Stereochemistry of Nucleic Acids and Their Constituents. XIII.[1] The Crystal and Molecular Structure of 3′-O-Acetyladenosine. Conformational Analysis of Nucleosides and Nucleotides with *syn* Glycosidic Torsional Angle

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Abstract: The structure of 3′-O-acetyladenosine, a synthetic analog of 3′-O-aminoacyladenosine, has been accurately determined using a total of 1189 reflections measured on a diffractometer. The structural solution was obtained by direct application of the tangent method for noncentrosymmetric crystals. The orthorhombic space group of the compound is $P2_12_12_1$ with four molecules in the unit cell. The cell dimensions are: $a = 12.312$, $b = 12.609$, and $c = 8.824$ Å; $D_{\text{calcd}} = 1.504$ g cm^{-3} and $D_{\text{obsd}} = 1.502$ g cm^{-3}. The bond distances and bond angles have estimated standard deviations of 0.006 Å and 0.3°, respectively. The molecule is in the *syn* conformation, $\chi = -139.9°$, with the intramolecular hydrogen bond O(5′)-H···N(3) = 2.768 Å. This constrains the conformation about the C(4′)-C(5′) bond to *gauche-gauche* and the conformation of the sugar to C(2′)-*endo*. For steric reasons the *gauche-gauche* conformation will not be allowed for the 5′ nucleoside mono-, di-, and triphosphates in the *syn* conformation. The acetate group is planar and the carbonyl group is *cis* to the C(3′)-H(3′) bond. The acetate group, the base nitrogen N(1) and the sugar O(1′) are not involved in hydrogen bonding. There are neither any interbase hydrogen bonds nor base stacking. However, the shortest intermolecular contact O(1′)···N(1) = 3.17 Å, involving the atoms not involved in hydrogen bonding. The dimensions of the neutral adenine base are compared with the N(1) protonated bases. A conformational analysis of *syn* nucleosides is also presented.

The crystal structure of 3′-O-acetyladenosine was determined as part of a program of studies to provide accurate information on the molecular conformation and intermolecular interactions involving the terminal aminoacyladenylate of the charged transfer ribonucleic acids (tRNAs). In this connection the structure of puromycin[3] was reported earlier from this laboratory. A considerable number of reasonably accurate studies have been carried out in the past on neutral adenine groups—*viz.*, 9-methyladenine[4] (in complex with 1-methylthymine), 9-methyladenine,[5] 9-ethyladenine[6] (in complex with 1-methyluracil), deoxyadenosine monohydrate,[7] and 2′-amino-2′-deoxyadenosine.[8] However, the present study represents the most precise analysis of a neutral adenine nucleoside to date. A brief report on the structure of 3′-O-acetyladenosine has been published.[9]

Experimental Section

Crystalline samples of 3′-O-acetyladenosine ($C_{12}N_5O_5H_{15}$, mol wt = 309) were obtained from Dr. C. B. Reese of Cambridge University, England. From these a crystal with approximate dimensions 0.2 × 0.25 × 0.3 mm^3 was chosen for the X-ray structure analysis. Oscillation and Weisenberg photographs established the crystal system to be orthorhombic. The systematic absences $h00$, $h = 2n + 1$; $0k0$, $k = 2n + 1$; and $00l$, $= 2n + 1$, indicated the space group to be $P2_12_12_1$. The cell constants were determined from measurements on medium angle reflections on a Picker automatic diffractometer with Cu Kα ($\lambda = 1.5418$ Å) radiation and were found to be $a = 12.312 \pm 0.002$, $b = 12.609 \pm 0.002$, and $c = 8.824 \pm 0.001$ Å. The density of the crystal determined by the method of flotation in a mixture of carbon tetrachloride and benzene was 1.502 g cm^{-3}, and is in excellent agreement with the calculated density of 1.504 g cm^{-3} for four molecules in the unit cell. Complete three-dimensional intensity data up to $2\theta = 133°$ were collected on the Picker automatic diffractometer with Ni-filtered Cu radiation employing the 2θ scan technique. The crystal was mounted along the c axis and parallel to the ϕ axis of the goniostat and 1372 reflections were recorded. The standard deviation in the intensities $\sigma(I)$ were computed using the counting statistics and an electronic instability of 2%. An analysis of the intensities for the systematically absent reflections showed that a reflection can be considered observed if $I > 1.4\sigma(I)$. Based on this criterion 1189 reflections were considered observed, and were used in the analysis of the structure. The intensities were corrected for Lorentz and polarization factors but not for absorption.

Structure Determination. The structure was solved by the application of direct methods as applied to the noncentrosymmetric case.[10] The Σ_2 relations were set up for 285 of the strongest reflections with $E > 1.2$ and the process of phase determination was initiated with phases assigned to four reflections to define the origin and enantiomorph (Table I). No other symbolic phase was found necessary. These phases were then used with the tangent formula,[11] which determined new phases as well as refined the phases already

Table I. Reflections Used to Define the Origin and Enantiomorph

h	k	l	$\lvert E\rvert$	Phase
12	3	0	2.79	0
3	7	0	2.18	$\pi/2$
0	10	1	2.06	0
7	0	2	1.99	0

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[1] Part XII of this series is the preceding paper by D. Rohrer and M. Sundaralingam, *J. Amer. Chem. Soc.*, **92**, 4956 (1970).

[2] To whom reprint requests should be addressed.

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[4] K. Hoogsteen, *Acta Crystallogr.*, **16**, 907 (1963).

[5] R. F. Stewart and L. H. Jensen, *J. Chem. Phys.*, **40**, 2071 (1964).

[6] F. S. Mathews and A. Rich, *J. Mol. Biol.*, **8**, 89 (1964).

[7] D. G. Watson, D. J. Sutor, and P. Tollin, *Acta Crystallogr.*, **19**, 111 (1965).

[8] See ref 1.

[9] S. T. Rao, M. Sundaralingam, S. K. Arora, and S. R. Hall, *Biochem. Biophys. Res. Commun.*, **38**, 496 (1970).

[10] J. Karle and I. Karle, *Acta Crystallogr.*, **21**, 849 (1966).

[11] J. Karle and H. Hauptman, *ibid.*, **9**, 635 (1956).

