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Molecular and Crystal Structure of the Modified Nucleoside 2′-*O*-Methyladenosine. A Novel 2′-*exo*-3′-*endo* ($_2T^3$) Sugar Pucker*

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The crystal structure of 2′-*O*-methyladenosine, a modified constituent of RNA's, has been determined from three-dimensional X-ray intensity data. The nucleoside crystallizes in the noncentrosymmetric space group $P2_1$ with unit-cell dimensions $a=4{\cdot}694\pm0{\cdot}005$, $b=32{\cdot}811\pm0{\cdot}045$, $c=8{\cdot}652\pm0{\cdot}010$ Å and $\beta=109{\cdot}1\pm0{\cdot}1°$. There are two independent molecules in the crystallographic asymmetric unit. The structural solution was obtained by application of the Nordman vector search program and refined by the method of least-squares to an R of 0·089. Both molecules are in the *anti* conformation with glycosyl torsion angles χ_{CN} of 15° (molecule *A*) and 0·5° (molecule *B*). The exocyclic C(4′)–C(5′) bond torsion, defined with respect to the backbone atoms C(3′)–C(4′)–C(5′)–O(5′), is *gauche*$^+$ in the former molecule and *trans* in the latter. The furanose rings of the two molecules have different conformations: in *A* it is the preferred 3′-*endo*-2′-*exo* ($^3T_2$) with a pseudorotation phase angle (P) of 11·1°, while in *B* it is 2′-*exo*-3′-*endo* ($_2T^3$) with a P value of −10·1°. The latter conformation has been observed for the first time in a nucleic acid constituent. The correlation between the novel 2′-*exo* sugar pucker, the low χ_{CN} and the *trans* conformation about the C(4′)–C(5′) bond in *B* has been demonstrated by semiempirical conformational energy calculations. The crystal structure also discloses an unusual triple base-pairing configuration involving both the Watson–Crick and Hoogsteen bonding sites of adenine. The bases stack in sheets parallel to the crystallographic $\bar{1}71$ planes with an interplanar separation of 3·38 Å.

Introduction

Considerable interest has been focused in recent years on the study of the molecular structures of the modified nucleosides and nucleotides of tRNA's (for review see Sundaralingam, 1972). Although a number of crystal structures of modified bases have been reported, there were no structural data available on a modified sugar nucleoside when this work was completed (Sundaralingam & Prusiner, 1973). Recently Hingerty, Bond & Langridge (1974) have reported the crystal structure of 2′-*O*-methylcytidine. The only modification that has been found in the sugar-phosphate backbone chains of nucleic acids is the methylation at the 2′ position. This is naturally restricted to only the RNA systems. 2′-*O*-Methylated nucleosides have been isolated from a wide variety of sources including bacterial and animal tRNA (Hall, 1964), ribosomal RNA (Wagner, Penman and Ingram, 1967), and rhapidosomal RNA (Correll, 1968). The structure–function relationship of the 2′-methylated derivatives is not clear although it is well known that in polymer systems methylation at 2′ confers stability to alkali and ribonuclease treatment (Smith & Dunn, 1959; Honjo, Kanai, Furukawa, Mizuno & Sanno, 1964). It has been also found that the thermal stability of poly *A* is markedly enhanced by 2′-methylation. Spectral studies do not show any significant structural differences between the two homopolymers (poly *A* and poly 2′-*O*-methyl *A*) (Bobst, Rottman & Cerutti, 1969; Pilet, Rottman & Brahms, 1973). In tRNA the 2′-methylated nucleosides are mainly distributed in the exposed loop regions of the molecule although in some cases they are also found in the double helical stems. The purpose of this X-ray investigation was to elucidate the effect of 2′-methylation on the conformations of nucleosides and nucleotides in addition to providing structural information that might be useful in the X-ray studies of polyribonucleotides containing the 2′-methylated constituents.

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Experimental section

Several attempts to crystallize the title compound from various solvent mixtures failed. Small plate-like crystals of 2′-*O*-methyladenosine were finally obtained by slow evaporation of an 80% aqueous butanol solution of the compound. A crystal with approximate dimensions $0{\cdot}15\times0{\cdot}10\times0{\cdot}05$ mm was used for the X-ray structure analysis. It was mounted about the a axis. Weissenberg photographs and diffractometer data indicated the systematic absences $k=2n+1$ for $0k0$. Thus, the space group is uniquely defined as $P2_1$ since the compound is optically active. The unit-cell dimensions were found to be $a=4{\cdot}694\pm0{\cdot}005$, $b=32{\cdot}811\pm0{\cdot}045$, $c=8{\cdot}652\pm0{\cdot}010$ Å and $\beta=109{\cdot}1\pm0{\cdot}1°$ as determined by a least-squares refinement of the angles 2θ, ω and χ and 12 reflections measured in the 2θ range 40–60° on a Picker FACSI diffractometer. $D_x=1{\cdot}482$ g cm^{-3} for two independent molecules in the crystallographic asymmetric unit. The density of the crystals was not measured since suitable crystals were not available.

Three-dimensional X-ray intensity data up to a 2θ limit of 127° were collected on a Picker FACSI automated diffractometer using Cu $K\alpha$ ($\lambda=1{\cdot}5418$ Å) radia-

tion employing the θ–2θ scan mode and a scan speed of 2° min^{-1}. Backgrounds were measured both at the beginning and end of each scan for 10s. Three standard reflections were scanned throughout the data collection at intervals of every 50 reflections. The data were corrected for background, variation in standards, and Lorentz and polarization effects. There were 1681 reflections of the total of 1888 measured that $I > 1{\cdot}5\sigma(I)$. Only these reflections were used in the structure analysis.

Determination of structure

The structure was solved by application of both Patterson and direct methods. The coordinates of the base of one of the molecules (molecule *B*) were obtained by employing the vector search programs of Nordman (Schilling, Hoge & Nordman, 1970). These coordinates were then used as the phasing model to refine the phases of 334 $|E|$'s $> 1{\cdot}3$ (where $|E|$ is the normalized structure factor) by the tangent formula using the program X-RAY 70 (Stewart, Kundell & Baldwin, 1970). The refinement converged in 20 iterations giving an $R(E)$ (Karle & Karle, 1966) of 0·18. The *E* map revealed the structure of the two independent molecules and also showed that the initial orientation of the base *B* derived from the vector-search method was approximately 180° from the final orientation as shown in Fig. 1. It is seen that only 7 of the 11 atoms of the initial base are within 0·3 Å of the actual atomic positions. Nevertheless the model was a sufficient phasing model when used in conjunction with the powerful tangent technique.

