/2+z	10	1-x	-1/2+y	1/2-z
5	1/2+x	1/2-y	-z	11	1-x	-1/2+y	-1/2-z
6	1/2+x	1/2-y	-1-z				

TABLE 5. SODIUM COORDINATION[a] DISTANCES FOR DISODIUM GMP

		SYMMETRY CODE	DISTANCE (Å)
Na(1)	W(1)	1	2.346(5)
	W(7)	11	2.423(5)
	W(8)	3	2.491(6)
	W(10)	1	2.358(5)
	$N(7)_a$	3	2.415(6)
	$N(7)_b$	11	2.613(5)
Na(2)	W(1)	1	2.382(5)
	W(2)	5	2.468(5)
	W(5)	6	2.393(6)
	W(6)	1	2.421(5)
	W(9)	1	2.422(6)
	W(10)	1	2.746(6)
Na(3)	W(2)	1	2.373(5)
	W(3)	1	2.476(5)
	W(4)	4	2.334(5)
	W(7)	1	2.328(5)
	W(8)	8	2.379(6)
	W(10)	9	2.611(6)
Na(4)	W(5)	6	2.803(6)
	W(6)	1	2.315(6)
	W(12)	5	2.281(9)
	W(13)	5	2.623(11)
	$O(2')_b$	1	2.492(5)
	$O(3')_b$	1	2.305(6)

[a]The symmetry operations are as defined in Table 4

pseudo-twofold symmetry resulting from this hydrogen bonding pattern and the above mentioned N(7)-sodium ion coordination is shown in Figure 5. The view of Figure 5 is down the a axis; the pseudo-twofold axis is normal to the plane of the figure at the position of Na(1). Figure 6 shows the relation of the pseudo-twofold axes to the crystallographic symmetry operations, and the resulting pseudo-twofold screw axis.

Figure 7 shows the sodium ion coordination. Each of the four sodium ions is coordinated to six ligands. The coordination shells about sodium ions 1 and 3 are fairly regular octahedra, while those about sodium ions 2 and 4 are more distorted. The mechanisms by which metal ions interact with nucleotides has been recently reviewed by Sternglanz, Subramanian, Lacey and Bugg (1976) in their paper reporting the structure of barium adenosine-5'-monophosphate heptahydrate. In that structure the barium ion was found to be completely hydrated, forming an outer sphere complex with the nucleotide. In the present structure, two of the sodium ions are completely hydrated (2 and 3) and the other two (1 and 4) form inner sphere complexes with N(7)a and N(7)b and with O(2')b and O(3')b, respectively. There is no direct sodium ion coordination to phosphate oxygens.

From the preponderance of purine structures involving a stacked arrangement of the aromatic bases, it is reasonable to conclude that such base stacking is highly favored in achieving an energy minimum in the molecular packing of guanine nucleosides and nucleotides. More generally, any molecule with a sufficiently large and dominant planar substituent would tend to pack with the aromatic portions stacked. The packing scheme achieved by disodium GMP heptahydrate is indicative of

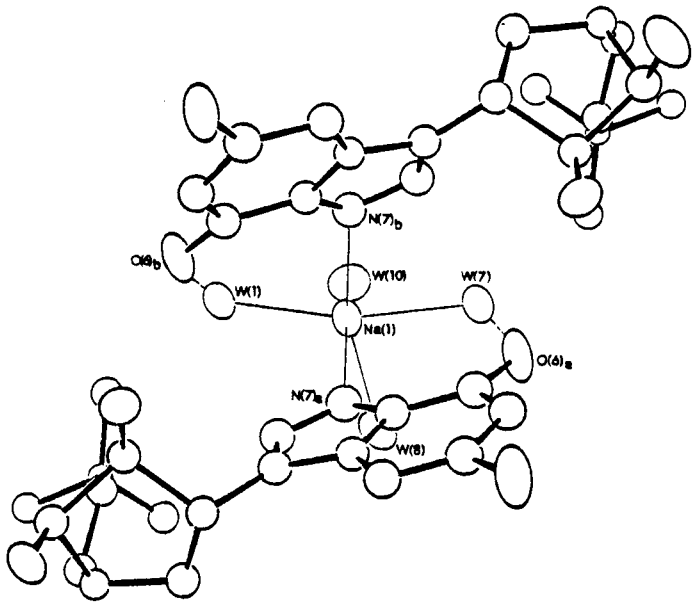


Figure 5. View of the Nearly Symmetric Coordination of Na(1) in the Structure of Disodium GMP Heptahydrate. The view is down the a axis. The thin solid lines represent coordination bonds. The dotted lines represent hydrogen bonds.

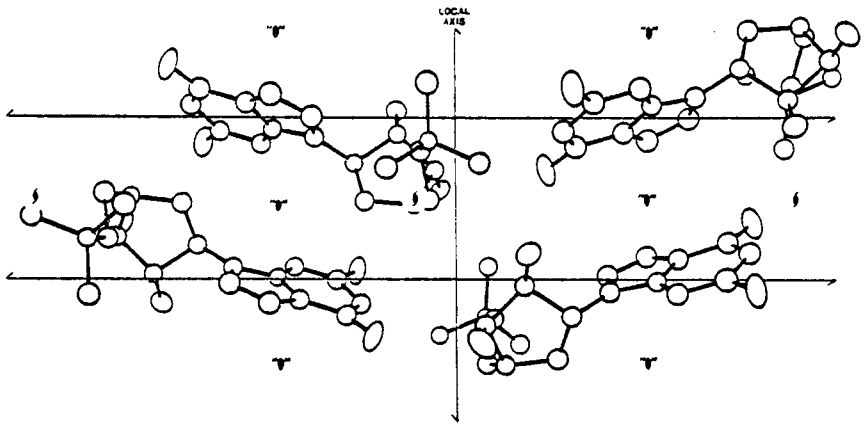


Figure 6. Relation of Pseudo Symmetry Elements to Crystallographic Symmetry Axes in the Structure of Disodium GMP. The crystallographic symmetry operations are indicated by standard symbols; the pseudo symmetry elements are indicated by symbols in quotes, or labelled "LOCAL AXIS."

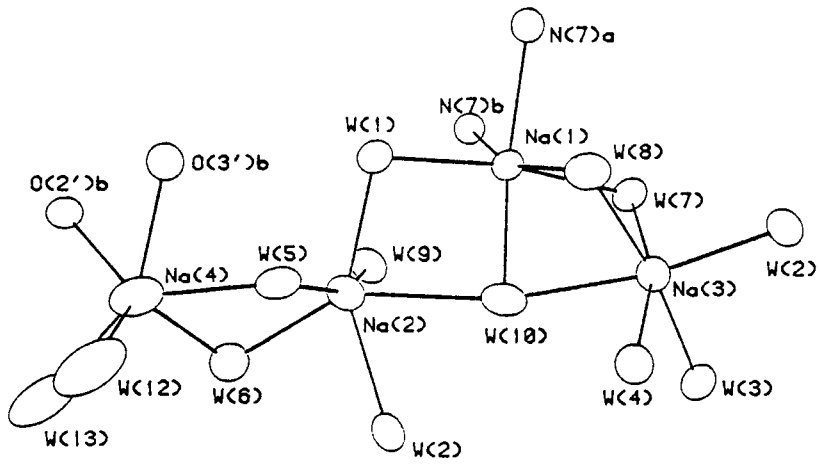


Figure 7. Sodium Ion Coordination Polyhedra for Disodium GMP. The bonds indicate coordination interactions.

alternative interactions possible with this nucleotide. Similar interactions with solvent and cations were observed in the structure of monosodium inosine-5'-phosphate hexahydrate (Rao and Sundaralingam, 1969), suggesting the importance of the degree of hydration in determining the mode of packing. Such interactions with solvent and cations must be considered in a complete description of polynucleotide structures in aqueous environments.

CHAPTER IV

STRUCTURE OF 4-THIOPSEUDOURIDINE

Introduction

Pseduouridine is unique among the nucleosides in ribonucleic acids in having a carbon-carbon "glycosidic" bond, which gives it structural properties different from those of uridine. The synthetic nucleoside 4-thiopseudouridine has been shown to inhibit the growth of Escherica coli (strain B5RU) when the cells are grown on pseudouridine (Wigler, Bindslev and Breitman, 1974). Wigler et al. have proposed that a metabolite of 4-thiopseudouridine, presumably the nucleotide, is an inhibitor of the enzyme pseudouridylate synthetase, which catalyzes the key reaction in the only known salvage pathway for pseudouridine-5'-phosphate (Solomon and Breitman, 1971), the conversion of pseudouridine-5'-phosphate to uridine-5'-phosphate. This study was undertaken to determine the conformational features of the 4-thio substituted pseudouridine for comparison with pseudouridine and with other thio-substituted pyrimidines. Our interest in this project was heightened when preliminary film studies indicated the presence of three independent molecules in the asymmetric unit, for space group $P2_1$, which according to our own experience and a recent survey (Belsky and Zorkii, 1977) is an uncommon occurrence.

Cell Data

Crystals of 4-thiopseudouridine ($C_9H_{12}N_2O_5S$) are monoclinic, space group $P2_1$, with $\underline{a}$ = 15.434(2), $\underline{b}$ = 7.4381(5), $\underline{c}$ = 14.818(2) Å, β = 110.45 (1)°. Calculated and observed densities are 1.627 and 1.62 g cm^{-3}, respectively, for Z=6.

Experimental

4-Thiopseudouridine was synthesized according to Wigler, Bindslev and Breitman (1974) and purified by chromatography (Uziel, Koh and Cohn, 1968). Recrystallization from aqueous ethanol produced well formed yellow monoclinic prisms, elongated along $\underline{b}$. A specimen 0.35x0.2x0.1 was selected for single crystal crystal diffraction study. Following initial determination of the space group and approximate cell dimensions by film methods in our laboratory, accurate cell dimensions and intensities were measured by Dr. Eric J. Gabe of the National Research Council of Canada, Ottawa, on a modified Picker diffractometer using crystal monochromatized Mo Kα radiation in the θ-2θ scanning mode. Of the 5020 independent observations recorded, 3972 were considered observed ($I<3\sigma(I)$).

The structure determination was approached using MULTAN 78 (Main, Hull, Lessinger, Germain, Declercq and Woolfson, 1978). The phase set with the highest combined figure of merit gave a plausible fraction of the structure, which produced more of the structure when used in the Karle recycling routine. The remaining non-hydrogen atoms were located using weighted Fourier syntheses, though one ribose appeared to be disordered. At this point, the model seemed reasonable, featuring hydrogen bonded

riboses and stacked ribbons of hydrogen bonded bases. However, refinement of the model, with one molecule included as disordered, converged to an R of 0.14, a result obviously inconsistent with the quality of the data. Examination of a model of the structure led to the observation that the stacked arrangement of the independent molecules results in a set of three reasonable structures differing primarily in the location of the origin along c. A shift of the origin of the false solution by c/3 gave a set of parameters which eventually refined to an R of 0.043. This structural pseudosymmetry is remarkably similar to that observed by van Zoeren, Oonk and Kroon (1978) in the structure of L-pyroglutamic acid in the space group $P2_12_12_1$, with Z=12.

Anisotropic refinement of the non-hydrogen atoms was performed by least-squares methods with the matrix in six blocks. The hydrogen atoms were located by difference Fourier syntheses during the later stages of refinement, and their idealized locations were updated between cycles of refinement. Atoms related by the approximate, non-crystallographic translational symmetry operation 0,0,1/3, were assigned to the same block during refinement. Each intensity was assigned a variance, $\sigma^2(I)$, based on counting statistics, plus a term, $(0.04\ I)^2$, empirically derived during refinement. The final values of R, the weighted R $\{[\Sigma w(F_o-F_c)^2/\Sigma wF_o^2]^{1/2}\}$, and σ, the goodness of fit $\{[\Sigma w(F_o-F_c)^2/(n-p)]^{1/2}$, where n=3972 reflections and p=460 variables$\}$, were 0.043, 0.047 and 1.40, respectively. The average shift on the final round of refinement was less than 20% of the estimated standard deviation. All refinements were performed with the XRAY system (Stewart, Kruger, Ammon, Dickinson and Hall, 1972). The

final atomic parameters are given in Table A-3. The final anisotropic thermal parameters are given in Table B-3.

Structural Details

A drawing of the molecules, including bond distances and angles, is shown in Figure 8. Differences in pseudouridine ring bond angles in comparison to those observed in other diketo pyrimidine structures are attributable to the substitution of the C(5)-C(1') bond for the normal N(1)-C(1') glycosidic bond, and are comparable to those observed in α-pseudouridine (Rohrer and Sundaralingam, 1970). The C-S bond distances are in the short end of the range reported for other thio substituted pyrimidines: 1.645-1.684Å, in a review of several structures (Saenger and Suck, 1971); 1.669Å, 1-methyl-4-thiouracil (Hawkinson, 1975); 1.677 Å, 2-thiouridine (Hawkinson, 1977). This difference may reflect an increased degree of double bond character in the C(4)-S bond in this structure.

