Tautomerism and conformation of the promutagenic analogue *N*⁶-methoxy-2',3',5'-tri-*O*-methyladenosine⁺George I. Birnbaum¹, Borys Kierdaszuk² and David Shugar^{2,3}¹Division of Biological Sciences, National Research Council of Canada, Ottawa K1A 0R6, Canada,²Department of Biophysics, Institute of Experimental Physics, University of Warsaw, 02-089, and³Institute of Biochemistry and Biophysics, Polish Academy of Sciences, 02-523 Warsaw, Poland

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ABSTRACT

*N*⁶-Methoxy-2',3',5'-tri-*O*-methyladenosine crystallizes in space group $P2_12_12_1$ with cell dimensions $a = 4.693$, $b = 11.412$, $c = 31.741$ Å. Least-squares refinement of diffractometer data converged at $R = 0.038$. The location of a hydrogen atom at N1 and the observed bond lengths and bond angles indicate unequivocally the imino tautomer of the adenine moiety. The *N*⁶-methoxy group is oriented *syn* to N1 and the glycosidic torsion angle χ_{CN} is -3.6° , i.e. in the *anti* range. The furanose ring has a C2'-*exo*/C3'-*endo* pucker ($P = 0.9^\circ$) and is unusually flattened ($\tau_m = 30.0^\circ$). The conformations of the *O*-methyl groups of the ribose ring are compared with those of monomethylated nucleosides, including the biologically important 2'-*O*-methyl nucleosides. Evidence is presented for the existence of C-H...N intermolecular hydrogen bonds between adenine moieties. Bearing in mind that *N*⁶-methoxyadenosine is a promutagenic analogue, the results are compared with those for the corresponding promutagenic *N*⁴-methoxycytidine. They are also discussed in relation to the tautomerism, the conformation of the *N*⁶-methoxy group, and the associated base-pairing abilities in the absence and presence of polymerases.

INTRODUCTION

The mutagenic effects of hydroxylamine (NH₂OH) and *O*-methylhydroxylamine (methoxyamine, NH₂OCH₃) were originally attributed to their conversion of cytosine and 5-substituted cytosine residues in DNA to *N*⁴-hydroxycytosine and *N*⁴-methoxycytosine, respectively,¹⁻³ and the resulting associated changes in base pairing. It was subsequently found that these mutagens also react, albeit at a lower rate, with adenine residues to give the corresponding *N*⁶-hydroxyadenine and *N*⁶-methoxyadenine.⁴ This accounts for the earlier observation that *N*⁶-hydroxyadenine is promutagenic in the rII mutant system of phage T4,⁵ in which it behaves like both adenine and guanine. Furthermore, it has recently been shown that in the *in vitro* transcription, by DNA-dependent RNA polymerase, of synthetic copolymers containing *N*⁶-methoxyadenine residues, the latter paired with both U and C, the inference being that the *N*⁶-methoxyadenine residues exist in two

tautomeric forms.⁶ Such dual functional properties in base pairing are reminiscent of the similar behaviour of N⁴-hydroxycytosine residues, both as substrates,⁷ and as components of a template.⁸

Tautomerism of N⁶-methoxyadenosine has been demonstrated by spectroscopic methods⁹ and shown to be markedly dependent on solvent polarity.¹⁰ In the latter investigation it was also demonstrated that, at the monomer level in apolar medium, the tautomeric equilibrium was appreciably shifted on addition of a potentially complementary base.

However, as we have elsewhere discussed in detail,¹¹ the base pairing properties of N⁶-methoxyadenine (like those of N⁴-methoxycytosine) are necessarily dependent on the orientation of the exocyclic N-methoxy group, which may be oriented syn or anti to the adenine ring N1 (see Scheme).

The present study describes the solid state structure and conformation of the tri-O-methylated homologue of N⁶-methoxyadenosine, their relevance to the results obtained in solution, and associated biological aspects.

Furthermore, the use of this analogue, in which all three sugar hydroxyls are methylated (in order to enhance solubility in non-polar solvents for solution studies), has provided conformational data for comparison with those of published monomethyl analogues, including the biologically important 2'-O-methyl nucleosides.

