

Structure and Thermal Vibrations of Adenosine from Neutron Diffraction Data at 123 K

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Abstract

The crystal structure of adenosine, $C_{10}H_{13}N_5O_4$, $M_r = 267.24$, has been refined by full-matrix least-squares methods using single-crystal neutron diffraction data ($\sin\theta/\lambda < 0.79 \text{ \AA}^{-1}$) measured at 123 K. Crystal data at 123 K: monoclinic, $P2_1$, $Z = 2$, $a = 4.7885(8)$, $b = 10.240(2)$, $c = 11.772(2) \text{ \AA}$, $\beta = 99.59(2)^\circ$, $V = 569.2 \text{ \AA}^3$, $\lambda = 1.0402(1) \text{ \AA}$, $D_n = 1.559 \text{ Mg m}^{-3}$, $\mu = 191.1 \text{ m}^{-1}$, $F(000) = 175.72 \text{ fm}$, $R(F^2) = 0.044$, $wR(F^2) = 0.055$, $S = 1.13$ for 2450 reflections with $F^2 > 0.0$. Bond lengths and angles have e.s.d.'s less than 0.002 \AA and 0.1° for non-H atoms and less than 0.004 \AA and 0.3° for H atoms. Values are in general agreement with those previously obtained from room-temperature X-ray data and with those obtained for 9-methyladenine by neutron diffraction at 126 K. Small displacements of amino H atoms from the least-squares plane through the non-H atoms of the adenine part appear to be the result of hydrogen bonding. There is an intermolecular interaction $C2-H2 \cdots O2'$ with an $H \cdots O$ distance of 2.20 \AA . In its thermal vibrations, the adenosine molecule behaves as two rigid segments with a torsional libration about the glycosidic bond of 14.4 deg^2 at 123 K. The force constant for this libration, as determined from the diffraction data, is $73(10) \text{ J mol}^{-1} \text{ deg}^{-2}$.

Introduction

The crystal structure of adenosine was first determined by Lai & Marsh (1972) using room-temperature X-ray diffraction data ($\sin\theta/\lambda < 0.63 \text{ \AA}^{-1}$). This was a very careful analysis leading to the determination of heavier-atom bond lengths and angles with e.s.d.'s of 0.003 \AA and 0.2° . We now report a neutron diffraction study undertaken to provide accurate positional and thermal-vibrational

parameters for the atomic nuclei, including H, at reduced temperature (123 K). The primary aim was to be able to use these in a future study of the electron density distribution in the crystal with X-ray data collected at the same reduced temperature. We have also analysed the nuclear anisotropic thermal parameters U^{ij} for adenosine in terms of the overall rigid-body motion of the molecule, the torsional libration about the glycosidic bond and the internal vibrations of the H nuclei. The analysis procedure (He & Craven, 1985; He, 1989) involved a least-squares refinement in which experimentally determined U^{ij} were the observables and the force constants for selected bond torsions were included among the variables. This has resulted in the experimental determination of the force constant for torsional libration about the glycosidic bond in adenosine. The value $f = 73(10) \text{ J mol}^{-1} \text{ deg}^{-2}$ which we obtain from neutron diffraction at 123 K is in good agreement with the value $f = 70(12) \text{ J mol}^{-1} \text{ deg}^{-2}$ we obtain in the same way using the U^{ij} derived by Lai & Marsh (1972) from X-ray diffraction data at room temperature.

Experimental

Prismatic crystals of adenosine (Sigma Chemical Co.) were grown from aqueous solution by slow evaporation. A 3.26 mg crystal with dimensions $5.50 \times 0.70 \times 0.66 \text{ mm}$, volume 2.15 mm^3 , exhibiting forms $\{100\}$, $\{011\}$, $\{001\}$ was selected for diffraction measurements. The data were collected at the Brookhaven High Flux Beam Reactor with a monochromated neutron beam obtained by reflection from Be (002) planes and with wavelength $1.0402(1) \text{ \AA}$ calibrated against a standard KBr crystal ($a_0 = 6.6000 \text{ \AA}$). Details of the experimental methods and procedures of measurements have previously been described (McMullan, Benci & Craven, 1980). The adenosine crystal, which was enclosed under helium in an aluminium canister, was aligned with the $[100]$ direction within $\sim 8^\circ$ of the diffractometer φ axis.

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The crystal temperature was held at 123 ± 0.5 K using a closed-cycle helium refrigerator.* The lattice constants were determined by a least-squares fit to $\sin^2\theta$ data for 32 reflections with $50 < 2\theta < 56^\circ$. Intensity data for reflections ($hk \pm l$; $h \leq 7$, $k \leq 16$, $|l| \leq 18$) were measured by the $\omega/2\theta$ step-scan method. Fixed scan widths of 3.2° in $\Delta 2\theta$ were taken for $\sin\theta/\lambda < 0.44 \text{ \AA}^{-1}$, and beyond this value the scan widths was varied as $\Delta 2\theta = (2.17 + 2.13 \tan\theta)^\circ$ up to $(\sin\theta/\lambda)_{\max} = 0.79 \text{ \AA}^{-1}$. Between 60 and 90 steps (N) were counted per scan taking at least 10% of N at each end of the scan as background counts. Counts were accumulated at each step for a preset count of the direct beam requiring ~ 1.4 s. The intensities of two reflections (085, 248), remeasured at 3 h intervals during the 8 days of data collection, showed no differences from the averages greater than 2%. The integrated intensity of each reflection was obtained by subtracting from the total scan count the background estimated from the two extreme 10% parts of the scan. The variances in intensities were derived from counting statistics. Absorption corrections (Templeton & Templeton, 1973; de Meulenaer & Tompa, 1965) were applied using $\mu = 191.1 \text{ m}^{-1}$ evaluated from the effective mass absorption coefficient ($2.474 \text{ m}^2 \text{ kg}^{-1}$) for chemically bonded hydrogen at $\lambda = 1.0402 \text{ \AA}$ (McMullan & Koetzle, 1980). The correction factors ranged from 1.12 to 1.19. The averages of 241 symmetry-related F_o^2 values for (0, k , $\pm l$) gave an agreement factor $\sum | \langle F_o^2 \rangle - F_o^2 | / \sum \langle F_o^2 \rangle = 0.02$. Of the 2852 reflections recorded, 2496 were symmetry-independent; 46 had negative F_o^2 values, four of which were of magnitude more than $3\sigma(F_o^2)$.