Torsion angles descriptive of the molecular conformation are given in Table 6. We follow the definition of the angle χ (C(6)-C(5)-C(1')-O(1')) established by Rohrer and Sundaralingam (1970), as this allows comparison with values determined for other nucleosides. By this definition, all three molecules are in the anti conformation. The sugars are intermediate in conformation between C(3')-endo and C(4')-exo. The C(3')-endo conformation has been commonly observed in pyrimidine nucleosides (Sundaralingam, 1975). Indeed, in the structure of uridine (Green, Rosenstein, Shiono and Abraham; Trus and Marsh, 1957) the values of χ

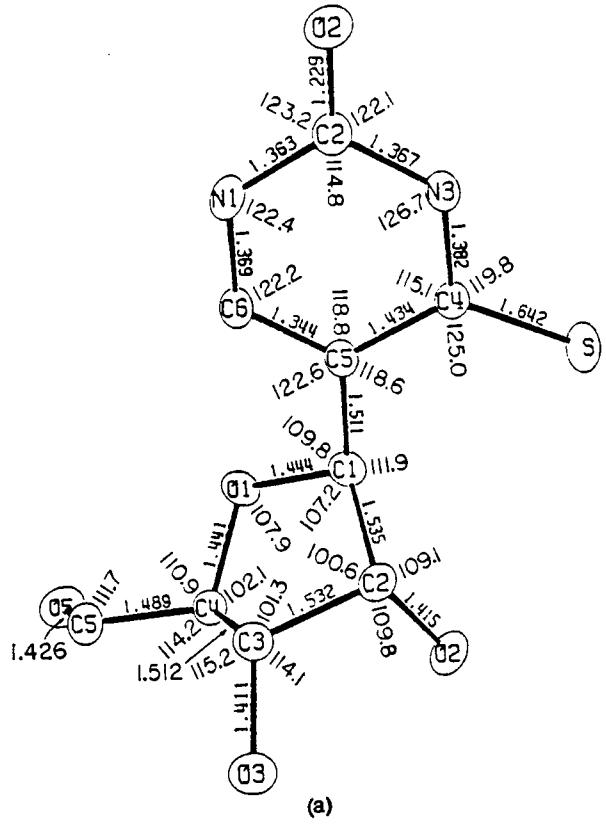
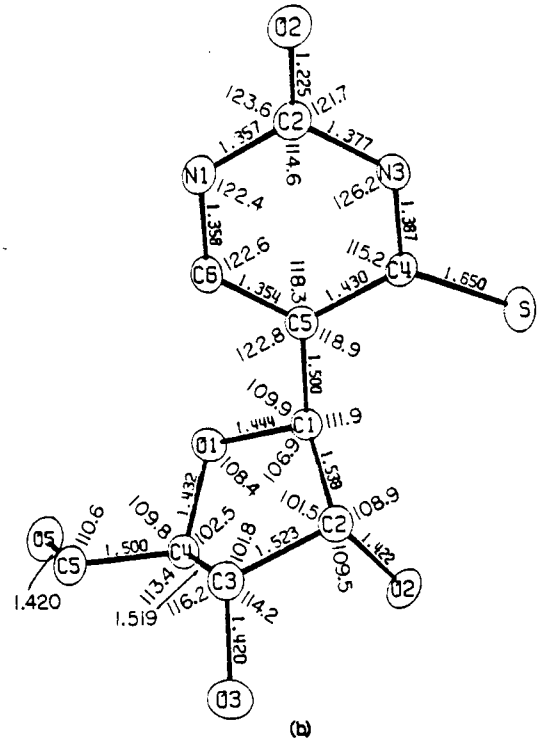


Figure 8. Bond distances (Å) and Angles (°) for 4-Thiopseudouridine. (a) Molecule A. (b) Molecule B. (c) Molecule C.



(a)

Figure 8. (Continued)

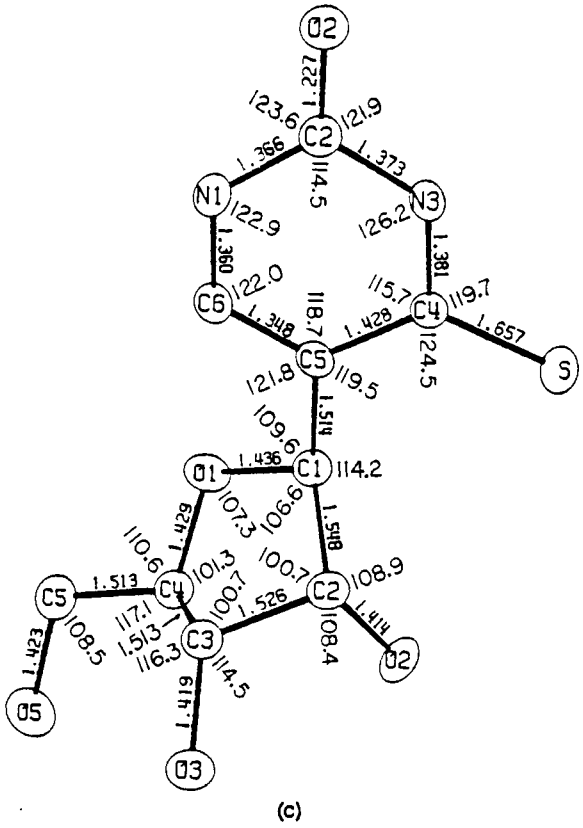


Figure 8. (Continued)

TABLE 6. TORSION ANGLES AND RIBOSE PSEUDO-ROTATION PARAMETERS FOR MOLECULES A, B AND C OF 4-THIOPSEUDOURIDINE

		A	B	C
χ	O(1')-C(1')-C(5)-C(6) (ribose anti to S^4)	18.4(3)	16.8(3)	21.4(3)
τ_0	C(4')-O(1')-C(1')-C(2')	- 9.3(2)	- 10.8(2)	-15.9(2)
τ_1	O(1')-C(1')-C(2')-C(3')	- 19.1(2)	- 16.6(2)	-14.6(2)
τ_2	C(1')-C(2')-C(3')-C(4')	38.9(2)	36.0(2)	37.5(2)
τ_3	C(2')-C(3')-C(4')-O(1')	- 45.7(2)	- 43.6(2)	-48.4(2)
τ_4	C(3')-C(4')-O(1')-C(1')	34.3(2)	33.9(2)	40.2(2)
ψ	O(5')-C(5')-C(4')-C(3')	-172.5(2)	179.7(2)	-70.7(3)
ψ'	C(5')-C(4')-C(3')-O(3')	71.0(3)	73.3(3)	66.8(3)
	Ribose pseudo-rotation parameters			
ϕ	Phase angle from ^3_2T	28°	30°	35°
Q	Amplitude of pucker	0.44Å	0.42Å	0.46Å
	Conformational descriptor	$^3T_4$	$^3T_4$	^3_4T

for two independent molecules, both with C(3')-*endo* riboses, were 18.3° and 24.3°, which values overlap the values found in this structure.

Nucleosides are flexible in conformation about the C(4')-C(5') exocyclic bond of the ribose. All three possible conformations defined by the angle C(3')-C(4')-C(5')-O(5'), *gauche*$^+$ (about 60°), *trans* (about 180°) and *gauche*$^-$ (about 300°), have been observed. In this structure, molecules A and B are *trans*, while molecule C is *gauche*$^-$, and this difference, which constitutes the major conformational dissimilarity among the three molecules, may have implications in the packing scheme discussed below.

Packing Details

Figure 9 is a stereo view of the packing interactions. The head-to-head, tail-to-tail packing of the nucleoside molecules results in alternating layers of hydrogen bonded riboses and stacked, hydrogen bonded bases. The hydrogen bonded bases form two crystallographically distinct ribbons, both parallel to the *b* axis. Figure 10 shows part of the ribbon composed of screw related molecules A. Figure 11 shows the second kind of ribbon, composed of alternating molecules B and C, arranged along a pseudo twofold screw axis. The major conformational difference between molecules B and C is the torsion angle about the C(4')-C(5') bond, the molecules being otherwise quite similar.

A careful examination of the figures reveals that the best position for the pseudo twofold screw axis relating molecules B and C is not quite on the *c* axis, but that the pseudo symmetry is good enough that relocation of the origin to 0,0,1/6 or 0,0,1/3 would result in structures closely

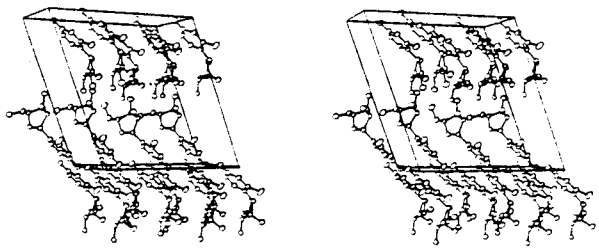


Figure 9. Stereo View of Packing Interactions for 4-Thiopseudouridine. The view is down the b axis.

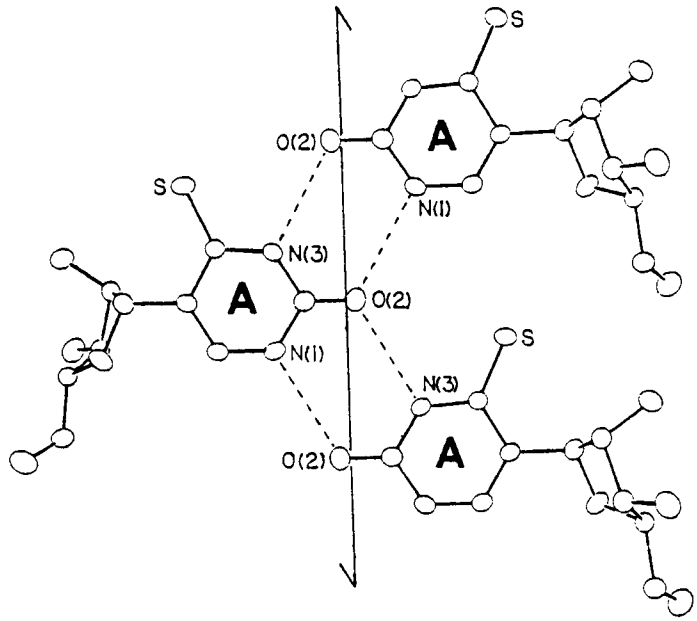


Figure 10. Ribbon Composed of Screw Related Molecules A of 4-Thiopseudouridine. The dotted lines represent hydrogen bonds.

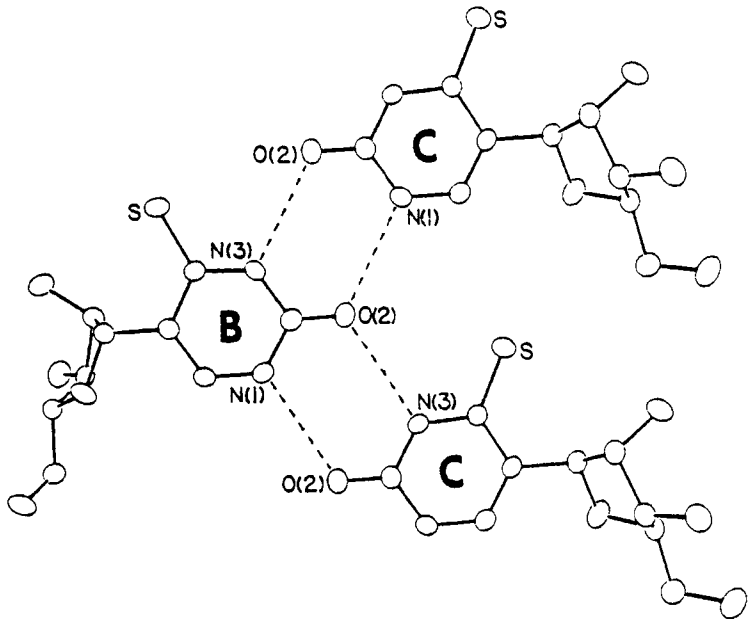


Figure 11. Ribbon Composed of Pseudo Twofold Screw Related Molecules B and C of 4-Thiopseudouridine. The dotted lines represent hydrogen bonds.

resembling the true structure. Such a false solution caused the initial difficulty with the structure determination. The pseudo translational symmetry relating molecules A, B and C is further illustrated in Figure 12. Note that the intermolecular vectors C A and A B are very nearly identical, but that B C does not exactly overlap the other two. Were molecule C not shifted, with the concomitant ribose conformational change, the result would be a new unit cell 1/3 the volume of the observed cell. The packing would then be identical to that found in a number of pyrimidine nucleoside structures.

The hydrogen bond distances and angles are given in Table 7. The sequence of hydrogen bonds involving ribose hydrogens O(2')H--O(3')H--O(5')H--X. In many nucleoside structures the O(5')H--X interaction is weak, as in for example, 2-thiouridine (Hawkinson, 1977), where X is sulfur. In the present case, there are three different types of hydrogen bonds O(5')H--X, where X is O(2'), O(3') or S, and all three interactions are longer than normal. This change in structure must be responsible for a slight lowering in energy, resulting in the crystal structure having three molecules per asymmetric unit rather than one.

Conclusions

It has been previously noted that the substitution of sulfur for oxygen in pyrimidine nucleosides has little effect on the conformational details of the nucleoside (Hawkinson, 1977). As the structure of the beta isomer of pseudouridine has not been reported in the literature, a direct comparison with the present structure is not possible, though the "normal" conformational parameters of 4-thiopseudouridine would seem

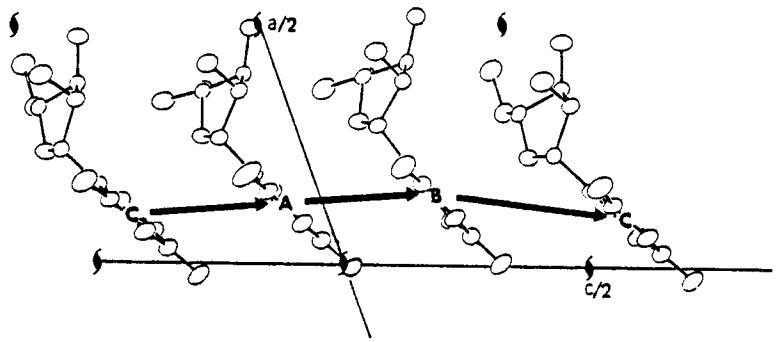


Figure 12. Illustration of the Intermolecular Vectors in the Structure of 4-Thiopseudouridine.