EXPERIMENTAL SECTION

2',3',5'-Tri-0-methyladenosine was prepared as described elsewhere.¹² The compound was then treated with methoxyamine, as described by Budowsky *et al.*⁷ for the reaction of adenosine with methoxyamine, to obtain N⁶-methoxy-2',3',5'-tri-0-methyladenosine, C₁₄H₂₁N₅O₅. The product was crystallized from a saturated solution in isopropanol by very slow cooling from 60°C to room temperature.

Precession photographs indicated the orthorhombic space group $P2_12_12_1$. A colorless crystal, measuring 0.15 × 0.25 × 0.40 mm, was mounted on a CAD-4 diffractometer. The unit cell dimensions were determined from angular settings of 22 reflections with θ in the range 40–49°. The following data were obtained: $a = 4.693(2)$, $b = 11.412(2)$, $c = 31.741(4)$ Å, $V = 1699.9$ Å³, $D_x = 1.33$ g cm⁻³, $Z = 4$ (20°C; Cu K α_1 , $\lambda = 1.54056$ Å), $F(000) = 720$, $\mu(\text{Cu K}\alpha) = 11.7$ cm⁻¹.

Intensities were measured with Ni-filtered Cu K α radiation up to $2\theta = 150^\circ$, using the $\omega/2\theta$ scan technique with $\Delta\omega = 1.5 + 0.2 \tan \theta$ and a maximum scan time of 210 s per reflection. Three reflections, monitored every 100 min, showed a 21% decrease of intensity during the data collection; the data were scaled accordingly. Of the 2077 unique reflections, 313 (15%) had $I < 3\sigma(I)$, and were considered unobserved. The intensities were corrected for Lorentz and polarization factors; absorption corrections were unnecessary.

The structure was determined by direct methods with the aid of the computer program MULTAN78.¹³ Of the 96 starting sets subjected to tangent refinement, the solution with the highest combined figure of merit yielded an E map on which all but one carbon atom were located. The atomic parameters were refined by the block-diagonal least-squares method with anisotropic temperature parameters. All hydrogen atoms were located on difference Fourier maps and refined with isotropic temperature parameters. The scattering factors were taken from the "International Tables for X-Ray Crystallography"¹⁴ and the oxygen curve was corrected for anomalous dispersion. Throughout the refinement the function $\sum w(|F_o| - |F_c|)^2$ was minimized and a factor of 0.8 was applied to all shifts. The following weighting scheme was used during the final stages: $w = w_1 \cdot w_2$, where $w_1 = 1$ for $|F_o| < 7$, $w_1 = 7/|F_o|$ for $|F_o| > 7$, $w_2 = \sin^2\theta/0.8$ for $\sin^2\theta < 0.8$, and $w_2 = 1$ for $\sin^2\theta > 0.8$. This scheme made the average values of $w(\Delta F^2)$ independent of $|F_o|$ and $\sin^2\theta$. After the final cycle the average parameter shift equalled 0.1σ and the largest (for an H atom) was 1.0σ . Seven

Table 1. Final atomic parameters.

	<u>x</u>	<u>y</u>	<u>z</u>	<u>U_{eq}/U</u>
N(1)	9087(5)	30882(17)	74922(5)	645
C(2)	7169(6)	25966(22)	77549(7)	689
N(3)	5643(5)	31311(17)	80364(6)	655
C(4)	6219(5)	43110(20)	80387(6)	588
C(5)	8043(5)	49174(20)	77890(6)	593
C(6)	9687(6)	42909(20)	74801(6)	617
N(6)	11466(6)	47853(18)	72277(6)	710
O(61)	12787(6)	39189(17)	69670(6)	878
C(61)	14474(10)	45205(33)	66700(10)	999
N(7)	8031(6)	60954(17)	78931(6)	680
C(8)	6191(7)	61700(21)	82042(7)	701
N(9)	5050(5)	51148(16)	83133(5)	595
C(1')	2820(5)	48554(22)	86255(6)	628
O(1')	2064(4)	59072(16)	88313(5)	735
C(2')	3835(5)	39900(21)	89635(6)	585
O(2')	1535(4)	33099(16)	91093(5)	710
C(21')	1150(8)	22558(27)	88797(10)	858
C(3')	4824(5)	48229(22)	93149(6)	615
O(3')	4539(4)	44042(18)	97300(5)	721
C(31')	6572(7)	35267(29)	98298(8)	805
C(4')	2932(6)	58836(24)	92631(7)	681
C(5')	4432(11)	70086(28)	93766(10)	983
O(5')	2442(11)	79189(25)	93797(11)	1420
C(51')	3636(27)	89827(37)	95004(16)	1779
H(1)	1003(9)	263(3)	731(1)	62(9)
H(2)	685(8)	180(3)	773(1)	50(8)
H(8)	576(7)	689(3)	837(1)	45(7)
H(61)	1318(9)	491(3)	652(1)	66(10)
H(62)	1557(11)	396(4)	651(1)	89(13)
H(63)	1572(15)	521(5)	681(2)	118(18)
H(1')	120(8)	459(3)	847(1)	51(8)
H(2')	523(6)	351(2)	887(1)	27(5)
H(21')	-37(10)	184(3)	904(1)	73(11)
H(22')	280(10)	182(3)	890(1)	76(11)
H(23')	95(9)	236(3)	861(1)	68(10)
H(3')	678(5)	499(2)	928(1)	24(5)
H(31')	653(9)	285(3)	960(1)	74(10)
H(32')	623(9)	334(3)	1011(1)	66(10)
H(33')	854(10)	378(3)	981(1)	78(11)
H(4')	132(6)	579(2)	941(1)	38(6)
H(5')	505(9)	690(3)	965(1)	68(10)
H(5'')	613(8)	734(3)	920(1)	61(9)
H(51')	455(16)	894(5)	982(2)	133(19)
H(52')	246(20)	955(6)	959(2)	167(26)
H(53')	536(17)	924(6)	931(2)	140(22)