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

Atom	$x \times 10^4$	$y \times 10^4$	$z \times 10^4$	$\beta_{11} \times 10^4$	$\beta_{22} \times 10^4$	$\beta_{33} \times 10^4$	$\beta_{12} \times 10^4$	$\beta_{13} \times 10^4$	$\beta_{23} \times 10^4$
N(1)	−66 (3)	5078 (3)	−7538 (5)	44 (3)	57 (3)	98 (5)	1 (2)	−12 (3)	−3 (4)
C(2)	653 (4)	4290 (4)	−7445 (6)	55 (4)	50 (3)	112 (7)	5 (3)	−13 (5)	−9 (4)
N(3)	1263 (3)	4000 (3)	−6261 (4)	49 (3)	45 (3)	92 (5)	7 (2)	−15 (4)	−11 (3)
C(4)	1077 (4)	4618 (3)	−5056 (5)	36 (3)	35 (3)	94 (6)	−1 (3)	−14 (4)	6 (4)
C(5)	375 (4)	5468 (4)	−4997 (5)	34 (3)	34 (3)	101 (7)	−5 (3)	−4 (4)	3 (4)
C(6)	−229 (3)	5696 (3)	−6325 (6)	37 (3)	37 (3)	124 (8)	−2 (3)	−6 (4)	17 (4)
N(6)	−941 (3)	6493 (3)	−6435 (5)	54 (3)	61 (3)	107 (6)	16 (3)	−7 (4)	17 (4)
N(7)	377 (3)	5926 (3)	−3544 (5)	49 (3)	44 (3)	114 (6)	4 (2)	−0.(4)	−0 (4)
C(8)	1085 (4)	5351 (4)	−2790 (5)	49 (3)	38 (3)	111 (7)	3 (3)	−12 (4)	−1 (4)
N(9)	1525 (3)	4546 (3)	−3626 (4)	43 (3)	30 (2)	86 (5)	2 (2)	−12 (3)	2 (3)
C(1′)	2259 (3)	3735 (3)	−3057 (5)	34 (3)	39 (3)	94 (6)	0 (2)	−8 (4)	2 (4)
C(2′)	1760 (4)	2625 (3)	−2974 (5)	44 (3)	37 (3)	90 (6)	−2 (3)	−9 (4)	4 (4)
O(2′)	1118 (3)	2560 (2)	−1650 (4)	50 (2)	47 (2)	124 (5)	−9 (2)	13 (3)	2 (3)
C(3′)	2770 (4)	1934 (4)	−3056 (5)	43 (3)	36 (3)	91 (6)	−1 (3)	−13 (4)	5 (4)
O(3′)	3273 (2)	1849 (2)	−1582 (3)	51 (2)	40 (2)	93 (5)	−0 (2)	−22 (3)	4 (3)
CA (1)	3039 (4)	965 (4)	−765 (5)	54 (4)	44 (3)	92 (7)	12 (3)	11 (4)	8 (4)
OA	2379 (3)	317 (3)	−1144 (4)	78 (3)	47 (3)	133 (6)	−14 (2)	−5 (4)	8 (3)
CA (2)	3704 (4)	928 (4)	651 (5)	65 (4)	84 (4)	80 (6)	2 (4)	−5 (5)	15 (5)
C(4′)	3531 (3)	2555 (3)	−4065 (5)	41 (3)	35 (3)	114 (7)	4 (3)	−5 (4)	−0 (4)
C(5′)	3573 (4)	2170 (4)	−5687 (6)	51 (4)	55 (4)	92 (6)	8 (3)	2 (4)	−9 (4)
O(5′)	2536 (3)	2192 (2)	−6374 (4)	68 (3)	51 (3)	116 (5)	16 (2)	−30 (3)	−23 (3)
O(1′)	3138 (2)	3645 (2)	−4078 (3)	39 (2)	35 (2)	105 (5)	−3 (2)	−1 (3)	5 (3)
		$\times 10^3$							
H(1)	75 (4)	380 (3)	−843 (5)[b]						
H(2)	−108 (4)	693 (4)	−543 (5)						
H(3)	−127 (4)	653 (3)	−724 (5)						
H(4)	119 (4)	555 (4)	−162 (5)						
H(5)	244 (4)	399 (3)	−194 (5)						
H(6)	134 (4)	255 (4)	−389 (5)						
H(7)	57 (4)	190 (4)	−166 (5)						
H(8)	252 (4)	123 (4)	−345 (5)						
H(9)	334 (4)	41 (4)	133 (5)						
H(10)	414 (4)	163 (3)	87 (6)						
H(11)	431 (3)	65 (3)	44 (4)						
H(12)	425 (5)	250 (4)	−375 (5)						
H(13)	409 (4)	266 (4)	−639 (5)						
H(14)	400 (4)	155 (4)	−625 (5)						
H(15)	200 (4)	272 (4)	−626 (5)						

[a] Standard deviations are given in parentheses and refer to the least significant digit. [b] All hydrogen atoms are isotropic with $B = 3$ Å^2. The anisotropic temperature factor is of the form $\exp[-(\beta_{11}h^2 + \cdots + 2\beta_{12}hk + \cdots)]$.

determined.[12] When complete convergent was achieved, it was found that the root mean square (rms) value of the change in phase in the last two cycles was less than 1° for the 285 reflections. An E map was computed using these phases and the entire structure was solved from this map, the 22 nonhydrogen atomic sites being found from among the 23 strongest peaks in the map.

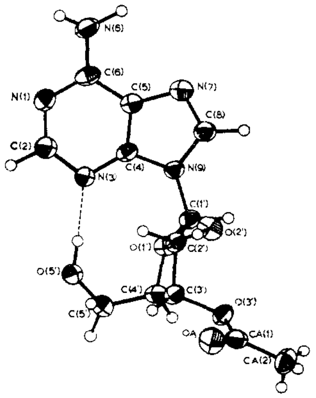

Figure 1. The ellipsoids of thermal vibrations of the nonhydrogen atoms projected on the base plane.

Structure Refinement. The coordinates of the 22 atoms of the molecule as read from the E map were subjected to three cycles of isotropic refinement using the full-matrix least-squares program[13] modified for use on the Univac 1108 computer.[14] This was followed by two cycles of anisotropic refinement which lowered the R value to 0.08. A difference electron density map computed at this stage clearly showed the positions of all the 15 hydrogen atoms in the structure. These hydrogen atoms were included in the refinement with fixed isotropic thermal parameters ($B = 3$ Å^2) and their positional parameters were refined along with the positional and thermal parameters of the heavy atoms. Convergence was achieved at the end of three cycles, the average shift in the parameters being less than 0.30σ, where σ is the estimated standard deviation in the atomic parameters. The refinement was terminated at this stage with an R value of 0.041 for 1189 observed reflections. The R value for all the data (1372 reflections, both observed and unobserved) is 0.054. The maximum electron density in a final difference electron density map was 0.09 e Å^{-3}.

The weighting scheme used was that deduced from the counting statistics. The scattering factors for carbon, nitrogen and oxygen were taken from the International Tables[15] and that of hydrogen was from Stewart, *et al.*[16]

The atomic parameters are listed in Table II. The observed and calculated structure factors are listed in Table III which has been deposited with the NAPS-ASIS agency.[17] The thermal ellipsoids of the atoms projected on the plane containing the base are represented by the drawing[18] in Figure 1.

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[12] S. R. Hall, Abstract IX-43, VIIIth International Union of Crystallography Congress, Stonybrook, N. Y., Aug 1969.