Refinement of structure

The positional and thermal parameters of the 40 nonhydrogen atoms of the two molecules were refined by the full-matrix least-squares program of Busing, Martin & Levy (1962) modified for use on the UNIVAC 1108 computer by Rao (1968). Four cycles of refinement using isotropic temperature factors lowered the agreement index $R\ (=\sum||F_o|-|F_c||/\sum|F_o|)$ from 0·23 to 0·14. In the initial refinement cycles, the weights were computed from counting statistics using an electronic instability factor of 0·02 (Stout & Jensen, 1968). In the later cycles a Hughes (1941) scheme was applied where $1/\sqrt{w} = 1{\cdot}0$ for $|F_o| < 24{\cdot}4$ and $1/\sqrt{w} = |F_o|/24{\cdot}4$ for $|F_o| \geq 24{\cdot}4$. After two cycles of refinement using anisotropic temperature factors a difference electron density map was computed. Nineteen of the expected 30 hydrogen atoms were located. These hydrogen atoms and the remaining 11 obtained by geometrical criteria were fixed in the subsequent two cycles of refinement and were given the temperature factors of the atoms to which they were attached. At the conclusion of the refinement the *R* value was 0·089 for the 1681 reflections. The final shift in the atomic parameters of the nonhydrogen atoms averaged $0{\cdot}08\sigma$ with a maximum of $0{\cdot}42\sigma$ where σ is the estimated standard deviation in the parameters. The scattering factors of C, N and O used throughout the refinement are from Cromer &

Table 1. *Positional parameters of the nonhydrogen atoms in molecule A and molecule B of 2′-O-methyladenosine*

Parameters have been multiplied by 10^4. Standard deviations in parentheses refer to the least significant digits. C(9) refers to the carbon atom of the methyl group.

	x	*y*	*z*
N(1)*A*	3999 (20)	8310 (3)	3121 (10)
C(2)*A*	1973 (28)	8019 (4)	2955 (13)
N(3)*A*	1445 (19)	7787 (3)	4123 (9)
C(4)*A*	3336 (20)	7888 (3)	5583 (10)
C(5)*A*	5558 (21)	8186 (3)	5938 (10)
C(6)*A*	5890 (23)	8400 (3)	4637 (11)
N(6)*A*	7887 (20)	8706 (3)	4742 (9)
N(7)*A*	7136 (17)	8203 (2)	7612 (9)
C(8)*A*	5732 (22)	7917 (3)	8219 (10)
N(9)*A*	3573 (17)	7727 (2)	7110 (8)
C(1′)*A*	1521 (21)	7399 (3)	7319 (10)
O(1′)*A*	1795 (16)	7392 (2)	9021 (7)
C(2′)*A*	2464 (22)	6969 (3)	6940 (11)
O(2′)*A*	−187 (17)	6739 (2)	6409 (9)
C(9)*A*	−320 (40)	6467 (5)	5160 (15)
C(3′)*A*	4498 (22)	6829 (3)	8563 (11)
O(3′)*A*	4993 (17)	6406 (2)	8736 (10)
C(4′)*A*	3038 (20)	7009 (3)	9754 (10)
C(5′)*A*	4854 (23)	7057 (3)	11480 (13)
O(5′)*A*	7639 (18)	7253 (3)	11599 (9)
N(1)*B*	2736 (17)	9037 (2)	7571 (9)
C(2)*B*	4035 (24)	9349 (3)	7011 (11)
N(3)*B*	6401 (20)	9585 (3)	7774 (9)
C(4)*B*	7650 (22)	9460 (3)	9363 (10)
C(5)*B*	6544 (22)	9141 (3)	10109 (10)
C(6)*B*	4023 (20)	8932 (2)	9165 (10)
N(6)*B*	2644 (18)	8638 (2)	9721 (8)
N(7)*B*	8382 (17)	9110 (2)	11726 (8)
C(8)*B*	10431 (23)	9396 (3)	11922 (11)
N(9)*B*	10026 (17)	9616 (2)	10519 (8)
C(1′)*B*	12047 (19)	9952 (3)	10362 (11)
O(1′)*B*	14331 (14)	9998 (2)	11825 (7)
C(2′)*B*	10423 (23)	10360 (3)	9980 (12)
O(2′)*B*	12260 (20)	10610 (2)	9323 (9)
C(9)*B*	10484 (51)	10890 (7)	8057 (21)
C(3′)*B*	10748 (22)	10513 (3)	11667 (12)
O(3′)*B*	10331 (18)	10944 (2)	11796 (10)
C(4′)*B*	13775 (22)	10360 (3)	12681 (11)
C(5′)*B*	14180 (32)	10251 (4)	14446 (13)
O(5′)*B*	17086 (20)	10107 (3)	15288 (10)

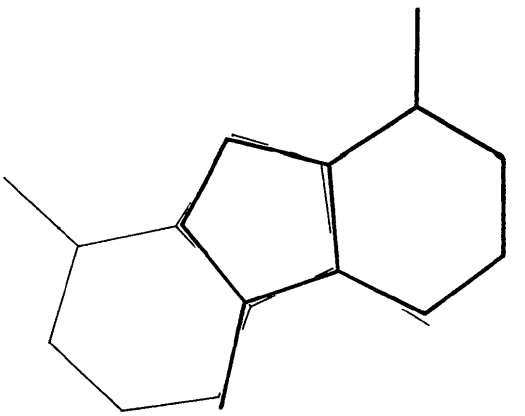

Fig. 1. A view normal to the base showing the relationship between the actual atomic sites (thick lines) and those derived from the vector space search (thin lines).

Waber (1965), while those of H are from Stewart, Davidson & Simpson (1965).