For non-hydrogen atoms the x coordinates and U_{eq} were multiplied by 10⁴, y and z by 10³. For hydrogen atoms all values were multiplied by 10³.

strong, low-order reflections suffered from extinction effects and were given zero weights. The final conventional residual index R is 0.038 and the weighted index R_w is 0.047 for 1757 observed and 32 unobserved

Fig. 1. Stereoscopic view of N⁶-methoxy-2',3',5'-tri-O-methyladenosine.

reflections with $|F_o| < |F_c|$. The coordinates are listed in Table 1. Lists of anisotropic temperature parameters and of structure factors are available from the first author. The overall conformation and the numbering scheme are shown in Fig. 1.

RESULTS AND DISCUSSION

Purine moiety.

One of the primary objectives of this study was to determine whether the N⁶-methoxyadenine exists in the amino or the imino form. This can be ascertained from the presence of a proton at either N6 or N1 as well as

Fig. 2. Bond lengths (Å) and bond angles (deg). The esd's are 0.003-0.004 Å and 0.2-0.3°.

from the geometry of the adenine moiety. The location of the proton at N1 was unequivocal, and refinement of its position gave rise to normal distances and angles. In addition, the shift to an imino tautomer produced profound changes in the geometry of the pyrimidine ring. Fig. 2 shows the bond lengths and bond angles in the structure as well as deviations from recently calculated mean values.¹⁵ It should be noted that deviations exceeding 0.014 Å and 0.9° are statistically highly significant. As expected, the largest differences are in the N1-C6-N6 region, but the rest of the ring is affected as well. The bond lengths indicate a loss of aromaticity and a localization of double bonds at C2=N2, C4=C5, and C6=N6. The latter is identical in length to the C4=N4 bond in 1,5-dimethyl-N⁴-hydroxycytosine (1.288 Å),¹¹ also in the imino form, but is significantly shorter than the partial double bond C4...N4 in the hydrochloride of 1-methyl-N⁴-hydroxycytosine.¹⁶ The deviations in bond lengths are qualitatively similar to, but much more pronounced than, those observed upon N1 protonation of adenines.^{15,17} Such protonation gives rise to two resonance forms, one imino and the other amino.

This paper is the first presentation of the exact geometry of a 9-substituted adenine in the "pure" uncharged imino form, and the results should therefore be of theoretical interest, e.g. in the choice of bond lengths and bond angles for the rare imino form in quantum chemical calculations of tautomeric equilibria.¹⁸

Another problem to be resolved was the conformation of the N⁶-methoxy group. As mentioned above, the N6-O6 bond could be either syn or anti with respect to N1-C6. The results show the former orientation (Fig. 1). The methoxy group is almost coplanar with the pyrimidine ring, the deviations being 0.007 and 0.151 Å for O61 and C61, respectively. The syn conformation has also been found in other N⁶-substituted adenine derivatives,¹⁹ and it may be rationalized on the basis of steric interactions. If the methoxy conformation was anti, then, given the same bond lengths and bond angles, the N7...O61 distance would be only 2.52 Å, i.e. about 0.53 Å shorter than the sum of van der Waals radii. In the syn conformation the H1...O61

Table 2. Selected torsion angles (in degrees).

