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Conformational Analysis of 8-Azanucleosides. Crystal and Molecular Structure of 8-Azatubercidin Monohydrate, a Nucleoside Analogue Exhibiting the "High Anti" Conformation

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Abstract: The crystal structure of 8-azatubercidin monohydrate, $C_{10}N_5O_4H_{14}\cdot H_2O$, a cytotoxic analogue of tubercidin, has been determined. The unit cell constants are $a = 7.458$ (2) Å, $b = 9.744$ (3) Å, $c = 17.267$ (5) Å, with $Z = 4$ and space group $P2_12_12_1$. The structure was solved by direct methods and refined by least squares to an R value of 0.040 using the intensities of 1112 reflections with $I > 1.50\sigma$. The ribose exhibits a nearly symmetrical C(1′)-exo-C(2′)-endo pucker (^2_1T), and the conformation about the exocyclic C(4′)-C(5′) bond is trans ($\psi = 179.5°$). The glycosyl conformation is in the "high-anti" range ($\chi = 102.4°$). There are close intramolecular contacts between the base ring nitrogen N(8) and the ribose C(2′) and H(2′) atoms of 2.81 and 2.49 Å, respectively. All available sites on the ribose and pyrazolopyrimidine base rings, except N(8), are involved in intermolecular hydrogen bonds. These include an N(6)-H(6)···O(1′) hydrogen bond of 2.99 Å involving the ribose ring oxygen atom. The screw-related bases exhibit a head-to-tail stacking with partial overlap of the rings and a minimum interplanar separation of 3.6 Å.

Adenine is outstanding among the common nucleic acid bases as precursor to a wide range of biologically important substances. In addition to its role as a component of DNA and RNA, it is essential in the varied functions of energy metabolism (ATP), hormonal control (cAMP), and antibiotic action. It is likely that adenine was of central evolutionary importance in the primordial development of biochemical systems. Among the antibiotic derivatives of adenosine (II) is the series of compounds generated by substitution of nitrogen for carbon or vice versa at one or more sites within the imidazole ring. 4-Amino-1-(β-D-ribofuranosyl)pyrazolo[3,4-*d*]pyrimidine (I) is one such compound. Because of its similarity to tubercidin, it is hereafter referred to as 8-azatubercidin. To facilitate comparison with other purine systems, we adopt the purine numbering throughout this work. The structure and conformation of similar compounds have also been the subject of much interest in our own and other laboratories. Structural studies of formycin monohydrate (VI),[2] tubercidin (III),[3] toyocamycin (IV),[4] formycin hydrobromide,[5] oxoformycin B, formycin B,[6] 2-methylformycin,[7] and 8-azaadenosine (VII)[8] have been published.

The conformational properties of such compounds include a number of noteworthy features. Outstanding among these is the tendency to assume the "high-anti"[2,8] glycosyl conformation (χ) in the solid state. The high-anti conformation, although unusual, is allowed in normal nucleosides and has been observed in the crystal structures of guanosine and inosine.[9,10] The preference for the high χ values in 8-azapurine and 6-azapyrimidine nucleoside analogues is influenced by the presence of the unprotonated and electronegative nitrogen atom N(8) or N(6) as will be discussed below in greater detail.

The antibiotic action of 8-azatubercidin (II)[11] appears analogous to that of adenosine[12] in its inhibition of uridine biosynthesis. In contrast to the 8-deaza analogues tubercidin (III) and sangivamycin (V), 8-azatubercidin (II), 8-azaadenosine (VII), and formycin (VI) are substrates for adenosine deaminase[11,13] although they bind less effectively to the enzyme than adenosine itself. The x-ray crystallographic analysis of 8-azatubercidin was undertaken to elucidate the effect of the pyrazolopyrimidine ring on the overall molecular conformation.

Experimental Section

Intensity Data Collection. A sample of 8-azatubercidin ($C_{10}N_5O_4H_{14}$) provided by Dr. Sidney Hecht was crystallized by slow evaporation from a solution of 50% aqueous ethanol. The crystals formed parallelepipeds elongated in the a direction and preliminary photographic work showed that they belong to the orthorhombic space group $P2_12_12_1$. Unit cell dimensions were measured by a least-squares refinement based on the goniostat angles θ, χ, and ϕ of 10 accurately centered reflections with $30° \leq 2\theta \leq 60°$. They are $a = 7.458$ (2) Å, $b = 9.744$ (3) Å, and $c = 17.267$ (5) Å. There are four molecules in the unit cell and the calculated density is 1.517 g cm^{-3}. Intensity data were collected on a Picker FACS-I diffractometer with Cu Kα radiation using a crystal measuring 0.5 mm × 0.15 mm × 0.15 mm mounted with the a axis coincident with the ϕ axis of the goniostat. Reflections (1231) were measured to a 2θ limit of 127° using a θ-2θ scan technique at scan rate of 2° min^{-1} with 10-s background counts taken at each scan limit. Of these reflections, 1119 had intensities greater than 1.5σ and were considered observed. Three moderately strong reflections were monitored at regular intervals during the course of data collection and displayed no significant change in intensity. The data were corrected for Lorentz and polarization effects and an empirical absorption correction based on the variation of the 400 reflection at $\chi = 90°$ as a function of ϕ (maximum variation, 7% on intensity) was also applied.