The refinement was carried out with the full-matrix least-squares program of Lundgren (1982). The function $\sum w | F_o^2 - (kF_c)^2 |^2$ was minimized with weights $w = \{ [\sigma(F) \sin 2\theta]^2 + (0.01 F_o^2)^2 \}^{-1}$, using the 2450 independent reflections with $F_o^2 > 0.0$. The initial atomic parameters were coordinates from Lai & Marsh (1972) and assumed isotropic U values of 0.010 \AA^2 for C, N, O and 0.019 \AA^2 for H. The coherent neutron-scattering lengths used were those given by Koester (1977). The y coordinate of atom N9 was held constant in order to define the unit-cell origin. A total of 289 parameters (p) were refined consisting of coordinates and anisotropic thermal factors for the 32 independent atoms, the scale factor k and the isotropic secondary-extinction coefficient g for a type I crystal with Lorentzian mosaic spread (Becker & Coppens, 1974). The indices of fit at convergence [$\Delta p_i / \sigma(p_i) < 0.02$] were $R(F^2) = 0.044$,

$wR(F^2) = 0.055$, $S = 1.13$.* In the final $\Delta\rho$ map, the largest residual error $|\rho|$ was $< 1.3\%$ of the maximum peak height in the ρ map (at N). The final value for the extinction coefficient was $g = 4.6(2) \times 10^{-3} \text{ rad}^{-1}$. The largest extinction correction was 1.30 for reflection (011), while fewer than 1% of the reflections had corrections greater than 1.10. The final positional and anisotropic thermal parameters are given in Tables 1 and 2 respectively.† An additional refinement including third-order temperature factors according to the Gram-Charlier formalism (Johnson & Levy, 1974) gave no values significantly different from zero. Figs. 1 and 2 show the molecule and the cell contents respectively.

Results and discussion

Molecular geometry and packing

The structure obtained by neutron diffraction at 123 K is very similar to that obtained by Lai & Marsh (1972) by X-ray diffraction at room temperature. The ribose ring is puckered with C3'-endo, C2'-exo and the configuration at the glycosidic N9—C1' bond is low *anti*, so that the overall molecular conformation conforms to that of the adenosine moieties in double-helical RNA (Rosenberg, Seeman, Kim, Suddath, Nicholas & Rich, 1973). Puckering coordinates (Cremer & Pople, 1975; Norrestam, 1981) for the ribose ring at 123 K are $q = 0.363(2) \text{ \AA}$ and $\varphi = 58.9(2)^\circ$, indicating a twist conformation with an approximate twofold axis passing through O1' and the midpoint of the C2'—C3' bond. Corresponding values derived for the room-temperature structure are $q = 0.361(2) \text{ \AA}$ and $\varphi = 60.6(3)^\circ$. The ideal twist conformation has $\varphi = 54^\circ$. Torsion angles at the glycosidic bond (C8—N9—C1'—O1') are $\chi = 8.7(2)^\circ$ at 123 K and $9.9(3)^\circ$ at room temperature. The adenine ring system is approximately planar (Table 3), with small but significant atomic displacements from the plane which are very similar to those observed at room temperature. Best least-squares planes through the heavy atoms of the imidazole and pyrimidine rings make a dihedral angle of $1.50(6)^\circ$. The imidazole ring is planar within experimental error. The pyrimidine ring adopts a very flattened boat conformation with N1 and C4 at bow and stern positions. As noted by Lai & Marsh (1972), the amino H atoms are displaced from the pyrimidine plane toward their hydrogen-bonding acceptor atoms in neighbouring

* $R(F^2) = \sum | F_o^2 - F_c^2 | / \sum F_o^2$; $wR(F^2) = [\sum w | F_o^2 - F_c^2 |^2 / \sum (w F_o^2)^2]^{1/2}$; $S = \{ \sum w | F_o^2 - F_c^2 |^2 / (n - p) \}^{1/2}$.

† Lists of structure factors have been deposited with the British Library Document Supply Centre as Supplementary Publication No. SUP 53699 (18 pp.). Copies may be obtained through The Technical Editor, International Union of Crystallography, 5 Abbey Square, Chester CH1 2HU, England.

* Air Products and Chemicals, Inc., Displex Model CS-202. The reported temperature includes a correction of $+2.0$ K based on a prior calibration with reference to the magnetic phase transition at $T_N = 78.38(1) \text{ K}$ in FeF_2 (Hutchings, Schulhof & Guggenheim, 1972).

Table 1. Nuclear positional parameters

	x	y	z
N1	0.92835 (23)	-0.08975 (13)	0.18573 (8)
C2	0.79764 (33)	-0.07368 (16)	0.27682 (12)
N3	0.63884 (23)	0.02665 (13)	0.30034 (8)
C4	0.62035 (27)	0.11809 (15)	0.21736 (10)
C5	0.74980 (28)	0.11654 (15)	0.11985 (10)
C6	0.91387 (27)	0.00496 (15)	0.10460 (10)
N7	0.68054 (21)	0.22815 (13)	0.05444 (7)
C8	0.51447 (30)	0.29364 (15)	0.11218 (11)
N9	0.47084 (19)	0.23265 (0)	0.21164 (7)
N10	1.05054 (21)	-0.01363 (13)	0.01595 (8)
C1'	0.30659 (27)	0.27599 (15)	0.29917 (10)
C2'	0.50172 (28)	0.31226 (15)	0.41212 (10)
C3'	0.54611 (26)	0.45809 (15)	0.39420 (11)
C4'	0.25798 (27)	0.50127 (15)	0.32859 (10)
C5'	0.27099 (30)	0.61674 (15)	0.24958 (11)
O1'	0.15178 (32)	0.38850 (17)	0.26043 (12)
O2'	0.35388 (40)	0.29717 (18)	0.50517 (13)
O3'	0.64015 (34)	0.52933 (17)	0.49604 (13)
O5'	-0.00471 (37)	0.64317 (17)	0.18868 (13)
H2	0.82175 (101)	-0.15303 (37)	0.33922 (34)
H8	0.41365 (87)	0.38587 (35)	0.08679 (31)
H10A	1.14051 (76)	-0.10208 (33)	0.00796 (28)
H10B	1.03694 (73)	0.05195 (33)	-0.05021 (26)
H1'	0.16305 (65)	0.19572 (29)	0.31361 (26)
H2'	0.69947 (69)	0.25579 (32)	0.42430 (27)
H3'	0.70658 (61)	0.46949 (33)	0.33770 (25)
H4'	0.11500 (62)	0.35233 (32)	0.39065 (24)
H5A'	0.35577 (91)	0.70115 (34)	0.30167 (33)
H5B'	0.41832 (78)	0.59449 (40)	0.18879 (30)
HO2'	0.33694 (83)	0.20282 (31)	0.51633 (28)
HO3'	0.52974 (75)	0.50657 (35)	0.55679 (25)
HO5'	-0.02007 (79)	0.73884 (32)	0.17585 (27)

Table 2. Nuclear anisotropic thermal parameters (\AA^2)

The temperature factor is: $T = \exp(-2\pi^2 \sum_i \sum_j h_i h_j a_i^* a_j^* U_{ij}')$.