C8-N9-C1'-O1'	-3.6	N9-C1'-O1'-C4'	-112.1
C8-N9-C1'-C2'	-122.5	C3'-C4'-C5'-H5'	-57
C4-N9-C1'-C2'	64.3	H4'-C4'-C5'-O5'	-47
N9-C1'-C2'-C3'	94.6	O1'-C4'-C5'-O5'	69.6
O1'-C1'-C2'-C3'	-24.9	C3'-C4'-C5'-H5''	67
C1'-C2'-C3'-C4'	30.0	C1'-C2'-O2'-C21'	90.6
C2'-C3'-C4'-O1'	-25.7	H2'-C2'-O2'-C21'	-33
C3'-C4'-O1'-C1'	10.3	C2'-C3'-O3'-C31'	73.1
C4'-O1'-C1'-C2'	9.7	H3'-C3'-O3'-C31'	-48
O1'-C1'-C2'-O2'	91.3	C4'-C5'-O5'-C51'	177.3
O2'-C2'-C3'-C4'	-86.7	H5'-C5'-O5'-C51'	64
O2'-C2'-C3'-O3'	32.4	H5''-C5'-O5'-C5'	-50
O3'-C3'-C4'-C5'	89.2	C6-N6-O61-C61	174.0

The esd's are 0.2-0.3° for angles not involving hydrogen atoms and 1.5-2.5° for those which do.

distance is more favorable, viz. 2.25 Å, or only 0.25 Å shorter than the sum of van der Waals radii. Furthermore, while N7 and O61 would be subject to a repulsive interaction, H1 and O61 are capable of forming an intramolecular hydrogen bond, by analogy with the situation in N⁴-hydroxycytosines^{11,16} (see below).

Glycosidic conformation.

The glycosidic torsion angle χ_{CN} [C(8)-N(9)-C(1')-O(1')] is -3.6° (Table 2), which corresponds to the lower range of the anti conformation. Such values of χ_{CN} are extremely rare.²⁰ In general, low values of χ_{CN} have been correlated with a C2'-exo/C3'-endo pucker of the ribose ring,²¹ and this relationship is observed in the present structure as well (see below). With such ring pucker, a small χ_{CN} angle results in a longer H8...H3' and a shorter H8...O1' distance. While the former interaction is definitely repulsive, the latter may be regarded as attractive since C8 is bonded to two electronegative nitrogen atoms.²² We note that the H8...O1' distance is 2.53(3) Å (i.e. less than the sum of van der Waals radii) and the C8-H...O1' angle is 95(2)°. Although the N9-C8-H8 angle (121°) is smaller than the N7-C8-H8 angle (126°), implying a bending of H8 toward O1', this difference in angles may not be significant.

Ribose moiety.

The conformation of the sugar ring is quite unusual in two respects. The phase angle of pseudorotation (P) is 0.9°,²³ corresponding to a C2'-exo/C3'-endo pucker. A survey of 178 β-D-furanoside nucleosides revealed only five with smaller values of P.²⁰ Interestingly, one of them is 2'-O-methyladenosine with P = -10.0° (one of two independent molecules). The

other exceptional feature is the extreme flattening of the ring ($\tau_m = 30.0^\circ$). None of the nucleoside and nucleotides surveyed was found to have a furanose ring as flat as this one. The question that must be raised is whether this unusual conformation can be attributed to O'-methylation. In addition to 2'-O-methyladenosine,²⁴ crystal structures have been determined of 2'-O-methylcytidine²⁵ (also with two molecules in the asymmetric unit) and of 3'-O-methylarabinofuranosylcytosine.²⁶ The ρ and τ_m values in these five molecules are as follows: -10.0, 36.2; 11.1, 37.4; 170.8, 38.5; 174.9, 39.0; 168.0, 38.5°. Apart from the first ρ value, all others show commonly observed C3'-endo and C2'-endo conformations with normal amplitude of ring puckering. Thus, there is no crystallographic evidence that O'-methylation affects the conformation of the furanose ring. This is consistent with results on the solution conformation of nucleosides, where NMR data demonstrate only minimal changes in conformation following methylation of one or more hydroxyls of the sugar ring.²⁷