[13] W. R. Busing, K. A. Martin, and H. A. Levy, Oak Ridge National Laboratory Report ORNL-TM-305, Oak Ridge, Tenn., 1962.

[14] S. T. Rao, unpublished results.

[15] "International Tables for Crystallography," Vol. III, Kynoch Press, Birmingham, 1962.

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

[17] Table III has been deposited as Document No. NAPS-01001 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.

Results and Discussion

Bond Distances and Bond Angles. The bond lengths and bond angles involving the nonhydrogen atoms are given in Tables IV and V. The bond lengths and bond angles in the neutral adenine moiety are generally in good agreement, within the experimental errors, with the values found in other nucleosides; deoxyadenosine monohydrate[7] and α-D-2′-amino-2′-deoxyadenosine monohydrate,[8] Table V,[19] and are in agreement with the average values for the neutral adenine base.[20,21] However, as has been noted earlier,[22] there are significant points of difference between the neutral adenine base and the N(1)-protonated base. These differences in the bond distances and bond angles are particularly large for the bond sequence N(3)–C(2)–N(1)–C(6)–N(6) in the vicinity of protonation. The C(6)–N(1) and N(1)–C(2) bond distances in acetyladenosine are smaller than those of 3′-AMP, while the C(2)–N(3) and C(6)–N(6) bond distances are larger in the neutral base. The variations in the bond distances among the neutral adenine or the protonated adenine are probably attributable to differences in hydrogen bonding and intermolecular interactions involving the bases. For instance, N(1) of the neutral adenine base in acetyladenosine and N(3) of 2′-amino-2′-deoxyadenosine monohydrate are not involved in hydrogen bonding, while in deoxyadenosine all the potential hydrogen bonding sites are involved in hydrogen bonding. In the protonated derivative, adenosine 3′-phosphate, all the sites are involved in hydrogen bonding, but the N(3) atom of the adenine moiety in adenosine 2′,5′-uridine phosphate[6] is not involved in hydrogen bonding, Figure 2. The effect of hydrogen bonding on the bond distances of the barbiturates was recently reported.[23]

Table IV. Some Bond Lengths and Bond Angles Involving the Heavy Atoms[a]

Atoms	Bond length	Atoms	Bond angle
N(9)–C(1′)	1.454 (5)	O(1′)–C(1′)–C(2′)	105.3 (3)
C(1′)–C(2′)	1.529 (6)	C(4′)–O(1′)–C(1′)	108.9 (3)
C(2′)–C(3′)	1.520 (6)	C(4)–N(9)–C(1′)	127.6 (3)
C(2′)–O(2′)	1.413 (5)	C(8)–N(9)–C(1′)	125.6 (3)
C(3′)–C(4′)	1.511 (6)	N(9)–C(1′)–C(2′)	114.2 (3)
C(3′)–O(3′)	1.445 (5)	N(9)–C(1′)–O(1′)	108.2 (3)
O(3′)–CA(1)	1.358 (6)	C(1′)–C(2′)–C(3′)	101.2 (3)
CA(1)–OA	1.200 (6)	C(2′)–C(3′)–C(4′)	103.7 (3)
CA(1)–CA(2)	1.494 (7)	C(3′)–C(4′)–O(1′)	106.7 (3)
C(4′)–C(5′)	1.513 (7)	C(1′)–C(2′)–O(2′)	108.5 (3)
C(4′)–O(1′)	1.457 (5)	C(3′)–C(2′)–O(2′)	117.6 (3)
O(1′)–C(1′)	1.413 (5)	C(2′)–C(3′)–O(3′)	110.5 (3)
C(5′)–O(5′)	1.414 (6)	C(4′)–C(3′)–O(3′)	107.6 (3)
		C(3′)–O(3′)–CA(1)	116.6 (3)
		O(3′)–CA(1)–OA	123.7 (3)
		O(3′)–CA(1)–CA(2)	110.6 (3)
		OA–CA(1)–CA(2)	125.7 (3)
		C(3′)–C(4′)–C(5′)	114.4 (3)
		O(1′)–C(4′)–C(5′)	107.9 (3)
		C(4′)–C(5′)–O(5′)	111.6 (3)

[a] Standard deviations are given in parentheses and refer to the least significant digit.

Table V. Comparison of the Bond Distances and Bond Angles in the Neutral Adenine Group of 3′-O-Acetyladenosine (**1**), 2′-Amino-2′-deoxyadenosine (**2**),[a] Deoxyadenosine (**3**),[b] and in the Protonated Adenine Group of Adenosine 3′-Phosphate (**4**),[c] and Adenosine 2′,5′-Uridine Phosphate (**5**)[d]

	Neutral, Å			Protonated, Å	
Bond	1	2	3	4	5
N(1)–C(2)	1.333	1.354	1.333	1.349	1.350
N(1)–C(6)	1.340	1.341	1.336	1.363	1.370
C(2)–N(3)	1.337	1.328	1.326	1.306	1.306
N(3)–C(4)	1.339	1.329	1.346	1.353	1.351
C(4)–C(5)	1.378	1.369	1.392	1.381	1.382
C(4)–N(9)	1.380	1.386	1.369	1.355	1.358
C(5)–C(6)	1.418	1.416	1.414	1.401	1.401
C(6)–N(6)	1.336	1.328	1.331	1.319	1.320
C(5)–N(7)	1.406	1.386	1.375	1.384	1.389
N(7)–C(8)	1.314	1.302	1.307	1.312	1.313
C(8)–N(9)	1.367	1.372	1.361	1.368	1.396
σ bond	0.006	0.006_5	0.011	0.006	0.010 Å

	Neutral, Å			Protonated, Å	
Angle	1	2	3	4	5
N(1)–C(2)–N(3)	128.7	129.2	128.8	125.9	125.2
C(2)–N(3)–C(4)	111.4	110.3	111.0	111.6	112.4
N(3)–C(4)–C(5)	126.2	128.1	126.9	127.2	126.8
C(4)–C(5)–C(6)	117.1	116.3	115.4	118.2	118.4
C(5)–C(6)–N(1)	117.6	117.9	118.1	113.7	113.5
C(6)–N(1)–C(2)	118.9	117.9	119.8	123.3	123.6
C(5)–N(7)–C(8)	103.7	103.6	104.4	103.3	104.8
N(7)–C(8)–N(9)	113.5	113.9	113.2	113.4	111.9
C(8)–N(9)–C(4)	106.6	105.6	106.8	106.6	106.6
N(9)–C(4)–C(5)	105.5	105.1	104.8	105.4	106.2
C(4)–C(5)–N(7)	110.7	111.6	110.7	111.2	110.5
N(1)–C(6)–N(6)	118.5	119.1	119.2	120.2	120.8
C(5)–C(6)–N(6)	123.8	122.9	122.7	126.1	125.7
σ angle	0.3	0.4	1.0	0.3	0.8

[a] Reference 8. [b] Reference 7. [c] Reference 19. [d] E. Shefter, M. Barlow, R. A. Sparks, and K N. Trueblood, *Acta Crystallogr.*, *B*, **25**, 895 (1969).