The atomic parameters together with their e.s.d.'s are given in Tables 1, 2 and 3. Since only small crystals were available as pointed out earlier, a large proportion of the reflections were weak and had low intensities. This accounts for the rather high standard deviations in the atomic positions. The observed and calculated structure amplitudes are given in Table 4.* The molecular conformations of the independent molecules and atom number of the nonhydrogen atoms are shown in Fig. 2.

Table 2. *Hydrogen atom parameters*

Positional parameters have been multiplied by 10^3. Atoms whose positions were fixed by geometrical criteria are denoted by *. In general the numbers in parentheses indicate the heavy-atom numbering (see Fig. 2). The exceptions are cases where more than one hydrogen atom is attached to the heavy atoms. The two hydrogen atoms on C(5′) are labelled H(51) and H(52); the three on C(9) are H(91), H(92) and H(93) and the two on the amino group are H(61) and H(62).

	x	*y*	*z*	*B* (Å²)
H(2)*A*	49	800	177	3·9
*H(61)*A*	790	885	373	3·3
H(62)*A*	958	875	573	3·3
*H(8)*A*	700	785	919	2·9
H(1′)*A*	−26	741	638	2·2
*H(2′)*A*	352	700	611	2·8
H(91)*A*	−201	655	402	5·5
*H(92)*A*	180	646	495	5·5
*H(93)*A*	−81	617	550	5·5
H(3′)*A*	621	689	916	2·7
H(O3′)*A*	337	626	787	3·9
H(4′)*A*	133	685	942	2·5
*H(51)*A*	360	717	1210	3·6
H(52)*A*	556	680	1201	3·6
H(O5′)*A*	849	740	1258	4·8
H(2)*B*	246	941	601	3·3
H(61)*B*	387	859	1060	2·9
H(62)*B*	73	851	885	2·9
H(8)*B*	1234	943	1282	3·0
*H(1′)*B*	1302	993	950	2·6
H(2′)*B*	867	1034	915	3·2
H(91)*B*	1068	1081	690	8·6
*H(92)*B*	817	1087	799	8·6
*H(93)*B*	1130	1119	836	8·6
*H(3′)*B*	912	1039	1203	2·9
H(O3′)*B*	1212	1092	1269	4·1
H(4′)*B*	1511	1050	1326	2·7
H(51)*B*	1262	1001	1428	4·4
*H(52)*B*	1380	1050	1503	4·4
H(O5′)*B*	1766	999	1619	6·0

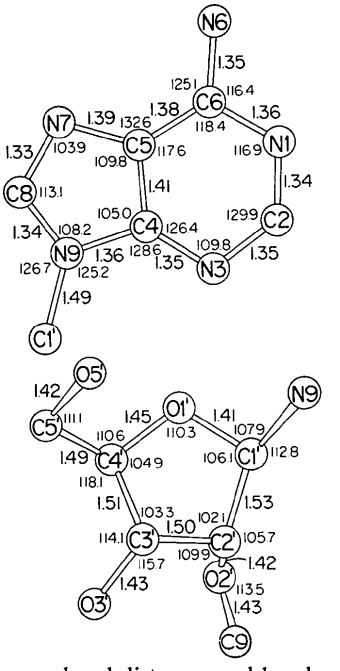

Fig. 3. The average bond distances and bond angles involving the non-hydrogen atoms.

Discussion

Bond distances and bond angles

The average standard deviations in bond distances and bond angles are 0·01 Å and 0·7°. The dimensions of the two independent molecules agree within three

* Table 4 has been deposited with the British Library Lending Division as Supplementary Publication No. SUP 31144 (9 pp.). Copies may be obtained through The Executive Secretary, International Union of Crystallography, 13 White Friars, Chester CH1 1NZ, England.

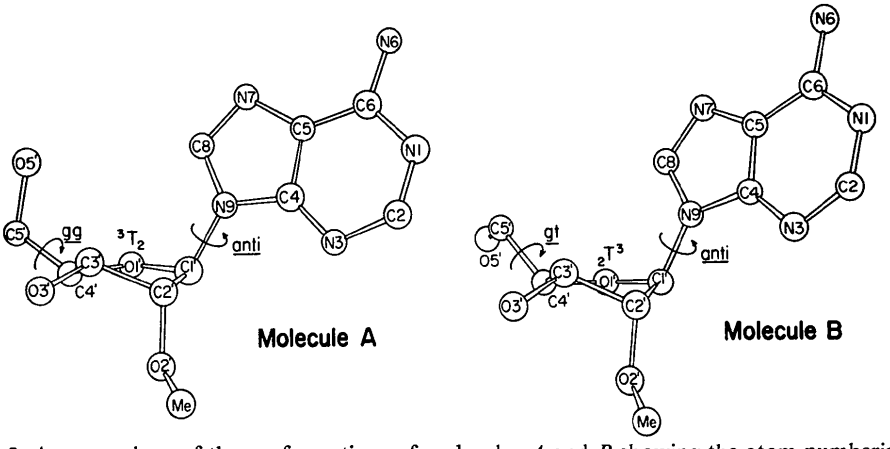

Fig. 2. A comparison of the conformations of molecules *A* and *B* showing the atom numbering.

standard deviations. In Fig. 3 the average bond distances and bond angles are shown whereas the individual values are given in Table 5. The distances and angles for the hydrogen atoms found in the difference maps are in the usual range and are not tabulated. The average bond distances and bond angles of the base are in agreement with the values found for the neutral adenine base in 3′-acetyladenosine (Rao & Sundaralingam, 1970), deoxyadenosine monohydrate (Watson, Sutor & Tollin, 1965; Lin & Sundaralingam, 1970; the latter is a redetermination of the structure) and adenosine (Lai & Marsh, 1970). Similarly the geometry of the ribose is in agreement with previous determinations (Sundaralingam & Jensen, 1965; Sundaralingam, 1973). The C(2′)–O(2′) and O(2′)–C(9) bond lengths of the methoxy group of both molecules are equal within experimental error. The average C–O distance is 1·43 Å and the average angle at O(2′) is 113·5°.

Planarity of the base

Table 6 shows the deviations of atoms from the least-squares planes through bases *A* and *B*. Both purine rings were found to be planar within the errors of the structure determination. The substituents N(6) and C(1′) of molecule *A* show significant deviations on the same side of the base plane whereas they are displaced on opposite sides in molecule *B*.