	U^{11}	U^{22}	U^{33}	U^{12}	U^{13}	U^{23}
N1	0.0219 (6)	0.0100 (4)	0.0149 (4)	0.0058 (3)	0.0087 (3)	0.0027 (2)
C2	0.0271 (7)	0.0108 (5)	0.0153 (5)	0.0081 (5)	0.0110 (5)	0.0045 (4)
N3	0.0214 (4)	0.0099 (4)	0.0128 (3)	0.0050 (3)	0.0084 (3)	0.0028 (3)
C4	0.0119 (5)	0.0073 (4)	0.0091 (4)	0.0010 (4)	0.0037 (4)	0.0002 (3)
C5	0.0121 (5)	0.0076 (4)	0.0095 (4)	0.0006 (4)	0.0033 (4)	-0.0001 (4)
C6	0.0124 (5)	0.0083 (4)	0.0100 (4)	0.0015 (4)	0.0037 (4)	-0.0005 (3)
N7	0.0173 (4)	0.0111 (3)	0.0108 (3)	0.0039 (3)	0.0061 (3)	0.0024 (3)
C8	0.0170 (6)	0.0097 (5)	0.0115 (5)	0.0041 (4)	0.0054 (4)	0.0027 (4)
N9	0.0131 (4)	0.0079 (3)	0.0096 (3)	0.0019 (3)	0.0037 (3)	0.0000 (3)
N10	0.0175 (4)	0.0126 (4)	0.0128 (4)	0.0030 (3)	0.0074 (3)	0.0000 (3)
C1'	0.0112 (5)	0.0070 (4)	0.0101 (4)	0.0009 (4)	0.0035 (4)	-0.0005 (4)
C2'	0.0143 (5)	0.0084 (5)	0.0095 (4)	0.0032 (4)	0.0016 (4)	-0.0008 (4)
C3'	0.0099 (5)	0.0082 (4)	0.0114 (5)	0.0008 (4)	0.0016 (4)	-0.0007 (4)
C4'	0.0099 (5)	0.0072 (4)	0.0115 (4)	0.0013 (4)	0.0024 (4)	-0.0002 (4)
C5'	0.0154 (6)	0.0087 (5)	0.0152 (5)	0.0004 (4)	0.0026 (4)	0.0020 (4)
O1'	0.0097 (5)	0.0092 (5)	0.0122 (6)	0.0021 (5)	0.0001 (5)	-0.0014 (4)
O2'	0.0299 (8)	0.0097 (6)	0.0114 (6)	0.0020 (6)	0.0086 (6)	-0.0005 (5)
O3'	0.0153 (6)	0.0109 (6)	0.0126 (6)	-0.0023 (5)	0.0016 (5)	-0.0023 (5)
O5'	0.0177 (7)	0.0103 (6)	0.0156 (6)	0.0042 (5)	0.0000 (5)	0.0011 (5)
H2	0.0583 (24)	0.0256 (14)	0.0353 (17)	0.0172 (16)	0.0246 (17)	0.0140 (13)
H8	0.0465 (20)	0.0210 (13)	0.0335 (15)	0.0168 (14)	0.0156 (14)	0.0096 (12)
H10A	0.0343 (16)	0.0212 (12)	0.0283 (13)	0.0085 (12)	0.0129 (12)	-0.0013 (11)
H10B	0.0316 (15)	0.0241 (13)	0.0220 (12)	0.0029 (12)	0.0101 (11)	0.0050 (10)
H1'	0.0225 (13)	0.0187 (11)	0.0268 (12)	-0.0044 (10)	0.0085 (11)	-0.0006 (10)
H2'	0.0235 (13)	0.0255 (14)	0.0270 (12)	0.0102 (11)	-0.0013 (11)	-0.0006 (10)
H3'	0.0176 (11)	0.0270 (13)	0.0248 (12)	-0.0020 (10)	0.0086 (10)	-0.0020 (11)
H4'	0.0206 (12)	0.0244 (13)	0.0240 (12)	0.0015 (10)	0.0086 (10)	-0.0040 (10)
H5A'	0.0421 (19)	0.0194 (13)	0.0393 (17)	-0.0063 (13)	-0.0064 (16)	-0.0014 (12)
H5B'	0.0289 (16)	0.0370 (18)	0.0311 (15)	0.0060 (13)	0.0131 (13)	0.0111 (13)
HO2'	0.0411 (18)	0.0158 (11)	0.0267 (13)	0.0000 (12)	0.0103 (13)	0.0050 (10)
HO3'	0.0339 (15)	0.0261 (13)	0.0206 (12)	-0.0062 (13)	0.0085 (11)	-0.0004 (10)
HO5'	0.0355 (16)	0.0166 (11)	0.0274 (12)	0.0079 (12)	0.0042 (12)	0.0041 (11)

molecules. From neutron diffraction, H10A is directed towards N7 of one molecule and H10B towards O5' of another with displacements 0.107 and 0.031 \AA respectively. The torsion angles N1—C6—N10—H10A and C5—C6—N10—H10B are 7.0 and -2.9° respectively. Atom H8 is displaced by 0.025 \AA from the imidazole plane toward atom N10

to give an intermolecular H...N distance of 2.56 \AA and C—H...N angle of 142.6° . We consider that this is better described as a van der Waals interaction than as a C—H...N hydrogen bond. Atom H8 also makes a short intramolecular approach of 2.57 \AA with the ring oxygen O1'. This occurs close to the plane of the imidazole ring. Atom H2 does not deviate significantly from the pyrimidine-ring plane. In the crystal structure of 9-methyladenine as determined by neutron diffraction at 126 K (McMullan, Benci & Craven, 1980), atoms of the adenine ring are also subject to small but significant deviations from coplanarity but in a different way from those presently reported, presumably because of the differences in adenine packing and hydrogen bonding in the two crystal structures.

As shown in Table 4, the values found for bond distances and angles involving the heavy atoms are in general agreement with those of Lai & Marsh (1972) when corrected for thermal-vibration effects (see

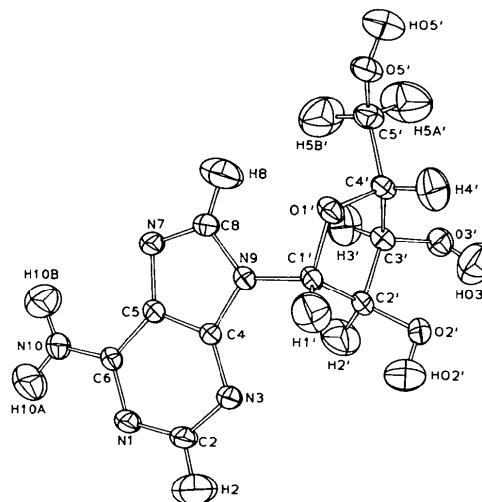


Fig. 1. Adenosine with atomic notation as used by Lai & Marsh (1972). Thermal ellipsoids (123 K) are shown at 90% probability level (Johnson, 1976).