TABLE 7. HYDROGEN BONDING DISTANCES AND ANGLES IN THE STRUCTURE OF 4-THIOPSEUDOURIDINE

Donor (D)	Acceptor (A)	In molecule at	D-A(Å)	H-A(Å)	D-H-A(°)
O(2')a	O(3')a	1-x,½+y,1-z	2.725(3)	1.9	170
O(3')a	O(5')b	1-x,½+y,-z	2.775(4)	2.0	157
O(5')a	S(3)	x,-1+y,-1+z	3.408(3)	2.7	144
O(2')b	O(3')c	1-x,½+y,1-z	2.762(3)	1.9	164
O(3')b	O(5')a	1-x,½+y,-z	2.709(4)	1.9	157
O(5')b	O(2')b	x,-1+y,z	3.039(3)	2.3	140
O(2')c	O(3')b	1-x,½+y,1-z	2.796(3)	2.0	175
O(3')c	O(5')c	1-x,½+y,1-z	2.833(4)	2.0	161
O(5')c	O(3')b	1-x,-½+y,1-z	3.083(3)	2.3	160
N(1)a	O(2)a	-x,-½+y,-z	2.984(4)	2.1	176
N(3)a	O(2)a	-x,½+y,-z	2.955(3)	2.1	177
N(1)b	O(2)c	-x,-½+y,1-z	2.981(4)	2.1	169
N(3)b	O(2)c	-x,½+y,1-z	2.967(4)	2.1	177
N(1)c	O(2)b	-x,-½+y,1-z	2.954(4)	2.1	176
N(3)c	O(2)b	-x,½+y,1-z	2.997(4)	2.2	176

to support this conclusion. The activity of 4-thiopseudouridine as an inhibitor of pseudouridine metabolism may correlate with the greater bond length of the C-S bond, the larger Van der Waals radius for sulfur, or the decreased ability of sulfur to participate as an acceptor in hydrogen bonding, compared to oxygen, or with an altered conformation of the nucleotide as it is bound to the enzyme active site.

PART II: REACTION PRODUCTS OF CHEMICAL MUTAGENS WITH CYTOSINE

CHAPTER I

INTRODUCTION

Bisulfite and a number of nitrogen nucleophiles, including hydroxylamine and semicarbazide, have been shown to be mutagenic in microbial systems (Hayatsu, 1976a; Budowsky, 1976). Bisulfite is a common industrial pollutant, though it is probable that human exposure to bisulfite is primarily from dietary intake of bisulfite as a food additive (Shapiro, 1977). Hydroxylamine and semicarbazide may serve as models for nitrogen nucleophiles of more environmental importance (Hayatsu, 1977).

The mechanism of the mutagenesis caused by these reagents probably involves direct reaction with cytosine residues in polynucleotides, at least in part. Structure determinations of reaction products of these mutagens with cytosine may help to elucidate their mode of action.

CHAPTER II

SODIUM 5,6-DIHYDROURACIL-6-SULFONATE MONOHYDRATE

Introduction

The title compound was obtained as a by-product of the synthesis of N^4-semicarbazido-5,6-dihydrocytosine-6-sulfonate. Nitrogen nucleophiles, such as semicarbazide, and bisulfite are mutagenic in combination, and are believed to induce mutations by reaction with cytosine residues in polynucleotides (Hayatsu, 1976a; Budowsky, 1976). Recently, Hayatsu (1977) has reported a "cooperative" action in the induction of mutations by combinations of bisulfite and several nitrogen nucleophiles, including semicarbazide. As the mechanism of mutation is in question, structure determinations of reaction products of these mutagens with cytosine can be important.

N^4-semicarbazido-5,6-dihydrocytosine-6-sulfonate was synthesized according to Hayatsu (1976b). The identity of the resulting finely divided powder was confirmed by the UV spectrum: UV, in water, λ_{max} = 243 nm, λ_{min} = 214 nm; in 0.1 N NaOH, λ_{max} = 265 nm, λ_{min} = 227 nm. Attempts to recrystallize the material from 0.2 M $NaHSO_3$-Na_2SO_3, pH 7, as suggested by Hayatsu, resulted, in our hands, in well formed crystals of 5,6-dihydrouracil-6-sulfonate. Recrystallization from water and from neutral solutions of semicarbazide also gave the elimination product. The identity of the crystalline material was established by its UV spectrum in water and by its reversal to uracil upon treatment with alkali (Hayatsu, 1976b).

As the apparent lability of the semicarbazide group suggests a significant role for the deaminated product in the mutagenic activity of semicarbazide-bisulfite reactions with cytosine, a structure determination was undertaken.

Cell Data.

Crystals of sodium 5,6-dihydrouracil-6-sulfonate monohydrate ($C_4H_5N_2O_5SNa \cdot H_2O$) are monoclinic, space group $P2_1/c$, with $\underline{a}$ = 5,668(1), $\underline{b}$ = 11.026(2), c = 13.261(2)A, β = 94.62(2) . Calculated and observed densities are 1.884 and 1.87 g cm^{-3}, respectively, for Z = 4.

Experimental

A crystal 0.30x0.35x0.30 mm was selected for diffraction study. The space group and approximate cell dimensions were determined from precession and Weissenberg photographs. More accurate cell dimensions were obtained by least-squares refinement of the first moments of 40 observed 2θ's in the range $45<2\theta<53$ [$rms(\theta_o-\theta_c)=0.026^\circ$], measured with an Oak Ridge computer controlled diffractometer (Busing, Ellison, Levy, King and Roseberry, 1968) using Mo $K\bar{\alpha}$ radiation, λ = 0.71073. The density of the crystals was determined by flotation in mixtures of chloroform and dibromomethane. Intensity data were collected on the diffractometer with Nb-filtered Mo $K\bar{\alpha}$ radiation using the θ-2θ scan technique. Of 1916 reflections with $2\theta<55$, 1735 were considered observed ($|F_o|>3\sigma(F_o)$). Each intensity was assigned a variance, $\sigma^2(I)$, based on counting statistics, plus a term $(0.04\ I)^2$ empirically derived during refinement. No absorption correction was applied (μ=0.45 mm^{-1}).

No significant changes were observed in two standard reflections during the course of the data collection.

The structure was solved with the aid of the direct methods program MULTAN 78 (Main, Hull, Lessinger, Germain, Declercq and Woolfson, 1978), and the hydrogen atoms were located from a difference Fourier synthesis. The atomic parameters were refined by full-matrix least-squares methods. The non-hydrogen atoms were refined with anisotropic thermal parameters and the hydrogen atoms were refined with isotropic thermal parameters. The final values of R, the weighted R $\{[\Sigma w(F_o-F_c)^2/\Sigma wF_o^2]^{1/2}\}$, and σ, the goodness of fit $\{[\Sigma w(F_o-F_c)^2/(n-p)]^{1/2}$, where n = 1735 reflections and p = 155 variables$\}$, were 0.032, 0.042 and 1.54, respectively. All refinements were performed with the XRAY system (Stewart, Kruger, Ammon, Dickinson and Hall, 1972). The final atomic parameters are given in Table A-4. The final anisotropic thermal parameters are given in Table B-4.

Discussion

A drawing of the anion, including bond distances and angles, is shown in Figure 13. The bond distances and angles about the six-membered ring are constant with those observed for outer dihydro-diketo pyrimidine structures [dihydrouracil (Rohrer and Sundaralingam, 1970); dihydrouridine (Sundaralingam, Rao and Abola, 1971); 5,6-dihydro-2-thiouracil (Kojic-Prodic, Ruzic-Toros and Coffou, 1976); 5,6-dihydro-2-thiouracil-6-sulfonate (Jain, Lee, Mertes and Pitman, Pitman, 1978]. As in 5,6-dihydro-2-thiouracil-6-sulfonate, the

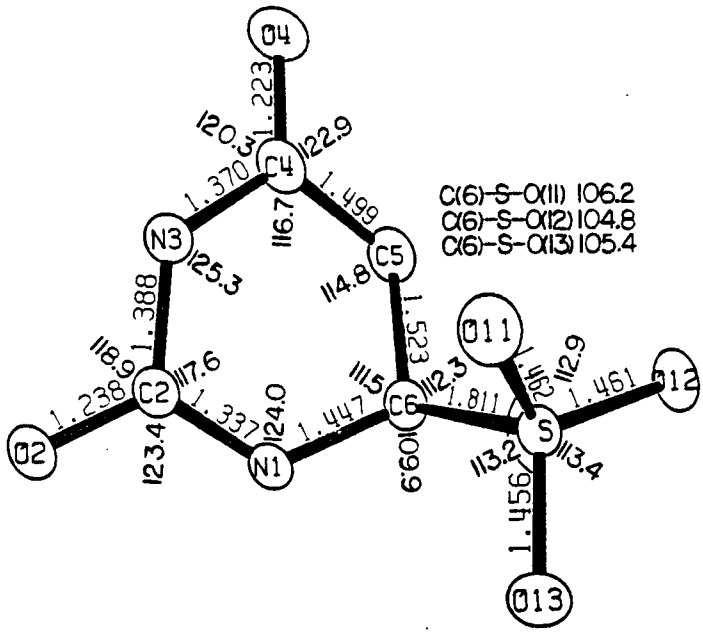


Figure 13. Bond Distances (Å) and Angles (°) for 5,6-Dihydrouracil-6-Sulfonate.

sulfonate group is attached axially to the puckered ring. The C-S and S-O bond distances compare well with those reported for 5,6-dihydro-2-thiouracil-6-sulfonate; 1.818(2) and 1.461(2), 1.464(2) and 1.447(2)Å. In both structures, the sulfonate oxygen atoms are nearly staggered with respect to the substituents on C(6).

That the six-membered ring is significantly puckered is reflected in the torsion angles given in Table 8. The best four atom least-squares displacement plane is through atoms C(2), N(3), C(4) and C(5). The rms deviation of these atoms from the plane is 0.007Å. N(1) and C(6) are displaced on the same side of this plane 0.206 and 0.763Å, compared to 0.197 and 0.729Å and 0.133 and 0.759Å for two independent molecules of dihydrouridine (Sundaralingam, Rao and Abola, 1971). The ring pucker is similar to that observed in 5,6-dihydro-2-thiouracil-6-sulfonate (Jain, Lee, Mertes and Pitman). The rms deviation of atoms N(1), C(2), N(3) and C(4) from their least-squares displacement plane is 0.042Å in the present structure and 0.021Å in 5,6-dihydro-2-thiouracil-6-sulfonate. Atoms C(5) and C(6) are displaced to opposite sides of this plane by 0.167 and 0.486Å in the present structure, and by 0.323 and 0.198A in 5,6-dihydro-2-thiouracil-6-sulfonate.

Figure 14 is a view of the packing interactions. The hydrogen bonds and sodium ion coordination are given in Tables 9 and 10. The anions are packed as hydrogen bonded pairs, related by a center of symmetry. The sodium ion coordination and hydrogen bonds to the water molecule bridge adjacent pairs.

TABLE 8. TORSION ANGLES FOR SODIUM 5,6-DIHYDROURACIL-6-SULFONATE*

A-B-C-D	
C(6)-N(1)-C(2)-N(3)	10.6(2)
N(1)-C(2)-N(3)-C(4)	7.9(2)
C(2)-N(3)-C(4)-C(5)	- 1.3(2)
N(3)-C(4)-C(5)-C(6)	-21.2(2)
C(4)-C(5)-C(6)-N(1)	35.5(2)
C(5)-C(6)-N(1)-C(2)	-31.9(2)

*The positive sense of rotation is clockwise while looking along the BC bond

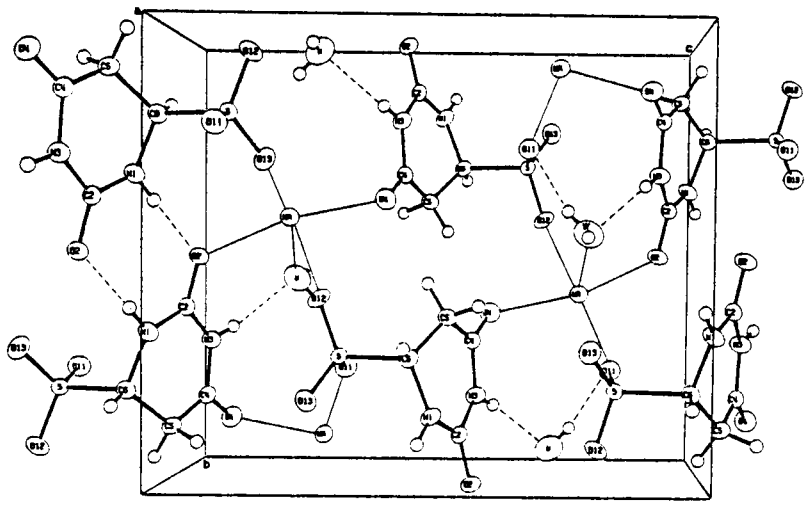


Figure 14. Packing Interactions for Sodium 5,6-Dihydrouracil-6-Sulfonate Monohydrate. The dotted lines represent hydrogen bonds. The fine solid lines represent sodium ion coordination.

