Stereochemistry of Nucleic Acids and Their Constituents. XV.[1] Crystal and Molecular Structure of 2-Thiocytidine Dihydrate, a Minor Constituent of Transfer Ribonucleic Acid

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Abstract: 2-Thiocytidine crystallized from aqueous ethanol solution as the dihydrate, $C_9H_{13}N_3O_4S \cdot 2H_2O$, in the form of triclinic crystals, space group $P1$ with $a = 9.595$ Å, $b = 7.135$ Å, $c = 5.030$ Å, $\alpha = 95.58°$, $\beta = 99.81°$, and $\gamma = 104.65°$; $Z = 1$. The crystal structure has been determined from 1103 three-dimensional X-ray intensity data (Cu Kα radiation) obtained by the use of a four-circle diffractometer. The structure was solved by the heavy-atom method and refined by full-matrix least squares to an R of 0.039. The average estimated standard deviations in bond distances is 0.005 Å and in bond angles 0.4° for nonhydrogen atoms. The glycosidic torsional angle, 20.3°, is anti. The glycosidic C(1′)–N bond distances in the pyrimidine derivatives show a tendency to increase with a decrease in this angle. An analysis of the deviations of the base atoms from the least-squares plane of the base reveals that the base is flexible. The mode of puckering of the base is regulated by the molecular environment. The sugar conformation is C(3′)-endo–C(2′)-exo ($^3T_2$). The conformation about the C(4′)–C(5′) bond is gauche–gauche. The sulfur atom is hydrogen bonded both to a water molecule and the amino group of an adjacent molecule. The latter hydrogen bond is the only interbase hydrogen bond. There is also a water–water hydrogen bond of 2.74 Å.

The thio derivatives of pyrimidine and purine bases and nucleosides have been a subject of considerable interest in recent years. Up to date, the crystal structure of 2-thiouracil,[2] 2,4-dithiouracil,[3] 2-thiocytosine,[4] 4-thiouridine,[5] 4-thiouridine disulfide,[6] 2,4-dithiouridine,[7] 6-mercaptopurine,[8,9] 2-mercapto-6-methylpurine,[10] 6-thioguanine,[11] and 6-thioinosine[12] have been reported. Moreover, the thio derivatives such as 4-thiouridine,[13–15] 5-methylaminomethyl-2-thiouracil,[16] N^6-(3-methyl-2-butenyl)-2-methylthioadenosine,[17] and 2-thiocytidine[16] are found as minor constituents in several *E. coli* transfer ribonucleic acids (tRNAs), while 6-thioinosine and 6-thioguanosine are effective as enzyme inhibitors and antitumor agents.[18] The function of the thio analogs of pyrimidine and purine nucleosides in tRNAs is not yet known. The present investigation of the molecular structure of 2-thiocytidine dihydrate is part of a continuing study in these laboratories to provide information on the conformation, base stacking, and hydrogen bonding characteristics of the modified constituents of nucleic acids.

Experimental Section

A sample of 2-thiocytidine, $C_9H_{13}N_3O_4S$, was provided by Professor Tohru Ueda of Hokaido University, Japan. The compound crystallized as the dihydrate from aqueous ethanol solution. Oscillation and Weissenberg photographs showed that the crystals belong to space group $P1$. The cell constants were determined from medium- and high-angle reflections measured on a Picker four-angle diffractometer with Cu Kα radiation ($\lambda = 1.5418$ Å) and were $a = 9.595 \pm 0.003$, $b = 7.135 \pm 0.002$, $c = 5.030 \pm 0.001$ Å, $\alpha = 95.58 \pm 0.01$, $\beta = 99.81 \pm 0.01$, and $\gamma = 104.65 \pm 0.01°$. The density calculated for one molecule of 2-thiocytidine dihydrate, $C_9H_{13}N_3O_4S \cdot 2H_2O$, per unit cell is 1.510 g cm^{-3}, which agrees with 1.513 g cm^{-3} obtained by flotation in a mixture of carbon tetrachloride and chloroform.

Three-dimensional intensity data were collected on the Picker four-circle diffractometer with Ni-filtered Cu Kα radiation. Intensity measurements were made by scanning reflections in the 2θ mode at a rate of 2°/min and a scan range of 3.4°. Background counts of 20 sec were taken at each end of the scan. Data were collected for 1117 reflections to a maximum 2θ of about 133°. A reflection with intensity smaller than 1.5 times its standard deviation was considered unobserved. There were only 14 such unobserved reflections. The data were corrected for Lorentz polarization effects, but no corrections for absorption were made.