Glycosyl torsion angle, χ_{CN}

In both molecules the disposition of the base is *anti* (Donohue & Trueblood, 1960), with glycosyl torsion angles (Sundaralingam, 1969) of 14·5° (*A*) and 0·5° (*B*). The χ angle in molecule *A* is close to that usually found for 3′-*endo* nucleosides (Sundaralingam, 1973), whereas that of molecule *B* is close to 0° and is correlated with the mode of sugar pucker (Prusiner, Yathindra & Sundaralingam, 1974).

The sugar conformation

Furanose rings in nucleosides and nucleotides generally fall into two broad conformational categories, designated as 2′-*endo* and 3′-*endo* (Sundaralingam,

Table 3. *Thermal parameters of the nonhydrogen atoms in molecules A and B of 2′-O-methyladenosine*

Parameters have been multiplied by 10^4. The anisotropic temperature factors are of the form $\exp[-(\beta_{11}h^2+\ldots 2\beta_{12}hk+\ldots)]$. Standard deviations in parentheses refer to the least significant digits.

	β_{11}	β_{22}	β_{33}	β_{12}	β_{13}	β_{23}
N(1)*A*	573 (56)	10 (1)	115 (12)	−16 (6)	139 (22)	5 (3)
C(2)*A*	677 (78)	11 (1)	124 (19)	−25 (8)	141 (32)	5 (4)
N(3)*A*	503 (48)	8 (1)	100 (12)	−16 (5)	117 (19)	5 (3)
C(4)*A*	355 (48)	6 (1)	84 (14)	4 (5)	88 (21)	2 (3)
C(5)*A*	396 (48)	6 (1)	99 (14)	1 (6)	135 (22)	2 (3)
C(6)*A*	546 (56)	6 (1)	92 (13)	−2 (6)	130 (22)	−1 (3)
N(6)*A*	622 (53)	9 (1)	89 (12)	−26 (6)	141 (21)	−2 (2)
N(7)*A*	419 (41)	6 (1)	106 (11)	−13 (4)	129 (18)	4 (2)
C(8)*A*	462 (60)	8 (1)	72 (14)	−2 (6)	107 (25)	2 (3)
N(9)*A*	470 (47)	5 (1)	77 (9)	−6 (4)	108 (17)	3 (2)
C(1′)*A*	394 (49)	7 (1)	78 (11)	−19 (5)	122 (19)	1 (3)
O(1′)*A*	620 (44)	7 (1)	104 (10)	8 (4)	186 (18)	5 (2)
C(2′)*A*	392 (49)	7 (1)	111 (15)	4 (5)	142 (22)	−7 (3)
O(2′)*A*	578 (46)	8 (1)	178 (12)	−19 (5)	160 (19)	−12 (2)
C(9)*A*	1248 (96)	16 (1)	108 (18)	−54 (12)	114 (41)	−3 (4)
C(3′)*A*	503 (58)	6 (1)	122 (16)	5 (5)	198 (26)	0 (3)
O(3′)*A*	610 (43)	5 (1)	196 (14)	17 (4)	152 (20)	6 (2)
C(4′)*A*	364 (49)	6 (1)	94 (14)	6 (5)	114 (21)	7 (3)
C(5′)*A*	445 (62)	9 (1)	160 (18)	−3 (6)	175 (27)	4 (3)
O(5′)*A*	640 (48)	17 (1)	144 (13)	−44 (6)	207 (20)	−16 (3)
N(1)*B*	472 (43)	7 (1)	102 (11)	−16 (4)	111 (18)	−6 (2)
C(2)*B*	566 (60)	9 (1)	94 (13)	−2 (6)	123 (23)	1 (3)
N(3)*B*	610 (53)	8 (1)	94 (11)	−7 (5)	138 (20)	−1 (3)
C(4)*B*	505 (55)	6 (1)	80 (12)	5 (5)	94 (22)	0 (3)
C(5)*B*	477 (53)	6 (1)	88 (13)	11 (5)	88 (22)	4 (2)
C(6)*B*	410 (49)	5 (1)	100 (13)	−5 (5)	122 (21)	1 (2)
N(6)*B*	495 (45)	6 (1)	93 (11)	−14 (5)	102 (18)	−3 (2)
N(7)*B*	448 (43)	7 (1)	96 (11)	−8 (5)	124 (18)	−2 (2)
C(8)*B*	468 (52)	7 (1)	94 (13)	−17 (6)	66 (22)	−2 (2)
N(9)*B*	489 (44)	6 (1)	82 (10)	−2 (4)	104 (18)	3 (2)
C(1′)*B*	310 (41)	6 (1)	139 (14)	0 (5)	147 (22)	5 (3)
O(1′)*B*	436 (33)	7 (1)	131 (11)	−2 (4)	140 (16)	−7 (2)
C(2′)*B*	466 (55)	6 (1)	122 (14)	−17 (6)	96 (23)	7 (3)
O(2′)*B*	941 (59)	7 (1)	194 (14)	−19 (5)	270 (23)	12 (2)
C(9)*B*	1462 (94)	22 (2)	227 (32)	−50 (17)	−12 (60)	36 (7)
C(3′)*B*	451 (52)	6 (1)	155 (16)	−9 (5)	176 (25)	0 (3)
O(3′)*B*	660 (49)	8 (1)	258 (16)	−1 (5)	279 (24)	−5 (3)
C(4′)*B*	490 (54)	6 (1)	119 (14)	−5 (5)	172 (23)	−2 (3)
C(5′)*B*	916 (91)	12 (1)	117 (16)	−21 (8)	164 (31)	−1 (4)
O(5′)*B*	662 (54)	22 (1)	181 (15)	−10 (7)	145 (24)	19 (4)

Table 5. *Bond distances* (Å), *bond angles* (°) *and their estimated standard deviations for molecules A and B*