TABLE 9. HYDROGEN BONDING DISTANCES AND ANGLES IN THE STRUCTURE OF SODIUM 5,6-DIHYDROURACIL-6-SULFONATE

X-H···Y	X···Y(Å)	H···Y(Å)	X-H···Y(°)
N(1)-H(1)···O(2)[a]	2.803(2)	1.96(3)	177(3)
N(3)-H(3)···O(w)[b]	2.880(3)	2.12(3)	155(3)
O(w)-H(w,1)···O(11)[c]	2.943(2)	2.28(7)	149(6)
O(w)-H(w,2)···O(12)[d]	2.925(2)	2.26(4)	147(4)

[a]Symmetry code: -x,1-y,-z

[b]Symmetry code: 1-x,1/2+y,-1/2-z

[c]Symmetry code: 1-x,-y,-z

[d]Symmetry code: -x,-y,-z

TABLE 10. SODIUM COORDINATION DISTANCES FOR SODIUM 5,6-DIHYDROURACIL-6-SULFONATE

Na-O(2)[a]	2.394(2)
Na-O(4)	2.418(2)
Na-O(11)[b]	2.378(2)
Na-O(12)[c]	2.391(2)
Na-O(13)[d]	2.448(2)
Na-O(w)	2.423(2)

[a]Symmetry code: 1-x,-1/2+y,-1/2-z

[b]Symmetry code: x,1/2-y,-1/2+z

[c]Symmetry code: 1-x,-y,-z

[d]Symmetry code: 1+x,1/2-y,-1/2+z

CHAPTER III

SODIUM CYTOSINE-5-METHYLENESULFONATE TRIHYDRATE

Introduction

Bisulfite has been shown to be mutagenic in a number of microbial systems (Hayatsu, 1976a; Shapiro, 1977). Because bisulfite catalyzes the deamination of cytosine by reversibly adding across the C(5)-C(6) double bond, and because most mutations attributable to bisulfite have been characterized as guanine-cytosine to adenine-thymine base pair transitions, cytosine deamination is believed to be the mechanism of bisulfite mutagenesis.

One system in which bisulfite mediated mutations have been observed, and characterized as guanine-cytosine to adenine-thymine transitions, is the rII system of T4 bacteriophage (Summer and Drake, 1971). However, bacteriophage T4, as do other T-even bacteriophages, contains hydroxymethylcytosine in place of cytosine in its genome. A deaminated product of the reaction of bisulfite with hydroxymethylcytosine has been suggested as the ultimate mutagen in the T4 system (Shapiro, 1977).

It has recently been shown that the major product of the reaction of bisulfite with hydroxymethylcytosine under conditions similar to those employed for the modification of cytosine, namely acid pH and high bisulfite concentration, is cytosine-5-methylenesulfonate rather than a C(6) substituted dihydropyrimidine sulfonate (Hayatsu and Shiragami, 1979).

Cell Data

Crystals of sodium cytosine-5-methylenesulfonate trihydrate ($C_5H_6N_3O_4SNa \cdot 3H_2O$) are monoclinic, space group $P2_1/c$, with $\underline{a}$ = 5.235(1), $\underline{b}$ = 22.843(5), $\underline{c}$ = 9.141(2)Å, β = 90.93(1)°. Calculated and observed densities are 1.709 and 1.71 g cm^{-3}, respectively, for Z=4.

Experimental

Cytosine-5-methylenesulfonate was synthesized according to Hayatsu and Shiragami (1979), and recrystallized from aqueous ethanol as the sodium salt. A crystal 0.32x0.35x0.30 mm was selected for diffraction study. The space group and approximate cell dimensions were determined from precession and Weissenberg photographs. More accurate cell dimensions were obtained with the use of the observed setting angles for 12 Mo $K\alpha_1$ reflections, in the range $40<2\theta<50°$, measured with an Oak Ridge computer controlled diffractometer (Busing, Ellison, Levy, King and Roseberry, 1968). The density of the crystals was determined by flotation in mixtures of chloroform and dibromomethane. Intensity data were collected on the diffractometer with Nb-filtered Mo $K\bar{\alpha}$ radiation using the θ-2θ scan technique. Of 2515 reflections with $2\theta<55°$, 2160 were considered observed ($|F_o|>3\sigma(F_o)$). Each intensity was assigned a variance, $\sigma^2(I)$, based on counting statistics, plus a term, $(0.04\ I)^2$, empirically derived during refinement. No absorption correction was applied (μ=0.37 mm^{-1}). No significant changes in two standard reflections were observed during the course of the data collection.

The structure was solved with the aid of the direct methods program MULTAN 78 (Main, Hull, Lessinger, Germain, Declercq and Woolfson, 1978). The hydrogen atoms were located from a difference electron density map. The atomic parameters were refined by full-matrix least-squares methods, with anisotropic thermal parameters for the non-hydrogen atoms and isotropic thermal parameters for the hydrogen atoms. The final values of R, the weighted $R\{[\Sigma w(F_o-F_c)^2/\Sigma wF_o^2]^{1/2}\}$, and σ, the goodness of fit $\{[\Sigma w(F_o-F_c)^2/(n-p)]^{1/2}$, where n = 2160 reflections and p = 203 variables$\}$, were 0.038, 0.043 and 1.28, respectively. The average shift on the final cycle of refinement was 2% of the estimated standard deviation. All refinements were performed with the XRAY system (Stewart, Kruger, Ammon, Dickinson and Hall, 1972). The final atomic parameters are given in Table A-5. The final anisotropic thermal parameters are given in Table B-5.

Discussion

A drawing of the anion, including bond distances and angles, is shown in Figure 15. The bond distances and angles about the six-membered ring and substituents O(2) and N(4) are consistent with the average values reported in a review for several neutral cytosine structures (Voet and Rich, 1970). The C(7)-S bond length, and the bond lengths and angles about the sulfonate group are comparable to those reported for other sulfonate structures: C-S bond length, 1.774(3) and 1.818(2)Å; average S-O bond length, 1.455 and 1.457Å;

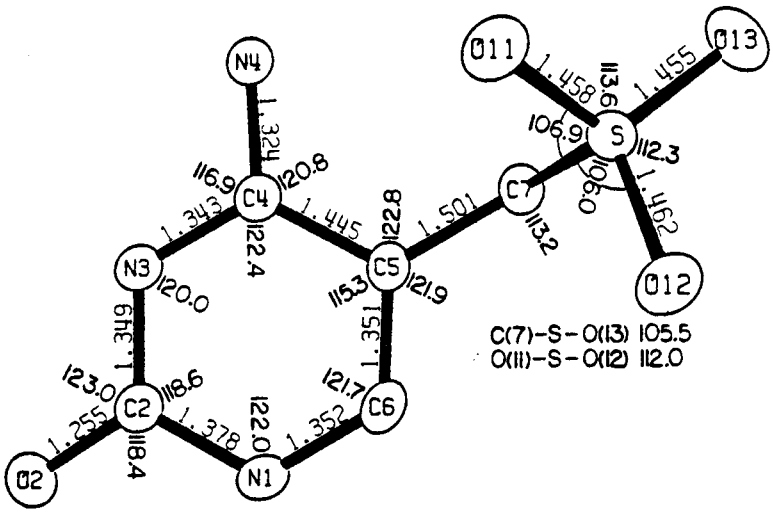


Figure 15. Bond Distances (Å) and Angles (°) for Cytosine-5-Methylenesulfonate.

average C-S-O angle, 105.2 and 105.8°; average O-S-O angle, 113.3 and 112.9°; for, respectively average O-S-O angle, 113.3 and 112.9°; for, respectively, orthanilic acid (Hall and Maslen, 1967) and sodium 5,6-dihydro-2-thiouracil-6-sulfonate (Jain, Lee, Mertes and Pitman, 1978).

Atoms O(2), N(4), C(7) and the ring atoms are nearly co-planar; the standard deviation of these atoms from their least-squares displacement plane is 0.027Å. The sulfonate group is rotated out of the plane. The torsion angles C(4)-C(5)-C(7)-S and C(6)-C(5)-C(7)-S are 90.2(2)° and -91.9(2)°. The sulfonate oxygen atoms are nearly staggered with respect to the substituents on C(7). The angles C(5)-C(7)-S-O are -51.5(2)° for O(11), 68.1(2)° for O(12), and -172.7(1) for O(13).

Figure 16 is a view of the packing interactions. The anions are packed with the aromatic rings stacked, forming columns along the a direction. The closest contacts are N(3)---C(6), 3.446(3)A, N(4)---C(7), 3.431(3)Å, and O(11)---O(12), 3.573(2)Å. The columns of anions are bridged by hydrogen bonds to the water molecules (Table 11) and by coordination to the sodium ion (Table 12). There are no anion-to-anion hydrogen bonds.

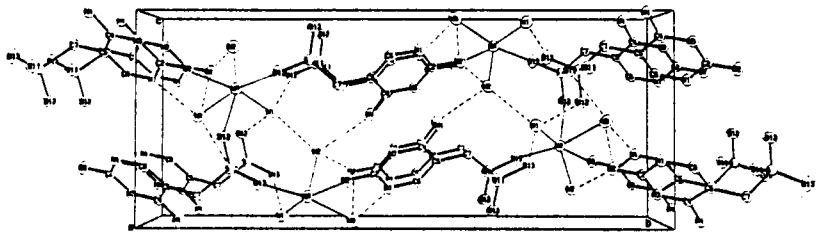


Figure 16. Packing Interactions for Sodium Cytosine-5-Methylene-sulfonate Trihydrate. The dotted lines represent hydrogen bonds. The fine solid lines represent sodium ion coordination.

TABLE 11. HYDROGEN BONDING DISTANCES AND ANGLES IN THE STRUCTURE OF SODIUM CYTOSINE-5-METHYLENESULFONATE

			Distance (Å)		D-H-A
Donor (D)	Acceptor (A)	In molecule at	D-A	H-A	Angle (°)
N(1)	O(w3)	1-x,1-y,1-z	2.802(3)	1.95(3)	169(3)
N(4)	O(w2)	2-x,1-y,1-z	2.870(3)	2.13(3)	140(2)
O(w1)	O(13)	1-x,1-y,1-z	2.909(3)	2.12(4)	155(3)
O(w1)	O(12)	1-x,-1/2+y,1/2-z	2.935(2)	2.11(4)	165(4)
O(w2)	O(2)	-1+x,y,z	2.882(2)	2.06(4)	170(4)
O(w2)	O(w1)	x,y,z	2.786(3)	1.89(5)	172(5)
O(w3)	O(11)	-1+x,y,1+z	2.846(3)	2.04(3)	171(3)
O(w3)	O(2)	2,x1-y,1-z	2.958(3)	2.24(4)	150(4)

TABLE 12. SODIUM COORDINATION DISTANCES FOR SODIUM CYTOSINE-5-METHYLENESULFONATE

Na-O(2)	2.471(2)
Na-O(12)[a]	2.391(2)
Na-O(13)[b]	2.323(2)
Na-O(w1)[c]	2.475(2)
Na-O(w2)	2.344(2)
Na-O(w3)[d]	2.652(2)

[a]Symmetry code: 2-x,1-y,-z

[b]Symmetry code: 2-x,-1/2+y,1/2-z

[c]Symmetry code: x,1/2-y,-1/2+z

[d]Symmetry code: 1-x,1-y,1-z

CHAPTER IV

BISULFITE AND HYDROXYLAMINE MUTAGENESIS: STRUCTURES OF N^4-HYDROXY-5,6-DIHYDROCYTOSINE-6-SULFONATE AND 1-METHYL-N^4-HYDROXY-5,6-DIHYDROCYTOSINE-6-SULFONATE

Introduction

Bisulfite and hydroxylamine (and related nitrogen nucleophiles) have been widely employed for the modification of nucleic acids. Both reagents have been shown to be mutagenic in microbial systems, inducing primarily guanine-cytosine to adenine-thymine base pair transitions (Hayatsu, 1976a; Budowsky, 1976). Bisulfite is known to catalyze the deamination of cytosine, yielding uracil, under mildly alkaline conditions in aqueous media. The mechanism of hydroxylamine mutagenesis is less clear, but has been proposed to involve a modified cytosine residue which resembles uracil or thymine in hydrogen bonding and base pairing capabilities. A problem arises in that hydroxylamine reacts with cytosine to yield more than one product (Budowsky, 1976).

Recently, Hayatsu (1977) has demonstrated a "cooperativity" in the actions of bisulfite and hydroxylamine, both *in vivo* and *in vitro*. The *in vitro* modification of cytosine with a combination of bisulfite and hydroxylamine proceeds at a much higher rate than the reaction with either reagent alone, and yields a single major product. Hayatsu observed a concomitant increase in the mutagenicity of the combination of reagents, relative to that of either reagent alone, in a bacteriophage system.

We have determined the structure of N^4-hydroxy-5,6-dihydrocytosine-6-sulfonate, the product of the action of bisulfite and hydroxylamine on cytosine, and therefore the putative intermediate in the mutations caused by this combination of reagents. The structure of 1-methyl-N^4-hydroxy-5,6-dihydrocytosine-6-sulfonate has also been determined.

Experimental

N^4-Hydroxy-5,6-dihydrocytosine-6-sulfonate (CYTOS) and 1-methyl-N^4-hydroxy-5,6-dihydrocytosine-6-sulfonate (MCYTOS) were prepared essentially by the method of Hayatsu (1977). One millimole of cytosine or 1-methylcytosine was added to 15 milliliters of a solution 1.0 molar with respect to both Na_2SO_3 and $H_2NOH \cdot HCl$, adjusted to pH 6.2. The reaction mixture was refrigerated, and in the case of CYTOS, crystals formed from the solution after several days. MCYTOS did not crystallize after an extended period in the cold, but when the solution was warmed to room temperature, crystals formed overnight. Both compounds were recrystallized from water by slow evaporation at room temperature.