Determination and Refinement of the Structure

The structure was solved by the heavy-atom method. The position of the sulfur atom was assumed to be at the origin, and a three-dimensional electron density map was calculated. The peaks in the map were not readily interpretable in terms of the structure because of the introduction of a spurious center of symmetry by placement of the sulfur atom at the origin. Fortunately, the four highest peaks which were later identified as O(1′), O(2′), C(1′), and O(W1) together with the S at the origin when used as input for a second three-dimensional electron density map revealed the positions of C(2′) and the remaining base atoms in the structure. Two cycles of isotropic full-matrix least-squares refinement of the

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Table I. Final Positional Parameters and Anisotropic Thermal Parameters of the Nonhydrogen Atoms[a-c]

Atom	$X \times 10^4$	$Y \times 10^4$	$Z \times 10^4$	β_{11}	β_{22}	β_{33}	β_{12}	β_{13}	β_{23}
S(2)	10000 (0)	0 (0)	10000 (0)	70 (1)	93 (2)	361 (4)	41 (1)	13 (2)	10 (2)
N(4)	9908 (5)	6240 (6)	15002 (8)	113 (5)	135 (8)	376 (17)	36 (5)	7 (7)	−52 (9)
N(1)	8230 (3)	2178 (4)	8295 (7)	57 (4)	74 (6)	302 (14)	19 (4)	37 (6)	11 (7)
C(2)	9343 (4)	1989 (5)	10286 (8)	45 (4)	85 (7)	278 (15)	10 (4)	26 (6)	27 (8)
N(3)	9913 (4)	3398 (5)	12450 (7)	57 (4)	103 (7)	308 (14)	19 (4)	19 (5)	12 (8)
C(4)	9350 (4)	4932 (5)	12759 (8)	70 (4)	83 (7)	323 (17)	17 (4)	44 (7)	10 (9)
C(5)	8192 (4)	5140 (6)	10762 (9)	77 (5)	94 (8)	369 (19)	33 (4)	39 (8)	20 (9)
C(6)	7683 (4)	3764 (6)	8572 (8)	71 (5)	83 (7)	372 (18)	34 (5)	35 (7)	22 (9)
C(1′)	7595 (4)	637 (5)	5852 (7)	60 (4)	84 (7)	263 (16)	17 (4)	40 (7)	12 (8)
C(2′)	6459 (4)	−1127 (5)	6451 (8)	67 (4)	69 (7)	284 (15)	13 (4)	30 (6)	6 (8)
C(3′)	5035 (4)	−555 (5)	5616 (8)	51 (4)	92 (8)	285 (15)	5 (4)	43 (6)	4 (8)
C(4′)	5307 (3)	438 (5)	3143 (8)	47 (4)	107 (8)	257 (15)	11 (4)	28 (6)	25 (8)
O(1′)	6864 (3)	1439 (4)	3781 (6)	48 (3)	124 (6)	262 (10)	13 (3)	28 (4)	54 (6)
O(2′)	6515 (3)	−2753 (4)	4667 (7)	90 (4)	84 (5)	500 (15)	22 (3)	73 (6)	−42 (7)
O(3′)	3754 (3)	−2165 (4)	4836 (6)	68 (3)	141 (6)	320 (12)	−24 (4)	27 (5)	15 (7)
C(5′)	4436 (4)	1902 (6)	2412 (9)	69 (5)	136 (9)	340 (17)	33 (5)	21 (7)	22 (10)
O(5′)	4615 (3)	3388 (4)	4637 (7)	71 (3)	118 (6)	454 (15)	25 (3)	41 (5)	3 (7)
O(W1)	2075 (3)	2739 (5)	6518 (7)	69 (3)	214 (8)	377 (14)	16 (4)	10 (6)	14 (8)
O(W2)	2599 (5)	−3548 (6)	9200 (7)	144 (5)	198 (8)	363 (15)	−28 (5)	19 (7)	19 (8)

[a] Standard deviations are given in parentheses. [b] Anisotropic thermal parameters and their standard deviations have been multiplied by 10^4. [c] The temperature factor is of the form $\exp[-(\beta_{11}h^2 + \cdots + 2\beta_{12}hk + \cdots)]$.

known atoms using the ORFLS program of Busing, Martin, and Levy[19] gave a reliability index, R, of 0.33 for 1103 observed reflections, where $R = \Sigma||F_o| - |F_c||/\Sigma|F_o|$; F_o is the observed and F_c is the calculated structure factor. The isotropic thermal parameter B for all atoms was initially set at 1.82 Å^2 which was obtained from a Wilson plot.[20] The positions of the remaining nonhydrogen atoms were obtained from a difference Fourier map. Two cycles of isotropic least-squares refinement of all the nonhydrogen atoms followed by two cycles of anisotropic refinement reduced the R value to 0.052. The difference Fourier map clearly revealed the positions of all the hydrogen atoms. Two cycles of isotropic refinement of the hydrogen atoms reduced the R to 0.046. The weighting scheme used in the above refinements is that of Evans.[21] However, a plot of $|\Delta F|$ *vs.* $|F_o|$ revealed that Cruickshank's[22] weighting scheme $1/w = 1.79 + 0.0011|F_o| + 0.00024|F_o|^2$ would be more appropriate. Consequently, two additional least-squares cycles were performed, with anisotropic temperature factors for nonhydrogen atoms and isotropic temperature factors for hydrogen atoms, using the new weighting scheme. The R value for the 1103 observed reflections was 0.039 and the final shift/σ is less than 0.24 for all parameters.

The scattering factors for S, O, N, and C atoms are from Cromer and Waber[23a] and for H atoms from Stewart, Davidson, and Simpson.[23b]

Results

The observed and calculated structure amplitudes have been deposited.[24] The final positional and thermal parameters for nonhydrogen atoms are given in Table I and those for hydrogen atoms are given in Table II.