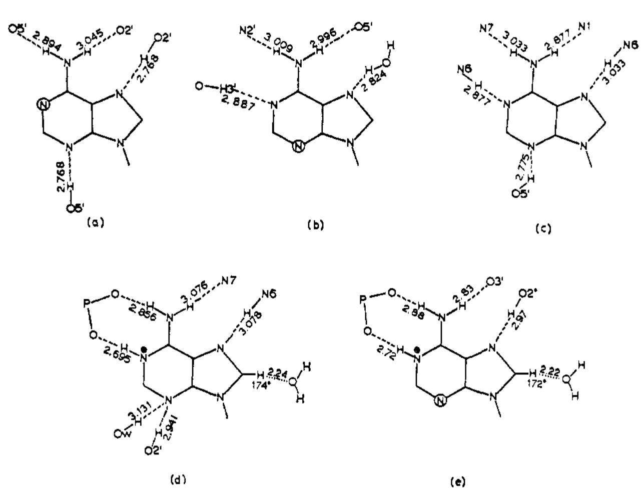

Figure 2. The hydrogen bonding differences of the base in the neutral adenine derivatives: (a) 3′-O-acetyladenosine, (b) 2′-amino-2′-deoxy-α-D-adenosine, (c) deoxyadenosine and the protonated adenine derivatives, (d) adenosine 3′-phosphate, and (e) adenosine 2′,5′-uridine phosphate. Note that the protonated adenines exhibit a C(8)–H···O hydrogen bonding; apparently the protonation at N(1) increases the polarization of the C(8)–H bond. The differences in the hydrogen bonding apparently alters the electronic configuration of the base sufficiently to cause noticeable differences in the bond distances (Table V).

More profound differences are observed in the bond angles. The bond angle at N(1) of acetyladenosine

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

(19) M. Sundaralingam, *Acta Crystallogr.*, **21**, 495 (1966).

(20) S. T. Rao and M. Sundaralingam in "Synthetic Procedures in Nucleic Acid Chemistry," Vol. II, W. W. Zorbach and R. S. Tipson, Ed., Wiley, New York, N. Y., in press.

(21) J. Donohue, *Arch. Biochem. Biophys.*, **128**, 59 (1968).

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

(23) B. M. Craven, *Acta Crystallogr.*, *A*, **25**, S138 (1969).

is 118.9 ± 0.3° while in 3′-AMP, where N(1) is protonated, the angle has increased to 123.3 ± 0.3°, Table V. Similar large differences are also observed in the angles at C(2) and C(6). The exocyclic angles at C(6) are larger in the protonated base. These differences in the molecular geometry of the protonated and unprotonated adenine rings are sufficiently large to indicate whether the base is protonated or not, even if the X-ray data does not reveal the proton at N(1).

The bond distances and angles in the ribose moiety are in agreement with the values generally found.[22,24] The largest and the smallest bond angles C(3′)-C(2′)-O(2′) = 117.6° and C(1′)-C(2′)-C(3′) = 101.2°, respectively, involve the puckered carbon atom C(2′).

The bond angle at the acetate ester oxygen atom O(3′) is 116.6° and is significantly smaller than the corresponding values observed in the 2′ and 3′-nucleotides, Table VI. The bond angle at the ester oxygen atom of the 5′-nucleotides shows a larger range, 114–122° (average 117.4°), than that of the 3′-nucleotides, range 118.5–121.2° (average 119.9°). In acetyladenosine the C(3′)-O(3′) bond distances of 1.445 ± 0.005 Å is longer than the normal (methanol) C-O bond of 1.427 Å[25] and shows the same trend as the distances observed in cytidine 3′-phosphate[26,27] where the sugar also exhibits a C(2′) *endo* pucker.

Table VI. A Comparison of the Ester C-O-P Bond Angles Involving the Primary and Secondary Carbon Atoms of the Sugar

	C(5′)-O(5′)-P
Adenosine 5′-phosphate monohydrate[a]	114.7 (0.5°)
Inosine 5′-phosphate$\cdot 7H_2O$[b]	114 (1.3°)
Thymidine 5′-phosphate$\cdot Ca \cdot 7H_2O$[c]	119 (1.4°)
Uridine 5′-phosphate$\cdot Ba \cdot 8H_2O$[d]	122 (2°)
	C(3′)-O(3′)-P
Adenosine 3′-phosphate dihydrate[e]	119.1 (0.2°)
Cytidine 3′-phosphate (orthorhombic)[f]	121.2 (0.4°)
Cytidine 3′-phosphate (monoclinic)[g]	118.5 (1.0°)
	C(2′)-O(2′)-P
Adenosine 2′,5′-uridine phosphate tetrahydrate[h]	124.3 (0.5°)

[a] J. Kraut and L. H. Jensen, *Acta Crystallogr.*, **16**, 79 (1963). [b] S. T. Rao and M. Sundaralingam, *J. Amer. Chem. Soc.*, **91**, 1210 (1969). [c] K. N. Trueblood, P. Horn, and V. Luzzati, *Acta Crystallogr.*, **14**, 965 (1961). [d] E. Shefter and K. N. Trueblood, *ibid.*, **18**, 1067 (1965). [e] Reference 19. [f] Reference 26. [g] Reference 27. [h] See Table V, footnote *d*.

The bond lengths and bond angles involving the hydrogen atoms are reasonable and are shown in Table VII.

Planarity of the Base. The least-squares plane through the nine ring atoms was calculated and the displacements of the atoms from the plane are listed in Table VIII (plane I). In this and all other calculations of least-squares planes, all the atoms fitted to the plane were given the same weights. It is seen that the atoms C(5), N(1), and N(9) are displaced from the plane by 0.022, 0.017, and 0.016 Å, respectively, which are only significant at the 2σ level (95% confidence level). But there are some known cases where the deviations from planarity are observed for the purine bases in nucleosides and nucleotides,[3] and the bases themselves.[28] The substituent atoms N(6) and C(1′) on the base are displaced on opposite sides of plane I by 0.006 and 0.133 Å, respectively. Such large deviations of the C(1′) atom from the base appear to be quite common.