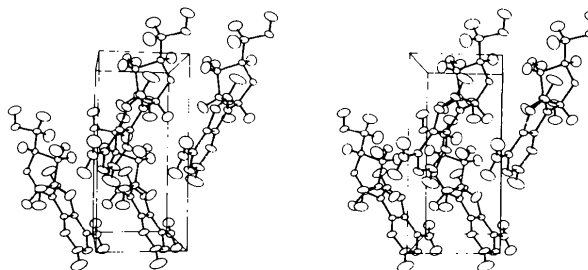


Fig. 2. Stereo diagram of molecular packing viewed approximately down c^* with $+a$ to right in right-handed system. Thermal ellipsoids are at 75% probability level. The four molecules shown are in positions: x, y, z ; $-1 + x, y, z$; $2 - x, 0.5 + y, 1 - z$; and $1 - x, 0.5 + y, 1 - z$.

Table 3. Least-squares planes through selected atoms

The planes are referred to the crystal axes and are of the form $Ax + By + Cz = D$, where x , y and z are fractional coordinates and the coefficients are in Å. The following planes were used (excluding H atoms): (1) The adenine framework. (2) The imidazole ring. (3) The pyrimidine ring.

Plane coefficients

	<i>A</i>	<i>B</i>	<i>C</i>	<i>D</i>
(1)	3.5044	4.6584	4.4544	3.6860
(2)	3.4630	4.7789	4.4913	3.6909
(3)	3.5344	4.5480	4.4481	3.7091

Distances of the atoms from the planes (Å × 10³)

E.s.d.'s are 0.002 Å for non-H atoms, 0.004 Å for H atoms.

(a) Atoms used in calculation of the planes; distances involving other atoms are in square brackets

	(1)	(2)	(3)
N1	-23	[-71]	-10
C2	-1	[-38]	6
N3	15	[-2]	6
C4	6	-2	-13
C5	18	1	4
C6	6	[-33]	9
N7	4	1	[-24]
C8	-15	-2	[-56]
N9	-9	2	[-46]
N10	3	[-46]	[-13]
R.m.s. deviation	12	2	9

(b) Other atoms

Cl'	7		
H2	-8		8
H8	-52	-25	
H10A	-129		-107
H10B	-34		-31
N7d*	-571		
O1*	-184		
O5'e*	-339		

* N7d and O5'e are H bonded with H10A and H10B, respectively (see Table 5). O1' is involved in a short intramolecular distance with H8.

below). The largest difference in a bond length was 0.011 (4) Å for C4—C5 and the largest difference in an angle was 0.7 (2)° for C2—N1—C6. The C—H, N—H and O—H bond lengths presently determined are about 0.1 Å longer, as is generally found in comparing neutron and X-ray diffraction results. The distances and angles of the adenine group generally agree well with the corresponding values from the neutron study of 9-methyladenine at 126 K (McMullan, Benci & Craven, 1980). The largest difference for non-H atoms is 0.007 (2) Å for C8—N9 and 0.6 (2)° for C2—N1—C6. The largest differences involving the H atoms are 0.005 (4) Å for N10—H10A and 1.5 (3)° for C6—N10—H10B.

The hydrogen bonds in the crystal structure of adenosine link the molecules in a three-dimensional network involving all potential donor and acceptor groups with the exception of the ring oxygen O1'. Base-pairing interactions as found in 9-methyladenine (McMullan, Benci & Craven, 1980) do not occur. Rather, only one of the five conventional hydrogen bonds (Table 5) is base-base, while three are base-sugar and one is sugar-sugar (Figs. 2 and 3). Distances and angles for these hydrogen bonds (Table 5) are not exceptional. However, as noted by Lai & Marsh (1972), there is an interesting intermolecular C2—H2...O2' interaction which might be

Table 4. Interatomic distances (Å) and angles (°)

(a) Bonds involving non-H atoms

(1) Values from neutron diffraction at 123 K, uncorrected. (2) Values from (1) with thermal-vibration corrections. (3) Values from X-ray diffraction at room temperature (Lai & Marsh, 1972) with thermal-vibration corrections. Uncorrected values have e.s.d.'s of 0.003 Å.

	(1)	(2)	(3)
N1—C2	1.339 (2)	1.340	1.342
N1—C6	1.355 (2)	1.357	1.356
C2—N3	1.334 (2)	1.335	1.332
N3—C4	1.345 (2)	1.348	1.355
C4—C5	1.393 (2)	1.394	1.383
C4—N9	1.370 (2)	1.371	1.377
C5—C6	1.415 (2)	1.416	1.418
C5—N7	1.387 (2)	1.390	1.391
C6—N10	1.335 (2)	1.335	1.334
N7—C8	1.313 (2)	1.314	1.310
C8—N9	1.373 (2)	1.375	1.367
N9—Cl'	1.465 (2)	1.466	1.468
Cl'—C2'	1.538 (2)	1.539	1.533
Cl'—O1'	1.404 (2)	1.406	1.414
C2'—C3'	1.528 (2)	1.529	1.531
C2'—O2'	1.408 (2)	1.409	1.413
C3'—C4'	1.530 (2)	1.532	1.526
C3'—O3'	1.412 (2)	1.413	1.420
C4'—C5'	1.512 (2)	1.514	1.512
C4'—O1'	1.449 (2)	1.450	1.452
C5'—O5'	1.419 (2)	1.421	1.424

(b) Bonds involving H atoms

	(1)	(1)	
C2—H2	1.089 (4)	C3'—H3'	1.103 (3)
C8—H8	1.080 (3)	C4'—H4'	1.103 (3)
N10—H10A	1.014 (3)	C5'—H5A'	1.098 (4)
N10—H10B	1.022 (3)	C5'—H5B'	1.109 (4)
Cl'—H1'	1.103 (3)	O2'—HO2'	0.980 (3)
C2'—H2'	1.098 (3)	O3'—HO3'	0.986 (3)
		O5'—HO5'	0.992 (3)

(c) Bond angles (°)