Structure Determination and Refinement. The structure was solved by direct methods with the program MULTAN.[14] The correct solution had a figure of merit of 1.08 and a residual $R(E)$ of 21.4%. A Fourier

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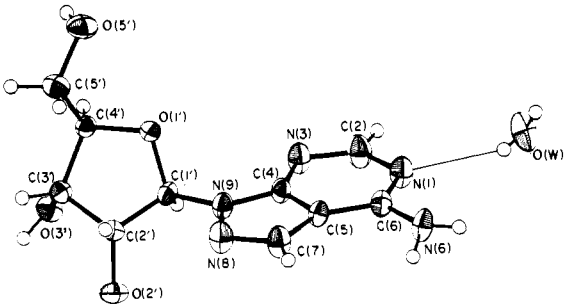

Figure 1. The ellipsoids of thermal vibration depicting 50% probability surfaces of the nonhydrogen atoms. The atomic numbering follows the purine convention. Although the recommended nomenclature for the ribose ring oxygen in nucleoside systems is O(4'),[41] the familiar O(1') designation is retained here.

synthesis calculated with 190 normalized structure factors, $|E| > 1.35$, yielded the positions of all pyrazolopyrimidine atoms and a portion of the ribose; the remaining atoms were subsequently found in a difference Fourier map. After two cycles of refinement of this model with isotropic temperature factors,[15] a solvent oxygen atom was discovered in a difference map. A third cycle of isotropic refinement reduced the R value to 0.12, where $R = \Sigma||F_o| - |F_c||/\Sigma|F_o|$, and a difference Fourier synthesis revealed the positions of all hydrogen atoms except those of the hydroxyl groups and the water. The positional parameters of these latter hydrogen atoms were fixed according to geometric criteria. Up to this stage unit weights were used. In subsequent cycles of least-squares refinement, weights derived from counting statistics using an electronic instability factor of 0.02 were used. The reflections 004, 013, 020, 024, 201, 210, and 400 were judged to be affected by secondary extinction and were given zero weight. Two cycles of refinement with anisotropic temperature factors for the nonhydrogen atoms reduced R to 0.068, and a difference Fourier synthesis revealed the solvent and hydroxyl hydrogen atoms. Examination of the torsion angles indicated that the incorrect enantiomorph had been chosen and this was corrected by reversing the signs of x coordinates. The nonhydrogen atoms were subjected to a final two cycles of anisotropic refinement resulting in the convergence of the positional and thermal parameters at an R of 0.045. The electron density corresponding to one of the hydrogens bound to the water oxygen (HW2) was diffuse. The position of this atom was therefore fixed, while the positional and thermal parameters of the rest of the hydrogen atoms were subjected to two cycles of isotropic refinement utilizing only the 394 low-angle reflections with $\sin\theta/\lambda < 0.4$. The final R values were 0.040 for the 1112 observed reflections used in the refinements and 0.051 for all 1231 measured reflections. The average ratios of final shifts to their estimated standard deviations were 0.08 for nonhydrogen and 0.28 for hydrogen atoms, with corresponding maximum values of 0.53 and 1.23, respectively. The scattering factors for carbon, nitrogen, and oxygen were those of Cromer and Waber[16] while those for hydrogen were from Stewart et al.[17]

Results

The positional parameters together with their esd's are listed in Table IA. The anisotropic thermal parameters for the nonhydrogen atoms and isotropic thermal parameters of the hydrogen atoms (given in Table IB) and the observed and calculated structure amplitudes (listed in Table II) are available in the microfilm edition of this journal (refer to the Supplementary Material Available paragraph at the end of this article). An ORTEP[18] drawing depicting 50% probability surfaces of the nonhydrogen atoms, including the hydrogen-bonded water molecule and the atomic numbering (the purine convention is used), is shown in Figure 1. Bond distances and angles with their estimated standard deviations in parentheses are given in Figure 2 and Table IV contains a number of pertinent conformational parameters.