Table II. Positional Parameters and Isotropic Thermal Parameters of Hydrogen Atoms[a]

Atom	$X \times 10^3$	$Y \times 10^3$	$Z \times 10^3$	B
H1(N4)	1061 (5)	604 (6)	1640 (8)	2.3 (0.9)
H2(N4)	959 (6)	726 (8)	1551 (11)	4.6 (1.2)
H(5)	791 (5)	636 (7)	1126 (9)	2.8 (1.0)
H(6)	683 (4)	374 (6)	714 (7)	1.5 (0.7)
H(1′)	842 (6)	37 (7)	504 (10)	3.3 (1.2)
H(2′)	660 (6)	−140 (8)	865 (11)	4.2 (1.2)
H(3′)	504 (4)	48 (5)	704 (7)	1.3 (0.8)
H(4′)	507 (4)	−60 (5)	141 (7)	1.2 (0.7)
H1(5′)	473 (7)	272 (9)	76 (12)	4.6 (1.2)
H2(5′)	349 (6)	128 (7)	195 (10)	3.6 (1.2)
H(O2′)	581 (7)	−365 (9)	468 (12)	5.0 (1.4)
H(O3′)	345 (5)	−243 (7)	622 (10)	3.5 (1.2)
H(O5′)	379 (7)	320 (9)	525 (13)	4.8 (1.8)
H1(W1)	138 (5)	275 (7)	510 (9)	2.7 (1.0)
H2(W1)	214 (10)	169 (13)	729 (17)	7.8 (1.9)
H1(W2)	256 (7)	−500 (9)	908 (17)	4.8 (1.4)
H2(W2)	321 (17)	−335 (21)	1021 (27)	14.0 (3.3)

[a] Standard deviations are given in parentheses.

Discussion

Bond Distances and Bond Angles. The intramolecular bond lengths and bond angles involving the nonhydrogen atoms are shown in Figure 1. It is of interest to compare the molecular dimensions of 2-thiocytidine with the common nucleoside cytidine.[25] The C(2)–N(3) bond in 2-thiocytidine is 1.342 Å while in cytidine it is 1.361 Å. The difference can be considered to be only marginally significant. However, the exocyclic angles show considerably larger differences, a maximum difference of about 3° occurring in the glycosidic bond. In 2-thiocytidine the exocyclic angles at N(1) are almost identical and close to 120°, while in cytidine these

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Table III. The C=S Bond Distances (Å) and Some Bond Angles (Degrees) in Thio Derivatives (Estimated Standard Deviations Are Given in Parentheses)

Pyrimidine compd	C(2)–S(2)	C(4)–S(4)	N(1)–C(2)–S(2)	N(3)–C(2)–S(2)	N(3)–C(4)–S(4)	C(5)–C(4)–S(4)	N(1)–C(2)–N(3)	N(3)–C(4)–C(5)
2-Thiouracil[a]	1.669 (5)		123	123			114	
2-Thiocytosine[b]	1.701 (1)							
2-Thiocytidine dihydrate (this investigation)	1.696 (4)		120.4 (0.3)	120.1 (0.3)			119.5 (0.3)	
2,4-Dithiouracil[c]	1.645 (6)	1.684 (6)	125.9 (0.5)	121.7 (0.4)	119.3 (0.4)	123.2 (0.4)	112.4 (0.5)	117.4 (0.5)
2,4-Dithiouridine monohydrate[d]	1.653 (5)	1.660 (5)	124.1 (0.3)	121.1 (0.3)	120.0 (0.3)	126.3 (0.3)	114.9 (0.3)	113.7 (0.3)
4-Thiouridine monohydrate[e]		1.662 (7)			121.4 (0.5)	124.1 (0.5)		114.5 (0.5)
Purine compds	C(6)–S(6)		N(1)–C(6)–S(6)	C(5)–C(6)–S(6)	N(1)–C(6)–C(5)			
6-Thioinosine[f]								
Molecule 1	1.671 (9)		121.7 (0.4)	126.7 (0.4)	111.6 (0.4)			
Molecule 2	1.667 (9)		122.1 (0.4)	127.3 (0.4)	110.5 (0.4)			
6-Mercaptopurine[g,h]	1.676 (2)		122.6 (0.1)	127.0 (0.1)	110.4 (0.1)			
	1.679 (1)		122.2 (0.1)	126.6 (0.1)	111.2 (0.1)			
6-Thioguanine[i]	1.690 (1)		122.0 (0.3)	126.6 (0.3)	111.3 (0.3)			

[a] See ref 2. [b] See ref 4. [c] See ref 3. [d] See ref 7. [e] See ref 5. [f] See ref 12. [g] See ref 8. [h] See ref 9. [i] See ref 11.

