Stereochemistry of Nucleic Acids and Their Constituents. XI. The Molecular Structure and Conformation of α-Pseudouridine Monohydrate, an Unusual Nucleoside with a "Glycosidic" Carbon–Carbon Bond[1]

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Abstract: The crystal structure of α-pseudouridine, an isomer of the naturally occurring β-pseudouridine found as a minor component in tRNA, has been determined. The "glycosidic" C–C bond in pseudouridine makes it a unique nucleoside. α-Pseudouridine crystallizes in the monoclinic system, space group $P2_1$, with unit cell dimensions $a = 14.032$, $b = 6.3076$, $c = 6.455$ Å and $\beta = 92.964°$. The structure was solved by a combination of the Harker–Patterson synthesis and the application of the tangent formula, and refined by least squares to an R index of 0.041 using 1038 intensities measured on a diffractometer. The pertinent conformational features are as follows. The glycosidic torsional angle of +3.2° is different from the values observed for the common nucleosides, the conformation of the sugar is C(2′)-*exo*, and the conformation about the C(4′)–C(5′) bond is *gauche-trans*. The *exo* puckering of the sugar is quite characteristic of the α-nucleosides. The only interbase hydrogen bond involves N(1)–H and O(4) of adjacent molecules; N(3)–H of the base is hydrogen bonded to the ribose hydroxy O(2′)–H group of a symmetry related molecule. All three hydroxy groups of the sugar are involved in a donor and an acceptor hydrogen bond, and the water is involved in three hydrogen bonds. The "base stacking" is such that there is only a partial overlap of the pyrimidine rings involving the N(1)–H atoms of adjacent bases rotated 180° to each other; thus, the adjacent bases are antiparallel.

Transfer RNAs contain about 10–15 minor nucleotides, most of which are modified in the base moiety. The presence of these modified nucleotides is unique to tRNA and may play an important role in establishing the tertiary structures of tRNAs. The tertiary structure is what makes them suitable for their biological function as specific amino acid carriers. The determination of the structure of pseudouridine was undertaken as part of a continuing study on the stereochemistry of the unusual components found in tRNAs. The carbon–carbon "glycosidic" linkage rather than the normal N-glycosidic linkage makes pseudouridine unique even among the unusual nucleotides. It occurs in the "loop" containing the nucleotide sequence, ribothymidine–pseudouridine–cytidine, a triplet of nucleotides which is present in all of the tRNAs that have been sequenced to date. Also, the anticodon loop often contains one and sometimes two pseudouridine residues. In this paper is presented the detailed crystal and molecular structure of α-pseudouridine, an isomer of the natural β-pseudouridine.

According to the specifications of Sigma Chemical Co., the material used in this work was mostly the β isomer. However, the crystal structure analysis revealed that the structure was the α isomer. The crystals were grown from aqueous solution (see below) in the presence of a small amount of $NiCl_2$. Therefore, it is not clear to us whether we had fractional crystallization of the isomers or that the $NiCl_2$ produced the interconversion of isomers. It will be of considerable interest to know if the α or β configurations might be selectively used in tRNA.

Experimental Section

Grade II pseudouridine, Lot No. 96B-1320, was purchased from Sigma Chemical Co., St. Louis, Mo. Attempts to crystallize it from water, water–ethanol mixtures, dimethyl sulfoxide, and acetone only yielded small crystals which were inadequate for the X-ray investigation. During the course of preparation of metal complexes of nucleic acid components, certain metal salts, *e.g.*, $NiCl_2$, were found to promote the growth of large single crystals of the nucleic acid components rather than form complexes with them.[3] Thus, single crystals of pseudouridine were obtained as very thin plates by slow evaporation from an aqueous 0.005 *M* $NiCl_2$ solution. An irregularly shaped crystal measuring 0.19 × 0.18 × 0.04 mm³ was mounted along the *b* axis. The preliminary film data gave approximate unit-cell constants and showed the systematic absences $0k0$, $k = 2n + 1$; therefore the crystals belong to the monoclinic system with the acentric space group $P2_1$ since the molecule is optically active. The accurate unit-cell dimensions measured on a Picker four-angle diffractometer are $a = 14.032 \pm 0.002$ Å, $b = 6.3076 \pm 0.0007$ Å, $c = 6.455 \pm 0.001$ Å, and $\beta = 92.964 \pm 0.004°$, $D_{\text{calcd}} = 1.526$ g cm^{-3}, assuming two molecules of pseudouridine monohydrate ($C_7N_2O_2H_{12} \cdot H_2O$, formula weight 262.17). The density measured, D_{obsd}, by flotation in CCl_4–$CHCl_3$ mixture is 1.591 g cm^{-3}. The rather large value for the observed density was due to the presence of $NiCl_2$ on the surface of the crystals. Altogether 1092 independent reflections were measured on a diffractometer using the θ–2θ scan mode and Ni-filtered Cu Kα radiation to a maximum 2θ of 134°. Of these, 1038 were considered above background and were used in the structure analysis.