Discussion

Geometry of the Base. The bond distances in the pyrimidine portion of the pyrazolopyrimidine ring agree well with the corresponding values for the neutral adenine base in adenosine,[19] 3'-*O*-acetyladenosine,[10] 2'-amino-α-2'-deoxyadenosine,[20] and deoxyadenosine monohydrate[21] and are all within 0.015 Å of those for the pyrimidine moiety of tubercidine.[3] The major departure in the molecular geometry of the pyrazolopyrimidine ring from that of adenine systems is observed in the pyrazolo ring. The distance C(7)–N(8) is comparable to that in allopurinol[22] and is about 0.02 Å longer than the N(7)–C(8) bond in adenosine. The C(5)–C(7) and C(4)–C(5) bond lengths are similar to those of tubercidin, and the C(4)–C(5) bond is longer than the corresponding distance in normal purines, indicating less double-bond character. The endocyclic angle at the unprotonated N(8) atom is, in accordance with the earlier observation of Prusiner et al.,[2] smaller by 4–8° than that at C(8)–H in purine systems. A concomitant increase in the angles at N(9) and C(7) is observed, while the angle C(4)–C(5)–N(7) is diminished, effects also observed in formycin monohydrate.

The geometry about the glycosyl bond is similar to that exhibited in normal syn purine nucleosides. The C(1')–N(9) glycosyl bond length is 1.460 Å, which is comparable to that observed in the common purine nucleosides such as adenosine[19] (1.466 Å). The exocyclic angles C(4)–N(9)–C(1') of 127.4° and N(8)–N(9)–C(1') of 121.1° are remarkably similar to the corresponding angles in formycin monohydrate[2] of 128.2 and 121.9°, respectively, even though the atom at position 9 is a carbon in the latter. The exocyclic angles about the glycosyl bond are sensitive to the glycosyl conformation.[10] In common purine nucleosides adopting the classical anti glycosyl con-

Table IA. Positional Parameters of Atoms in 8-Azatubercidin[a]

Atom	x	y	z
N(1)	−1360(6)	2061(4)	−976(2)
C(2)	−1231(8)	3438(5)	−1075(2)
N(3)	−1172(6)	4425(3)	−548(2)
C(4)	−1244(6)	3902(4)	174(2)
C(5)	−1342(6)	2523(4)	372(2)
C(6)	−1427(6)	1592(4)	−247(2)
N(6)	−1572(6)	231(3)	−135(2)
C(7)	−1335(7)	2510(4)	1192(2)
N(8)	−1253(6)	3767(3)	1483(2)
N(9)	−1210(5)	4625(3)	847(2)
C(1')	−1180(6)	6114(4)	938(2)
O(1')	603(4)	6588(3)	811(2)
C(2')	−1615(6)	6604(4)	1747(2)
O(2')	−3457(4)	6531(3)	1941(2)
C(3')	−829(6)	8050(4)	1739(2)
O(3')	−2066(4)	9015(3)	1419(2)
C(4')	777(5)	7901(4)	1195(2)
C(5')	2566(6)	7938(5)	1612(2)
O(5')	4019(4)	7783(3)	1077(2)
O(W)	−1018(4)	352(3)	−2308(2)
H(2)	−114(7)	370(5)	−162(3)
H(7)	−133(7)	172(5)	153(3)
H(6)1	−180(7)	−9(5)	42(3)
H(6)2	−184(7)	−20(5)	−50(3)
H(1')	−189(7)	648(5)	57(3)
H(2')	−92(7)	601(5)	216(3)
H(O2')	−403(7)	697(5)	163(3)
H(3')	−26(7)	840(5)	230(3)
H(O3')	−261(7)	926(5)	182(3)
H(4')	77(6)	867(5)	81(3)
H(5')1	268(7)	719(5)	207(3)
H(5')2	263(6)	887(4)	198(3)
H(O5')	414(8)	850(6)	92(3)
H(W)1	−10	−19	−223
H(W)2	−132	83	−199

[a] Positional parameters of nonhydrogen atoms have been multiplied by 10^4. Positional parameters of hydrogen atoms have been multiplied by 10^3. Standard deviations in parentheses refer to the least significant digits. Positional parameters of H(W)1 and H(W)2 were not refined.