C2—N1—C6	120.1 (1)	C2'—C3'—C4'	102.6 (1)
N1—C2—N3	128.3 (1)	C2'—C3'—O3'	114.8 (1)
N1—C2—H2	116.0 (2)	C4'—C3'—O3'	114.4 (1)
N3—C2—H2	115.8 (2)	C2'—C3'—H3'	108.3 (2)
C2—N3—C4	110.9 (1)	C4'—C3'—H3'	109.0 (2)
N3—C4—C5	127.3 (1)	O3'—C3'—H3'	107.6 (2)
N3—C4—N9	127.0 (1)	C3'—C4'—C5'	114.2 (1)
C5—C4—N9	105.7 (1)	C3'—C4'—O1'	104.7 (1)
C4—C5—C6	116.5 (1)	C5'—C4'—O1'	109.2 (1)
C4—C5—N7	110.5 (1)	C3'—C4'—H4'	109.2 (2)
C6—C5—N7	132.9 (1)	C5'—C4'—H4'	110.4 (2)
N1—C6—C5	116.9 (1)	O1'—C4'—H4'	108.9 (2)
N1—C6—N10	118.4 (1)	C4'—C5'—O5'	109.2 (1)
C5—C6—N10	124.7 (1)	C4'—C5'—H5A'	108.7 (2)
C5—N7—C8	104.1 (1)	C4'—C5'—H5B'	109.4 (2)
N7—C8—N9	113.6 (1)	O5'—C5'—H5A'	111.0 (2)
N7—C8—H8	125.8 (2)	O5'—C5'—H5B'	110.6 (2)
N9—C8—H8	120.6 (2)	H5A'—C5'—H5B'	107.8 (3)
C4—N9—C8	106.2 (1)	Cl'—O1'—C4'	110.9 (1)
C4—N9—Cl'	124.2 (1)	C2'—O2'—HO2'	106.0 (2)
C—N9—Cl'	129.6 (1)	C3'—O3'—HO3'	111.1 (2)
C6—N10—H10A	118.1 (2)	C5'—O5'—HO5'	107.7 (2)
C6—N10—H10B	121.7 (2)		
H10A—H10—H10B	119.4 (3)		
N9—Cl'—C2'	111.2 (1)		
N9—Cl'—O1'	109.7 (1)		
C2'—Cl'—O1'	107.1 (1)		
C2'—Cl'—H1'	110.6 (2)		
N9—Cl'—H1'	107.7 (2)		
O1'—Cl'—H1'	110.7 (2)		
Cl'—C2'—C3'	101.3 (1)		
Cl'—C2'—O2'	109.5 (1)		
C3'—C2'—O2'	108.3 (1)		
Cl'—C2'—H2'	111.8 (2)		
C3'—C2'—H2'	113.3 (2)		
O2'—C2'—H2'	112.0 (2)		

(d) Torsion angles (°)

Sign is positive if, viewed from second to third atom, clockwise motion superimposes first on fourth atom.

C6—N1—C2—N3	1.9 (2)	C4—N9—Cl'—H1'	-52.2 (2)
C6—N1—C2—H2	-178.9 (3)	C8—N9—Cl'—C2'	-109.5 (2)
C2—N1—C6—C5	-1.9 (2)	C8—N9—Cl'—O1'	8.7 (2)
C2—N1—C6—N10	179.0 (1)	C8—N9—Cl'—H1'	129.2 (2)
N1—C2—N3—C4	-0.1 (2)	N9—Cl'—C2'—C3'	91.6 (1)
H2—C2—N3—C4	-179.3 (3)	N9—Cl'—C2'—O2'	-154.2 (1)

Table 4 (cont.)

C2—N3—C4—C5	-1.8 (2)	N9—C1'—C2'—H2'	-29.4 (2)
C2—N3—C4—N9	178.6 (1)	O1'—C1'—C2'—C3'	-28.2 (1)
N3—C4—C5—C6	1.7 (2)	O1'—C1'—C2'—O2'	86.0 (2)
N3—C4—C5—N7	-180.0 (1)	O1'—C1'—C2'—H2'	-149.2 (2)
N9—C4—C5—C6	-178.6 (1)	H1'—C1'—C2'—C3'	-148.9 (2)
N9—C4—C5—N7	-0.3 (2)	H1'—C1'—C2'—O2'	-34.6 (2)
N3—C4—N9—C8	-179.9 (1)	H1'—C1'—C2'—H2'	90.2 (3)
N3—C4—N9—C1'	1.3 (2)	N9—C1'—O1'—C4'	-112.1 (1)
C5—C4—N9—C8	0.4 (1)	C2'—C1'—O1'—C4'	8.7 (2)
C5—C4—N9—C1'	-178.5 (1)	H1'—C1'—O1'—C4'	129.3 (2)
C4—C5—C6—N1	0.3 (2)	C1'—C2'—C3'—C4'	35.8 (1)
C4—C5—C6—N10	179.4 (1)	C1'—C2'—C3'—O3'	160.5 (1)
N7—C5—C6—N1	-177.6 (1)	C1'—C2'—C3'—H3'	-79.3 (2)
N7—C5—C6—N10	1.5 (2)	O2'—C2'—C3'—C4'	-79.3 (1)
C4—C5—N7—C8	0.0 (2)	O2'—C2'—C3'—O3'	45.4 (2)
C6—C5—N7—C8	178.0 (2)	O2'—C2'—C3'—H3'	165.6 (2)
N1—C6—N10—H10A	7.0 (3)	H2—C2'—C3'—C4'	155.8 (2)
N1—C6—N10—H10B	176.2 (3)	H2—C2'—C3'—O3'	-79.6 (2)
C5—C6—N10—H10A	-172.1 (3)	H2—C2'—C3'—H3'	40.6 (3)
C5—C6—N10—H10B	-2.9 (3)	C1'—C2'—O2'—HO2'	72.9 (3)
C5—N7—C8—N9	0.3 (2)	C3'—C2'—O2'—HO2'	-177.5 (3)
C5—N7—C8—H8	-178.6 (3)	H2—C2'—O2'—HO2'	-51.8 (3)
N7—C8—N9—C4	-0.4 (2)	C2'—C3'—C4'—C5'	-151.3 (1)
N7—C8—N9—C1'	178.3 (1)	C2'—C3'—C4'—O1'	-31.9 (1)
H8—C8—N9—C4	178.5 (3)	C2'—C3'—C4'—H4'	84.5 (2)
H8—C8—N9—C1'	-2.7 (3)	O3'—C3'—C4'—C5'	83.7 (2)
C4—N9—C1'—C2'	69.0 (2)	O3'—C3'—C4'—O1'	-156.9 (1)
C4—N9—C1'—O1'	-172.7 (1)	O3'—C3'—C4'—H4'	-40.5 (2)
H3'—C3'—C4'—C5'	-36.7 (2)	O1'—C4'—C5'—H5B	-61.3 (3)
H3'—C3'—C4'—O1'	82.7 (2)	H4'—C4'—C5'—O5'	-59.8 (2)
H3'—C3'—C4'—H4'	-160.9 (3)	H4'—C4'—C5'—H5A'	61.5 (3)
C2'—C3'—O3'—HO3'	-46.2 (3)	C4'—C4'—C5'—H5B	179.1 (3)
C4'—C3'—O3'—HO3'	72.1 (3)	C3'—C4'—O1'—C1'	14.8 (2)
H3'—C3'—O3'—HO3'	-166.8 (3)	C5'—C4'—O1'—C1'	137.5 (1)
C3'—C4'—C5'—O5'	176.7 (1)	H4'—C4'—O1'—C1'	-101.9 (2)
C3'—C4'—C5'—H5A'	-62.0 (3)	C4'—C5'—O5'—HO5'	147.2 (3)
C3'—C4'—C5'—H5B	55.5 (3)	H5A'—C5'—O5'—HO5'	27.3 (4)
O1'—C4'—C5'—O5'	59.9 (2)	H5B'—C5'—O5'—HO5'	-92.4 (3)
O1'—C4'—C5'—H5A'	-178.8 (3)		