Structure Determination and Refinement

The structure was solved by a combination of Patterson and direct methods. An origin-removed Patterson was calculated using $E^2 - 1$ as the coefficient of the Fourier summation, where E is the normalized structure amplitude. The distribution of the peaks around the origin of the section at $y = 0$ fitted the pattern for a six-membered pyrimidine ring almost perfectly. The Harker section at $y = {}^1/_2$ clearly revealed the center-to-center interaction involving the 2_1 symmetry related

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[1] Part X of this series by C. E. Bugg, J. Thomas, M. Sundaralingam, and S. T. Rao, *Biopolymers*, in press.

[2] To whom reprint requests should be addressed at: Department of Biochemistry, University of Wisconsin, Madison, Wisconsin 53706.

[3] M. Sundaralingam and J. A. Carrabine unpublished results, (1968).

Table I. Atomic Positional and Thermal Parameters^a

Atom	x/a	y/b	z/c	β_{11}	β_{22}	β_{33}	β_{12}	β_{13}	β_{23}
N(1)	−01336 (20)	−07593 (86)	53242 (42)	30 (1)	166 (9)	83 (6)	6 (4)	−3 (2)	−8 (9)
C(2)	−08751 (23)	−07602 (97)	38864 (50)	26 (2)	130 (9)	115 (7)	2 (5)	−7 (3)	−4 (9)
N (3)	−06062 (20)	−07604 (92)	18817 (41)	22 (1)	222 (9)	81 (6)	11 (4)	−16 (2)	−5 (9)
C(4)	03174 (23)	−07649 (101)	12177 (47)	27 (1)	170 (9)	84 (7)	12 (5)	−11 (2)	2 (9)
C(5)	10606 (22)	−08219 (94)	28581 (45)	21 (1)	150 (9)	88 (7)	2 (4)	−14 (2)	−5 (9)
C(6)	08005 (23)	−07884 (95)	48312 (49)	26 (1)	138 (9)	112 (8)	2 (5)	−18 (3)	−6 (10)
O(2)	−17185 (16)	−07439 (00)	43349 (37)	24 (1)	266 (9)	149 (6)	5 (4)	−1 (2)	−10 (8)
O(4)	04598 (16)	−07588 (86)	−06295 (34)	32 (1)	337 (10)	84 (5)	14 (4)	−5 (2)	1 (8)
C(1′)	20872 (24)	−08496 (104)	23095 (56)	24 (1)	212 (11)	133 (8)	−6 (5)	−9 (3)	−35 (12)
C(2′)	23880 (25)	−27612 (93)	10308 (56)	20 (2)	207 (12)	107 (9)	2 (4)	−4 (3)	−11 (9)
C(3′)	34602 (27)	−28676 (99)	16558 (62)	21 (2)	192 (12)	162 (9)	2 (4)	−4 (3)	−8 (9)
C(4′)	35008 (26)	−21531 (93)	39179 (58)	24 (2)	187 (13)	151 (9)	−5 (4)	−21 (3)	−15 (10)
C(5′)	43562 (27)	−08071 (107)	45544 (66)	22 (2)	208 (12)	242 (10)	6 (6)	−16 (3)	−67 (14)
O(1′)	26590 (18)	−09556 (95)	42244 (41)	23 (1)	430 (13)	164 (7)	24 (4)	−33 (2)	−137 (10)
O(2′)	19004 (19)	−46653 (80)	14909 (42)	29 (1)	207 (9)	121 (6)	−9 (3)	−16 (2)	−20 (7)
O(3′)	38101 (22)	−49389 (84)	13502 (54)	31 (1)	277 (11)	297 (10)	37 (4)	−50 (3)	−107 (9)
O(5′)	44487 (21)	−04669 (84)	67213 (54)	32 (1)	281 (13)	246 (9)	14 (3)	−35 (3)	−99 (8)
O$_w$	31727 (33)	−78014 (105)	82390 (62)	76 (2)	384 (16)	289 (12)	72 (6)	−62 (4)	−156 (11)
				B (Å^2)					
H(1)	−0313 (25)	−0515 (90)	6546 (57)	2.14					
H(3)	−0940 (27)	−0708 (99)	1073 (54)	2.17					
H(6)	1322 (25)	−0745 (91)	5759 (53)	2.22					
H(1′)	2288 (28)	0472 (84)	1629 (64)	2.38					
H(2′)	2326 (25)	−2586 (81)	−0514 (59)	1.98					
H(3)	3084 (28)	−1947 (79)	0825 (62)	2.60					
H(4′)	3502 (29)	−3418 (79)	4729 (62)	2.44					
H(5′)	4941 (31)	−1377 (76)	4299 (61)	3.07					
H′(5′)	4231 (28)	0586 (83)	3644 (66)	3.07					
H(O)(2′)	1852 (31)	−4847 (85)	2567 (67)	2.36					
H(O)(3′)	4267 (34)	−4877 (93)	1748 (77)	3.63					
H(O)(5′)	4130 (36)	0024 (105)	7147 (81)	3.45					
H(O$_w$)	3354 (43)	−6887 (113)	8828 (89)	5.27					
H′(O$_w$)	2680 (37)	−6798 (91)	7181 (77)	5.27					