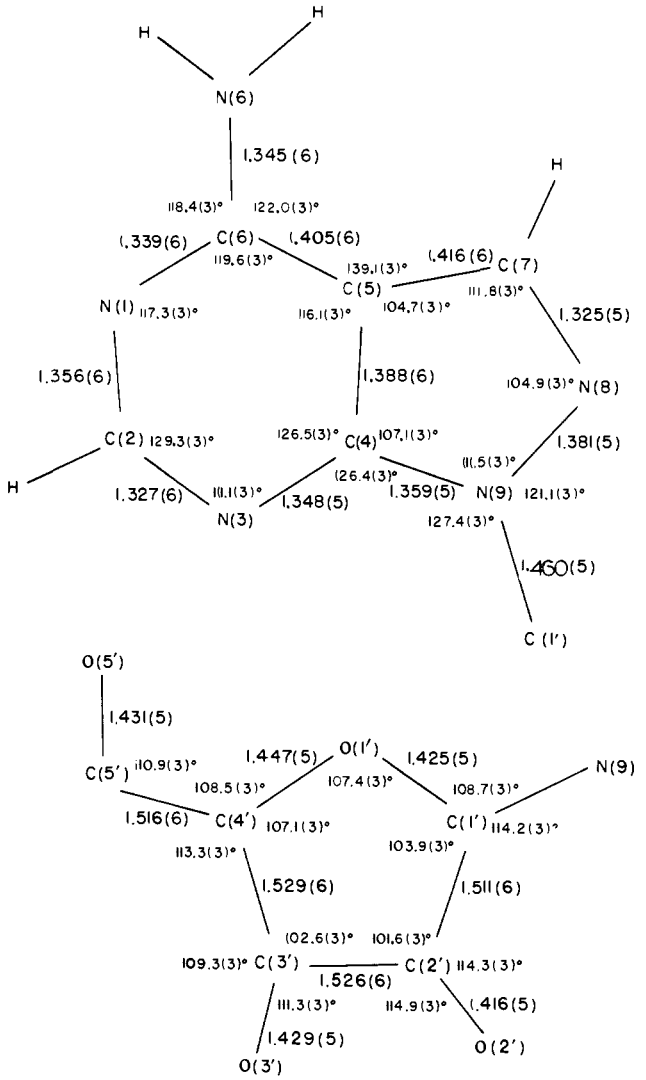

Figure 2. The bond lengths and bond angles involving the nonhydrogen atoms. Estimated standard deviations in parentheses refer to the least significant digit.

formation, the C(4)-N(9)-C(1′) angle is 2-6° smaller than the C(8)-N(9)-C(1′) angle. For nucleosides in the syn conformation, this trend is reversed (Table III). A similar trend appears to be evident in the 8-azapurine nucleosides exhibiting the high anti and syn conformations. The substantial differences between exocyclic angles as a function of the glycosyl torsion χ indicate that variation of valence angles should be considered in calculations of conformational energy barriers around the glycosyl bond.[23]

Planarity of the Base. The least-squares plane through the nine atoms of the base ring is given by the equation $-0.998X + 0.059Y - 0.007Z = 1.142$ where X, Y, and Z are atomic coordinates in angstroms referred to the crystallographic a, b, and c axes. All atoms of the base are within 0.018 Å of this plane, but N(6) and C(1′) show significant deviations of 0.044 and 0.080 Å, respectively, and lie on the same side of the base. The pyrimidine and pyrazolo ring planes form a dihedral angle of 1.2°. Similar values are usually found in purines and their analogues.[25,26] The amino nitrogen atom is pyramidal rather than planar, such that the dihedral angle between the purine ring and the three-atom plane of the amino group is 23°. The displacements of the amino hydrogen atoms from the least-squares plane of the base are observed to promote the linearity of the hydrogen bonds to the acceptor atoms O(3′) and O(1′) in adjacent molecules.

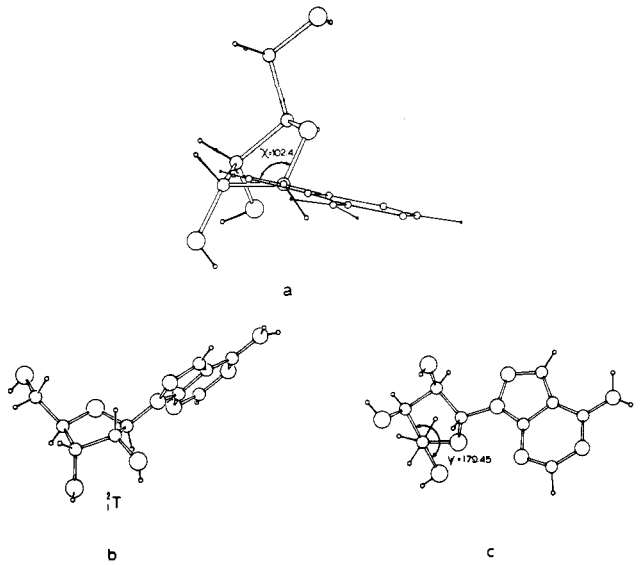

Figure 3. Views of the molecule: (a) along the glycosyl N(8)-C(1′) bond showing the high anti conformation; (b) illustrating the ^2_1T ribofuranose; (c) along the C(4′)-C(5′) bond depicting the trans conformation.