In previous studies of 2'- and 3'-O-methylated nucleosides²⁴⁻²⁶ the O-CH₃ bond was observed to be either gauche⁺ or gauche⁻ to the C-H bond, but not ideally staggered. The H-C-O-CH₃ torsion angles were found to be in the 23-40° range. In the present structure both torsion angles are in the gauche⁻ conformation: H2'-C2'-O2'-C21' -33°, H3'-C3'-O3'-C31' -48° (Table 2). It has been suggested²⁴ that the most stable conformation is one in which "the methyl groups are nearly eclipsed to H2'." The new data indicate that a torsion angle in the range 30-40° is the most likely.

The conformation of the side chain is anti (Table 2). Although not as common as gauche⁺, this rotamer is also favored by a C5'-O5'/C4'-O1' gauche interaction. On the basis of NMR data Hruska *et al.*²⁸ suggested that in purine nucleosides with a type N sugar ring pucker the gauche⁺ side chain conformation is destabilized. Our analysis of crystallographic results did not reveal any evidence for such a correlation.²⁹ The O5'-C51' bond is trans to C4'-C5'; being gauche to two C-H bonds, this is the most stable conformation.

The bond lengths in the ribose moiety are normal and require no comments. Endocyclic bond angles in furanose rings are known to depend on ring conformation.³⁰⁻³² In view of the unusual puckering in this structure it is of interest to examine a previously proposed correlation which takes both ρ and τ_m into account.^{31,32} The calculated values for the angles at O1', C1', ...C4' are 110.8, 107.3, 102.8, 103.7, 106.2°. Apart from the angle at C2', the agreement is excellent. While exocyclic angles generally

depend on intermolecular interactions (e.g. hydrogen bonds), the large C2'-C3'-O3' angle (116.1°) may be a consequence of O-methylation.

Hydrogen bonding and packing.

The molecules are linked to one another by two hydrogen bonds: N1 donates its proton to N7 while C2 donates its hydrogen atom to the exocyclic N6. The geometry of these hydrogen bonds was calculated after normalizing the covalent N-H and C-H distances to their standard values (1.03 and 1.09 Å, respectively), as determined by neutron diffraction.^{33, 34} The following distances and angles were obtained: H1...N7 1.95(4), N1...N7 2.915(3) Å, N1-H...N7 154(3)°; H2...N6 2.29(3), C2...N6 3.272(3) Å, C2-H...N6 148(2)°. The first set of values indicates a normal, strong N-H...N bond. The second bond, however, is most unusual. A recent survey of C-H...O, C-H...N, and C-H...Cl hydrogen bonds in crystal structures determined by neutron diffraction did not reveal any such bonds involving C2 of a purine moiety.³⁵ Our own search of X-ray analyses disclosed one other crystal structure of a nucleoside with a short C2-H...N contact. In adenosine hydrochloride an intermolecular H2...N7 distance was found to be 2.36(4) Å (uncorrected),³⁶ but the authors did not consider this to be a hydrogen bond. The present structure analysis may therefore be the first crystallographic evidence for the participation of H2 in base pairing of adenosines. The evidence for the existence of the C2-H...N6 bond is very strong. In the survey mentioned above,³⁵ the (C-)H...N distances were found to be in the range 2.522-2.721 Å. The H2...N6 distance is significantly shorter than those distances and very much shorter than 2.75 Å, the sum of the van der Waals radii of H and N. The C-H...N angle is well within the previously observed range (124.6-157.3°). The ability of H2 to participate in hydrogen bonds can be attributed to decreased electron density at C2, caused by the inductive effect of two adjacent nitrogen atoms.³⁵

The arrangement of molecules in the crystal is shown in Fig. 3. The hydrogen bonds link the purine moieties into ribbons parallel to b. These hydrophobic ribbons alternate, along c, with hydrophilic regions formed by ribose moieties.

In the absence of intermolecular hydrogen bonds, the imino form is capable of forming an intramolecular N1-H...O61 hydrogen bond. In the present structure the H1...O61 distance is 2.22(4) Å and the N1-H...O61 angle is 99(2)°. These values are very similar to those in 1,5-dimethyl-N⁴-hydroxycytosine (2.17 Å, 100°), where the presence of an intramolecular

Fig. 3. Stereoscopic view of the contents of a unit cell.