[a] The positional and anisotropic parameters (β) have been multiplied by 10^5, while the positional parameters of the hydrogen atoms have been multiplied by 10^4. Estimated standard deviations are in parentheses.

Table III. Bond Lengths and Angles Not Involving Hydrogen Atoms^a

	Length, Å			Angle, deg	
Atom	Pseudouridine	5-Methyl-uridine	Atom	Pseudouridine	5-Methyl-uridine
N(1)–C(2)	1.358 (4)	1.377^b	N(1)–C(2)–N(3)	114.1 (2)	114.2^b
C(2)–N(3)	1.367 (4)	1.376	C(2)–N(3)–C(4)	127.0 (3)	127.2
N(3)–C(4)	1.386 (4)	1.384	N(3)–C(4)–C(5)	115.0 (3)	115.4
C(4)–C(5)	1.448 (4)	1.437	C(4)–C(5)–C(6)	118.2 (3)	117.6
C(5)–C(6)	1.343 (4)	1.345	C(5)–C(6)–N(1)	122.2 (3)	124.2
C(6)–N(1)	1.365 (4)	1.361	C(6)–N(1)–C(2)	123.5 (2)	121.6
C(2)–O(2)	1.233 (4)	1.196	N(1)–C(2)–O(2)	123.4 (2)	123.0
C(4)–O(4)	1.219 (4)	1.223	N(3)–C(2)–O(2)	122.5 (2)	122.9
C(5)–C(1′)	1.501 (4)	1.481^c	N(3)–C(4)–O(4)	120.4 (3)	119.2
C(1′)–C(2′)	1.533 (7)		C(5)–C(4)–O(4)	124.6 (3)	125.4
C(2′)–C(3′)	1.539 (5)		C(4)–C(5)–C(1′)	119.4 (3)	116.8^d
C(3′)–C(4′)	1.526 (6)		C(6)–C(5)–C(1′)	122.3 (2)	121.5^e
C(4′)–O(1′)	1.424 (6)		C(1′)–C(2′)–C(3′)	100.7 (3)	
O(1′)–C(1′)	1.440 (4)		C(2′)–C(3′)–C(4′)	103.0 (3)	
C(2′)–O(2′)	1.421 (7)		C(3′)–C(4)–O(1′)	107.4 (3)	
C(3′)–O(3′)	1.413 (8)		C(4′)–O(1′)–C(1′)	109.5 (3)	
C(4′)–C(5′)	1.510 (6)		O(1′)–C(1′)–C(2′)	105.6 (3)	
C(5′)–O(5′)	1.415 (5)		O(1′)–C(1′)–C(5)	107.3 (3)	
			C(2′)–C(1′)–C(5)	115.5 (3)	
			C(1′)–C(2′)–O(2′)	113.8 (3)	
			C(3′)–C(2′)–O(2′)	112.5 (3)	
			C(2′)–C(3′)–O(3′)	110.2 (3)	
			C(4′)–C(3′)–O(3′)	114.2 (3)	
			C(3′)–C(4′)–C(5′)	114.6 (3)	
			O(1′)–C(4′)–C(5′)	108.5 (3)	
			C(4′)–C(5′)–O(5′)	112.7 (3)	

[a] The bases have been given the same numbering in pseudouridine and 5-methyluridine. Estimated standard deviations are in parentheses. [b] The average estimated standard deviation in bond distances is 0.006 Å and in bond angles is 0.5°. [c] Glycosidic C–N bond distance. [d] Valence angle C(2)–N(1)–C(1′). [e] Valence angle C(6)–N(1)–C(1′).