Table IV. The Glycosidic Bond Distance and Glycosidic Torsional Angles in Some Pyrimidine Derivatives[a]

	C(1′)–N, Å	χ_{CN}, deg[l]
Cytidine[b]	1.497 (6)	18.4
Cytidine 3′-phosphate[c] (orthorhombic form)	1.475 (6)	42.1
Cytidine 3′-phosphate[d] (monoclinic form)	1.485 (9)	39.3
Deoxycytidine hydrochloride[e]	1.508 (3)	0.0
Deoxycytidine 5′-phosphate monohydrate[f]	1.510 (4)	−5.9
2-Thiocytidine dihydrate	1.494 (4)	20.3
Thymidine[g]	1.480 (6)	40.0
5-Fluoro-2′-deoxyuridine[h]	1.482 (9)	58.7
5-Methyluridine[i]	1.481 (6)	29.4
Adenosine-2′,5′-uridine-phosphoric acid[j] tetrahydrate uridine residue	1.497 (6)	4.9
2,4-Dithiouridine monohydrate[k]	1.492 (6)	19.5
Mean	1.491	

[a] Only those structures with estimated standard deviations in bond distances less than 0.01 Å are given. [b] See ref 25. [c] See ref 36. [d] C. E. Bugg and R. E. Marsh, *J. Mol. Biol.*, **25**, 67 (1967). [e] D. J. Hunt and E. Subramanian, *Acta Crystallogr., Sect. B*, **25**, 2144 (1969). [f] M. A. Viswamitra, B. Swaminatha Reddy, Hung-Yin Lin, and M. Sundaralingam, *J. Amer. Chem. Soc.*, in press. [g] D. W. Young, P. Tollin, and H. R. Wilson, *Acta Crystallogr., Sect. B*, **25**, 1423 (1969). [h] See ref 34. [i] See ref 35. [j] See ref 37. [k] See ref 7. [l] The definition of the glycosidic torsion angle, χ_{CN}, used here is that given in ref 27. Note that the angles 180–360° in ref 27 are referred to as −180 to 0° here.

angles differ by almost 5°. The expansion of the valence angle C(2)–N(1)–C(1′) in 2-thiocytidine as compared to that of cytidine is attributable to the nonbonded interaction between the sulfur and C(1′). The bond distances and bond angles involving hydrogen atoms are normal.

C–S Bond Distance. A comparison of the C–S bond distances in the known thio derivatives is given in Table III. The C–S bond distances lie in the range 1.64–1.70 Å and the average of the 13 values in Table III is 1.673 Å. These values are characteristic of the carbon–sulfur double bond indicating that the thione form (rather than the mercapto form) is indeed the preferred tautomer even in the thio analogs of the nucleic acid constituents.

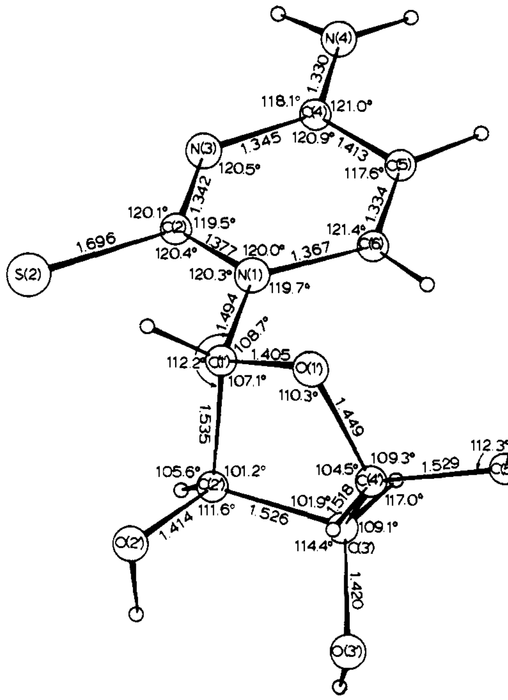

Figure 1. Bond distances and bond angles in 2-thiocytidine. Average estimated standard deviations are 0.004 Å for the C–S bond, 0.005 Å for C–C, C–N, and C–O bonds, and 0.3° for all bond angles.

The C(2)–S(2) bond distances in 2-thiocytidine and 2-thiocytosine[4] are close to 1.70 Å which is significantly longer than the corresponding values in 2,4-thiouridine[7] (1.65 Å), 2,4-dithiouracil[3] (1.65 Å), and 2-thiouracil[2] (1.67 Å). The average C(4)–S(4) bond distance is 1.668 Å, which is close to the average of 1.671 Å for the C(6)–S(6) bond distance in the purine derivatives.

Both the exocyclic and ring angles involving the C–S bonds are also shown in Table III. In all cases, except 2-thiocytidine, the exocyclic angles are unequal. On an average a difference of about 4° is observed.

Table V. Least-Squares Planes through the Base and Sugar[a,b]

Atom	Deviation from plane I, Å	Atom	Deviation from plane II, Å	Deviation from plane III, Å
S(2)	−0.069	C(1′)	**−0.026** (0.003)	**0.000** (0.003)
N(4)	−0.033	C(2′)	**0.015** (0.003)	0.127
N(1)	**0.001** (0.003)	C(3′)	−0.585	−0.487
C(2)	**−0.016** (0.003)	C(4′)	**−0.017** (0.003)	**0.000** (0.003)
N(3)	**0.019** (0.003)	O(1′)	**0.027** (0.003)	**0.000** (0.002)
C(4)	**−0.006** (0.003)	C(5′)	−0.792	−0.823
C(5)	**−0.010** (0.003)	N(1)	−1.280	−1.267
C(6)	**0.012** (0.003)			
C(1′)	−0.019			
Rms Δ[c]	0.012		0.022	0.000
σ(rms Δ)	0.003		0.003	0.003

[a] Equations of planes: plane I, $0.657x + 0.581y - 0.480z = 3.031$; plane II, $0.474x - 0.509y - 0.718z = 1.120$; plane III, $0.458x - 0.560y - 0.690z = 1.068$. [b] Displacements in bold type denote the atoms used in the calculation of the planes. For these atoms the estimated standard deviations are given in parentheses. [c] Root mean square deviation of fitted atoms from plane.