Table III. Exocyclic Glycosyl Bond Angles Involving the Base in 8-Azanucleosides and Normal Nucleosides in the Syn and Anti Conformations

Compound	Bond angle, deg[a] C(4)-X(9)-C(1′)	C(8)-X(9)-C(1′)	χ, deg	Ref
Formycin B[b]	124.8	124.8	30.4	6
8-Azatubercidin	127.4	121.1	102.4	
Formycin·H_2O	128.2	121.9	109.8	2
Oxoformycin	131.1	119.6	195.9	6
2-Methylformycin	132.0	122.8	202.5	7
Formycin·HBr	131.0	123.2	210.5	5
Purine nucleosides[c]	129.3	124.7	200-276	
Purine nucleosides	125.9	127.5	35-73	
Purine nucleosides	124.6	128.4	0-30	

[a] X is a nitrogen in the 8-aza system and a carbon in the normal purines. All values are given in degrees. [b] Formycin B is the only example to date of an 8-azanucleoside adopting a "classical" anti conformation in the solid state. The exocyclic angles about the N(9) atom in this compound are emphasized for comparison with the corresponding angles in the high-anti and syn 8-azanucleoside analogues. [c] These values are averages over a sample of normal purine nucleoside and nucleotide crystal structures solved in the last 10 years. All structures incorporated in the sample were refined to an $R \leq 0.06$ and show estimated standard errors in bond angles of less than 0.6°. The syn (200° < χ < 276°) values were derived from nine independent determinations (one with two molecules per asymmetric unit, the "mid" anti (35° < χ < 73°) derived from eight independent determinations; one with two molecules per asymmetric unit) and the "low" anti (0° < χ < 30°) values were derived from seven independent determinations (one with two molecules per asymmetric unit and one with three molecules per asymmetric unit).

Glycosyl Conformations. The glycosyl torsion angle χ, C(1′)-O(1′)-N(9)-N(8), in 8-azatubercidin is 102.4° which is in the "high-anti" range[2] (Figure 3a), i.e., intermediate between the classical anti and syn regions, viz., $-10° \lesssim \chi \lesssim 80°$ and $200° \lesssim \chi \lesssim 260°$, respectively. Glycosyl conformations in the high-anti, "mid-anti" ($40° \lesssim \chi \lesssim 90°$), and syn ranges are generally displayed by nucleosides and nucleotides

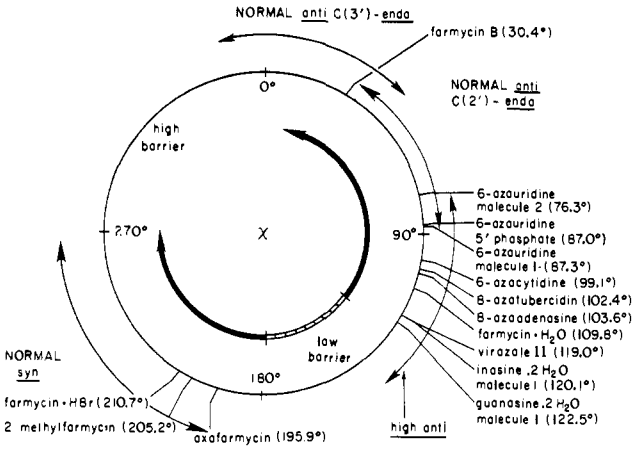

Figure 4. Conformation circle showing the glycosyl torsions assumed by the azanucleosides. For comparison, inosine and guanosine[9] are indicated (see text). The normal ranges for anti and syn and the high "anti" are indicated. The bold arrow within the circle illustrates the broad range in glycosyl angles accessible to the aza series of nucleosides. Note that the high anti values form a continuum with the "low" (C(3')-endo) and "mid" anti (C(2')-endo) χ values. The energy barrier between the syn and the "low" anti conformations is considerably higher than that between the high anti and classical syn regions. Consequently, the syn ↔ anti interconversion would be expected to proceed via the "low-energy" path.[36] Note: χ angles slightly greater than 270° may be referred to as low syn by analogy to the high anti χ angles ($\gtrsim$90) which are slightly greater than the conventional anti values.