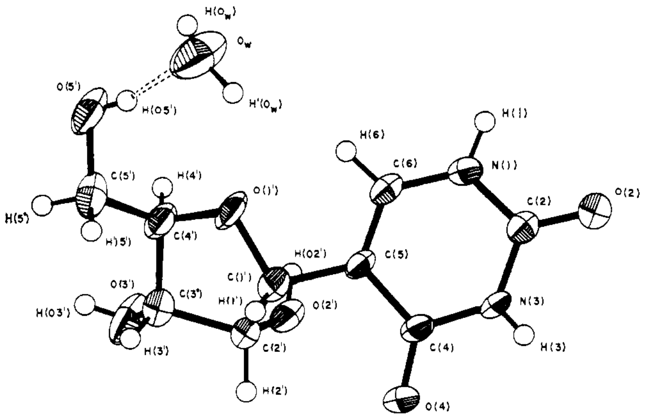

Figure 1. The thermal ellipsoids of the nonhydrogen atoms and the atom numbering.

bases, thus permitting the determination of preliminary atomic coordinates for the six pyrimidine ring atoms. These atoms were used to generate starting phases for refinement by the tangent formula[4] on the 247 largest E values, $E > 1.8$. The resulting E map clearly showed all but the three atoms O(2'), O(3'), and the water oxygen. These atoms were obtained by structure factor–Fourier calculations. Least-squares isotropic and anisotropic refinement lowered the R index, defined as $\Sigma||F_o| - |F_c||/\Sigma|F_o|$, to 0.065. The fourteen hydrogen atoms were located in a difference electron density map. When these were included in the refinement, varying their positions, but holding their temperature factors fixed, the R index dropped to the final value of 0.041. The hydrogen atoms were assigned the isotropic temperature factors of the atoms to which they were attached.

The final positional coordinates and thermal parameters are given in Table I. The observed and calculated structure factors are listed in Table II, which has been deposited with the NAPS–ASIS agency.[5] The plot of the thermal ellipsoids and the numbering of the molecule are shown in Figure 1.

The scattering factors of carbon, nitrogen, and oxygen were from the International Tables of Crystallography,[6] while that of hydrogen was from Stewart, *et al.*[7] The weighting scheme used was based on counting statistics.

Description of the Structure

Bond Distances. The bond distances and angles involving the nonhydrogen atoms are shown in Table III and Figure 2, while those involving the hydrogen atoms are given in Table IV.

Of interest is the "glycosidic" C–C bond distance of 1.501 ± 0.004 Å which is, as expected, 0.029 Å longer than the average value of 1.472 Å found for the glycosidic C–N bond distance. The bond distances in the uracil moiety of α-pseudouridine are compared with those of 5-methyluridine[8] in Table III. It is seen that the largest differences are in the N(1)–C(2) and C(4)–C(5) bonds, the former bond is adjacent to the glycosidic C–N bond in the common β-nu-

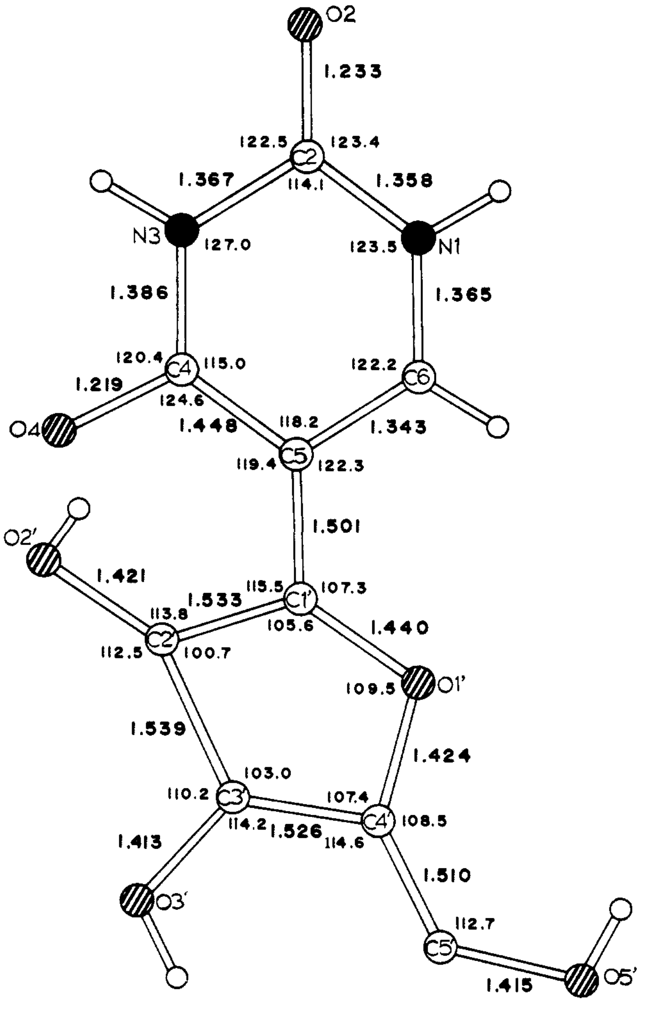

Figure 2. A schematic drawing showing the bond distances and bond angles not involving the hydrogen atoms.