Table VI. Planarity of the Pyrimidine Bases in Nucleosides and Nucleotides

		Displacement (× 10³ Å) of atoms which determine the least-squares plane						Displacement (× 10³ Å) of substituents from ring plane						
	Rms Δ	N(1)	C(2)	N(3)	C(4)	C(5)	C(6)	O(2)	O(4)	N(4)	C(7)	X(5)	C(1′)	O(1′)
Aminopyrimidines														
Cytidine[b]	16	0	19	−27	14	6	−13	58		39			22	−396
Cytidine 3′-phosphate[c] (orthorhombic form)	16	23	−12	−11	24	−14	9	−53		104			−5	−950
Cytidine 3′-phosphate[d] (monoclinic form)	9	1	0	−8	15	13	6	130		45			114	−651
Deoxycytidine hydrochloride[e]	18	25	−29	12	10	−15	−2	−86		29			−67	−170
Keto- (and thio-)pyrimidines														
2-Thiocytidine	12	−1	11	−19	6	10	12	69[a]		33			19	−439
Calcium thymidine 5′-phosphate[f]	7	−11	9	−3	0	−3	9	109	67		39		−51	−1006
5-Methyluridine[g]	6	0	−8	8	−1	−6	7	−1	13		−13		82	−499
5-Fluoro-2′-deoxyuridine[h]	11	1	−12	20	−15	4	2	−40	−34			14	−144	−1393
5-Bromouridine–dimethyl sulfoxide complex[i]	28	39	−27	−11	36	−26	−11	−52	131			−134	111	−1111
5-Iodouridine[j]	20	28	−32	12	11	−16	−3	−117	4			−26	5	−1236
Barium uridine 5′-phosphate[k]	34	22	−42	52	−39	18	−11	−141	−41				119	−752
Adenosine 2′,5′-uridine phosphate[l] (uridine residue)	24	31	−32	4	25	−28	1	−58	104				−12	−198
Cyclic 3′,5′-uridine monophosphate[m]														
Molecule 1	12	12	5	−13	5	12	−20	−4	262				−99	−1490
Molecule 2	12	−11	16	−5	−12	18	−7	28	−78				−33	−1843
2,4-Dithiouridine[n]	16	−17	26	−14	−8	18	−5	143[a]	−21[a]				−94	−595

[a] Displacement of substituent sulfur atoms. [b] See ref 25. [c] See ref 36. [d] See Table IV; footnote *d*. [e] See Table IV, footnote *e*. [f] K. N. Trueblood, P. Horn, and V. Luzzati, *Acta Crystallogr.*, **14**, 965 (1961). [g] See ref 35. [h] See ref 34. [i] J. Iball, C. H. Morgan, and H. R. Wilson, *Proc. Roy. Soc., Sect. A*, **302**, 225 (1968). [j] N. Camerman and J. Trotter, *Acta Crystallogr.*, **18**, 203 (1965). [k] See ref 33. [l] See ref 37. [m] C. L. Coulter, *Acta Crystallogr., Sect. B*, **25**, 2055 (1969). [n] See ref 7. Note that the direction of displacement of the atoms is given relative to that of O(1′).

The Glycosidic C(1′)–N Bond. The glycosidic bond distance of 1.494 ± 0.004 Å is in agreement with those observed in other structures, Table IV. The average value of the glycosidic bond, 1.491 Å, in the pyrimidine nucleosides and nucleotides is slightly longer than the average, 1.46 Å, in the purine derivatives.[26] This difference is attributed to the differences in the nonbonded interactions between the base and sugar; in the purines a five-membered heterocycle is attached to the sugar while in the pyrimidines a six-membered heterocycle is attached. That the glycosidic bond distance is correlated to the glycosidic torsional angle is indicated in Table IV. There is a tendency for the C(1′)–N bond to increase as the torsional angle decreases (Figure 2). This is consistent with the increase in steric interaction between the base and sugar as the torsional angle decreases. The bond distances and bond angles in the ribose residue are within the usual range.

Conformation of the Nucleoside

Glycosidic Torsional Angle. The torsional angle[27] about the glycosidic bond, 20.3°, is anti,[27,28] as is usually

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(26) M. Sundaralingam and J. Abola, *Acta Crystallogr.*, in press.

(27) M. Sundaralingam, *Biopolymers*, 7, 821 (1969).