Base

	Molecule	
	A	*B*
N(1)–C(2)	1·32 (1)	1·35 (1)
N(1)–C(6)	1·35 (1)	1·36 (1)
C(2)–N(3)	1·35 (1)	1·34 (1)
N(3)–C(4)	1·33 (1)	1·36 (1)
C(4)–C(5)	1·39 (1)	1·41 (1)
C(5)–C(6)	1·38 (1)	1·38 (1)
C(6)–N(6)	1·36 (1)	1·34 (1)
C(5)–N(7)	1·39 (1)	1·39 (1)
N(7)–C(8)	1·35 (1)	1·31 (1)
C(8)–N(9)	1·30 (1)	1·37 (1)
N(9)–C(4)	1·39 (1)	1·33 (1)
N(9)–C(1′)	1·49 (1)	1·49 (1)

	Molecule	
	A	*B*
C(2)–N(1)–C(6)	118·8 (7)	116·9 (7)
N(1)–C(2)–N(3)	128·8 (7)	131·0 (7)
C(2)–N(3)–C(4)	109·9 (7)	109·7 (7)
N(3)–C(4)–C(5)	127·4 (7)	125·4 (7)
C(4)–C(5)–C(6)	117·0 (7)	118·0 (7)
C(5)–C(6)–N(1)	117·9 (7)	118·9 (6)
N(1)–C(6)–N(6)	116·4 (7)	116·3 (6)
C(5)–C(6)–N(6)	125·6 (7)	124·6 (6)
C(4)–C(5)–N(7)	111·4 (6)	108·4 (6)
C(5)–N(7)–C(8)	102·4 (6)	105·4 (6)
N(7)–C(8)–N(9)	114·0 (6)	112·2 (6)
C(8)–N(9)–C(4)	108·6 (6)	107·7 (6)
N(9)–C(4)–C(5)	103·6 (6)	106·4 (6)
C(4)–N(9)–C(1′)	122·2 (6)	128·0 (6)
C(8)–N(9)–C(1′)	129·2 (6)	124·2 (6)
C(6)–C(5)–N(7)	131·5 (7)	133·6 (7)
N(3)–C(4)–N(9)	128·9 (7)	128·2 (7)

Ribose

	Molecule	
	A	*B*
C(1′)–C(2′)	1·54 (1)	1·52 (1)
C(2′)–C(3′)	1·49 (1)	1·50 (1)
C(3′)–C(4′)	1·53 (1)	1·49 (1)
C(4′)–O(1′)	1·44 (1)	1·47 (1)
C(1′)–O(1′)	1·43 (1)	1·37 (1)
C(2′)–O(2′)	1·40 (1)	1·43 (1)
O(2′)–C(9)	1·39 (2)	1·46 (2)
C(3′)–O(3′)	1·41 (1)	1·44 (1)
C(4′)–C(5′)	1·47 (1)	1·52 (1)
C(5′)–O(5′)	1·43 (1)	1·40 (1)

	Molecule	
	A	*B*
O(1′)–C(1′)–C(2′)	105·5 (6)	106·7 (6)
C(1′)–C(2′)–C(3′)	102·8 (7)	101·4 (6)
C(2′)–C(3′)–C(4′)	103·1 (7)	103·5 (7)
C(3′)–C(4′)–O(1′)	104·2 (6)	105·5 (6)
C(4′)–O(1′)–C(1′)	110·6 (6)	109·9 (6)
C(1′)–C(2′)–O(2′)	106·0 (7)	105·3 (7)
O(2′)–C(2′)–C(3′)	111·7 (7)	108·1 (7)
C(2′)–O(2′)–C(9)	114·3 (8)	112·7 (9)
C(2′)–C(3′)–O(3′)	115·9 (7)	115·4 (7)
C(4′)–C(3′)–O(3′)	113·8 (7)	114·4 (7)
O(1′)–C(4′)–C(5′)	111·7 (7)	109·5 (7)
C(3′)–C(4′)–C(5′)	119·2 (7)	116·9 (7)
C(4′)–C(5′)–O(5′)	109·6 (7)	112·5 (8)
O(1′)–C(1′)–N(9)	106·9 (6)	108·9 (6)
C(2′)–C(1′)–N(9)	113·1 (4)	112·4 (6)

Table 6. *Least-squares planes for bases A and B and deviations of atoms from the planes* (Å)

Atoms used in fitting the least-squares planes are denoted by asterisks. Plane I: $0{\cdot}726X-0{\cdot}679Y-0{\cdot}100Z=18{\cdot}064$. Plane II: $0{\cdot}717X-0{\cdot}645Y-0{\cdot}265Z=-21{\cdot}392$.

	Molecule *A* Plane I	Molecule *B* Plane II
N(1)*A*	−0·005*	0·001*
C(2)*A*	0·002*	0·013*
N(3)*A*	0·003*	−0·011*
C(4)*A*	0·000*	0·007*
C(5)*A*	−0·007*	−0·002*
C(6)*A*	0·003*	−0·011*
N(6)*A*	−0·028	−0·085
N(7)*A*	0·009*	0·004*
C(8)*A*	−0·006*	−0·006*
N(9)*A*	0·000*	0·008*
C(1′)*A*	−0·026	0·026
R.m.s. deviation of fitted atoms	0·005	0·008

1969). In terms of the pseudorotation model (Altona & Sundaralingam, 1972), these have phase angles, *P*, of 0–36° (mean 13·0°) and 144–180° (mean 161·1°) respectively. The mean *P* values were obtained by averaging 34 nucleoside and nucleotide crystal structures in the former set and 67 structures in the latter set (Prusiner, 1974). In 2′-*O*-methyladenosine, molecule *A* exhibits the common 3′-*endo*-2′-*exo* ($^3T_2$) (Sundaralingam & Jensen, 1965; Sundaralingam, 1965, 1969) puckering with a phase angle of 11·1° while molecule *B* displays an important variant of this conformation *viz* 2′-*exo*-3′-*endo* ($_2T^3$) conformation with a *P* value of −10·1° (or 349·9°). This is the first time that the 2′-*exo* conformation has been observed for a nucleoside possessing a common base. We have observed this puckering earlier in virazole, a synthetic antiviral agent containing a five-membered triazole base ring (Prusiner & Sundaralingam, 1973). However the 2′-*exo* puckering is fairly common in α-nucleosides and nucleotides (Sundaralingam, 1971).