Table IV. Ranges and Average Values of the Bond Distances and Bond Angles Involving Hydrogen Atoms[a]

Bond	Range	Av, Å
N–H (trigonal)	0.68–0.85	0.77
C–H (trigonal)		0.92
C–H (tetrahedral)	0.92–1.06	0.98
O–H (tetrahedral)	0.62–1.13	0.77
Angle	**Range**	**Av, deg**
C–N–H (trigonal)	112–123	117
C–C–H (trigonal)		112
N–C–H (trigonal)		126
C–C–H (tetrahedral)	105–116	110
O–C–H (tetrahedral)	101–115	107
C–O–H (tetrahedral)	103–119	112
H–C–H (tetrahedral)		110
H–O–H (tetrahedral)	93	93

[a] The average estimated standard deviation in the bond distance is 0.04 Å and in the bond angle is 1°.

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(4) K. Karle and I. L. Karle, *Acta Crystallogr.*, 21, 849 (1966).

(5) Table II has been deposited as Document No. NAPS-00999 with the ASIS National Auxiliary Publication Service, c/o CCM Information Corp., 909 3rd Ave., New York, N. Y. 10022. A copy may be secured by citing the document number and by remitting $1.00 for microfiche or $3.00 for photocopies. Advance payment is required. Make checks or money orders payable to: ASIS–NAPS.

(6) "International Tables for X-ray Crystallography," Vol. III, Kynoch Press, Birmingham, 1962.

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

(8) D. J. Hunt and E. Subramanian, *Acta Crystallogr.*, B25, 2144 (1969).

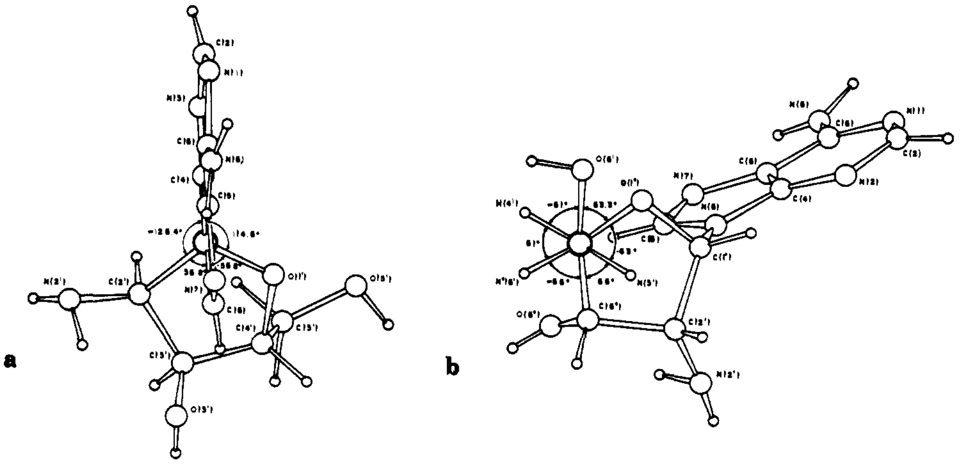

Figure 3. Conformation of the molecule as viewed down the (a) glycosidic bond and (b) the C(5′) → C(4′) bond.

cleoside, while the latter bond is adjacent to the "glycosidic" C–C bond in the α-nucleoside.

The carbonyl C(2)–O(2) bond distance of 1.233 ± 0.004 Å is longer than the carbonyl C(4)–O(4) bond distance of 1.219 ± 0.004 Å by 3.5σ, where σ is the estimated standard deviation in the bond distance. Since the bond distances have not been corrected for thermal motion it is difficult to evaluate the significance of this difference. However, it is noteworthy that O(2) is involved in two hydrogen bonds while O(4) is involved in only one. Furthermore, in the common nucleosides the reverse trend is observed in the carbonyl bond distances, *i.e.*, C(2)–O(2) < C(4)–O(4). The ribose C–C bond distances and the C–OH bond distances are within the limits of values generally observed.[9, 10]