Table VII

	Range	Mean	Esd
C-H bonds	0.85–1.08 Å	1.01 Å	0.05 Å
N-H bonds	0.82–1.05 Å	0.94 Å	0.05 Å
O-H bonds	0.95–1.07 Å	1.01 Å	0.05 Å
C-C-H angle (tetrahedral)	107–130°	108°	2°
O-C-H angle (tetrahedral)	104–112°	109°	2°
C-O-H angle (tetrahedral)	108–114°	110°	2°
N-C-H angle (trigonal) C-N-H	110–118°	115°	2°
H-C-H angle (tetrahedral)	87–132°	107°	2°
H-N-H angle (trigonal)		127°	2°

Table VIII. Least-Squares Planes for the Base and Deviation of the Atoms from the Planes[a,b]

Atoms	Plane I, Å	Plane II, Å	Plane III
N(1)	**0.017 A**	**0.005 Å**	0.024
C(2)	**0.004**	**−0.003**	0.002
N(3)	**−0.012**	**−0.004**	−0.021
C(4)	**−0.009**	**0.009**	**−0.016**
C(5)	**−0.022**	**−0.006**	**−0.019**
C(6)	**0.000**	**−0.001**	0.010
N(6)	−0.006	−0.011	0.013
N(7)	**0.001**	0.030	**0.004**
C(8)	**0.006**	0.047	**0.001**
N(9)	**0.016**	0.051	**0.005**
C(1′)	0.133	0.117	0.111
H(1)	0.04	0.03	0.04
H(2)	0.04	0.05	0.07
H(3)	0.05	0.04	0.08
H(4)	0.06	0.11	0.06
Rms deviation of fitted atoms	0.012	0.005	0.010

[a] Atoms used in fitting the least-squares plane are shown in bold type. The dihedral angle between planes II and III is 0.9°. [b] Plane I, $-0.731X - 0.615Y + 0.295Z = -5.859$; plane II, $-0.727X - 0.615Y + 0.306Z = -5.919$; plane III, $-0.736X - 0.610Y + 0.294Z = -5.823$.

Sugar Puckering. The five possible four-atom planes were calculated for the ribose and the deviations of the atoms from the base plane are given in Table IX. C(2′) is displaced by 0.565 Å on the same side of the plane as C(5′). Thus, the sugar displays the C(2′)-*endo* pucker. Again here the four atoms comprising the plane show marked deviation from the plane. Hence, the displacement of the atoms C(2′) and C(3′) from the plane through the three remaining ring atoms was calculated, plane V. It is seen that both C(2′) and C(3′) are displaced on the same side of the plane as C(5′). Therefore, the conformation of the sugar described in terms of the three atom plane is C(2′)-*endo*-C(3′)-*endo*,[24] Figure 3. This conformation, although less common appears to be one of the preferred modes of puckering of the sugar, and hitherto it has not been considered in model building studies of the nucleic acids.

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(24) M. Sundaralingam, *J. Amer. Chem. Soc.*, **87**, 599 (1965).

(25) P. Venkateswarlu and W. Gordy, *J. Chem. Phys.*, **23**, 1200 (1955).

(26) M. Sundaralingam and L. H. Jensen, *J. Mol. Biol.*, **13**, 914 (1965).

(27) C. E. Bugg and R. E. Marsh, *ibid.*, **25**, 67 (1967).

(28) J. Sletten and L. H. Jensen, *Acta Crystallogr.*, *B*, **25**, 1608 (1969).

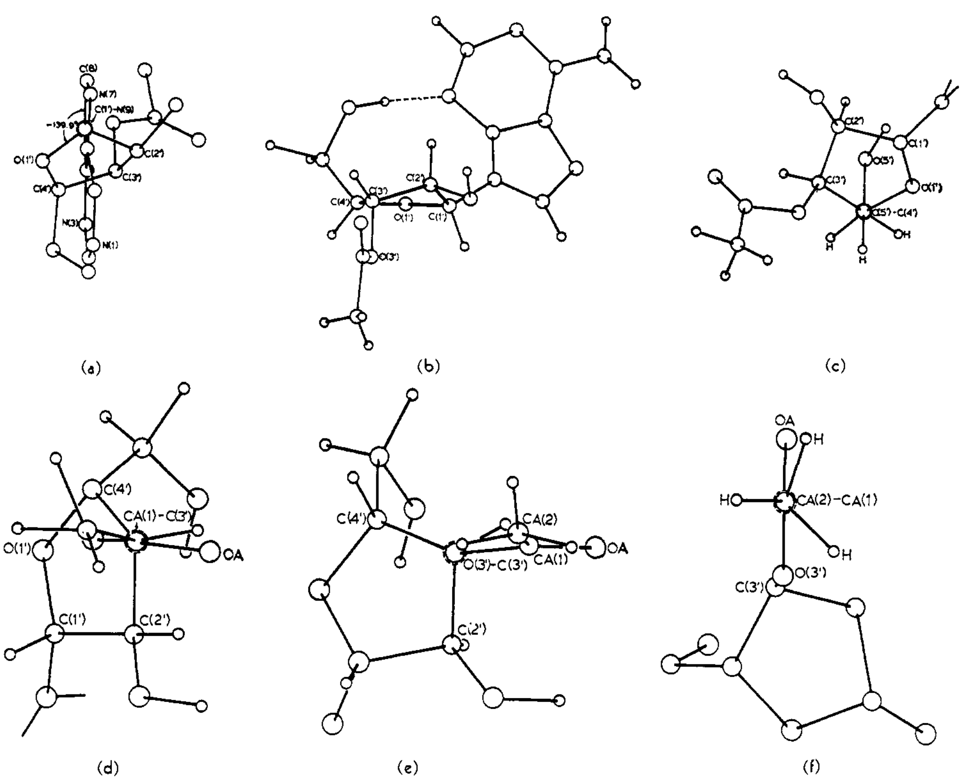

Figure 3. (a) A view down the glycosidic bond showing the *syn* conformation, (b) the sugar viewed end on parallel to the three-atom plane C(1')-O(1')-C(4') showing the displacement of atoms C(2') and C(3') on the same side of C(5'); thus, the conformation of the sugar is C(2')-*endo*-C(3')-*endo*. A conformation that has been noticed sufficiently frequently,[19,24] and should be considered as a possible mode of puckering of the sugar in the nucleic acids. Therefore, this mode of puckering should be considered hereafter in nucleic acid model building studies. (c) A projection down the C(5')-C(4') bond showing the most preferred *gauche-gauche* conformation, and the only conformation possible for the nucleoside to be involved in an intramolecular O(5')-H···N(3) hydrogen bond (see (b)). Views of the conformation of the acetate group, (d) projected down the virtual bond C(Al)-C(3'), showing the nearly *cis* arrangement of the C(3')-H(3') and C(Al)=O(A) bond; (e) projected down the ester bond O(3')-C(3') showing the nearly *cis* arrangement of the C(3')-H(3') and O(3')-C(Al) bond; and (f) projection down the C(Al)-C(A2) bond showing the partial staggering of the methyl hydrogens with respect to the carbonyl oxygen O(A). These conformations represent the preferred conformation for the acetate group, and are very likely to be the same for the animo-acylated (charged) tRNAs.