The difference of 22·2° in the *P* values of molecule *A* produces a 12° increase in the backbone torsion ψ′ O(3′)–C(3′)–C(4′)–C(5′) in molecule *B* (see Table 8) and also increases the probability of occurrence of the *trans* conformation about the C(4′)–C(5′) bond (Prusiner *et al.*, 1974). Thus the 2′-*exo* puckering and its effect on the backbone should be considered in the analysis of nucleic acid conformations, particularly ribonucleic acids and polyribonucleotides.

In the crystal both the 2′-*O*-methyladenosine molecules have the 3′-*endo* type conformations ($^3T_2$, mole-

cule *A*; ${}_2T^3$, molecule *B*). In solution, n.m.r. studies show that there is an equilibrium between the 3′-*endo* and 2′-*endo* conformations with the latter predominating (69%) (Hruska, 1974). In contrast, in 2′-*O*-methylcytidine (Hingerty *et al.*, 1974), the two independent molecules have the 2′-*endo* pucker in the crystal while in solution there is a slight bias towards the 3′-*endo* conformation (Hruska, Mak, Singh & Shugar, 1973). 2′-*O*-Methyluridine shows results similar to the latter solution (Hruska *et al.*, 1973). Thus the 2′-methylated ribosides, just as the ribosides themselves, favor the familiar 3′-*endo* and 2′-*endo* sugar conformations.

The conformation about the exocyclic C(4′)–C(5′) bond molecule *A* is *gauche* (g^+) and in molecule *B* it is *trans* (*t*).* Both of these conformations are observed for the nucleosides (Sundaralingam, 1969, 1973). It is found that the exocyclic bond conformation is correlated to the ring puckering, the 3′-*endo* favoring the g^+ and the 2′-*exo* favoring the *t* (Prusiner, Yathindra & Sundaralingam, 1974).

The methoxy group

One of the interests in this study was to obtain information on the conformation of the methoxy group. Three idealized staggered conformations might be visualized for the methoxy group, they are the g^+, g^- and *t*. In the first two, the methyl group is *gauche* to the H(2′) proton. In the 2′-*O*-methyladenosine both molecules exhibit the g^+ conformation. It may be noted that the torsion angle H(2′)–C(2′)–O(2′)–C(9) is 23° in molecule *A* and 28° in molecule *B* and they depart considerably from the ideal staggered arrangement. It appears that the most stable conformation is one where the methyl groups are nearly eclipsed to H(2′). The alternative g^- conformation appears to be also likely according to potential-energy calculations (Prusiner, Yathindra & Sundaralingam, 1974). It is of interest to compare the above values with the torsional angle H(3′)–C(3′)–O(3′)–C(9) of −20° in 3′-*O*-acetyladenosine (Rao, Sundaralingam, Arora & Hall, 1970; Rao & Sundaralingam, 1970). The methyl hydrogen atoms are staggered relative to the C(2′)–O(2′) bond.

Hydrogen bonding

Self-pairing of adenine

An interesting feature in this structure is the base-pairing configuration between the two independent molecules that has some resemblance to the Watson–Crick and Hoogsteen hydrogen-bonding model of adenine–uracil base pairs. In the present structure (Fig. 6) N(1), N(6) and N(7) of adenine are involved in the self pairing. Molecules *A′* and *B′* in Fig. 4 are related to molecules *A* and *B* by a translation along **c**. The two independent molecules *A* and *B* are related to each

* The conformational notations g^+ (~60°), *t* (~180°), and g^- (~−60°) are with reference to the backbone atoms C(3′)–C(4′)–C(5′)–O(5′).

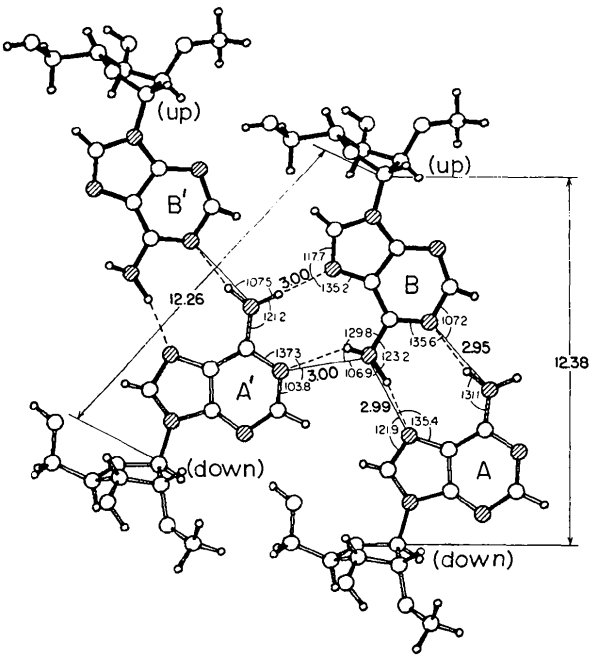

Fig. 4. A view normal to the crystallographic $\bar{1}71$ plane showing the interbase hydrogen bonding and geometry.

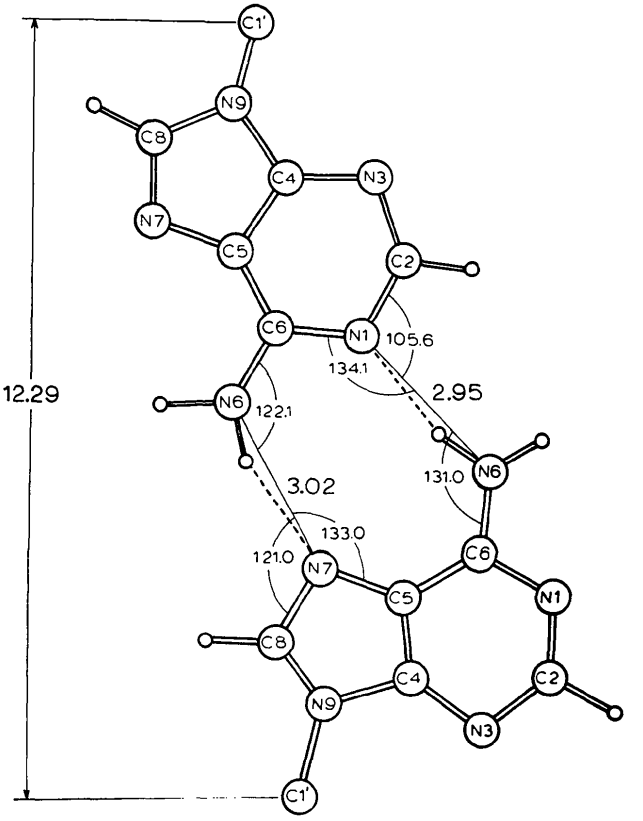

Fig. 5. Average base-pairing geometries for the hybrid Watson–Crick–Hoogsteen base pair.