Table V. Least-Squares Planes[a] of the Sugar and the Base

Atom	Sugar displacement, Å		Atom	Base displacement, Å	
C(1′)	**−0.001**	**0.000**	N(1)	**−0.011** (5)	**−0.006**
C(2′)	0.572	0.577	C(2)	**0.003** (5)	**0.007**
C(3′)	**0.001**	0.007	N(3)	**0.004** (5)	**0.002**
C(4′)	**−0.002**	**0.000**	C(4)	**−0.005** (6)	**−0.011**
O(1′)	**0.002**	**0.000**	C(5)	**0.017** (6)	**0.012**
C(5)	0.788	0.786	C(6)	**−0.005** (6)	**−0.004**
O(2′)	1.992	1.997	O(2)	**0.004** (0)	0.011
O(3′)	0.797	0.806	O(4)	**−0.007** (5)	−0.018
C(5′)	−1.186	−1.185	H(1)	−0.16	−0.16
			H(3)	−0.02	−0.02
			H(6)	−0.04	−0.04
			C(1′)	0.021	0.012
Rms deviation	0.001	0.000		0.008	0.009
σ (rms deviation)	0.005	0.000		0.005	0.005
l	0.490	0.489		−0.0100	−0.0122
m	0.835	0.836		−0.9999	−0.9999
n	−0.250	−0.247		−0.0039	−0.0004
d	0.578	0.579		0.4803	0.4882

[a] The equation of the plane is of the form $lx + my + nz = d$, where l, m, n are the direction cosines of the normal to the plane, and d (A) is the distance of the plane from the origin. x, y, z are coordinates in ångström units (axes a, b, c^*). Atoms shown in bold type were included in the calculation of the plane.

Bond Angles. It is worthwhile to point out that the pyrimidine ring angles at C(2) and C(4) are markedly smaller than the ideal trigonal value of 120°, while the angles at the nitrogen atoms are significantly greater. There is only a difference of 0.9° in the exocyclic angles involving the carbonyl group at C(2), but a significant difference, 4.2°, is found at the carbonyl group at C(4). Similar asymmetry in the exocyclic angles is also observed in the common nucleosides.

In general the bond angles of the pyrimidine system in pseudouridine and 5-methyluridine show good agreement, Table III. The largest differences are found in the angles C(5)–C(6)–N(1) and C(6)–N(1)–C(2); the former is 2.0° smaller in pseudouridine and the latter is 1.9° larger.

The valence angles in the sugar ring are all different; there is a difference of about 2° between the angles at C(2′) and C(3′), C(3′) and C(1′), C(1′) and C(4′), and C(4′) and O(1′). The smallest angle is associated with the puckered carbon atom C(2′) as is often the case.[9,10] The exocyclic valence angles involving the secondary hydroxy groups are greater than the tetrahedral value. Similarly, the angles C(2′)–C(1′)–C(5) and C(3′)–C(4′)–C(5′) are considerably larger than the tetrahedral value, while the angles O(1′)–C(1′)–C(5) and O(1′)–C(4′)–C(5′) are smaller than the tetrahedral value.

[9] M. Sundaralingam, *J. Amer. Chem. Soc.*, **87**, 599 (1965).

[10] M. Sundaralingam and L. H. Jensen, *J. Mol. Biol.*, **13**, 930 (1965).

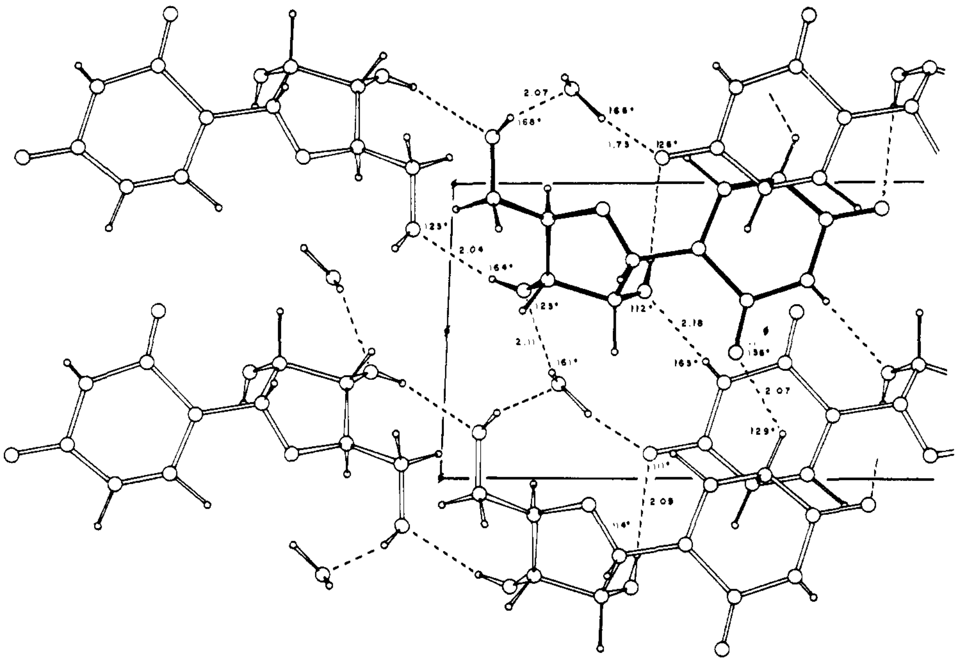

Figure 4. Hydrogen bonding in the crystal viewed along the *b* axis.