Table 5. Intermolecular distances (Å) and angles (°)

Hydrogen-bond interactions involving distances O...H and N...H < 2.2 Å

X—H...Y	X...Y	H...Y	X—H...Y
O5'—HO5'...N1(a)	2.735 (2)	1.779 (3)	166.4 (3)
O3'—HO3'...N3(b)	2.932 (2)	1.994 (3)	158.3 (3)
O2'—HO2'...O3'(c)	2.743 (2)	1.787 (3)	164.0 (3)
N10—H10A...N7(d)	3.113 (1)	2.123 (3)	165.0 (3)
N10—H10B...O5'(e)	2.873 (2)	1.863 (3)	169.0 (3)
C2—H2...O2'(c)	3.080 (2)	2.199 (4)	136.3 (3)

Other short distances (H...H < 2.5; O...H and N...H < 2.2; O...N < 3.2 Å)

H3'...H4'(g)	2.025 (4)	C8...H10A(h)	2.579 (3)
HO2'...HO3'(c)	2.317 (5)	O1'...H3'(i)	2.590 (3)
HO2'...H5A'(e)	2.389 (5)	C5'...H10B(j)	2.640 (3)
H10A...HO5'(e)	2.412 (4)	O2'...H5A'(e)	2.649 (4)
H2...HO5'(f)	2.446 (5)	C4'...H3'(i)	2.680 (3)
H2...HO2'(c)	2.466 (5)	C3'...HO2'(b)	2.740 (3)
C2...H13(f)	2.491 (3)	O3'...H4'(g)	2.767 (3)
N10...H8(e)	2.563 (4)	C4'...H1'(g)	2.773 (3)

Symmetry code: (a) $-1+x, 1+y, z$; (b) $1-x, 0.5+y, 1-z$; (c) $1-x, -0.5+y, 1-z$; (d) $2-x, -0.5+y, -z$; (e) $1-x, -0.5+y, -z$; (f) $1+x, -1+y, z$; (g) $1+x, y, z$; (h) $2-x, 0.5+y, z$; (i) $-1+x, y, z$; (j) $1-x, 0.5+y, -z$.

considered as a weak hydrogen bond. From neutron diffraction at 123 K we find the short distances C2...O2' and H2...O2' to be 3.080 (2) and 2.199 (4) Å (Table 5, Fig. 3) with angle C2—H2...O2' 136.3 (3)°.

Analysis of the molecular thermal vibrations

The nuclear anisotropic thermal parameters (Table 2) have been analysed in order to obtain a better understanding of the dynamical behavior of the adenosine molecule. The thermal vibrations which

can be studied most readily from such data are those involving the smallest restoring forces and the least inertia. Most importantly, they include the external or lattice vibrations of the molecule, torsional vibrations of almost rigid molecular segments about selected bonds and the internal or intramolecular vibrations of the lighter H atoms.

It can be seen in Fig. 1 that the thermal ellipsoids are consistently larger for the H atoms, presumably because of their internal vibrations. A rigid-bond test (Harel & Hirshfeld, 1975; Hirshfeld, 1976) showed that for every covalently bonded pair of heavier atoms, differences in mean-square amplitudes of vibration along the bond direction were too small to be experimentally significant, the largest difference being 0.0011 (7) Å² for C2'—C3'.* However, non-rigidity was strongly indicated for most covalent bonds involving H. The difference in mean-square amplitude along these bonds ranged in magnitude from $\Delta = 0.0038$ (17) Å² for C3'—H3' to $\Delta = 0.0076$ (18) Å² for C2—H2 and in significance level from 2.3 to 4.3 σ (Table 6b). Consequently, for the initial least-squares fitting of observed and calculated anisotropic thermal parameters assuming the rigid-body model of Schomaker & Trueblood (1968), all H atoms were omitted. The function which was minimized was $\sum w(U_{\text{obs}}^{ij} - U_{\text{cal}}^{ij})^2$ with $w = 1/\sigma^2(U_{\text{eq}})$. The fit gave $wR = 0.170$ and $S = 3.67$ and 93 degrees of freedom. When the rigid-bond test was extended to include intramolecular nonbonded pairs of heavier atoms (Rosenfield, Trueblood & Dunitz, 1978), some highly significant mean-square amplitude differences were obtained for pairs having one atom in the adenine-ring system and the other in the ribose. The largest difference [0.0040 (8) Å²] was for C6...C5'.

* The thermal-vibration analysis was carried out using a package of computer programs by Craven, He & Weber (1985) and a revised version of the program QNLS for quasnormal-mode analysis (He, 1989).

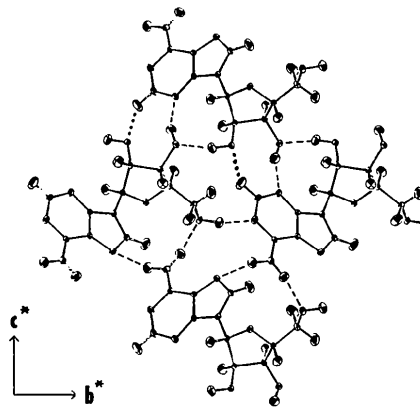


Fig. 3. H-bonding scheme viewed down the a axis. Dashed lines represent H bonds; dotted lines show C—H...O interactions.

This provided the justification to search for a model with nonrigid vibrations of the adenosine molecular framework.

The search was carried out using the method of quasinormal-mode analysis (He & Craven, 1985). This has been modified (He, 1989) so that a molecule in a crystal can be treated conveniently as a sequence of rigid segments with torsional librations permitted about the covalent bonds linking the segments and with all other internal vibrations frozen. In this respect, the procedure is similar to that of Dunitz & White (1973). However, our procedure differs in that quasinormal modes are constructed as linear combinations of the permitted bond torsions (Ω). It is assumed that mean-square amplitudes from internal and external molecular vibrations are additive. Observational equations can then be written which involve as variables the force constants for permitted bond torsional librations and also the rigid-body \mathbf{T} , \mathbf{L} and \mathbf{S} tensor components. An iterative least-squares refinement is necessary because these equations are nonlinear with respect to the elements of the force constant matrix, \mathbf{F} . As in the usual normal-mode analysis (Wilson, Decius & Cross, 1955) from which this procedure was derived, the frequencies associated with individual quasinormal modes can be calculated. It is also possible to make the bond-length and bond-angle corrections which arise from the correlated motions of the atoms both in the rigid-body librations and in the various quasinormal modes.