N-H...O bond was unequivocally established.¹¹ It is possible, therefore, to regard the H1...O61 contact as an attractive interaction in the solid state. In solution, this interaction would stabilize the N⁶-methoxy group in the syn orientation.

Solution conformation.

In a companion study on solution conformations (Stolarski *et al.*, in preparation), it was found that, in polar solvents, there are comparable populations of the C2'-endo and C3'-endo conformers of the sugar ring for both the N⁶-hydroxy and N⁶-methoxy adenosines; in a low-polar solvent like chloroform, there is a preference (60-70%) for the C2'-endo pucker for both amino and imino tautomers. Furthermore, the conformation of the exocyclic 5'-CH₂OCH₃ group exhibits a 40-50% population of the gauche⁺ rotamer, which increases to >80% in non-polar solvents.

BIOLOGICAL ASPECTS

Before proceeding to further discussion, it is pertinent to recall previous observations on the analogous N⁴-hydroxy and methoxy cytosines. Like 1,5-dimethyl-N⁴-hydroxycytosine and the cation of 1-methyl-N⁴-hydroxycytosine in the solid state,^{11,16} 1-methyl-N⁴-methoxycytosine in chloroform solution is predominantly, if not exclusively, in the imino form with the N⁴-OCH₃

group syn to the ring N3. The compound should, therefore, be unable to form a planar associate by Watson-Crick base pairing. And, in fact, it associates with a 9-substituted adenine only via reverse Watson-Crick base pairing, with N3 as donor and O2 as acceptor.³⁷ It is this tendency of the exocyclic N⁴-OH, or N⁴-OCH₃, to adopt the syn conformation which is undoubtedly responsible for the observed inability of poly(N⁴-hydroxycytidine) to form a double-stranded helical structure with either poly(A) or other potentially complementary synthetic homopolymers.³⁸ However, it was recently demonstrated by Spengler and Singer³⁹ that synthetic copolymers of uridine and N⁴-methoxycytidine (including up to 36% of the latter residues) could be annealed with poly(A) to form a double-stranded helix. The helix did exhibit a lower T_m and broader temperature profile than poly(A)·poly(U), consistent with many of the N⁴-methoxycytidine residues being in the imino form, with the N⁴-methoxy groups anti to N3, to permit pairing with poly(A). However, some looping-out of residues with the N⁴-methoxy groups syn to N3 cannot be excluded.

By contrast, when the foregoing copolymers were employed as templates in an RNA polymerase system, with only ATP as substrate, the resulting double helix exhibited a higher T_m , and a considerably narrower temperature profile, strongly suggestive of involvement of the enzyme in constraint of the N⁴-methoxy groups to the anti conformation to permit pairing with A residues in the complementary strand.

Both 1-substituted N⁴-methylcytosine⁴⁰ and 9-substituted N⁶-methyladenine⁴¹ exist in the amino form in solution, with the orientation of the exocyclic N-methyl groups predominantly, but not exclusively, in the syn conformation. In the solid state, N⁶-methyladenines, whether in the neutral or cationic forms, exist only with the N⁶-methyl syn to N3.¹⁹ For the corresponding N-methoxy analogues in solution, spectroscopic methods have revealed no detectable anti populations of the N-methoxy groups in either N⁴-methoxycytosine or N⁶-methoxyadenosine.³⁷ The foregoing is in agreement with the lack of activity, in an in vitro RNA polymerase system, of the 5'-triphosphate and a homopolymer of N⁶-methoxyadenosine as substrate and template, respectively.⁴²

However, as in the case of copolymers containing residues of N⁴-methoxycytidine (see above), it has now been shown⁶ that copolymers of C with N⁶-methoxyadenosine (the content of the latter ranging from 17-33%) are good templates for transcription by an in vitro RNA polymerase system, with incorporation of both U and C. Firstly, this is consistent with the

Fig. 4. Watson-Crick-type base pairing of the imino tautomer of adenosine with cytidine.