The space groups and approximate cell dimensions were determined from precession and Weissenberg photographs. More accurate cell dimensions were obtained by least-squares refinement with the use of the observed setting angles for eight (CYTOS) and 12 (MCYTOS) Mo $K\alpha_1$ reflections, in the range $45<2\theta<55$, measured with an Oak Ridge computer controlled diffractometer (Busing, Ellison, Levy, King and

Roseberry, 1968). The densities of the crystals were measured by flotation in mixtures of chloroform and dibromomethane. Crystal data are given in Table 13.

Intensity data were collected on the diffractometer with Nb-filtered Mo $K\bar{\alpha}$ radiation, using the θ-2θ scan technique. Of 2589 reflections measured for CYTOS and 2996 reflections measured for MCYTOS ($2\theta<55$), 2325 and 2624, respectively, met the criterion for observed data ($|F_o|>3\sigma(F_o)$). Each intensity was assigned a variance, $\sigma^2(I)$ based on counting statistics, plus a term $(0.04\ I)^2$ empirically derived during refinement. Absorption corrections were not applied [μ(CYTOS)=0.43 mm^{-1}, μ(MCYTOS)=0.43 mm^{-1}]. No significant changes were observed in standard reflections during the course of either data collection.

The structures were solved by direct methods, MCYTOS with MULTAN 78 (Main, Hull, Lessinger, Germain, Declercq and Woolfson, 1978), CYTOS with an earlier version of MULTAN (Germain, Main and Woolfson, 1971). Hydrogen atoms were located from difference Fourier syntheses. The atomic parameters were refined by full-matrix least-squares methods, with anisotropic thermal parameters for the non-hydrogen atoms and isotropic thermal parameters for the hydrogen atoms. All refinements were performed with the XRAY system (Stewart, Kruger, Ammon, Dickinson and Hall, 1972). The final agreement factors are given in Table 14. The final atomic and anisotropic thermal parameters are given in Tables A-6 and B-6.

TABLE 13. CRYSTAL DATA FOR CYTOS AND MCYTOS

	CYTOS	MCYTOS
Empirical Formulae	$C_4H_6N_3O_5SN_a \cdot H_2O$	$C_5H_8N_3O_5SN_a \cdot 4H_2O$
T	299±1°K	299±1°K
λ(Mo $K\alpha_1$)	0.70926Å	0.70926Å
Space Group	$P2_1/c$	$P2_1/c$
a	12.977(2)Å	6.491(1)Å
b	8.384(2)Å	11.129(2)Å
c	8.552(2)Å	17.883(3)Å
β	106.89(1)°	93.50(1)°
V	809.3Å^3	1289.4Å^3
Z	4	4
$D_{obs.}$	1.87 Mg m^{-3}	1.65 Mg m^{-3}
$D_{calc.}$	1.859 Mg m^{-3}	1.634 Mg m^{-3}

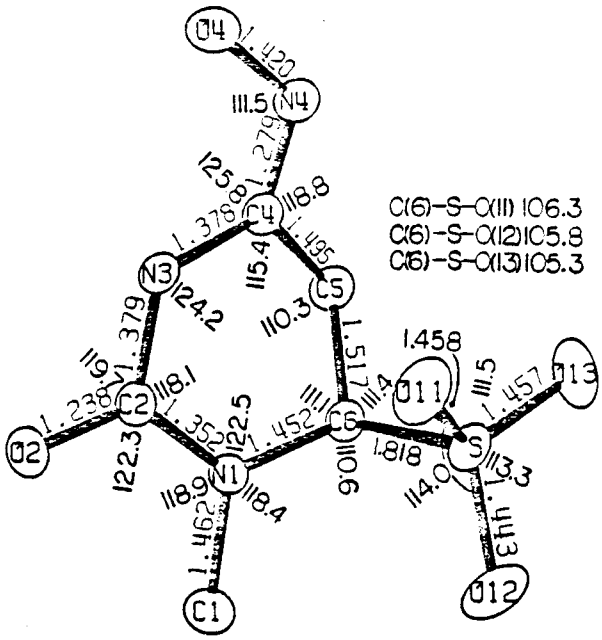
TABLE 14. REFINEMENT PARAMETERS FOR CYTOS AND MCYTOS

	CYTOS	MCYTOS
Reflections measured	2589	2996
Reflections included in refinement ($\|F_o\|>3\sigma(F_o)$) (p)	2325	2624
Number of parameters (p)	169	237
$[\Sigma\|F_o-F_c\|/\Sigma\|F_o\|]$	0.036	0.037
$[\Sigma w(F_o-F_c)^2/\Sigma wF_o^2]^{1/2}$	0.046	0.046
$[\Sigma w(F_o-F_c)^2/(n-p)]^{1/2}$	1.43	1.47
Average shift on the final round of refinement, as a percentage of the estimated standard deviation	7.1%	11.0%

Results

Drawings of the CYTOS and MCYTOS anions, including bond distances and angles, are shown in Figure 17. The bond distances and angles about the six-membered rings are comparable to those observed in several 5,6-dihydrouracil structures [dihydrouracil (Rohrer and Sundaralingam, 1970); dihydrouridine (Sundaralingam, Rao and Abola, 1971); 5-6-dihydro-2-thiouracil (Kojic-Prodic, Ruzic-Toros and Coffou, 1976); 5,6-dihydro-2-thiouracil-6-sulfonate (Jain, Lee, Mertes and Pitman, 1978)]. In particular, the N(3)-C(4) bond lengths and the C(2)-N(3)-C(4) bond angles are consistent with a single bond character for the N(3)-C(4) bond, and these values differ significantly from the average values for several neutral cytosine structures [1.339(7)Å and 120.5(1.3) (Voet and Rich, 1970)]. The C(4)-N(4) bond lengths are significantly shorter than in any of the above cytosine structures (average value, 1.324(20)Å), and compare well with the C=N bond lengths in other oxime structures [1.288Å, 1,5-dimethyl-N^4-hydroxycytosine (Shugar, Huber and Brinbaum, 1976); 1.302Å, 1-methyl-N^4-hydroxycytosine (Birnbaum, Kulikowsky and Shugar, 1979); 1.285Å, anti-4-pyrimidinecarboxaldehyde oxime (Martinez-Ripoll and Lorenz, 1974)].

Table 15 lists the torsion angles about the anions. The O(4) atoms are approximately syn to the ring N(3) atoms, as was also observed in 1,5-dimethyl-N^4-hydroxycytosine and 1-methyl-N^4-hydroxycytosine. The sulfonate groups are attached axially to the puckered rings, and the sulfonate oxygen atoms are nearly staggered with respect to the substituents on C(6).



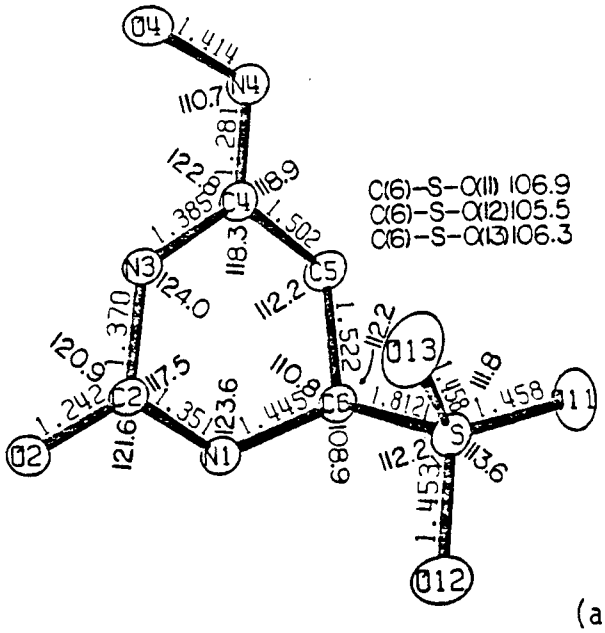


Figure 17. Bond Distances (Å) and Angles (°) for (a) CYTOS and (b) MCYTOS.

TABLE 15. TORSION ANGLES FOR CYTOS AND MCYTOS

Atom-Atom-Atom-Atom	CYTOS	MCYTOS
Ring Angles		
N(1)-C(2)-N(3)-C(4)	4.1(2)	8.1(2)
C(2)-N(3)-C(4)-C(5)	2.8(2)	13.4(2)
N(3)-C(4)-C(5)-C(6)	- 25.9(2)	- 41.1(2)
C(4)-C(5)-C(6)-N(1)	40.7(1)	47.9(2)
C(5)-C(6)-N(1)-C(2)	- 38.3(2)	- 30.1(2)
C(6)-N(1)-C(2)-N(3)	15.6(2)	1.4(2)
Exocyclic Angles		
C(1)-N(1)-C(2)-O(2)		- 2.9(2)
C(1)-N(1)-C(2)-N(3)		176.2(2)
O(2)-C(2)-N(3)-C(4)	-175.6(1)	-172.7(2)
C(2)-N(3)-C(4)-N(4)	179.6(1)	-168.8(2)
N(3)-C(4)-N(4)-O(4)	- 0.4(2)	0.2(3)
O(4)-N(4)-C(4)-C(5)	176.3(1)	177.9(1)
N(4)-C(4)-C(5)-C(6)	157.2(1)	141.0(2)
C(4)-C(5)-C(6)-S	- 81.3(1)	- 75.9(2)
C(5)-C(6)-S-O(11)	- 67.1(1)	70.1(1)
C(5)-C(6)-S-O(12)	171.7(1)	-168.4(1)
C(5)-C(6)-S-O(13)	52.4(1)	- 48.3(1)
C(6)-N(1)-C(2)-O(2)	-164.8(1)	-177.7(2)
O(11)-S-C(6)-N(1)	169.9(1)	- 54.1(1)
O(12)-S-C(6)-N(1)	48.7(1)	67.4(1)
O(13)-S-C(6)-N(1)	- 70.6(1)	-172.4(1)
S-C(6)-N(1)-C(2)	85.6(2)	94.2(2)
S-C(6)-N(1)-C(1)		- 80.6(2)
C(5)-C(6)-N(1)-C(1)		155.1(2)

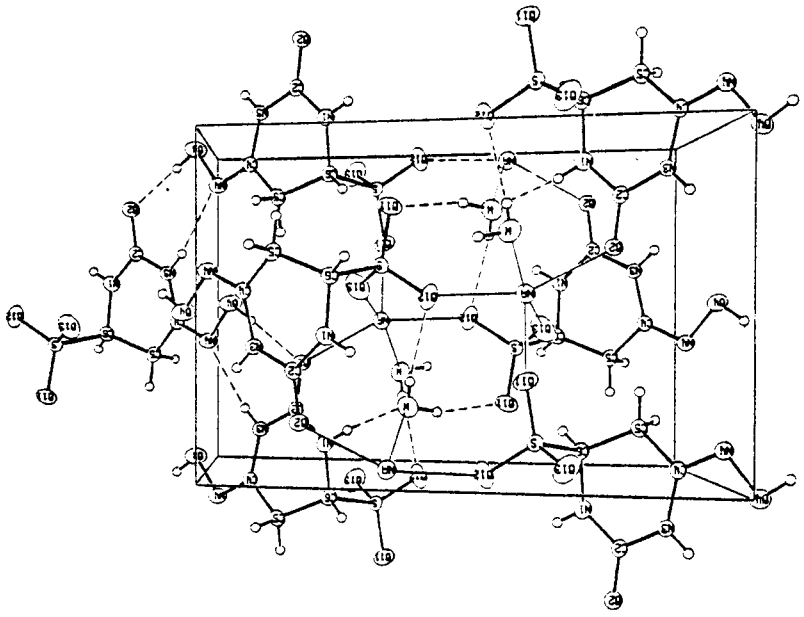
The ring pucker in CYTOS and MCYTOS is similar to that observed in the dihydrouracil structures listed above. Atoms C(5) and C(6) are displaced to opposite sides of the least-squares displacement plane of atoms N(1), C(2), N(3) and C(4) by 0.193 and 0.338A in CYTOS and by 0.459 and 0.164A in MCYTOS. For comparison, in two independent molecules of dihydrouridine, C(5) and C(6) are displaced 0.167 and 0.491A in molecule A and 0.022 and 0.653A in molecule B.

Figure 18 shows views of the packing interactions in CYTOS and MCYTOS. Hydrogen bond and sodium ion parameters are given in Tables 16 and 17. The CYTOS anions are packed as ribbons of screw related anions, along the b direction. Ribbons related by the c glide overlay in the c direction, resulting in layers of anions alternating with channels of solvent and sodium ions along the a direction.

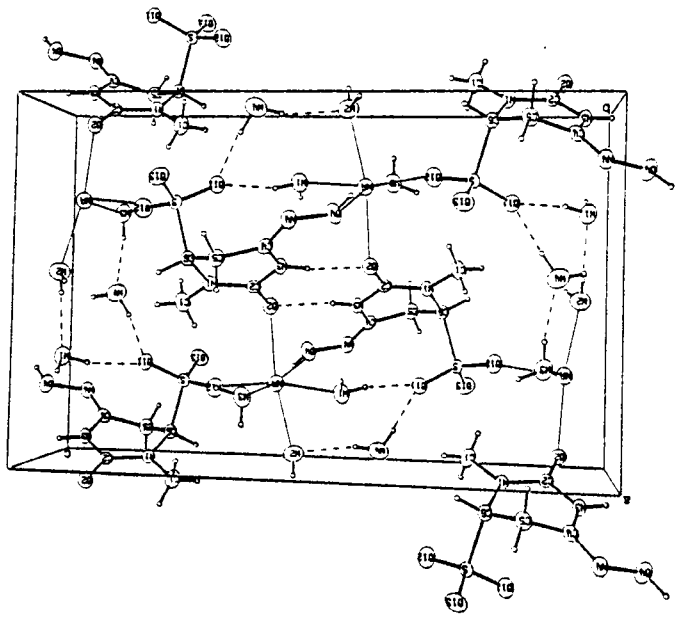
The greater hydration of the MCYTOS crystal, and the 1-methyl substituent, result in markedly different packing interactions. The MCYTOS anions are packed as discrete hydrogen bonded pairs, related by a center of symmetry. Adjacent pairs are bridged by a network of hydrogen bonds to the water molecules, and by coordination to the sodium ion.