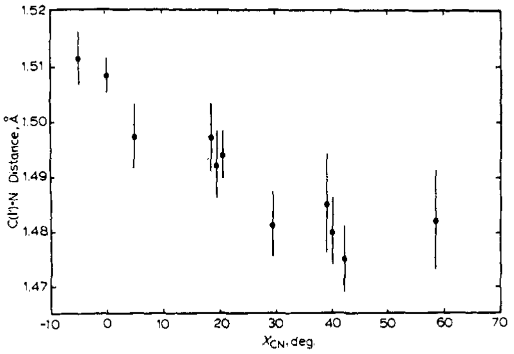

Figure 2. Graph showing the dependence of the glycosidic C(1′)-N bond distance on the glycosidic torsional angle χ(O(1′)-C(1′)-N-C(6)) in the pyrimidine derivatives. The line through each point represents ±1 estimated standard deviation.

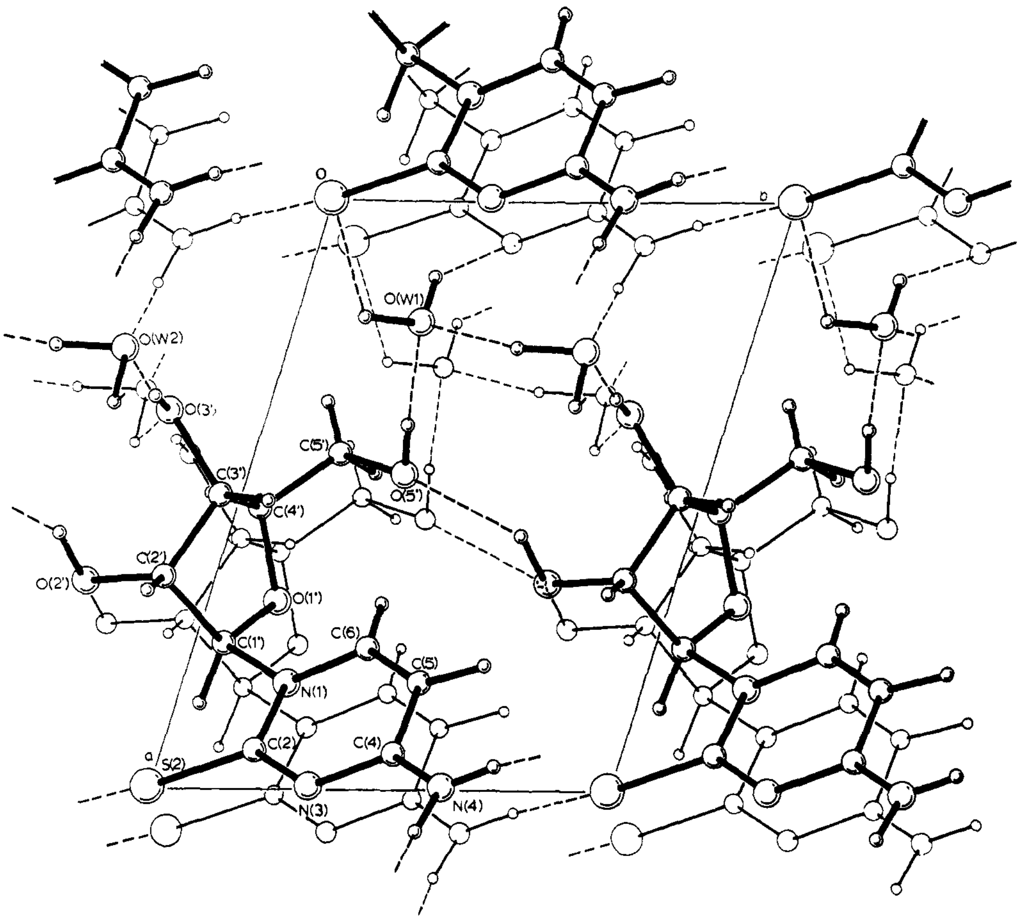

Figure 3. Intermolecular hydrogen bond scheme.

found in the pyrimidine nucleosides and nucleotides[27-29] (Table IV).

Planarity of the Base. The least-squares plane through the six atoms of the pyrimidine ring (plane I in Table V) shows that the ring atoms, N(3) and C(2), are probably significantly displaced on opposite sides from the plane. The substituents S(2) and N(4) show marked displacements on the same side of the ring. The adjacent substituents C(1′) and S(2) in thiocytidine are displaced on the same side from the ring though these substituents may also be displaced on opposite sides from the ring plane in the nucleosides and nucleotides.