Conformation of the Nucleoside

"Glycosidic" C–C Torsional Angle. The glycosidic torsional angle χ for pseudouridine is here defined as the angle made by the projection of the C(6)–C(5) bond with respect to the O(1′)–C(1′) bond. When viewed along the C(1′)–C(5) bond positive angles (0–180°) are for a clockwise rotation of the C(6)–C(5) bond relative to the O(1′)–C(1′) bond, while negative angles (0–180°) are for a counterclockwise rotation. The conformation is *anti* when $-90° \leq \chi \leq 90.0°$.[11] In pseudouridine the "glycosidic" C–C torsional angle is +3.2° and the conformation therefore is *anti*, Figure 3. However, the glycosidic C–N torsional angle in all other known α nucleosides is in the range $-50° \pm 15°$.[11] The difference in the glycosidic C–C and C–N torsional angles is probably due to the differences in the bond distances and valence angles involving the glycosidic bonds.

The Sugar Conformation. The sugar exhibits an envelope conformation with C(2′) atom displaced by 0.572 Å on the opposite side of C(5′), Table V. This conformation is also referred to as the C(2′)-*exo* conformation, and, although rarely observed for the natural β-nucleosides,[9,11] it appears to be one of the preferred conformations of α-nucleosides.[12] The four atoms of the sugar ring, excluding C(2′), are perfectly coplanar. The torsional angles about the individual sugar ring bonds are: O(1′) → C(1′), 23.0°; C(1′) → C(2′), −35.4°; C(2′) → C(3′), 34.2°; C(3′) → C(4′), −22.2°; C(4′) → O(1′), 0.3°. The Cs symmetry of the sugar ring is seen by the close equivalence of the pairs of torsional angles about C(1′) → C(2′) and C(2′) → C(3′) on one hand and about C(3′) → C(4′) and O(1′) → C(1′) on the other.

The conformation about the C(4′)–C(5′) bond is *gauche–trans* with the torsional angles C(3′)–C(4′)–C(5′)–O(5′) = −170.4° and O(1′)–C(4′)–C(5′)–O(5′) = 69.5°, Figure 3. This conformation has not been observed in the 5′-nucleotides.[11]

The ribose hydroxy oxygen atoms O(2′) and O(3′) form a torsional angle of 34.9° about the C(2′)–C(3′) bond. This value is considerably less than the values generally observed in the ribosides,[13] and is attributable to the C(2′) *exo* puckering of the sugar.

Planarity of the Base. The least-squares planes through the atoms of the uracil moiety and the six pyrimidine ring atoms are given in Table V. In the former plane the largest deviation is exhibited by atom C(5). The sugar ring atom C(1′) attached to the base is also markedly displaced on the same side of the base as C(5). The atoms of the ring lie approximately in a plane, and the substituent oxygen atom O(4) is significantly displaced from this plane.

Crystal Packing and Base Stacking

Hydrogen Bonding. Altogether there are seven different hydrogen bonds in the structure with all the potential donors and acceptors being involved, Table VI and Figure 4. The strong interbase hydrogen bond N(1)–H···O(4) = 2.700 Å forms hydrogen-bonded ribbons of bases running parallel to the *c* axis. Normally N(1) is not available for hydrogen bonding, being covalently bonded to the sugar. The second nitrogen of the base is involved in an approximately linear hydrogen bond N(3)–H···O(2′) = 2.847 Å to the ribose hydroxy group O(2′)–H, which participates in a donor hydrogen bond with the oxygen atom O(2) of the base. The latter atom also accepts a hydrogen bond from the water molecule. Thus, O(2) of the base is involved in two hydrogen bonds while O(4) is involved in only one. Both these atoms are involved in nonlinear hydrogen bonds.

The sugar–sugar hydrogen bond O(3′)–H···O(5′) between twofold screw axis (at 0,0,0; 0,0,1/2) related molecules generates a right-handed hydrogen bonded

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[11] M. Sundaralingam, *Biopolymers*, **7**, 821 (1969).

[12] M. Sundaralingam, submitted for publication.

[13] M. Sundaralingam, *Acta Crystallogr.*, **21**, 495 (1966).