existence of the N⁶-methoxyadenosine residues as an equilibrium mixture of the amino and imino forms. Observed incorporation of C was about 10% that of U, leading to the inference that the tautomeric equilibrium constant $K_T \sim 0.1$ for the imino form. However, this is probably only an apparent value in the light of the observation that, at the monomer level, the tautomeric equilibrium is appreciably shifted on addition of a potentially complementary base such as U or C.¹⁰ Secondly, the observed incorporation of U and C must also be taken to indicate that the exocyclic N⁶-methoxy syn rotamer is converted to the anti conformation, probably under the influence of the enzyme, since otherwise no base pairing (i.e. incorporation) would occur. An analogous situation prevails for N⁶-methyladenosine residues which, although exclusively syn to N3 in the solid state, and predominantly so in solution, behave at the polymer level in matrices, as well as in vivo, like adenosine residues.^{4,3} There are, however, no physico-chemical data available as to the conformation of such exocyclic N⁶-methyl groups at the polymer level in the absence of polymerases.

Our finding of an intermolecular hydrogen bond involving C2-H in the present crystal structure is in line with our observation that the imino form of N⁶-methoxyadenosine forms base pairs with itself, as well as with cytidine, in chloroform solution (manuscript in preparation). ¹³C NMR data indicate that the hydrogen bonds involved in the self-association are the same as those found in the crystal structure (N1-H...N7 and C2-H...N6); the base pairing with cytidine is of the Watson-Crick type (Fig. 4) with N1-H...N3, C2-H...O2, and O6...H-N4 hydrogen bonds. This scheme provides an interpretation for the A+G transitions observed by Singer and Spengler^{4,3} during transcription by an RNA polymerase system.

Finally, it should be noted that 9-(β -D-arabinofuranosyl)-N⁶-hydroxy-

adenine (N⁶-hydroxy-araA), originally synthesized as a potential antitumour agent because of its resistance to enzymatic deamination, unlike the parent araA, was subsequently found to exhibit significantly higher activity than araA as an antiherpetic agent, and conferred protection in mice infected with herpes simplex virus type 1.⁴⁴ Its mechanism of action as an antiviral agent has not been elucidated, but it would clearly be desirable to establish which of the two tautomeric forms is the active species, and what role in activity is played by the conformation of the exocyclic N⁶-hydroxy group.

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REFERENCES

1. Janion, C. and Shugar, D. (1965) *Biochem. Biophys. Res. Commun.* **18**, 617-622; *Acta Biochim. Polon.* **12**, 337-355.
2. Phillips, H.H. and Brown, D. (1967) *Prog. Nucleic Acid Res. Mol. Biol.* **7**, 349-368.
3. Kochetkov, N.K. and Budowsky, E.I. (1969) *Prog. Nucleic Acid Res. Mol. Biol.* **9**, 405-437.
4. Budowsky, E.I. (1976) *Prog. Nucleic Acid Res. Mol. Biol.* **16**, 125-188.
5. Freese, E.B. (1968) *Mutation Res.* **5**, 299-301.
6. Singer, B. and Spengler, S. (1982) *FEBS Lett.* **139**, 69-71.
7. Budowsky, E.I., Sverdlov, E.D. and Spasokukotskaya, T.N. (1971) *FEBS Lett.* **17**, 336-338.
8. Flavell, R.A., Sabo, D.L., Bundle, E.F. and Weissman, C. (1974) *J. Mol. Biol.* **89**, 255-272.
9. Morozov, Y.V., Savin, F.A., Chekov, V.O. and Budowsky, E.I. (1982) *J. Photochem.* **20**, 229-252.
10. Kierdaszuk, B., Stolarski, R. and Shugar, D. (1983) *FEBS Lett.* **158**, 128-130.
11. Shugar, D., Huber, C.P. and Birnbaum, G.I. (1976) *Biochim. Biophys. Acta* **447**, 274-284.
12. Kazimierczuk, Z., Darzynkiewicz, E. and Shugar, D. (1976) *Biochemistry* **15**, 2735-2740.
13. Main, P., Hull, S.E., Lessinger, L., Germain, G., Declercq, J.-P., Woolfson, M.M. MULTAN78, University of York, England, and University of Louvain, Belgium, 1978.
14. "International Tables for X-ray Crystallography", Ibers, J.A. and Hamilton, W.C., Eds., Vol. IV, Kynoch Press, Birmingham, England, 1974.
15. Taylor, R. and Kennard, O. (1982) *J. Mol. Struct.* **78**, 1-28.