In the case of the adenosine molecular framework, trial and error gave rise to only one model which allowed a satisfactory refinement. Thus, when torsion about the C4'—C5' bond was permitted, the force constant for this libration became very large with a large e.s.d. The model finally adopted for adenosine was the simplest possible, consisting of the adenine and ribose moieties as rigid bodies undergoing libration about the glycosidic bond, N9—C1'. All atoms of the framework are involved in this internal vibration subject to the condition that there is no change in the linear or angular momentum of the molecule.* Least-squares refinement converged with $wR = 0.107$ and $S = 2.31$ as shown in Table 6(a). From the least-squares inverse matrix, we find the largest correlation coefficient to be 0.85 involving S_{11} and S_{22} . The largest correlation involving the force constant (0.43) is with T_{11} . At 123 K, the mean-square amplitude for the libration about the glycosidic bond is $\Omega = 14.4 \text{ deg}^2$.

Anisotropic thermal parameters U_{cal}^i for the H nuclei were then calculated, assuming all H nuclei to

* The motion is different in the model of Dunitz & White (1973) where one molecular segment is chosen to be fixed while the other librates.

Table 6. *Molecular thermal motion*

(a) Molecular-framework vibrations

Rigid-body calculations were carried out with respect to the Cartesian axes corresponding to the principal moments of inertia of the molecule, and with the origin at the molecular center of mass. Values (1) are from neutron diffraction at 123 K, (2) from X-ray diffraction at room temperature (Lai & Marsh, 1972).

	(1)	(2)
Goodness of fit	2.311	4.539
R.m.s. value for $ (U_{ij})_{\text{obs}} - (U_{ij})_{\text{calc}} $, excluding H atoms (\AA^2)	0.0010	0.0028
wR	0.107	0.124
Ω^* (deg^2)	14.4	36.1
Force constant ($\text{J mol}^{-1} \text{deg}^{-2}$)	73 (10)	70 (12)
Translational tensor \mathbf{T} ($\text{\AA}^2 \times 10^4$)	$\begin{pmatrix} 105 (5) & 4 (3) & -7 (2) \\ & 80 (3) & 7 (2) \\ & & 75 (2) \end{pmatrix}$	$\begin{pmatrix} 276 (15) & 9 (9) & -31 (7) \\ & 225 (9) & 12 (6) \\ & & 214 (8) \end{pmatrix}$
Principal values (\AA^2)	0.0107, 0.0085, 0.0068	0.0289, 0.0231, 0.0195
Librational tensor, \mathbf{L} (deg^2)	$\begin{pmatrix} 0.9 (1) & -0.3 (1) & 0.2 (1) \\ & 0.7 (1) & 0.5 (1) \\ & & 4.7 (6) \end{pmatrix}$	$\begin{pmatrix} 1.9 (3) & -0.8 (3) & 1.0 (4) \\ & 1.3 (5) & 1.3 (5) \\ & & 11.3 (16) \end{pmatrix}$
Principal values (deg^2)	1.1, 0.4, 4.7	2.5, 0.5, 11.6
Cross tensor, \mathbf{S} ($\text{deg} \text{\AA} \times 10^3$)	$\begin{pmatrix} -4 (9) & -9 (3) & -13 (3) \\ -23 (4) & -14 (9) & -13 (4) \\ -45 (6) & -28 (7) & 19 \end{pmatrix}$	$\begin{pmatrix} -17 (27) & -22 (7) & -30 (10) \\ -44 (11) & -17 (29) & -28 (13) \\ -106 (21) & -78 (22) & 34 \end{pmatrix}$

(b) Intramolecular H-atom vibrations

For each H nucleus, ΔU_k ($k = 1, 2, 3$) in units $\text{\AA}^2 \times 10^4$ are the principle values for the difference tensor ($\mathbf{U}_{\text{obs}} - \mathbf{U}_{\text{cal}}$) where \mathbf{U}_{obs} has components U_{obs}^i from Table 2 and \mathbf{U}_{cal} has components calculated for the H nuclei assuming that they are carried rigidly on the heavy-atom framework of adenosine. See (a) for the model used to describe the framework vibrations. Angles ($^\circ$) given as $\angle(\Delta U_1)(\text{bond})$ and $\angle(\Delta U_2)(\text{perp})$ are, respectively, the angle between the vibration direction of ΔU_1 and the covalent bond X—H and the angle between the vibration direction of ΔU_2 and the normal to the plane of C—N—H, N—C—H, the ribose C—O—H group or the C5'-methylene group. Values Δ are the differences in mean-square amplitudes $\langle u^2 \rangle_{\text{H}} - \langle u^2 \rangle_{\text{X}}$ along the bond direction, as determined from \mathbf{U}_{obs} . Values of ν (cm^{-1}) are estimated X—H bond-stretching frequencies obtained from Δ .

	ΔU_1	ΔU_2	ΔU_3	$\angle(\Delta U_1)(\text{bond})$	$\angle(\Delta U_2)(\text{perp})$	Δ	ν
H2	72	198	131	10	16	76 (18)	2202
H8	41	269	160	7	4	46 (16)	3640
H10A	54	186	120	10	13	51 (15)	3285
H10B	50	117	143	4	19	51 (14)	3285
HO2'	41	82	194	25	19	43 (13)	3893
HO3'	14	221	130	19	12	50 (19)	3347
HO5'	36	74	123	14	38	53 (13)	3158
H1'	42	92	152	34	—	57 (15)	2936
H2'	27	232	135	17	—	52 (15)	3219
H3'	24	147	186	10	—	38 (17)	4404
H4'	43	184	121	6	—	57 (17)	2936
H5A'	59	164	425	18	13	67 (16)	2499
H5B'	62	158	354	20	10	71 (21)	2357

* Torsional libration about the glycosidic bond N9—C1'.

be carried rigidly on the vibrating framework of the heavier atoms. Differences ($U_{\text{obs}}^i - U_{\text{cal}}^i$) were transformed to local atomic axial systems and were analysed assuming that they represented the intramolecular vibrations of the H nuclei. The principal values for the difference tensors are shown in Table 6(b). The minimum principal value (ΔU_1) for each H nucleus has a vibration direction which makes an angle less than 20° with the covalent-bond direction (C—H, N—H or O—H) except for H1' (34°) and HO2' (25°). Thus we associate ΔU_1 with the mean-square amplitude of X—H bond stretching. Values obtained in this way are in good agreement with the Δ values obtained in carrying out the rigid-

bond test. As can be seen from Table 6(b), principal ΔU_2 values have vibration directions which are approximately perpendicular to the planes of pertinent local groups (N—C—H, C—N—H, C—O—H or H—C—H). Directions for ΔU_3 are close to the planes of these groups.* Estimated errors $\sigma(\Delta U)$ range from 0.0012 to 0.0024 Å². A comparison of chemically related H atoms shows the largest variations to involve ΔU_2 and ΔU_3 for the three hydroxyl H atoms. This might be expected since the hydroxyl H atoms are hydrogen bonded and subject to intermolecular interactions which are neglected in our thermal-vibration model. The agreement is better for the values of ΔU_1 . The average ΔU_1 values for the methine (0.0034 Å²) and methylene H nuclei (0.0061 Å²) are similar to those obtained for 12 methine (0.0050 Å²) and 32 methylene H nuclei (0.0057 Å²) in the crystal structure of cholesteryl acetate (Weber, Craven, Sawzik & McMullan, 1991).