Table IX. Least-Squares Planes for the (a) Sugar and (b) Acetate Group[a]

Atoms	Sugar[b] Plane IV, Å	 Plane V
C(1')	**0.017**	**0.000**
C(2')	−0.565	−0.659
C(3')	**−0.016**	−0.122
C(4')	**0.026**	**0.000**
O(1')	**−0.027**	**0.000**
C(5')	−1.128	−1.132
Rms deviation of fitted atoms	0.022	0.000

Atoms	Acetate group[c] Plane VI, Å
O(3')	**0.001**
CA(1)	**−0.002**
OA	**0.001**
CA(2)	**0.001**
C(3')	−0.139
Rms deviation of fitted atoms	0.001

[a] Atoms used in fitting the plane are shown in bold type. [b] Plane IV, $0.616X + 0.185Y + 0.765Z = 0.505$; plane V, $0.637X + 0.230Y + 0.736Z = 0.870$. [c] Plane VI, $-0.712X + 0.504Y + 0.489Z = -2.378$.

The torsional angles about the ring bonds are: τ_0,[29] −26.4; τ_1, 37.1; τ_2, −33.2; τ_3, 18.8; τ_4, 4.8.

The acetate group is planar (Table IV, Plane VI) and is rotated by about 6.6° about the O(3')-CA(1) bond, Figure 3. The torsional angle C(3')-O(3')-CA(1)-CA(2) is 173.9°, and the torsional angle H(3')-C(3')-O(3')-CA(1) is 20°. Similar conformations are exhibited by the nucleoside 3'-phosphates for the H(3')-C(3')-O(3')-P torsional angle.[19,29] A projection down the CA(2)→CA(1) bond and the torsional angles involving the methyl hydrogens are shown in Figure 3.

The C(5')-O(5') bond displays the preferred *gauche-gauche*[29,30] conformation with respect to the ribose ring bonds O(1')-C(4') and C(3')-C(4'), Figure 3. The

Table X. Some Torsional Angles[a]

Atoms	Torsional angle
C(8)-N(9)-C(1')-C(2')	110.1 (5)
C(4)-N(9)-C(1')-O(1')	−52.1 (5)
C(4)-N(9)-C(1')-C(2')	64.9 (5)
N(9)-C(1')-C(2')-C(3')	−155.7 (4)
N(9)-C(1')-O(1')-C(4')	149.1 (3)
O(2')-C(2')-C(3')-O(3')	−36.1 (5)
C(2')-C(3')-O(3')-CA(1)	97.8 (4)
C(1')-O(1')-C(4')-C(5')	−128.2 (4)
C(2')-C(3')-C(4')-C(5')	100.4 (4)

[a] Standard deviations are given in parentheses and refer to the least significant digits.

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

(30) See Table VI, footnote *d*.

Table XI. Purine Systems with *syn* Conformation

	Intra-molecular H bond	χ_{CN},[a] deg	Sugar puckering	Conformation about the C(5′)–C(4′) bond: ϕ_{OO},[f] deg O(1′)–C(4′)–C(5′)–O(5′)	Conformation about the C(5′)–C(4′) bond: ϕ_{OC},[f] deg C(3′)–C(4′)–C(5′)–O(5′)
3′-O-Acetyladenosine	Yes	−133	C(2′)-*endo*[c]	−61 (*gauche*)	58 (*gauche*)
8-Bromoguanosine[b,g]	Yes	−129	C(2′)-*endo*	−66 (*gauche*)	54 (*gauche*)
8-Bromoguanosine[b,h]	Yes	−130	C(2′)-*endo*	−65 (*gauche*)	54 (*gauche*)
8-Bromoadenosine[h]	Yes	−120	C(2′)-*endo*	−74 (*gauche*)	46 (*gauche*)
6-Thioinosine[i]					
Molecule 1	Yes	−135	C(2′)-*endo*	−63 (*gauche*)	55 (*gauche*)
Molecule 2	Yes	−144	C(2′)-*endo*	−63 (*gauche*)	57 (*gauche*)
2′-Deoxyguanosine[j]	Yes	−149	C(2′)-*endo*	−43 (*gauche*)	57 (*gauche*)
Formycin HBr[k]	No	−148	C(2′)-*endo*	(*gauche*)	(*trans*)
Cyclic 3′,5′-adenosine monophosphate[l]					
Molecule 2	No	−102	C(4′)-*endo*[d]	(*trans*)	(*gauche*)
Guanosine[m]					
Molecule 1[e]	No	123	C(2′)-*endo*	−51 (*gauche*)	8 (*gauche*)
Inosine[m]					
Molecule 1[e]	No	121	C(2′)-*endo*	−55 (*gauche*)	64 (*gauche*)

[a] Refers to the torsional angle O(1′)–C(1′)–N(9)–C(8). [b] These values are from two independent investigations of 8-bromoguanosine. [c] *endo* means the puckered carbon atom is displaced on the same side as C(5′). [d] This conformation differs very slightly from the C(3′)-*exo* conformation. [e] Molecule 2 is in the *anti* conformation. [f] See Table VI, footnote *d*. [g] C. E. Bugg and U. Thewalt, *Biochem. Biophys. Res. Commun.*, **37**, 623 (1969). [h] S. S. Tavale and H. M. Sobell, *J. Mol. Biol.*, **48**, 109 (1970). [i] E. Shefter, *J. Pharm. Sci.*, **57**, 1157 (1968). [j] A. E. V. Haschemeyer and H. M. Sobell, *Acta Crystallogr.*, **19**, 125 (1965). [k] G. Koyama, K. Maeda, H. Umezawa, and Y. Zitaka, *Tetrahedron Lett.*, **6**, 597 (1966). [l] Reference 33. [m] C. E. Bugg, U. Thewalt, and R. E. Marsh, *Biochem. Biophys. Res. Commun.*, **33**, 436 (1968), and private communications.