Table VI. The Hydrogen Bond Distances and Angles[a]

Symmetry no.[b]	Translation X	Y	Z	Atom	Angle, deg	Length, Å	Length from hydrogen, Å
1	0	0	−1	N(1)–H(1)···O(4)	129	2.700 (3)	2.07 (3)
				C(6)–N(1)···O(4)	88.5		
				C(2)–N(1)···O(4)	148.0		
				N(1)···O(4)–C(4)	152.6		
				H(1)···O(4)–C(4)	138		
2	0	−1	0	N(3)–H(3)···O(2′)	165	2.847 (4)	2.18 (3)
				C(2)–N(3)···O(2′)	121.8		
				C(4)–N(3)···O(2′)	109.5		
				N(3)···O(2′)–C(2′)	109.9		
				H(3)···O(2′)–C(2′)	112		
2	0	0	1	O(2′)–H(O2′)···O(2)	140	2.804 (4)	2.09 (4)
				C(2′)–O(2′)···O(2)	118.3		
				O(2′)···O(2)–C(2)	111.3		
				H(O2′)···O(2)–C(2)	111		
2	1	0	1	O(3′)–H(O3′)···O(5′)	164	2.705 (4)	2.04 (4)
				C(3′)–O(3′)···O(5′)	111.2		
				O(3′)···O(5′)–C(5′)	120.5		
				H(O3′)···O(5′)–C(5′)	123		
1	0	−1	0	O(5′)–H(O5′)···O(W)	168	2.678 (6)	2.07 (5)
				C(5′)–O(5′)···O(W)	115.3		
1	0	0	−1	O(W)–H(OW)···O(3′)	161	2.812 (6)	2.11 (6)
				O(W)···O(3′)–C(3′)	126.4		
				H(OW)···O(3′)–C(3′)	123		
2	0	0	1	O(W)–H′(OW)···O(2)	166	2.874 (5)	1.75 (5)
				O(W)···O(2)–C(2)	121.4		
				H′(OW)···O(2)–C(2)	126		

[a] Estimated standard deviations in parentheses. [b] Symmetry operations: (1) X, Y, Z; (2) $-X, 1/2 + Y, -Z$.

spiral. Furthermore, O(5′) donates its hydrogen atom to the water molecule[14] forming quite a strong hydrogen bond O(5′)–H···O_w = 2.678 Å, while O(3′) is involved in a second hydrogen bond by accepting a hydrogen atom from the water molecule. Hence, the three hydroxy groups of the sugar are each involved in a donor and an acceptor hydrogen bond and the water of crystallization is involved in two donor and one acceptor hydrogen bond. Only one of the sugar hydroxy groups is hydrogen bonded to the base, and also the water molecule forms a hydrogen bond to the base.

The sugar ring oxygen atom O(1′) is the only oxygen atom in the structure that is not involved in a hydrogen bond. This is a recurring feature in all of the known nucleosides and nucleotides.[15]

Base "Stacking." In pseudouridine a zigzag arrangement of base "stacking" down the screw axis at 1/2,0,0 is observed. The adjacent bases are inclined to each other at 1.4°. The overlap of the rings of the bases is meager, a feature that certainly appears to be the rule rather than the exception in the nucleic acids and their constituents.[1,16] The overlapping ring atoms are the N(1) atoms, and the adjacent bases are rotated 180° from each other. The shortest interbase distances are N(1)···N(1)··· = 3.206 Å, N(1)···C(6) = 3.272 Å, and C(2)···C(6) = 3.244 Å. The remaining interbase distances are greater than 3.4 Å.

The following programs were used in this analysis: (1) the full-matrix least-squares program of Busing, Martin, and Levy[17] modified for the Univac 1107 and 1108 computers;[18] (2) the direct phasing programs developed by Hall;[19] (3) ORTEP thermal ellipsoid plot program;[20] (4) the other programs were written by Rao[18] and others in these laboratories.

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(14) A survey (M. Sundaralingam (1968), unpublished results) of the hydrogen bonding features in nucleosides, 3′-nucleotides, and pyranosides reveals that the primary hydroxy group shows a marked preference for hydrogen bonding to water.

(15) M. Sundaralingam, *Biopolymers*, **6**, 189 (1968).

(16) M. Sundaralingam, S. T. Rao, C. E. Bugg, and J. Thomas, Abstracts, American Crystallographer's Association, Seattle, Wash., March 1969.

(17) W. R. Busing, K. A. Martin, and H. A. Levy, Oak Ridge National Laboratory Report ORNL-TM-305, Oak Ridge, Tenn., 1962.

(18) S. T. Rao, unpublished work.

(19) S. R. Hall, Abstract IX-43, VIIIth International Union of Crystallography Congress, Stonybrook, N. Y., Aug 1969.

(20) C. K. Johnson, Oak Ridge National Laboratory Report ORNL-3794, Oak Ridge, Tenn., 1965.