The deviations of the atoms (relative to the deviation of the furanose ring oxygen O(1′)) from the base plane are listed for several pyrimidine derivatives in Table VI. In general the ring atoms show much less deviation than the substituents. The substituents are not always displaced in the same manner relative to the base, indicating that the base has some flexibility and its mode of distortion is regulated by the molecular environment, hydrogen bonding, and van der Waals' forces. The purine bases also show similar flexibility.[8,30]

Ribose Conformation. Least-squares planes were calculated for all combinations of four ring atoms. The one defined by C(1′), O(1′), C(2′), and C(4′) (plane II in Table V) is the best among those planes, the deviations lying in the range from 0.015 to 0.027 Å. Atom C(3′) is displaced by 0.585 Å from this plane on the same side of C(5′). Thus, the conformation of the sugar is C(3′) endo. Based on the plane defined by C(1′), O(1′), and C(4′) (plane III in Table V), the sugar possesses a twist (T) conformation and is referred to as C(3′)-endo-C(2′)-exo ($^3T_2$). The torsional angle O(2′)-C(2′)-C(3′)-O(3′) of 43° is comparable to the values observed in similar structures. The conformation of the ring can also be described by means of a series of

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(28) J. Donohue and K. N. Trueblood, *J. Mol. Biol.*, 2, 363 (1960).
(29) A. E. V. Haschemeyer and A. Rich, *ibid.*, 27, 369 (1967).
(30) M. Sundaralingam and S. K. Arora, *Proc. Nat. Acad. Sci. U. S.*, **64**, 1021 (1969).

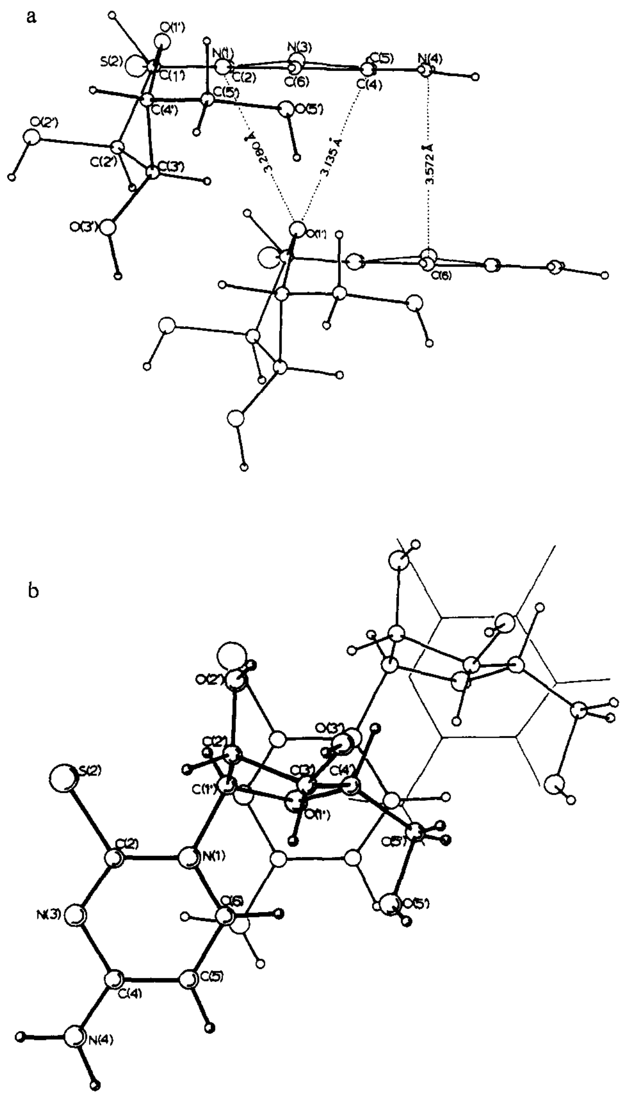

Figure 4. Projections (a) parallel and (b) perpendicular to the base plane showing the shortest base–base and sugar–base distances. Note the lack of base stacking in the structure. It has been observed that the majority of the known pyrimidine nucleosides and nucleotides show little tendency for overlap of the base rings; rather the preferred mode of stacking is the superposition of the carbonyl or amine function of the base with an adjacent base ring.[39,40]

torsional angles:[27,31,32] $\tau_0 = 4.9^\circ$, $\tau_1 = -26.8^\circ$, $\tau_2 = 37.1^\circ$, $\tau_3 = -35.3^\circ$, and $\tau_4 = 19.3^\circ$.[27]

The dihedral angles formed by the adjacent ring protons also provide information on the conformation of the furanose ring. Indeed the utility of proton magnetic resonance spectroscopy in the study of molecular conformation relies heavily on the relation between dihedral angles and coupling constants. The dihedral angles involving the ring hydrogen atoms are H(1′)-C(1′)-C(2′)-H(2′), 100°; H(2′)-C(2′)-C(3′)-H(3′), 49°; H(3′)-C(3′)-C(4′)-H(4′), −166°; H(4′)-C(4′)-C(5′)-H1(5′), −63°; and H(4′)-C(4′)-C(5′)-H2(5′), 59°. The estimated errors in these torsional angles are about 4°.

It would be expected that the conformation of the hydroxy group is influenced by the molecular environment. Indeed the hydroxy group may possess the gauche (cis) or the anti conformation; however, it has been noted that there is a preference for the gauche conformation. The torsional angles H–C–O–H are H(2′)-C(2′)-O(2′)-H(O2′), 66°; H(3′)-C(3′)-O(3′)-H(O3′), −39°; H1(5′)-C(5′)-O(5′)-H(O5′), 125°; and H2(5′)-C(5′)-O(5′)-H(O5′), 11°.