Discussion

Hydroxylamine causes primarily guanine-cytosine to adenine-thymine base pair transitions (Budowsky, 1976). Until recently, it has been assumed that a modified cytosine residue, resembling uracil in base pairing, was responsible for the nearly specific mutagenesis caused by



(a)



(b)

Figure 18. Packing Interactions for (a) CYTOS and (b) MCYTOS. The dotted lines represent hydrogen bonds. The fine solid lines represent sodium ion coordination.

TABLE 16. HYDROGEN-BONDING DISTANCES AND ANGLES FOR CYTOS AND MCYTOS

Donor (D)	Acceptor (A)	Symmetry Operation*	D-A (Å)	H-A (Å)	D-H-A (°)
		CYTOS			
N(1)	W(1)	x,-1/2-y,1/2+z	2.960(3)	2.14(3)	172(3)
N(3)	N(4)	2-x,-1/2+y,1/2-z	2.946(2)	2.16(3)	159(3)
O(4)	O(2)	2-x,1/2+y,1/2-z	2.731(2)	1.88(4)	171(3)
W(1)	O(11)	1-x,-y,-z	2.970(2)	2.15(3)	165(3)
W(1)	O(12)	x,y,z	3.065(3)	2.40(4)	170(5)
		MCYTOS			
N(3)	O(2)	1-x,1-y,1-z	2.953(2)	2.13(2)	170(2)
O(4)	W(3)	2-x,1-y,1-z	2.748(2)	1.81(3)	167(3)
W(1)	N(4)	-1+x,y,z	2.889(2)	2.10(3)	168(3)
W(1)	O(11)	x,y,z	2.812(2)	2.02(3)	163(3)
W(2)	W(1)	x,-1+y,z	2.834(2)	2.09(4)	168(4)
W(2)	W(4)	-1+x,1/2-y,1/2+z	2.840(3)	1.84(4)	168(3)
W(3)	W(4)	x,1/2-y,1/2+z	2.749(3)	1.96(4)	167(3)
W(3)	O(13)	2-x,-1/2+y,3/2-z	2.761(2)	1.93(3)	173(3)
W(4)	O(11)	x-3/2-y,-1/2+z	2.839(2)	2.02(4)	158(4)
W(4)	W(2)	1-x,1/2+y,1/2-z	2.823(3)	2.07(5)	162(5)

*The symmetry operation is applied to the acceptor atom coordinates given in Table A-6

TABLE 17. SODIUM COORDINATION DISTANCES FOR CYTOS AND MCYTOS

CYTOS			MCYTOS		
	Symmetry* Operation	Distance (Å)		Symmetry* Operation	Distance (Å)
Na--O(2)	x,y,z	2.382(2)	Na--O(2)	x,y,z	2.440(2)
Na--O(11)	x,-1+y,z	2.323(2)	Na--O(4)	1-x,1-y,1-z	2.494(2)
Na--O(12)	1-x,-1/2+y,1/2-z	2.353(2)	Na--O(12)	1-x,-1/2+y,3/2-z	2.332(2)
Na--O(13)	x,-1/2-y,1/2+z	2.289(2)	Na--W(1)	1-x,1-y,1-z	2.437(2)
Na--W(1)	x,y,z	2.451(2)	Na--W(2)	x,y,z	2.436(2)
			Na--W(3)	x,y,z	2.396(2)

*Symmetry operation applied to atom in Table A-6

this reagent. Such a molecule can be produced by substitution of hydroxylamine at position four of the cytosine ring, if the added N(4) hydroxyl group is located anti to the ring N(3) position, as shown in Figure 19. However, in both the structures reported of such modified cytosines, 1,5-dimethyl-N^4-hydroxycytosine and 1-methyl-N^4-hydroxycytosine, the hydroxyl has been located syn to N(3), as we have found in CYTOS and MCYTOS. Should the combination of bisulfite and hydroxylamine also be shown to cause guanine-cytosine to adenine-thymine transitions, the mechanism of such mutations may be similar to that of hydroxylamine alone. It appears unlikely that either case may be explained entirely by a change in base pairing of the type discussed above.

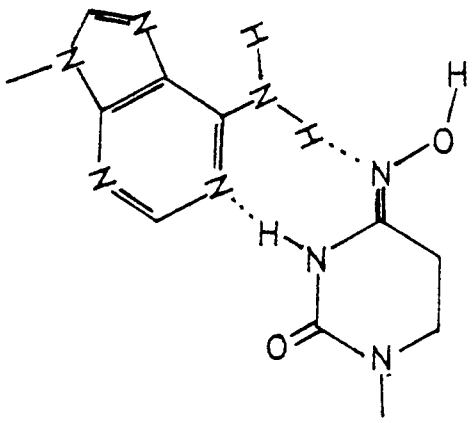


Figure 19. Hypothetical Trans Conformation of Hydroxylamine Modified Cytosine Residue Hydrogen Bonded to Adenine.

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APPENDICES

APPENDIX A

POSITIONAL PARAMETERS

TABLE A-1. POSITIONAL PARAMETERS ($X10^4$) FOR THE NON-HYDROGEN ATOMS OF ADENOSINE-5'-METHYLPHOSPHONATE HEMIHYDRATE

Atom	Molecule A x	Molecule A y	Molecule A z	Molecule B x	Molecule B y	Molecule B z
N(1)	13356(2)	4903(2)	9742(3)	1570(2)	6881(2)	1830(3)
C(2)	12222(2)	4130(3)	9434(3)	1092(2)	5487(3)	2317(3)
N(3)	11241(2)	4637(2)	8360(3)	1672(2)	4426(2)	3482(3)
C(4)	11480(2)	6057(2)	7555(3)	2843(2)	4914(2)	4146(3)
C(5)	12601(2)	6942(2)	7741(3)	3414(2)	6324(2)	3765(3)
C(6)	13630(2)	6319(2)	8912(3)	2706(2)	7391(2)	2568(3)
N(6)	14773(2)	6992(2)	9248(3)	3098(2)	8802(2)	2130(3)
N(7)	12481(2)	8329(2)	6697(3)	4609(2)	6426(2)	4708(3)
C(8)	11298(2)	8271(3)	5918(3)	4748(2)	5088(3)	5608(4)
N(9)	10648(2)	6916(2)	6372(3)	3716(2)	4120(2)	5341(3)
C(1')	9311(2)	6471(3)	5810(3)	3621(2)	2551(2)	6122(3)
C(2')	8304(2)	7264(3)	6419(3)	4597(2)	1705(2)	5485(3)
O(2')	7262(2)	6260(3)	6938(3)	3984(2)	563(2)	4716(2)
O(3')	7836(2)	8320(2)	4701(3)	5497(2)	1181(2)	7176(3)
O(3')	6579(2)	7865(2)	4641(3)	5881(2)	- 248(2)	7418(2)
O(4')	7973(2)	7469(2)	3310(3)	4708(2)	1223(2)	8679(3)
O(1')	9154(2)	6780(2)	3926(2)	3816(2)	2408(2)	7975(2)
O(5')	8040(2)	8333(3)	1444(3)	5465(3)	1482(3)	10357(3)
O(5')	8986(2)	9548(2)	1423(3)	6353(2)	2753(2)	9967(3)
P	10000(0)	10000(0)	0(0)	6647(1)	3846(1)	11294(1)
O(6)	11136(2)	10760(2)	853(3)	7382(4)	5153(3)	10325(3)
O(7)	10267(2)	8623(2)	- 554(2)	5398(2)	4089(3)	11891(3)
O(9)	9229(3)	11183(4)	-1864(4)	7621(3)	2889(4)	13162(4)

	Water x	Water y	Water z
O(W)	541(3)	1421(2)	4017(3)

TABLE A-2. POSITIONAL PARAMETERS (X 10^4) FOR NON-HYDROGEN ATOMS OF DISODIUM GMP HEPTAHYDRATE*

Atom	Molecule A			Molecule B		
	x	y	z	x	y	z
N(1)	4112(2)	- 627(2)	3187(5)	5924(2)	6711(2)	-1791(5)
C(2)	3681(2)	- 286(2)	2480(6)	6312(3)	6350(3)	-2603(6)
N(2)	3159(2)	- 590(2)	2259(8)	6847(2)	6609(2)	-2945(7)
N(3)	3753(2)	298(2)	2046(5)	6203(2)	5768(2)	-3014(5)
C(4)	4305(2)	516(2)	2354(6)	5643(2)	5587(2)	-2618(6)
C(5)	4772(2)	206(3)	3033(6)	5203(2)	5925(2)	-1887(6)
C(6)	4689(2)	- 423(3)	3487(7)	5339(2)	6543(2)	-1406(6)
O(6)	5046(2)	- 784(2)	4103(6)	5018(2)	6919(2)	- 753(6)
N(7)	5280(2)	586(2)	3121(6)	4683(2)	5577(2)	-1756(5)
C(8)	5111(3)	1109(3)	2497(7)	4815(3)	5041(3)	-2366(7)
N(9)	4528(2)	1101(2)	2041(5)	5392(2)	5013(2)	-2910(5)
C(1')	4219(2)	1620(2)	1294(6)	5673(2)	4496(2)	-3658(6)
C(2')	4190(2)	2211(2)	2236(6)	5708(2)	3895(2)	-2740(6)
O(2')	3669(2)	2185(2)	3157(4)	6230(2)	3915(2)	-1851(5)
C(3')	4191(2)	2731(2)	1076(6)	5711(2)	3396(2)	-3934(6)
O(3')	3587(2)	2802(2)	565(5)	6306(2)	3336(2)	-4449(5)
C(4')	4596(2)	2454(2)	- 105(6)	5298(2)	3669(2)	-5127(6)
O(1')	4556(2)	1781(2)	21(4)	5321(2)	4344(2)	-4928(4)
C(5')	5246(2)	2660(2)	- 78(6)	4661(2)	3449(3)	-5104(6)
O(5')	5521(2)	2544(2)	1341(4)	4392(2)	3552(2)	-3689(4)
O(6')	6360(2)	3296(2)	764(4)	3546(2)	2802(2)	-4213(4)
O(7')	6552(2)	2139(2)	589(4)	3379(2)	3957(2)	-4507(5)
O(8')	6366(2)	2635(2)	3083(4)	3541(2)	3496(2)	-1948(4)
P	6243(0)	2657(1)	1440(1)	1921(2)	3672(0)	-3586(1)

Atom	Solvent		
	x	y	z
NA(1)	6149(1)	579(1)	-5282(3)
NA(2)	7000(1)	1740(1)	-3382(3)
NA(3)	3869(1)	4509(1)	992(3)
NA(4)	7138(1)	3414(2)	-2943(5)
W(1)	6159(2)	1671(2)	-5001(5)
W(2)	2997(2)	3669(2)	2681(5)
W(3)	2416(2)	3832(2)	- 933(5)
W(4)	3077(2)	5128(2)	-3297(6)
W(5)	2377(2)	2499(2)	-4908(6)
W(6)	7308(2)	2514(2)	-1577(5)
W(7)	3820(2)	4455(2)	- 67(5)
W(8)	6705(2)	283(2)	2430(6)
W(9)	6314(2)	1299(2)	-1584(6)
W(10)	7163(2)	562(2)	-4527(6)
W(11)	7065(3)	5127(3)	- 774(9)
W(12)	2980(3)	1151(4)	3949(12)
W(13)	2569(5)	1066(5)	537(10)
W(14)	4129(4)	4949(4)	-5369(9)

*Standard deviations in units of the last significant digits are given in parentheses

TABLE A-3. POSITIONAL PARAMETERS (X 10^4) FOR NON-HYDROGEN ATOMS OF 4-THIOPSEUDOURIDINE*

Atom	x	y	z
		Molecule A	
N(1)	845(2)	- 653(3)	- 523(2)
C(2)	500(2)	880(4)	- 274(2)
O(2)	- 54(2)	879(3)	155(2)
N(3)	820(2)	2438(3)	- 535(2)
C(4)	1457(2)	2589(4)	- 994(2)
S	1749(1)	4585(0)	-1266(1)
C(5)	1821(2)	917(4)	-1188(2)
C(6)	1497(2)	- 626(4)	- 955(2)
C(1')	2572(2)	964(4)	-1623(2)
C(2')	3521(2)	1412(4)	- 871(2)
O(2')	4018(1)	2504(3)	-1302(1)
C(3')	3955(2)	- 465(4)	- 674(2)
O(3')	4924(1)	- 458(3)	- 210(1)
C(4')	3614(2)	-1272(4)	-1674(2)
O(1')	2651(1)	- 777(3)	-2020(1)
C(5')	3723(2)	-3258(4)	-1693(2)
O(5')	3503(1)	-3916(3)	-2650(2)
		Molecule B	
N(1)	960(2)	- 483(4)	2552(2)
C(2)	623(2)	1048(4)	2804(2)
O(2)	59(1)	1067(3)	3216(2)
N(3)	964(2)	2611(3)	2558(2)
C(4)	1638(2)	2736(4)	2140(2)
S	1926(1)	4923(1)	1838(1)
C(5)	2035(2)	1062(4)	2010(2)
C(6)	1669(2)	- 475(4)	2209(2)
C(1')	2856(2)	1083(4)	1689(2)
C(2')	3750(2)	1592(4)	2515(2)
O(2')	4291(1)	2725(3)	2147(2)
C(3')	4221(2)	- 230(4)	2777(2)
O(3')	5181(1)	- 134(3)	3321(1)
C(4')	3957(2)	-1132(4)	1798(2)
O(1')	2998(1)	- 685(3)	1363(1)
C(5')	4071(2)	-3137(4)	1856(2)
O(5')	3810(2)	-3896(3)	919(2)
		Molecule C	
N(1)	656(2)	- 514(4)	6308(2)
C(2)	305(2)	1030(4)	6544(2)
O(2)	- 245(1)	1054(3)	6975(2)
N(3)	618(2)	2581(3)	6261(2)