Table XII. Comparison of the Exocyclic Bond Angles at N(9) in *syn* and *anti* Structures

	C(4)–N(9)–C(1′), deg	C(8)–N(9)–C(1′), deg	$\sigma(\theta)$, deg	$\frac{\theta_1 - \theta_2}{\sigma}$[a]
syn,[b] (A)[f]				
3′-O-Acetyladenosine	127.7	125.3	0.3	+8.0
8-Bromoguanosine[b,c]	126.7	127.9	?	
8-Bromoguanosine[b,d]	126.8	127.8	0.6	−1.7
8-Bromoadenosine	127.1	128.3	1.0	−1.3
6-Thioinosine[e]				
Molecule 1	129.1	124.6	0.4	+11.1
Molecule 2	129.1	124.1	0.4	+12.5
2′-Deoxyguanosine[g]	131	124	2.5	+2.8
Cyclic 3′,5′-adenosine[h] Monophosphate				
Molecule 2[i]	137	*j*		
syn (B)[l]				
Guanosine[k]				
Molecule 1	126.2	128.2	0.3	−6.7
Inosine[k]				
Molecule 1	125.1	128.9	0.3	−12.7
anti				
Guanosine[k]				
Molecule 2	127.1	126.2	0.3	−3.0
Inosine[k]				
Molecule 2	128.3	125.9	0.3	−8.0
Deoxyadenosine[m]	123.2	129.6	1.0	−6.4
Adenosine 3′-phosphate dihydrate[n]	125.2	128.0	0.2	−14.0
Adenosine 2′,5′-uridine phosphate tetrahydrate	124.4	129.0	0.6	−7.7

[a] The difference in the two angles is significant if $|\Delta\sigma| > 3.0$. [b] These values are from two independent investigations. [c] See Table XI, footnote *g*. [d] See Table XI, footnote *h*. [e] See Table XI, footnote *i*. [f] Glycosidic torsional angle χ centered around −140°. [g] See Table XI, footnote *j*. [h] Reference 33. [i] Molecule 1 exhibits the *anti* conformation. [j] This value was not given in the paper (ref 33). [k] See Table XI, footnote *m*. [l] Glycosidic torsional angle χ centered around +122°. [m] Reference 7. [n] Reference 19.

torsional angles are O(1′)–C(4′)–C(5′)–O(5′) = −60.6°; C(3′)–C(4′)–C(5′)–O(5′) = 58.0°. The 5′ hydroxy bond O(5′)–H(O5′) has evidently rotated, torsional angle C(4′)–C(5′)–O(5′)–H(O5′) = 39.6°, from the usual *anti* conformation so as to form the intramolecular hydrogen bond with N(3) of the base. The dihedral angle between the purine ring (plane I) and the best four-atom plane of the sugar (plane IV) is 109.8°. This value may be compared with the considerably lower range 50–85° of values found for the *anti* nucleosides and nucleotides. The dihedral angle between the acetate group (plane VI) and the sugar (plane IV) is 88.4° while that between the acetate group plane (VI) and the base (plane I) is 69.2°.

The other conformational angles of interest are given in Table X.

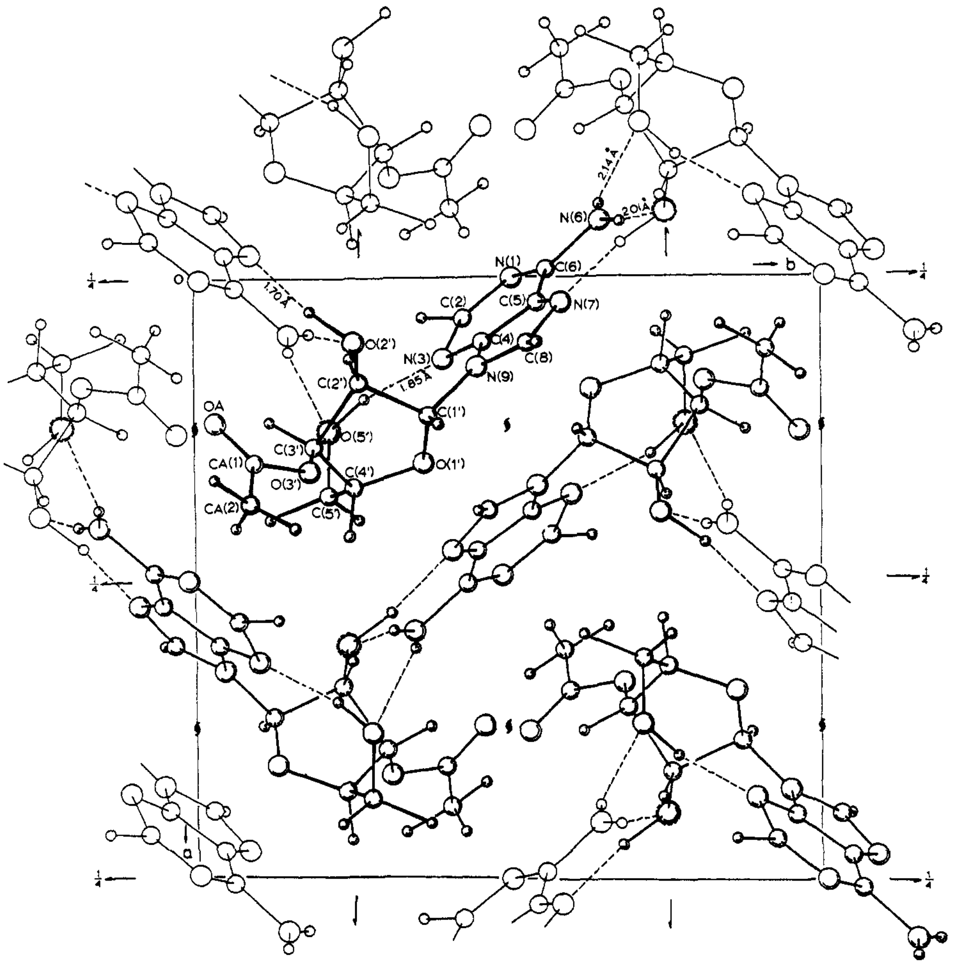

Figure 4. A view along the *c* axis showing the crystal packing and hydrogen bonding pattern.

Conformational Analysis of *syn* Nucleosides and Nucleosides

Glycosidic Torsional Angle. Of interest is the glycosidic torsional angle χ(O(1′)–C(1′)–N(9)–C(8)) which is −139.9° and the conformation is *syn*. This conformation is considerably more preferred in the purine nucleosides than in the pyrimidine nucleosides. A likely reason for this is the lower energy barrier to rotation about the glycosidic bond in the purine nucleosides.[29,31,32] To the knowledge of the authors there are ten purine systems exhibiting the *syn* conformation, and these are given in Table XI together with their main conformational features. All of them contain a ribose moiety with the exception of deoxyguanosine. Apparently, there is a striking correlation between the intramolecular O(5′)–H···N(3) hydrogen bond and the conformation of the nucleoside. The nucleoside is constrained to the *syn* glycosidic torsional angle χ = −140.0 ± 10°, the C(2′)-*endo* pucker for the sugar, the *gauche–gauche* conformation about the C(4′)–C(5′) bond, and the *gauche* conformation for C(4′)–C(5′)–O(5′)–H(O5′).