other by a pseudoscrew axis which passes through the point (2·43, 28·46, 4·95 Å) and makes angles of 62·2, 89·7 and 27·8° with the *a*, *b* and *c** axes. The mean and r.m.s. deviations from the ideal screw symmetry between equivalent pairs of nonhydrogen atoms, excluding O(5′) (see below), are 0·26 and 0·29 Å, respectively. The breakdown in the ideal screw relationship arises mainly from the conformational differences about the C(4′)–C(5′) bond (g^+ in molecule *A* and *t* in molecule *B*). There are also some differences in the sugar conformation (Table 7) and base-pairing geometries (Table 8).

Table 7. *Conformation angles for 2′-O-methyladenosine* (°)

	Molecule *A*	Molecule *B*
Glycosyl torsion angle, χ_{CN}	14·5 (*anti*)	0·5 (*anti*)
Conformation about C(4′)–C(5′)	*gauche–gauche*	*gauche–trans*
φ_{OO}	−73·0	59·5
φ_{OC}	48·7	179·3
Mode of ribose puckering	C(3′)-*endo*, C(2′)-*exo* ($^3T_2$)	C(2′)-*exo*, C(3′)-*endo* ($_2T^3$)
Backbone torsion angle ψ' [defined by O(3′)–C(3′)–C(4′)–C(5′)]	74·1	85·9
Pseudorotation parameters:		
Phase angle of pseudorotation *P*	11·1	349·9
Maximum amplitude of pseudorotation τ_{max}	37·3	36·2
τ_0	4·4	17·4
τ_1	−25·8	−32·7
τ_2	36·1	34·7
τ_3	−34·1	−25·7
τ_4	18·2	5·2
Torsion angles involving the methyl group		
C(1′)–C(2′)–O(2′)–C(9)	142·7	145·7
C(3′)–C(2′)–O(2′)–C(9)	−106·1	−106·5

The above scheme appears to be an important mode of pairing between neutral adenine bases. It has been observed twice in the present work (*A* to *B*, and *A′* to *B*) and previously in deoxyadenosine (Watson, Sutor & Tollin, 1965; Lin & Sundaralingam, 1970, the latter is a reinvestigation of the structure using diffractometer data), and in 9-methyladenine (Stewart & Jensen, 1964). This pairing configuration may be relevant in tertiary structures of nucleic acids such as tRNA and rRNA especially where two or more sugar–phosphate–sugar chains run close together with two of them being parallel in a mode similar to that of *A* and *A′* in Fig. 6, and the third is antiparallel as is *B* in Fig. 6 (Brennan & Sundaralingam, 1975). The average geometry for this type of base pair calculated from this and the two other known structures is shown in Fig. 5. The N(6)···N(1) and N(6)···N(7) hydrogen-bond distances are relatively constant with average deviations from the mean of 0·03 Å. It is also interesting that the

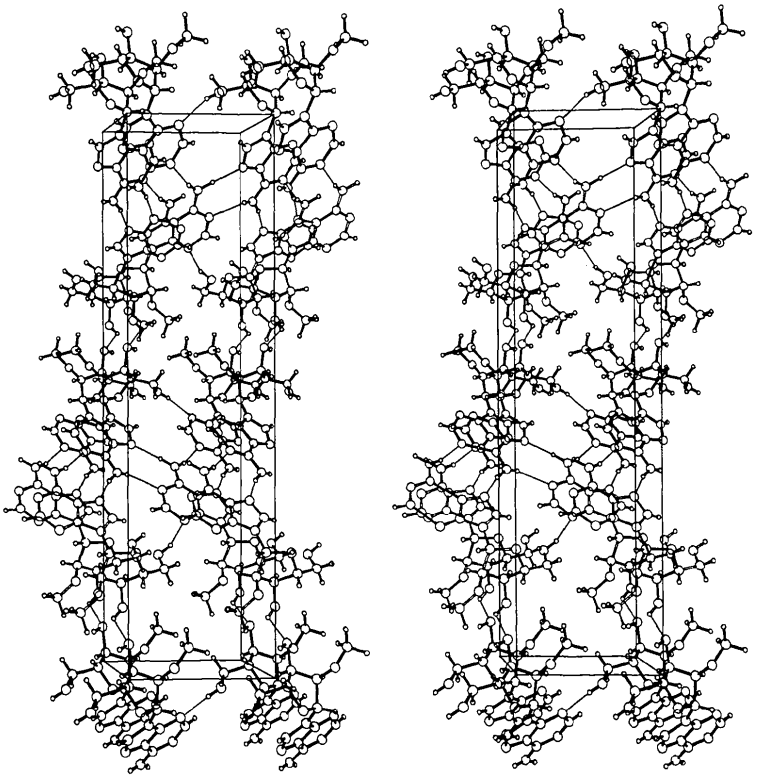

Fig. 6. A stereoscopic packing diagram along the *a** axis displaying the hydrogen bonding and crystal packing. The bonds of molecule *B* are shaded.

C(1′)···C(1′) distances observed in 2′-*O*-methyladenosine (*A*···*B*, 12·38 Å; *A*′···*B*, 12·26 Å) are strikingly similar to the values observed in deoxyadenosine (12·371 Å) and 9-methyladenine (12·18 Å) even though in the latter structures the molecules are related by a true crystallographic screw axis or glide plane. The hydrogen bonding angles, however, show considerably larger variation (Table 9). The angle C(6)–N(1)···N(6) shows the largest variation whereas the angle C(6)–N(6)···N(7) shows the smallest.