TABLE A-3 (Continued)

Atom	x	y	z
C(4)	1248(2)	2694(4)	5792(2)
S	1572(1)	5792(1)	5529(1)
C(5)	1585(2)	1019(4)	5577(2)
C(6)	1280(2)	- 516(4)	5848(2)
C(1')	2286(2)	1010(4)	5074(2)
C(2')	3293(2)	1356(4)	5751(2)
O(2')	3731(1)	2492(3)	5276(2)
C(3')	3703(2)	- 526(4)	5807(2)
O(3')	4684(1)	- 582(3)	6156(2)
C(4')	3221(2)	-1168(4)	4785(2)
O(1')	2282(1)	- 714(3)	4637(1)
C(5')	3306(2)	-3142(5)	4585(2)
O(5')	4231(2)	-3488(4)	4644(2)

*Standard deviations in units of the last significant digits are given in parentheses

TABLE A-4. POSITIONAL PARAMETERS (X 10^4) FOR NON-HYDROGEN ATOMS OF SODIUM 5,6-DIHYDROURACIL MONOHYDRATE*

Atom	x	y	z
N(1)	634(3)	3374(1)	- 38(1)
C(2)	2420(3)	3868(2)	- 496(1)
O(2)	2520(3)	4958(1)	- 712(1)
N(3)	4223(3)	3104(1)	- 753(1)
C(4)	4202(3)	1864(2)	- 683(1)
O(4)	5827(3)	1267(1)	- 980(1)
C(5)	2069(3)	1301(2)	- 275(1)
C(6)	650(3)	2148(2)	353(1)
S	1718(1)	2146(1)	1678(1)
O(11)	4217(2)	2484(1)	1714(1)
O(12)	1354(2)	904(1)	2016(1)
O(13)	267(3)	3032(1)	2157(1)
Na	6589(1)	836(1)	-2712(1)
O(w)	3222(3)	- 525(2)	-2842(2)

*Standard deviations in units of the last significant digits are given in parentheses

TABLE A-5. POSITIONAL PARAMETERS (X 10^4) FOR NON-HYDROGEN ATOMS OF SODIUM CYTOSINE-5-METHYLENE-SULFONATE TRIHYDRATE*

Atom	x	y	z
N(1)	9099(4)	4711(1)	1830(2)
C(2)	11176(4)	4469(1)	2552(2)
O(2)	11737(3)	3746(1)	2291(2)
N(3)	12506(3)	4804(1)	3511(2)
C(4)	11739(4)	5354(1)	3787(2)
N(4)	13075(4)	5651(1)	4780(2)
C(5)	9528(4)	5611(1)	3073(2)
C(6)	8287(4)	5261(1)	2108(2)
C(7)	8610(4)	6219(1)	3408(2)
S	10005(1)	6767(1)	2274(1)
O(11)	12763(3)	6682(1)	2355(2)
O(12)	8979(3)	6664(1)	799(2)
O(13)	9172(3)	7324(1)	2873(2)
Na	8751(2)	3179(1)	1416(1)
O(w1)	5203(4)	2400(1)	5331(2)
O(w2)	6135(4)	3329(1)	3438(2)
O(w3)	4121(3)	6007(1)	9868(2)

*Standard deviations in units of the last significant digits are given in parentheses

TABLE A-6. POSITIONAL PARAMETERS (X 10^4) FOR NON-HYDROGEN ATOMS OF CYTOS AND MCYTOS

Atom	MCYTOS x	MCYTOS y	MCYTOS z	CYTOS x	CYTOS y	CYTOS z
C(1)	5809(3)	4478(2)	7387(1)			
N(1)	7081(2)	5008(1)	6826(1)	7535(1)	- 822(2)	4266(2)
C(2)	6414(3)	4951(1)	6096(1)	8157(1)	-1802(2)	3691(2)
O(2)	4804(2)	4423(1)	5883(1)	8039(1)	-3289(2)	3703(2)
N(3)	7571(2)	5517(1)	5579(1)	8931(1)	-1152(2)	3096(2)
C(4)	9488(3)	6016(2)	5745(1)	9155(1)	466(2)	3142(2)
N(4)	10465(2)	6701(2)	5314(1)	9888(1)	1059(2)	2586(2)
O(4)	9320(2)	6902(1)	4622(1)	10413(1)	- 155(2)	1958(2)
C(5)	10497(3)	5673(2)	6488(1)	8548(1)	1553(2)	3957(2)
C(6)	8926(3)	5663(2)	7082(1)	7457(1)	871(2)	3937(2)
S	8276(1)	7178(1)	7365(1)	6441(1)	1214(1)	2006(1)
O(11)	7089(2)	7710(1)	6731(1)	6252(1)	2929(2)	1870(2)
O(12)	7132(3)	7043(1)	8027(1)	5503(1)	301(2)	2092(2)
O(13)	10249(3)	7786(1)	7509(1)	6891(1)	622(2)	739(2)
Na	4135(1)	2269(1)	5789(1)	6344(1)	-4606(1)	3144(1)
W(1)	4428(2)	7890(1)	5435(1)	5864(1)	-2943(2)	680(2)
W(2)	2836(3)	254(2)	5510(1)			
W(3)	7707(2)	2019(2)	6178(1)			
W(4)	8859(3)	5331(2)	981(1)			

TABLE A-6. POSITIONAL PARAMETERS (X 10^4) FOR NON-HYDROGEN ATOMS OF CYTOS AND MCYTOS

Atom	MCYTOS x	MCYTOS y	MCYTOS z	CYTOS x	CYTOS y	CYTOS z
C(1)	5809(3)	4478(2)	7387(1)			
N(1)	7081(2)	5008(1)	6826(1)	7535(1)	- 822(2)	4266(2)
C(2)	6414(3)	4951(1)	6096(1)	8157(1)	-1802(2)	3691(2)
O(2)	4804(2)	4423(1)	5883(1)	8039(1)	-3289(2)	3703(2)
N(3)	7571(2)	5517(1)	5579(1)	8931(1)	-1152(2)	3096(2)
C(4)	9488(3)	6016(2)	5745(1)	9155(1)	466(2)	3142(2)
N(4)	10465(2)	6701(2)	5314(1)	9888(1)	1059(2)	2586(2)
O(4)	9320(2)	6902(1)	4622(1)	10413(1)	- 155(2)	1958(2)
C(5)	10497(3)	5673(2)	6488(1)	8548(1)	1553(2)	3957(2)
C(6)	8926(3)	5663(2)	7082(1)	7457(1)	871(2)	3937(2)
S	8276(1)	7178(1)	7365(1)	6441(1)	1214(1)	2006(1)
O(11)	7089(2)	7710(1)	6731(1)	6252(1)	2929(2)	1870(2)
O(12)	7132(3)	7043(1)	8027(1)	5503(1)	301(2)	2092(2)
O(13)	10249(3)	7786(1)	7509(1)	6891(1)	622(2)	739(2)
Na	4135(1)	2269(1)	5789(1)	6344(1)	-4606(1)	3144(1)
W(1)	4428(2)	7890(1)	5435(1)	5864(1)	-2943(2)	680(2)
W(2)	2836(3)	254(2)	5510(1)			
W(3)	7707(2)	2019(2)	6178(1)			
W(4)	8859(3)	5331(2)	981(1)			

TABLE B-1. ANISOTROPIC THERMAL PARAMETERS ($\AA^2 \times 10^4$) FOR NON-HYDROGEN ATOMS OF ADENOSINE-5'-METHYLPHOSPHONATE HEMIHYDRATE

Atom	U_{11}	U_{22}	U_{33}	U_{12}	U_{13}	U_{23}
			Molecule A			
N(1)	270(9)	242(9)	305(9)	74(7)	0(7)	- 19(7)
C(2)	341(12)	240(10)	320(11)	41(8)	48(9)	- 17(8)
N(3)	295(9)	245(9)	334(10)	4(7)	30(8)	- 32(8)
C(4)	252(10)	242(10)	230(9)	28(8)	16(7)	- 28(8)
C(5)	267(10)	237(10)	250(10)	27(8)	41(8)	- 34(8)
C(6)	236(10)	252(10)	269(10)	32(8)	43(8)	- 76(8)
N(6)	258(9)	289(10)	460(12)	47(8)	-371(8)	- 35(9)
N(7)	263(9)	253(10)	369(11)	10(7)	22(8)	- 2(8)
C(8)	273(11)	267(11)	369(12)	21(9)	5(9)	19(9)
N(9)	219(8)	260(9)	300(9)	11(7)	- 8(7)	- 6(7)
C(1')	226(10)	332(12)	301(11)	- 23(8)	- 24(8)	- 26(9)
C(2')	221(10)	514(15)	235(10)	- 38(10)	17(8)	- 97(10)
O(2')	261(9)	803(16)	297(9)	-145(10)	4(7)	121(10)
C(3')	218(9)	264(10)	299(11)	- 10(8)	62(8)	- 97(8)
O(3')	253(8)	322(9)	451(10)	31(6)	85(7)	-153(7)
C(4')	225(9)	254(10)	243(10)	21(8)	11(8)	- 61(8)
O(1')	312(9)	471(10)	281(8)	152(8)	- 24(7)	-134(8)
C(5')	350(12)	304(11)	278(11)	- 17(9)	34(9)	- 55(9)
O(5')	617(13)	340(10)	420(11)	-169(9)	238(10)	-145(8)
P	327(3)	254(2)	261(3)	5(2)	21(2)	0(2)
O(6)	409(10)	322(9)	505(11)	- 28(8)	- 32(8)	- 90(8)
O(7)	405(10)	376(9)	293(8)	61(8)	- 19(7)	- 90(7)
C(9)	518(17)	441(16)	442(16)	107(13)	- 35(13)	94(13)
			Molecule B			
N(10)	294(9)	264(9)	280(9)	49(7)	- 7(7)	- 60(7)
C(2)	271(10)	308(11)	328(11)	21(9)	7(9)	-127(9)
N(3)	274(9)	252(9)	360(10)	- 8(7)	- 1(7)	- 89(8)
C(4)	263(10)	248(10)	263(10)	42(8)	36(8)	- 60(8)
C(5)	261(10)	255(10)	279(10)	8(8)	40(8)	- 75(8)
C(6)	311(11)	245(10)	240(10)	21(8)	43(8)	- 59(8)
N(6)	381(11)	254(10)	378(11)	- 16(8)	- 9(9)	- 42(8)
N(7)	278(10)	264(10)	385(11)	- 5(8)	- 6(8)	- 65(8)
C(8)	292(11)	288(11)	396(13)	22(9)	- 43(10)	- 66(10)
N(9)	279(9)	232(9)	346(10)	31(7)	- 3(8)	- 51(8)
C(1')	261(10)	232(10)	323(11)	0(7)	51(8)	- 45(8)
C(2')	276(10)	226(10)	265(10)	- 11(8)	62(8)	- 53(8)
O(2')	369(9)	339(9)	340(9)	- 61(7)	37(7)	-154(7)
C(3')	246(10)	216(9)	285(10)	- 4(8)	55(8)	- 71(8)
C(3')	437(10)	273(8)	348(9)	119(7(	62(7)	- 83(7)
C(4')	359(12)	205(10)	274(11)	21(8)	76(9)	- 49(8)

TABLE B-1 (Continued)

Atom	U_{11}	U_{22}	U_{33}	U_{12}	U_{13}	U_{23}
O(1')	457(10)	383(10)	298(9)	173(8)	95(8)	- 57(7)
C(5')	524(15)	253(11)	286(11)	6(10)	42(10)	- 68(9)
O(5')	570(12)	411(11)	343(10)	-120(9)	124(9)	-174(8)
P	490(4)	247(3)	200(2)	1(2)	- 23(2)	- 26(2)
O(6)	1388(28)	517(14)	303(11)	-473(17)	110(13)	- 66(10)
O(7)	608(13)	533(13)	369(10)	316(11)	-123(9)	-147(9)
C(9)	409(16)	646(21)	412(16)	144(14)	- 43(12)	- 12(14)
			Water			
O(w)	647(15)	357(11)	546(13)	- 9(10)	72(11)	- 92(9)