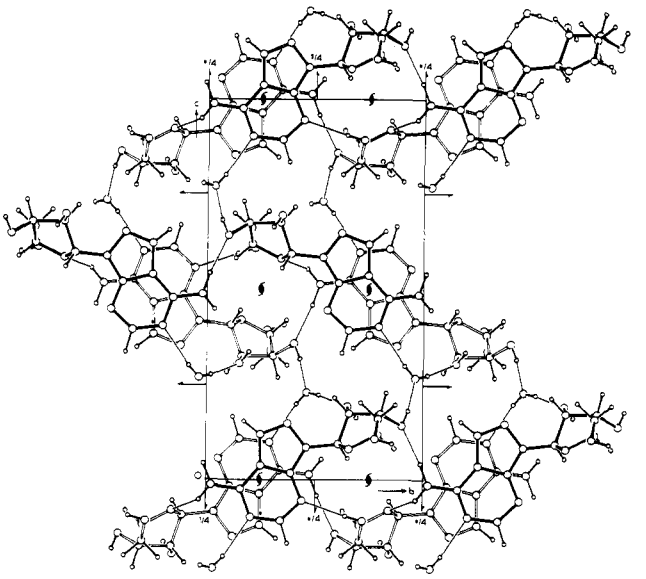

Figure 5. View down the *a* axis showing the hydrogen bonding and molecular packing in 8-azatubercidin.

Table IV. Selected Conformational Parameters[a]

Glycosyl angle	χ	102.4° (anti)
Ribose ring	τ_0	−35.3°
torsions	τ_1	41.9°
	τ_2	−32.1°
	τ_3	12.2°
	τ_4	14.2°
Ribose pucker	${}^2_1T$	
Pseudorotation parameters:		
Phase angle of pseudorotation	P	141.9°
Maximum amplitude of pucker	τ_m	−41.9°
C(4')-C(5') torsion		
C(3')-C(4')-C(5')-O(5')	ψ	179.5° (trans)
O(1')-C(4')-C(5')-O(5')		60.6°

[a] Conventions are as defined by Sundaralingam.[27]

adopting the C(2')-endo ribofuranose pucker whereas the "low-anti" ($-10° \lesssim \chi \lesssim 30°$) values are assumed by nucleosides in the C(3')-endo conformation (Figure 4).[27,28] In 8-azatubercidin as in the other high-anti nucleosides 8-azaadenosine and formycin monohydrate, the N(8) atom is unprotonated. Consequently, hindrance to rotation about the glycosyl bond at χ near 100° is reduced. Inosine and guanosine have been observed to adopt slightly higher values in χ[9] (120 and 123°, respectively), possibly to relieve the steric interactions between the C(8) proton of the base, and the C(2') and H(2') atoms of the sugar. The high-anti conformation in 8-azatubercidin may gain some stability from attractive van der Waals interactions resulting from the short intramolecular contacts between the electronegative N(8) atom of the base and C(2') and H(2') of the sugar of 2.815 and 2.49 Å, respectively. A similar interaction has been noted in the crystal structure of 8-azaadenosine[8] and analogous contacts involving N(6) with C(2') and H(2') are observed in 6-azacytidine[29] and 6-azauridine.[30]

The wide range of glycosyl conformations observed in the formycin series (Figure 4) results from the increased C–C-glycosyl bond length[31] and especially the N(3)-C(4)-C(9) exocyclic angle[2] which lift the bulky pyrimidine portion of the base away from the ribofuranose ring plane at χ near 160 ± 20° and thus increase the separation between the base N(3) and ribose ring oxygen O(1'). The consequent reduction in base–sugar steric interactions in this region of χ is corroborated by conformational energy calculations on formycin.[32,33] In the formycin series and the other 8-azanucleosides, conformations near $\chi \approx 0°$ are less favored because of repulsive interactions between the lone-pair electrons of the base N(8) and the ribose ring oxygen O(1'), whereas normal purine nucleosides with carbon at the 8 position do not experience this repulsion and assume low anti conformations.[27]