Since the intramolecular hydrogen bond is not possible in the 5′ nucleotides, the conformation about the C(4′)–C(5′) should be either *gauche–trans* or *trans–gauche* and not *gauche–gauche* because of steric interaction between the base and the phosphate. In fact, considerations of molecular models indicate that for the C(2′)-*endo* conformation of the sugar and for the normal *syn* range only the *trans–gauche* conformation appears to be allowed. However, for the C(3′)-*endo* sugar conformation both the *gauche–trans* and *trans–gauche* conformations appear likely. The only known *syn* nucleotide is the cyclic adenosine phosphate,[33] where the conformation about C(4′)–C(5′) is *trans–gauche* and the sugar exhibits a C(4′)-*endo* puckering. All of the known 5′-nucleotides on the other hand exhibit not only the *anti* conformation but also the *gauche–gauche* conformation about the C(4′)–C(5′) bond.[29] Even though three of the last four compounds in Table XI are not involved in intramolecular hydrogen bonding, the sugar still exhibits the C(2′)-*endo* pucker. In the 3′,5′-cyclic phosphate structure the sugar is constrained to the C(4′)-*endo* pucker.

There appears to be two distinct ranges for the *syn* conformation, the common range χ = −140 ± 10° (type A or − anticlinal (−ac) in the Klyne–Prelog notation[34]) and the uncommon range 120 ± 5° (type **B** or + anticlinal (+ac). In the latter category are the compounds guanosine (molecule 1) and inosine (molecule 1), where the intramolecular hydrogen bond is not possible, Table XI. Whatever the χ value,

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(31) J. Donohue and K. N. Trueblood, *J. Mol. Biol.*, **2**, 363 (1960).

(32) A. E. V. Haschemeyer and A. Rich, *ibid.*, **27**, 369 (1967).

(33) K. Watenpaugh, J. Dow, L. H. Jensen, and S. Furberg, *Science*, **159**, 206 (1968).

(34) W. Klyne and V. Prelog, *Experientia*, **16**, 521 (1960).

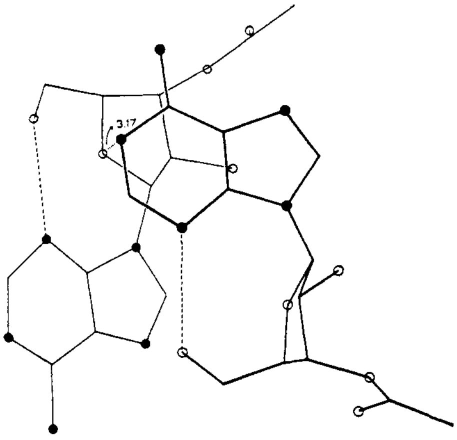

Figure 5. A view normal to the base plane (indicated in thick lines) showing the shortest contact between the ribose ring O(1′) and the base N(1). Contacts between sugar ring O(1′) and base are characteristic of the solid state structures of the nucleosides and nucleotides, and may be important in maintaining the stability of secondary and tertiary structures of the nucleic acid.

Table XIII. Hydrogen Bond Lengths and Angles[a]

Atoms	Distance, Å	Atoms	Angle, deg
O(5)···N(3) I	2.768	O(5′)–H(15)···N(3)	163.5
H(15)···N(3) I	1.85	H(15)–O(5′)···N(3)	10.9
		C(5′)–O(5′)···N(3)	120.8
		C(4)–N(3)···O(5′)	127.2
		C(2)–N(3)···O(5′)	121.0
N(6)···O(2′) II	3.045	C(6)–N(6)···O(2′)	108.3
H(2)···O(2′) II	2.01	N(6)–H(2)···O(2′)	168.4
		H(2)–N(6)···O(2′)	7.6
N(6)···O(5′) III	2.894	C(6)–N(6)···O(5′)	136.3
H(3)···O(5′) III	2.14	N(6)–H(3)···O(5′)	151.7
		H(3)–N(6)···O(5′)	20.6
O(2′)···N(7) II	2.768	C(2′)–O(2′)···N(7)	117.8
H(7)···N(7) II	1.70	O(2′)–H(7)···N(7)	171.8
		H(7)–O(2′)···N(7)	5.0
		C(5)–N(7)···O(2′)	111.1
		C(8)–N(7)···O(2′)	145.1

[a] Symmetry code: (I) x, y, z; (II) $-x, \frac{1}{2} + y, \frac{1}{2} - z$; (III) $-x, \frac{1}{2} + y, \frac{3}{2} - z$.

the sugar shows a marked preference for the C(2′)-*endo* puckering in the *syn* conformation, type A or B.

A further noteworthy point about the type A *syn* structures is that the bond angle C(4)–N(9)–C(1′) is greater than that of C(9)–N(9)–C(1′), in contrast to the *anti* structures which exhibit the reverse trend, Table XII. However, the type B *syn* structures, guanosine (molecule 1) and inosine (molecule 1) are the exceptions, show the same trend as the *anti* structures. It should be pointed out that the second molecule in the asymmetric unit of structure of guanosine (molecule 2) and inosine (molecule 2) exhibit the *anti* conformation.

Molecular Packing and Hydrogen Bonding. A packing diagram viewed down the *c* axis is given in Figure 4. The four hydrogen atoms H(2), H(3), H(7), and H(15) are involved in hydrogen bonds (Table XIII). The H(8) atom attached to the atom C(8) of the base is not involved in a C–H···O interaction as is generally observed for N(1) protonated adenines (Figure 2). The O(5′)–H is involved in an intramolecular hydrogen bond with N(3) atom of the base. The two hydrogen atoms of the amino N(6) atom are involved in donor hydrogen bonds to O(2′) and O(5′) of the sugar of neighboring molecules. The sugar hydroxyl group O(2′)–H is hydrogen bonded to N(7). None of the hydrogen bonds are perfectly linear, and the hydrogen bond angles are in the range 151–171°. This range of hydrogen bond angles is commonly observed in the crystal chemistry of the nucleic acid constituents. There are no interbase hydrogen bonds in this structure as in many others, and N(1) is not involved in hydrogen bonding. The acetate group is not involved in any hydrogen bonds and there are no other short contacts in the structure.

Sugar Base Contact. No base stacking interactions of any significance is found. On the contrary the closest van der Waals approach (3.17 Å) involves the sugar ring oxygen atom O(1′) and the base nitrogen atom N(1), Figure 5. It is interesting that neither of these atoms is involved in hydrogen bonding. The O(1′)···N(1) intermolecular contact distance is close to the sum of the van der Waals radii of the respective atoms; nevertheless, the distance is less than the values generally observed between interbase atoms[35,36] when the bases are in stacked arrangement.

The following programs were used in this analysis: (1) the full-matrix least-squares program of Busing, Martin, and Levy[13] modified[14] in this laboratory, (2) the direct phasing programs developed by S. R. Hall for the X-ray 67 system,[12] (3) ORTEP thermal ellipsoid plot program of C. K. Johnson.[15] Other programs were written by one of us (S. T. R.)[14] and other members of this laboratory.

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(35) M. Sundaralingam, S. T. Rao, and C. E. Bugg, Abstracts, American Crystallography Association Meeting, Seattle, Wash., March 1969.

(36) C. E. Bugg, J. Thomas, M. Sundaralingam, and S. T. Rao, *Biopolymers*, in press.