16. Birnbaum, G.I., Kulikowski, T. and Shugar, D. (1979) *Can. J. Biochem.* 57, 308-313.
17. Markowski, V., Sullivan, G.R. and Roberts, J.D. (1977) *J. Am. Chem. Soc.* 99, 714-718.
18. Mezey, P.G. and Ladik, J.J. (1979) *Theoret. Chim. Acta* 52, 129-145.
19. Sternglanz, H. and Bugg, C.E. (1978) *J. Cryst. Mol. Struct.* 8, 263-277.
20. de Leeuw, H.P.M., Haasnoot, C.A.G. and Altona, C. (1980) *Isr. J. Chem.* 20, 108-126.
21. Berman, H.M. (1981) in *Topics in Nucleic Acid Structure*, Neidle, S. Ed., Part 1, pp. 1-15, Macmillan Publishers Ltd., London.
22. Taylor, R. and Kennard, O. (1982) *J. Am. Chem. Soc.* 104, 5063-5070.
23. Altona, C. and Sundaralingam, M. (1972) *J. Am. Chem. Soc.* 94, 8205-8212.
24. Prusiner, P. and Sundaralingam, M. (1976) *Acta Crystallogr.*, Section B, 32, 161-169.
25. Hingerty, B., Bond, P.J., Langridge, R. and Rottman, F. (1977) *Acta Crystallogr.*, Section B, 33, 1349-1356.
26. Birnbaum, G.I., Darzynkiewicz, E. and Shugar, D. (1975) *J. Am. Chem. Soc.* 97, 5904-5908.
27. Ekiel, I., Darzynkiewicz, E. and Shugar, D. (1978) *Biochemistry* 17, 1530-1536.
28. Hruska, F.E., Wood, D.J. and Singh, H. (1977) *Biochim. Biophys. Acta* 474, 129-140.
29. Birnbaum, G.I. and Shugar, D. (1984) in *Topics in Nucleic Acid Structure*, Neidle, S. Ed., Part 3, Macmillan Publishers Ltd., London, in press.
30. Murray-Rust, P. and Motherwell, S. (1978) *Acta Crystallogr.*, Sect. B, 34, 2534-2546.
31. Westhof, E. and Sundaralingam, M. (1980) *J. Am. Chem. Soc.* 102, 1493-1500.
32. Birnbaum, G.I., Cygler, M., Dudycz, L., Stolarski, R. and Shugar, D. (1981) *Biochemistry* 20, 3294-3301.
33. Taylor, R., Kennard, O. and Versichel, W. (1983) *J. Am. Chem. Soc.* 105, 5761-5766.
34. McMullan, R.K., Benci, P. and Craven, B.M. (1980) *Acta Crystallogr.*, Sect. B, 36, 1424-1430.
35. Taylor, R. and Kennard, O. (1982) *J. Am. Chem. Soc.* 104, 5063-5070.
36. Shikata, K., Ueki, T. and Mitsui, T. (1973) *Acta Crystallogr.*, Sect. B, 29, 31-38.
37. Kierdaszuk, B., Stolarski, R. and Shugar, D. (1983) *Eur. J. Biochem.* 130, 559-564.
38. Janion, C. and Shugar, D. (1968) *Acta Biochim. Polon.* 15, 107-121.
39. Spengler, S. and Singer, B. (1981) *Biochemistry* 20, 7290-7294.
40. Shoup, R.R., Miles, H.T. and Becker, E.D. (1967) *J. Am. Chem. Soc.* 89, 6200-6204.
41. Engel, J.D. and von Hippel, P.H. (1974) *Biochemistry* 13, 4143-4158.
42. Budowsky, E.I., Sverdlov, E.D., Spasokukotskaya, T.N. and Koudelka, J. (1975) *Biochim. Biophys. Acta* 390, 1-13.
43. Singer, B. and Spengler, S. (1981) *Biochemistry* 20, 1127-1132.
44. Giner-Sorolla, A., Burchenal, J.H. and Lopez, C. (1981) in *Medicinal Chemistry Advances*, de las Heras, F.G. and Vega, S. Eds., pp. 55-67, Pergamon Press, Oxford.
45. Ahmed, F.R., Hall, S.R., Pippy, M.E. and Huber, C.P. (1973) *J. Appl. Crystallogr.* 6, 309-346.
46. Johnson, C.K. (1970) ORTEP Report ORNL-3794 (2nd revision), Oak Ridge National Laboratory, Oak Ridge, TN.