Geometry and Conformation of the Ribose. The bond distances and angles within the ribofuranose unit are in agreement with other known C(2')-endo puckered purine nucleosides.[28] The inequality of the ring C–O bond lengths persists in 8-azatubercidin as in the common purine and pyrimidine nucleosides.[34] The ribose assumes a nearly ideal C(1')-exo-C(2')-endo (${}^2_1T$) twist conformation with C(1') and C(2') displaced on opposite sides of the O(1')-C(4')-C(3') plane by 0.334 and 0.314 Å, respectively. The C(1') atom lies on the side opposite (exo) from the exocyclic C(5') atom (Figure 3b). The ring pucker at C(1') diminishes the endocyclic angle at C(1') (103.9°) and increases that at C(4') (107.1°) compared to the values of about 107 and 105° observed in ribofuranose rings exhibiting the familiar C(2') and C(3')-endo twist puckers (${}^2_3T$, ${}^3_2T$).[34,35] In terms of pseudorotation model (Table IV),[36] the ribofuranose exhibits a pseudorotation angle, P, of 141.9° and a maximum amplitude of pucker, τ_m, of −41.9°. Similar ribofuranose conformations are also typical of other high-anti purine nucleoside analogues (Table V). In contrast, both 6-azacytidine[29] and 6-azauridine[30] assume the C(3')-endo conformation. This suggests that the preference of pyrimidine nucleosides for the C(3')-endo ribose pucker and that of the purine nucleosides for the C(2')endo conformation[27,28] are conserved by the 6-azapyrimidine and 8-azapurine nucleosides as well. The conformation about C(4')-C(5') is trans (Figure 3c). This conformation appears to be correlated with the C(2')-endo ribose pucker and the high-anti glycosyl conformation as noted in several other 8-azapurine and normal purine nucleosides (Table V).

Hydrogen Bonding and Base Stacking. Figure 5 shows an *a* axis projection of the unit cell. The hydrogen bonding and

Table V. Conformational Parameters of Relevant Nucleoside Analogues

Purine analogues	χ[a]	ψ[b]	Sugar pucker	P[c]	τ_m	Ref
Toyocamycin	60.7	57.1 (g^+)	$^2T_3$	165.7	42.5	4
Tubercidin	71.8	180.0 (t)	$^2T_1$	148.1	44.4	3
Formycin·H_2O	109.8	175.8 (t)	$^2T_1$	148.3	39.6	2
8-Azaadenosine[d]	103.6	(t)	$^2T_1$			8
Virazole 2 (five-membered base ring)	119.9	−179.9 (t)	$_2T^1$	335.8	36.4	39
Normal purines						
Guanosine·$2H_2O$ molecule 1	122.5	67.9 (g^+)	^2E	161.4	36.2	9
Inosine·$2H_2O$ molecule 1	120.1	64.0 (g^+)	$^2T_3$	163.6	39.1	9
Pyrimidine analogues						
6-Azauridine						
Molecule 1	87.3	−170.3 (t)	$^3T_4$	27.9	37.5	
Molecule 2	76.3	−173.3 (t)	$^3T_4$	21.8	33.5	31
6-Azauridine 5′-phosphate[d]	87.0	−172.0 (t)	$^3T_2$			40
6-Azacytidine	99.1	55.4 (g^+)	^3E			30

[a] χ is the glycosyl torsion angle defined by O(1′)-C(1′)-X(9)-Y(8), where X is N (usually) or C, and Y is C (usually) or N. [b] ψ is the C(3′)-C(4′)-C(5′)-O(5′) torsion. [c] P = phase angle of pseudorotation and τ_m = maximum amplitude of pseudorotation. [d] Coordinates are not available for these structures.

Table VI. Hydrogen Bond Lengths (Angstroms) and Angles (Degrees)

Bond A-H···B	Symmetry operation for B	Distance A-H	Distance H···B	Distance A···B	Angle, deg A-H···B
N(6)-H(61)···O(3′)	$x, y-1, z$	1.02	1.94	2.958	172
N(6)-H(62)···O(1′)	$-\frac{1}{2}+x, \frac{1}{2}-y, -z$	0.81	2.38	2.989	133
O(2′)-H(O2′)···O(5′)	$x-1, y, z$	0.81	1.91	2.693	163
O(3′)-H(O3′)···O(W)	$-\frac{1}{2}-x, 1-6, \frac{1}{2}+z$	0.84	1.85	2.691	174
O(5′)-H(O5′)···N(3)	$\frac{3}{2}+x, -6, -z$	0.76	2.12	2.872	166
O(W)-H(W1)···O(2′)	$\frac{3}{2}+x, \frac{1}{2}-y, -z$	0.88	1.86	2.723	169
O(W)-H(W2)···N(1)	x, y, z	0.72	2.13	2.851	161

head-to-tail base stacking patterns are strikingly similar to those observed in formycin monohydrate. The stacking is slightly skewed, the dihedral angle between stacked base planes being 6.9°. The closest approach of stacked bases is the 3.585-Å contact of C(4) and N(6), suggesting only weak hydrophobic interaction between the pyrazolopyrimidine rings. All of the potential hydrogen bonding sites on the ribose and the base, except N(8), are involved in hydrogen bonds although there are no interactions between the pyrazolopyrimidine moieties. The solvent water is "trapped" in the lattice by three neighboring molecules which are linked to it by hydrogen bonds (Figure 5).