The torsional angles about the C(4′)-C(5′) bond denoted by ϕ_{oo}(O(1′)-C(4′)-C(5′)-O(5′)) and ϕ_{co}(C(3′)-C(4′)-C(5′)-O(5′))[33] are −63.8 and 54.6°, respectively. Thus, the conformation of the hydroxymethyl group is the preferred gauche–gauche conformation.[32,33]

The dihedral angle between the least-squares plane of the pyrimdine ring and that of the ribose ring excluding C(3′) is 68.9°. This angle is comparable to the values in other pyrimidine derivatives, 5-fluoro-2′-deoxy-β-uridine (71.9°),[34] 5-methyluridine (71.9°),[35] cytidine 3′-phosphate (62°),[36] 2,4-dithiouridine (63.6°),[7] and cytidine (75°).[25]

Hydrogen Bonding and Crystal Packing

The intermolecular hydrogen bond scheme is shown by broken lines in Figure 3. The hydrogen bond distances and angles are given in Table VII. Each molecule of 2-thiocytidine is involved in nine hydrogen bonds with surrounding molecules and the water. The water–water hydrogen bond distance is 2.74 Å. The sulfur atom, S(2), is hydrogen bonded to both N(4) and O(W1), and the only interbase hydrogen bond in the structure is N(4)···S(2) = 3.47 Å. The water molecules are involved in a tetrahedral scheme of hydrogen bonds, and they are directly attached to each other, the base, and the sugar. The hydrogen bonds to the base are O(W1)-H···N(3), O(W2)···H-N(4), and to the sugar are O(W1)···H-O(5′), O(W2)-H···O(3′), and O(W2)···H-O(3′). O(2′) is the only atom that is not directly hydrogen bonded to the water molecules. This and other crystal structures[7] of thio analogs of nucleic acid constituents indicate that the sulfur can participate in hydrogen bonding, although, from considerations of electronegativity differences, it will be expected that sulfur will form weaker hydrogen bonds than the corresponding oxygen analogs.

Projections parallel to the base plane and perpendicular to the base plane are shown in Figure 4a and 4b, respectively. There hardly is any overlap of the bases. The only atom that sticks under the π system of an adjacent base is one of the hydrogen atoms of the amino group. The interplanar distance between base planes is 3.32 Å and the shortest distance between bases is 3.572 Å for N(4)···C(6). On the other hand, the sugar ring oxygen points into the π system of the base from the opposite side of the middle nucleoside, Figure 4b. The shortest sugar–base contact distance is 3.135 Å for O(1′)···C(4). The preference of the sugar ring O(1′) to approach the π system of the base has already been pointed out.[37–40] The ring O(1′)

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Table VII. Hydrogen Bonds and Angles

Atom A	Atom B	Symmetry code[a]	Distance, Å	Bond	Angle, deg
S(2)	N(4)	II	3.467	C(2)-S(2)···N(4)	138.4
				C(4)-N(4)···S(2)	156.7
S(2)	H_2(N4)	II	2.75	C(2)-S(2)···H_2(N4)	129
				N(4)-H_2(N4)···S(2)	139
S(2)	O(W1)	III	3.276	C(2)-S(2)···O(W1)	81.3
S(2)	H_2(W1)	III	2.74	C(2)-S(2)···H_2(W1)	94
				O(W1)-H_2(W1)···S(2)	120
N(3)	O(W1)	IV	2.819	C(2)-N(3)···O(W1)	118.7
				C(4)-N(3)···O(W1)	122.4
N(3)	H_1(W1)	IV	1.94	C(2)-N(3)···H_1(W1)	112
				C(4)-N(3)···H_1(W1)	127
				O(W1)-H_1(W1)···N(3)	166
O(W2)	N4	V	3.01	C(4)-N(4)···O(W2)	127
O(W2)	H_1(N4)	V	2.11	N(4)-H_1(N4)···O(W2)	161
O(W1)	O(5′)	I	2.711	C(5′)-O(5′)···O(W1)	108.4
O(W1)	H(O5′)	I	1.83	O(5′)-H(O5′)···O(W1)	178
O(W2)	O(3′)	I	2.778	C(3′)-O(3′)···O(W2)	114.0
O(W2)	H(O3′)	I	1.96	O(3′)-H(O3′)···O(W2)	170
O(5′)	O(2′)	VI	2.890	C(5′)-O(5′)···O(2′)	119.0
				C(2′)-O(2′)···O(5′)	120.8
O(5′)	H(O2′)	VI	2.13	O(2′)-H(O2′)···O(5′)	157
				C(5′)-O(5′)···H(O2′)	122
O(3′)	O(W2)	VII	2.841	C(3′)-O(3′)···O(W2)	119.0
O(3′)	H_2(W2)	VII	2.32	C(3′)-O(3′)···H_2(W2)	113
				O(W2)-H_2(W2)···O(3′)	135
O(W1)	O(W2)	VI	2.737		
O(W1)	H_1(W2)	VI	1.87	O(W2)-H_1(W2)···O(W1)	141

[a] I x, y, z; II $x, y - 1, z - 1$; III $x + 1, y, z$; IV $x + 1, y, z + 1$; V $x - 1, y - 1, z - 1$; VI $x, y + 1, z$; VII $x, y, z - 1$.

shows a striking preference for hydrophobic interaction rather than hydrogen bonding.[38]

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