With the values of Δ obtained directly from the U_{obs}^i (Table 6b), we have calculated the frequencies ν for X—H bond stretching, using the expression $\Delta = (h/8\pi^2 m c \nu) \coth(hc\nu/2kT)$. The value for the reduced mass m of the system was assumed to be the mass of the H nucleus since X was considered to be rigidly connected to the rest of the molecule, thereby having a much larger effective mass than H. The resulting frequencies (Table 6b) lie in the range 2202 to 4404 cm⁻¹. All except the frequencies for HO2' (3893 cm⁻¹) and H3' (4404 cm⁻¹) lie within a broad absorption band which extends from 2000 to 3600 cm⁻¹ in the infrared spectrum of adenosine (Brown, 1972). The maximum absorption occurs near 3200 cm⁻¹ close to the frequencies which we obtain for H10A, H10B, H2' and HO5'.

The vibrational model for the adenosine framework as described above was also used to fit the anisotropic thermal parameters as determined by Lai & Marsh (1972) from their room-temperature X-ray diffraction data. The agreement between observed and calculated U^i values is almost as good, with $wR = 0.124$ and $S = 4.54$. Because of the higher temperature, atomic mean-square amplitudes of vibration are two to three times greater than at 123 K, and therefore the mean-square amplitudes obtained for the rigid-body librations are considerably greater. The mean-square amplitude of torsional libration about the glycosidic bond increases to $\Omega = 36.1$ deg². In Table 4, the corrections for external and internal thermal vibration derived from this model have been applied to the framework bond lengths obtained from both X-ray and neutron diffraction. The corrections for bond angles are small (< 0.1°) and have been neglected.

An outstanding feature of the thermal-motion analysis is the agreement obtained from neutron diffraction at 123 K and X-ray diffraction at 295 K for the force constant for torsional libration about the glycosidic bond. The values are 73 (10) and 70 (12) J mol⁻¹ deg⁻² respectively and the corresponding frequencies are 62 and 61 cm⁻¹. This result is gratifying, firstly, because the force constants are insignificantly different at the two temperatures, as might be expected for molecules in very similar environments, and secondly, because the two estimates come from quite different Bragg diffraction experiments. The determination of force constants from Bragg diffraction data has already been reported, but only in certain cases, such as the torsional librations of methyl groups (Trueblood & Dunitz, 1983). The analysis of anisotropic thermal parameters in terms of quasinormal modes requires further testing but the procedure seems promising for the study of a wider variety of crystal structures containing molecules which can be treated as segmented rigid bodies. In the crystal structure of adenosine where the molecular conformation is similar to that of the corresponding fragment in double-helical RNA, the experimental determination of the force constant for torsion about the glycosidic bond is of special interest. For example, such a result can be useful in constructing a force field for molecular-dynamics calculations involving RNA.

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* The distinction between ΔU_2 and ΔU_3 is not meaningful for the four methine H nuclei.

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Structural Studies of Thiophilic *N*-Chloroazasteroids

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Abstract

N-Chloroazasteroids form covalent S—N bonds with thiol groups and so are of interest as chemoselective irreversible binding agents for the active sites of steroid receptors and enzymes. The solid-state structures of *N*-chloro-3-methoxy-17-aza-*D*-homo-1,3,5(10)-estratrien-16-one (1), *N*-chloro-3-methoxy-17-aza-*D*-homo-1,3,5(10)-estratrien-17a-one (2) and *N*-chloro-3-methoxy-16-aza-1,3,5(10)-estratrien-17-one (3) were determined to obtain information about the spatial arrangement of the N—Cl groups. Crystal data: (1) $C_{19}H_{24}ClNO_2$, $M_r = 333.84$, orthorhombic, $P2_12_12_1$, $a = 8.0190$ (3), $b = 12.7175$ (5), $c = 16.5047$ (9) Å, $V = 1683.2$ (1) Å³, $Z = 4$, $D_x = 1.317$ g cm⁻³, $\lambda(Cu K\alpha) = 1.54178$ Å, $\mu = 20.9$ cm⁻¹, $F(000) = 712$, $T = 295$ K, $R = 0.045$ for 1786 observed reflections; (2) $C_{19}H_{24}ClNO_2$, $M_r = 333.84$, orthorhombic, $P2_12_12_1$, $a = 10.9853$ (7), $b = 11.8216$ (5), $c = 12.9851$ (9) Å, $V = 1686.3$ (2) Å³, $Z = 4$, $D_x = 1.315$ g cm⁻³, $\lambda(Cu K\alpha) = 1.54178$ Å, $\mu = 20.9$ cm⁻¹, $F(000) = 712$, $T = 295$ K, $R = 0.035$ for 1891 observed reflections; (3) $C_{18}H_{22}ClNO_2$, $M_r = 319.81$, monoclinic, $P2_1$, $a = 13.246$ (2), $b = 7.972$ (2), $c = 7.696$ (3) Å, $\beta = 90.24$ (2)°, $V = 812.7$ (4) Å³, $Z = 2$, $D_x = 1.307$ g cm⁻³, $\lambda(Cu K\alpha) = 1.54178$ Å, $\mu = 21.4$ cm⁻¹, $F(000) = 340$, $T = 295$ K, $R = 0.045$ for

1636 observed reflections. Conformational analysis and comparisons of molecules of (1), (2) and (3) are presented. If the phenolic rings of these molecules are superimposed, the orientation of their reactive *N*-chloro groups is similar in (1) and (2) and different in (3), due to the different five-membered *D* ring in (3) and to the different *B*-ring conformation of (3). The distances in this superposition between the Cl atom of steroid (3) and the Cl atoms of steroids (1) and (2) are 1.87 and 2.27 Å, respectively.

Introduction

Modified steroids containing alkylating or other reactive functional groups that are capable of bonding covalently to the active sites of their receptor proteins, or to those of enzymes that use them as substrates, are of biological and medicinal interest. They can be used as affinity labels, as enzyme inhibitors, and in the treatment of breast and prostatic carcinomas (Dence, 1980).

Jensen, Hurst, DeSombre & Jungblut (1967) demonstrated that thiol (sulfhydryl) groups in the estrogen receptor play a crucial role in the binding of estradiol and, presumably, other estrogens as well. Reactive estrogen analogues that are capable of selectively forming covalent bonds with thiol groups are therefore of special interest (Simons, Pons & Johnson, 1980; Chin & Warren, 1968).

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