Involvement of the ribose ring oxygen in hydrogen bonds is uncommon in nucleoside structures[37] but occurs in 8-azatubercidin and in the other high-anti crystal structures formycin monohydrate,[2] 8-azadenosine,[8] and 6-azauridine.[30] O(1′) is sometimes observed to partake in dipole-induced dipole stacking interactions with adjacent purine or pyrimidine bases.[38] These interactions appear to be common in nucleosides exhibiting anti glycosyl conformations in which the O(1′) atom lies approximately in the plane of the base, i.e., low χ. In these cases the O(1′) atom is sterically shielded from potential hydrogen-bonding interactions. However, in the high-anti conformation, the ribose oxygen is rotated from the base plane and is exposed to potential hydrogen-bond donors. In 8-azatubercidin the distances between O(1′) and the donor N(6) and the donor H(62) hydrogen atoms are 2.989 and 2.38 Å, respectively. The hydrogen bond angle O(1′)···H(62)-H(6) of 133° is characteristic of a weak hydrogen-bonded interaction (see Table VI).

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Supplementary Material Available: A listing of anisotropic thermal parameters for nonhydrogen atoms and isotropic thermal parameters for hydrogen atoms (Table IB), and the observed and calculated structure amplitudes (Table II) (6 pages). Ordering information is given on any current masthead page.

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Carbon Kinetic Isotope Effects on Pyruvate Decarboxylation Catalyzed by Yeast Pyruvate Decarboxylase and Models[1]

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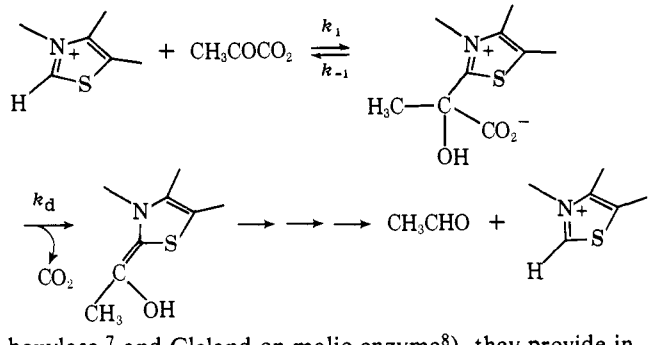
Abstract: Carbon-13 kinetic isotope effects were determined on pyruvate decarboxylation catalyzed by the enzyme yeast pyruvate decarboxylase and by thiamin, and in $CHDT^+Cl^-$ (2-(1-carboxy-1-hydroxyethyl)-3,4-dimethylthiazolium chloride). The $CHDT^+Cl^-$ gave an effect of 1.051 corresponding to the maximum isotope effect anticipated for CO_2 loss. Thiamin-catalyzed decarboxylation gave a pH-independent inverse isotope effect of 0.992 indicating that in that model decomposition of the covalent adduct formed between thiamin and pyruvate to reactants has a higher activation energy than the subsequent decarboxylation step. The enzymatic isotope effect was found to be normal varying from 1.002 at pH 7.5 to 1.011 at pH 5.0. At pH 5.00 the isotope effect was found to be temperature independent. The results were interpreted to mean that in the pH range employed decarboxylation is faster than the decomposition of the enzyme-bound thiamin–pyruvate covalent complex. A model is presented to account for the observed pH dependence of the enzymatic kinetic isotope effect.

Yeast pyruvate decarboxylase (EC 4.1.1.1) (PDCase) catalyzes the decarboxylation of pyruvate to acetaldehyde with the assistance of thiamin pyrophosphate and Mg(II) as cofactors.[2] Numerous studies have shown that pyruvate and the coenzyme form a covalent complex. In fact, 2-(α-hydroxy)-ethylthiamin pyrophosphate (the adduct of acetaldehyde and the coenzyme) can be isolated from the enzymic reaction.[3] Scheme I accounts for the known facts.

Carbon kinetic isotope effects on the release of CO_2 can elucidate whether the decarboxylation is the slow, rate-controlling step in the series of steps culminating with CO_2 release (assuming this last step to be essentially irreversible). While few such experiments have been reported (Seltzer et al. on oxaloacetate decarboxylose,[4] O'Leary on isocitrate dehydrogenase,[5] acetoacetate decarboxylase,[6] and glutamate decarboxylase,[7] and Cleland on malic enzyme[8]), they provide information not readily accessible by any other technique.

Scheme I



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