TABLE B-2. ANISOTROPIC THERMAL PARAMETERS (X 10^3) FOR NON-HYDROGEN ATOMS OF DISODIUM GMP HEPTAHYDRATE

Atom	U_{11}	U_{22}	U_{33}	U_{12}	U_{13}	U_{23}
N(2)a	37(3)	29(3)	78(4)	- 9(2)	- 12(3)	9(3)
O(6)a	45(3)	26(2)	77(4)	- 1(2)	- 25(3)	16(2)
O(2')a	34(2)	36(2)	29(2)	- 6(2)	6(2)	- 1(2)
(3')a	24(2)	35(2)	34(2)	8(2)	0(2)	10(2)
N(2)b	37(3)	28(2)	66(4)	- 11(2)	6(3)	10(3)
O(6)b	45(2)	29(2)	65(3)	0(2)	16(2)	-19(2)
O(2')b	35(2)	40(2)	35(2)	- 2(2)	- 5(2)	7(2)
O(3')b	30(2)	50(2)	55(3)	8(2)	4(2)	-21(2)
NA(1)	29(1)	29(1)	45(1)	- 1(1)	- 4(1)	5(1)
NA(2)	36(1)	47(1)	36(1)	- 6(1)	- 1(1)	0(1)
NA(3)	34(1)	36(1)	37(1)	3(1)	2(1)	1(1)
NA(4)	39(1)	71(2)	103(3)	- 6(1)	- 7(2)	38(2)
W(1)	32(2)	34(2)	43(2)	- 2(2)	- 1(2)	9(2)
W(2)	41(2)	33(2)	39(2)	11(2)	- 2(2)	- 2(2)
W(3)	32(2)	37(2)	43(2)	- 2(2)	7(2)	- 4(2)
W(4)	41(2)	40(2)	59(3)	3(2)	4(2)	- 2(2)
W(5)	24(2)	62(3)	45(2)	- 7(2)	- 1(2)	2(2)
W(6)	38(2)	51(3)	38(2)	1(2)	7(2)	1(2)
W(7)	33(2)	32(2)	47(2)	- 1(2)	- 1(2)	- 7(2)
W(8)	43(2)	53(3)	51(3)	- 7(2)	- 8(2)	16(2)
W(9)	40(2)	49(3)	54(32)	- 4(2)	- 6(2)	-12(2)
W(10)	35(2)	71(3)	52(3)	- 1(2)	- 10(2)	- 6(3)
W(11)	61(3)	77(4)	94(5)	9(3)	- 10(4)	13(4)
W(12)	60(4)	111(6)	179(10)	- 26(4)	- 18(5)	64(7)
W(13)	196(10)	161(9)	113(7)	-129(8)	103(7)	-77(7)
W(14)	90(5)	102(6)	94(6)	12(4)	- 1(5)	22(5)

TABLE B-3. ANISOTROPIC THERMAL PARAMETERS ($Å^2 \times 10^4$) FOR NON-HYDROGEN ATOMS OF 4-THIOPSEUDOURIDINE

Atom	U_{11}	U_{22}	U_{33}	U_{12}	U_{13}	U_{23}
			Molecule A			
N(1)	350(13)	194(13)	483(15)	- 38(10)	232(12)	4(11)
C(2)	267(13)	234(14)	335(15)	- 2(12)	121(11)	11(12)
O(2)	336(10)	324(12)	486(13)	- 35(10)	256(09)	- 13(11)
N(3)	290(12)	186(11)	437(14)	10(10)	204(11)	- 27(11)
C(4)	249(14)	199(14)	385(16)	- 5(11)	157(12)	- 3(12)
S	549(05)	183(04)	911(07)	- 6(04)	505(05)	26(05)
C(5)	244(13)	194(13)	357(15)	14(12)	132(11)	- 15(12)
C(6)	297(14)	187(14)	418(16)	6(11)	176(13)	- 18(12)
C(1')	223(12)	258(14)	289(13)	20(12)	97(10)	- 20(12)
C(2')	243(12)	245(14)	282(13)	- 40(11)	121(10)	- 55(11)
O(2')	330(11)	254(11)	429(12)	- 82(09)	168(09)	- 40(10)
C(3')	231(12)	278(14)	231(12)	14(12)	84(10)	21(12)
O(3')	244(09)	389(12)	311(11)	1(10)	58(09)	90(10)
C(4')	226(12)	214(13)	256(13)	11(11)	108(10)	5(11)
O(1')	220(09)	274(11)	316(10)	41(08)	62(08)	- 84(08)
C(5')	328(15)	222(13)	412(16)	15(12)	178(13)	21(13)
O(5')	344(11)	353(12)	537(13)	- 42(11)	171(10)	-164(12)
			Molecule B			
N(1)	304(12)	218(12)	403(13)	- 20(11)	176(10)	20(12)
C(2)	260(13)	258(14)	298(14)	- 24(12)	103(11)	2(13)
O(2)	388(12)	325(12)	526(13)	- 38(11)	304(10)	- 13(11)
N(3)	271(11)	204(12)	419(14)	17(10)	184(10)	- 32(11)
C(4)	224(12)	201(13)	309(14)	- 31(11)	95(11)	- 12(12)
S	463(04)	193(03)	643(05)	- 9(04)	323(04)	23(04)
C(5)	230(12)	227(13)	274(13)	5(12)	92(10)	- 12(12)
C(6)	278(13)	197(13)	367(15)	14(12)	139(11)	- 13(13)
C(1')	224(12)	222(13)	299(13)	6(12)	112(10)	- 16(12)
C(2')	265(13)	239(14)	286(14)	- 6(12)	105(11)	- 34(12)
O(2')	317(10)	252(11)	456(12)	- 89(09)	137(09)	- 11(10)
C(3')	284(13)	269(13)	259(13)	10(14)	143(11)	38(13)
O(3')	282(10)	420(13)	324(10)	15(11)	93(08)	78(11)
C(4')	254(14)	224(13)	316(15)	16(11)	156(12)	12(12)
O(1')	254(09)	267(12)	394(11)	24(08)	103(08)	-105(09)
C(5')	340(16)	233(14)	486(19)	2(13)	224(14)	7(14)
O(5')	496(13)	259(11)	646(15)	- 22(11)	306(12)	-152(12)
			Molecule C			
N(1)	321(12)	219(12)	428(14)	- 12(11)	192(11)	32(12)
C(2)	267(14)	276(14)	341(15)	- 9(13)	143(12)	3(13)
O(2)	362(11)	329(12)	491(13)	- 8(11)	278(10)	- 4(11)

TABLE B-3 (Continued)

Atom	U_{11}	U_{22}	U_{33}	U_{12}	U_{13}	U_{23}
N(3)	291(12)	198(11)	445(14)	22(10)	193(11)	- 13(11)
C(4)	220(13)	235(13)	339(15)	- 2(12)	120(11)	- 11(12)
S	438(04)	229(04)	646(05)	- 21(04)	311(04)	24(04)
C(5)	224(12)	253(13)	301(13)	34(12)	107(11)	- 15(13)
C(6)	287(14)	240(15)	384(16)	28(13)	159(12)	- 8(14)
C(1')	246(12)	280(14)	245(13)	- 6(12)	104(10)	- 33(12)
C(2')	239(13)	311(15)	311(14)	- 18(12)	118(11)	- 41(13)
O(2')	372(11)	271(11)	534(13)	-105(10)	188(10)	- 18(11)
C(3')	246(12)	302(15)	256(13)	11(12)	98(10)	44(13)
O(3')	244(10)	410(14)	439(12)	8(10)	80(09)	108(11)
C(4')	285(14)	294(15)	262(14)	6(12)	150(11)	- 2(12)
O(1')	256(10)	392(14)	357(11)	18(09)	87(08)	-146(10)
C(5')	395(17)	307(16)	420(18)	3(14)	196(14)	- 44(14)
O(5')	445(13)	462(15)	582(15)	117(12)	259(12)	- 38(13)

TABLE B-4. ANISOTROPIC THERMAL PARAMETERS ($\AA^2 \times 10^4$) FOR NON-HYDROGEN ATOMS OF SODIUM 5,6-DIHYDROURACIL-6-SULFONATE MONOHYDRATE

Atom	U_{11}	U_{22}	U_{33}	U_{12}	U_{13}	U_{23}
N1	316(8)	204(7)	252(7)	96(6)	86(6)	73(6)
C2	310(8)	188(7)	161(7)	47(6)	37(6)	17(6)
O2	440(8)	169(6)	266(7)	71(5)	132(6)	51(5)
N3	305(8)	190(7)	269(7)	44(6)	99(6)	28(6)
C4	325(9)	193(7)	159(7)	122(6)	78(6)	-11(5)
C5	379(9)	167(7)	220(8)	8(7)	27(7)	-18(6)
C6	248(8)	196(7)	197(7)	2(6)	15(6)	25(6)
S	245(2)	161(2)	166(2)	14(1)	20(1)	18(1)
O11	282(7)	272(6)	293(7)	- 49(5)	- 17(5)	1(5)
O12	324(7)	202(6)	286(6)	- 3(5)	31(5)	94(5)
O13	428(8)	294(7)	262(6)	124(6)	75(6)	-23(5)
NA	324(4)	200(3)	230(3)	34(3)	43(3)	- 7(3)
W1	330(8)	458(9)	549(11)	- 23(7)	163(7)	-93(8)

TABLE B-5. ANISOTROPIC THERMAL PARAMETERS ($\AA^2 \times 10^4$) FOR NON-HYDROGEN ATOMS OF SODIUM CYTOSINE-5-METHYLENE SULFONATE TRIHYDRATE

Atom	U_{11}	U_{22}	U_{33}	U_{12}	U_{13}	U_{23}
N1	296(9)	225(8)	291(9)	- 6(7)	-111(7)	- 32(7)
C2	219(9)	224(9)	219(9)	2(7)	- 7(7)	9(7)
O2	307(8)	222(7)	374(9)	36(6)	- 44(6)	- 62(6)
N3	212(8)	220(8)	236(8)	- 1(6)	- 37(6)	- 2(6)
C4	190(8)	207(9)	202(9)	-10(7)	9(7)	24(7)
N4	264(9)	221(9)	322(10)	30(7)	- 97(7)	- 26(7)
C5	199(9)	182(8)	227(9)	5(7)	- 3(7)	39(7)
C6	232(9)	251(10)	277(10)	17(7)	- 60(8)	47(8)
C7	215(9)	206(9)	233(10)	12(7)	11(7)	16(7)
S	200(2)	181(2)	223(2)	- 6(2)	- 9(2)	- 1(2)
O11	199(7)	352(9)	333(8)	-19(6)	- 31(6)	3(7)
O12	281(7)	338(8)	237(7)	-19(6)	- 35(6)	30(6)
O13	347(8)	199(7)	437(10)	- 5(6)	36(7)	- 46(6)
NA	304(4)	247(4)	288(4)	26(3)	8(3)	4(3)
W1	375(9)	356(9)	340(10)	30(7)	10(8)	- 35(7)
W2	378(9)	344(9)	337(9)	17(7)	26(7)	- 14(7)
W3	282(8)	378(9)	377(10)	-38(7)	- 6(7)	-106(7)

TABLE B-6. ANISOTROPIC THERMAL PARAMETERS ($\AA^2 \times 10^4$) FOR NON-HYDROGEN ATOMS OF CYTOS AND MCYTOS

Atom	U_{11}	U_{22}	U_{33}	U_{12}	U_{13}	U_{23}
			CYTOS			
N1	207(6)	207(6)	289(7)	29(5)	136(5)	43(5)
C2	150(6)	194(7)	227(7)	15(5)	60(5)	27(5)
O2	229(6)	177(5)	457(8)	1(4)	174(5)	37(5)
N3	185(6)	159(6)	303(7)	0(5)	129(5)	- 4(5)
C4	155(6)	182(7)	256(7)	5(5)	60(5)	5(5)
N4	238(7)	180(6)	401(8)	2(5)	162(6)	1(6)
O4	361(7)	199(6)	561(9)	- 20(5)	323(7)	- 10(6)
C5	166(6)	206(7)	304(8)	- 20(5)	69(6)	- 59(6)
C6	176(6)	183(6)	200(6)	14(5)	71(5)	- 12(5)
S	151(2)	171(2)	233(2)	14(1)	33(1)	- 13(1)
O11	352(7)	194(6)	344(7)	66(5)	27(5)	5(5)
O12	182(6)	416(9)	573(10)	- 75(6)	34(6)	101(7)
O13	380(8)	461(9)	236(6)	127(7)	49(5)	- 87(6)
NA	277(3)	233(3)	285(4)	- 13(3)	78(3)	35(3)
W1	277(7)	295(7)	380(8)	33(6)	50(6)	- 28(6)
			MCYTOS			
C1	318(10)	381(11)	224(9)	- 99(9)	35(8)	59(8)
N1	232(7)	236(7)	170(7)	- 61(6)	10(5)	16(6)
C2	197(7)	167(7)	200(8)	4(6)	- 2(6)	- 2(6)
O2	250(6)	284(7)	243(6)	- 85(5)	- 31(5)	14(5)
N3	216(7)	255(7)	146(6)	- 26(6)	- 3(5)	0(6)
C4	184(8)	222(8)	210(8)	8(6)	42(6)	- 45(6)
N4	215(7)	317(8)	209(7)	- 19(6)	20(6)	- 61(6)
O4	258(7)	397(8)	236(6)	- 50(6)	- 1(5)	78(6)
C5	188(8)	296(9)	236(9)	31(7)	16(7)	- 21(7)
C6	190(7)	213(8)	175(7)	3(6)	- 8(6)	- 6(6)
S	289(2)	199(2)	154(2)	13(2)	- 3(2)	- 15(2)
O11	417(8)	291(7)	237(6)	106(6)	- 62(6)	1(5)
O12	603(10)	364(8)	246(7)	79(7)	174(7)	- 14(6)
O13	428(9)	307(8)	428(8)	-115(6)	-101(7)	- 47(7)
NA	252(4)	285(4)	249(4)	- 14(3)	25(3)	- 9(3)
W1	274(7)	379(8)	279(7)	- 58(6)	- 35(6)	37(6)
W2	447(9)	320(8)	427(9)	- 23(7)	62(7)	- 20(7)
W3	303(7)	391(9)	285(7)	1(6)	- 20(6)	36(6)
W4	560(11)	351(8)	438(10)	52(8)	49(8)	- 69(8)

VITA

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