In addition to the above base-pairing scheme the other interesting hydrogen-bonding features are the intermolecular interactions between O(5′) and the N(3) atom of the bases in the two independent molecules even though they have different conformations about C(4′)–C(5′). These represent the only ribose···base hydrogen bonding in the structure. It is interesting that this hydrogen bonding was also observed in deoxyadenosine which has the *trans* conformation about C(4′)–C(5′). The ribose moieties in 2′-*O*-methyladenosine are connected by a single hydrogen bond between O(3′)–H of molecule *A* and O(3′) of molecule *B*. This constitutes the only hydrogen bonding that is not reciprocated between the two independent molecules in the crystal.

Molecular packing and base stacking

A stereoscopic view of the molecular packing (Fig. 6) and a projection view along **c*** (Fig. 7) vividly illustrate the molecular packing in the unit cell. The bases form a hydrogen-bonded network parallel to the crystallographic $\bar{1}71$ planes. The molecules form the alternating hydrophobic and hydrophilic zones of base and ribose moieties typical of most nucleoside crystal structures. The methyl groups of adjacent residues come within 3·30 Å of each other. The bases stack with partial overlap also typical of nucleoside and nucleotide crystal

Table 8. *Comparison of the base-pairing geometries in the adenine self base pairs*

	A···*B*	*A*′···*B*	dA	9MA	Range	Mean	Average deviation from mean
C(1′)···C(1′)	12·36 Å	12·26 Å	12·373 Å	12·18 Å	12·18 – 12·373 Å	12·29 Å	0·07 Å
N(6)···N(1)	2·95	3·00	2·890	2·96	2·890– 3·00	2·95	0·03
N(6)···N(7)	2·99	3·00	3·039	3·06	2·99 – 3·06	3·02	0·03
C(6)–N(6)···N(1)	131·1°	129·8°	135·0°	127·9°	127·9 –135·0°	131·0°	2·1°
C(6)–N(6)···N(7)	123·2	121·2	124·5	119·3	119·3 –124·5	122·1	1·8
C(5)–N(7)···N(6)	135·4	135·2	125·2	136·3	125·2 –136·3	133·0	3·9
C(8)–N(7)···N(6)	121·9	117·7	125·7	118·6	117·7 –125·7	121·0	2·8
C(2)–N(1)···N(6)	107·2	103·8	109·9	101·6	101·6 –109·9	105·6	2·9
C(6)–N(1)···N(6)	135·6	137·3	125·1	138·3	125·1 –138·3	134·1	4·5

Abbreviations: *A*···*B* and *A*′···*B*: 2′-*O*-methyladenosine *A*···*B* and *A*′···*B* pair, respectively; dA: deoxyadenosine; 9MA: 9-methyladenine.

Table 9. *Hydrogen-bond lengths* (Å) *and angles* (°) *of* 2′-*O-methyladenosine*

Standard deviations are given in parentheses. Symmetry operation: (1) x,y,z; (2) $-x,\frac{1}{2}+y,-z$.

Symmetry No.	Translation *x*	*y*	*z*		Angle	Length	Length from hydrogen
1	0	0	+1	N(6)*B*–H(61)*B*···N(1)*A*	138	2·99 (1)	2·35
1	+1	0	+1	O(5′)*A*–H(O5′)*A*···N(3)*A*	155	2·91 (1)	2·03
1	0	0	−1	N(6)*A*–H(61)*A*···N(7)*B*	173	3·00 (1)	2·01
1	+1	0	0	N(6)*A*–H(62)*A*···N(1)*B*	162	2·95 (1)	2·02
1	−1	0	0	N(6)*B*–H(62)*B*···N(7)*A*	168	2·99 (1)	1·95
2	+1	−1	+2	O(3′)*A*–H(O3′)*A*···O(3′)*B*	126	2·82 (1)	2·12
1	+1	0	+1	O(5′)*B*–H(O5′)*B*···N(3)*B*	145	2·84 (1)	2·12

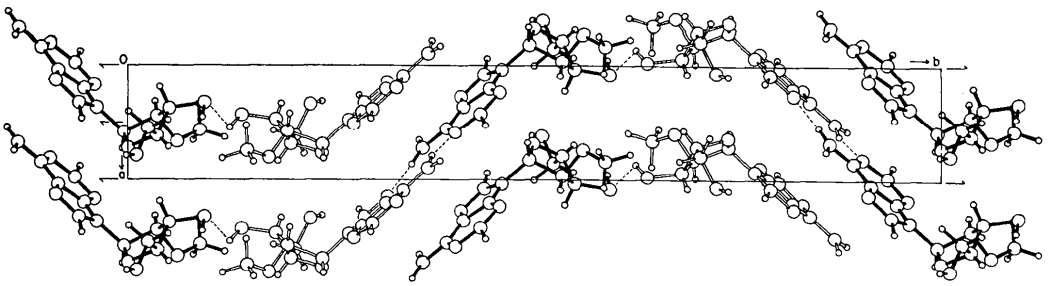

Fig. 7. A view along the *c** axis showing the alternating hydrophilic and hydrophobic zones and the zigzag pattern of the bases. The bonds of molecule *B* are shaded.

structures (Bugg, Thomas, Rao & Sundaralingam, 1971). The two different stacking patterns involving the independent molecules are shown in Fig. 8. In the case of molecule *A* the amino group is stacked directly over the pyrimidine ring of a translationally related base whereas in molecule *B* it lies over the imidazole ring of a neighboring molecule. The respective interplanar separations are 3·40 and 3·36 Å.

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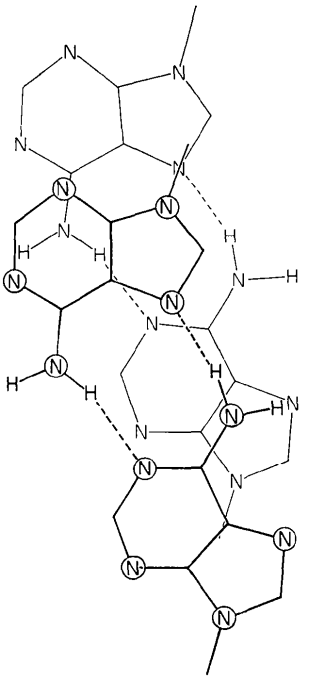

Fig. 8. A view normal to the base plane of molecule *A* illustrating the different base stacking patterns.

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