

The Crystal and Molecular Structure of 6-Methylmercaptapurine Riboside Monohydrate

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The crystal structure of 6-methylmercaptapurine riboside monohydrate has been determined by X-ray diffraction methods using 3988 observed reflections collected on a counter diffractometer. The crystals are monoclinic, space group $P2_1$, with cell dimensions $a = 7.912(2)$ Å; $b = 18.120(1)$ Å; $c = 4.848(1)$ Å; $\beta = 105.70(2)^\circ$. The structure was refined to a conventional R -factor of 0.040, the standard deviations in bond lengths and angles involving non-hydrogen atoms are 0.002 Å and 0.1°, respectively.

The molecular and crystal structure is discussed and the results compared to those obtained from magnetic resonance experiments.

Several sulfur containing nucleic acid analogues are effective metabolic inhibitors used in cancer chemotherapy. The adenosine analogue 6-methylmercaptapurine riboside (9- β -D-ribofuranosyl-6-methylthiopurine) is a strong inhibitor of several mouse tumors and is also effective against some human leukemias as well as other tumors which have become resistant to 6-mercaptapurine therapy.¹ It has been shown that the riboside is mistaken for adenosine at the enzymatic level and is incorporated into the nucleic acids.² The action of this drug, either administered alone or in combination with other agents, is to influence the purine metabolic pathways.

Previous investigators have found similarities between enzymatic reactions and reactions in the solid state due to ionizing radiation.³ In an attempt to study reactions induced by ionizing radiation in chemotherapeutic agents, magnetic resonance experiments (ESR and ENDOR) are being carried out on irradiated mercaptapurine crystals.^{4,5} Although the relative orientations of the purine rings in the unit cell of

the crystals can be predicted from the ESR/ENDOR data, a detailed stereochemical comparison of molecular directions cannot be made without a complete structural analysis of the crystals.

Using one of the crystals grown for the magnetic resonance experiments, an analysis of the crystal and molecular structure of 6-methylmercaptapurine riboside (6MeMPR) was undertaken.

EXPERIMENTAL

Powdered samples of 6MeMPR obtained from Nutritional Biochemical Company were recrystallized from water at a constant temperature of 30 °C. A crystal fragment of approximate dimensions 0.3 × 0.5 × 0.5 mm was cut from a larger crystal and used for the X-ray experiments. Data were collected on a SYNTEX PI four-circle diffractometer (graphite crystal monochromated $\text{MoK}\alpha$ radiation, $\lambda = 0.71069$).

The crystals are monoclinic; systematic absences are k odd for $(0k0)$ indicating $P2_1$ or $P2_1/m$ as possible space groups of which the latter could be ruled out for this optically active compound. Cell parameters were determined by a least-squares fit to the diffractometer settings for 15 general reflections.

Intensity data were recorded using the $\theta/2\theta$ scanning mode using a scan speed of 2° min^{-1} for reflections with $\sin \theta/\lambda$ below 0.65 \AA^{-1} and scan speeds varying from 1 to 2° min^{-1} depending on the intensity for reflections with $\sin \theta/\lambda$ between 0.65 and 0.95 \AA^{-1} . Reflections in the latter interval were measured only if a quick scan gave an intensity larger than a preset value. The scan range was from 1° below $2\theta(\alpha_1)$ to 1° above $2\theta(\alpha_2)$ and background counts were taken for half the scan time at each of the scan limits.

From the 4348 unique reflections recorded, 3988 with $I > 2.50\sigma(I)$ were retained for the structure analysis. The standard deviations for the intensities were calculated by $\sigma(I) = [C_T + (0.02C_N)^2]^{1/2}$ where C_T is the total number of counts and C_N is the scan count minus background count. The usual corrections were made for Lorentz and polarization effects, but no correction was applied for absorption or extinction.

Scattering factors used were those of Doyle and Turner for S, O, N, and C,⁶ and of Stewart, Davidson and Simpson for H.⁷ Description of the computer programs used are given in Refs. 8 and 9. The quantity minimized in the least-squares calculations was $\sum w\Delta F^2$ where w is the inverse of the variance of the observed structure factors.

CRYSTAL DATA

6-Methylmercaptapurine riboside monohydrate, $C_{11}H_{14}O_4N_4S \cdot H_2O$, monoclinic, $a = 7.912(2)$ Å; $b = 18.120(1)$ Å; $c = 4.848(1)$ Å; $\beta = 105.70(2)^\circ$; ($t = 18 \pm 1$ °C); $V = 669.1$ Å³; $M = 316.34$; $Z = 2$; $F(000) = 332$; $D_{\text{calc}} = 1.570$ g cm⁻³. Absent reflections: $(0k0)$ for k odd. Space group $P2_1$ (No. 4).

STRUCTURE DETERMINATION

The structure was solved by application of noncentrosymmetric direct methods using the

program assembly MULTAN.⁹ Phases for 175 reflections with $|E| > 1.5$ were determined with the best starting set of phases yielding an absolute figure of merit of 1.31. An E -map based on these phases revealed 26 peaks, of which 18 could be related to a molecule of the anticipated geometry. The remaining three non-hydrogen atoms were localized by the use of successive Fourier refinements; the positions of the hydrogen atoms were calculated from stereochemical considerations after a preliminary refinement. Using the data with $\sin \theta/\lambda < 0.65$ the positional parameters, anisotropic parameters for non-hydrogen atoms and isotropic parameters for hydrogen atoms were refined by least-squares methods to a conventional R -factor of 0.04. The goodness of fit, $S = [\sum w\Delta F^2 / (m - n)]^{1/2}$, was 3.53, however, and a weight analysis indicated systematic errors present for the low-angle data. The least-squares procedure was then repeated with all the data; the weights for the 1231 reflections with $\sin \theta/\lambda < 0.6$ were multiplied with a factor varying from 0 to 1 for reflections with $\sin \theta/\lambda$ varying from 0 to 0.60. The remaining 2757 reflections were given normal weights. The refinement converged to a conventional R -factor of 0.040, $R_w = 0.043$ and $S = 1.77$.

Table 1. Atomic parameters and their estimated standard deviations. Atoms marked with asterisks belong to the ribose moiety. The anisotropic temperature factor has the form $\exp -2\pi^2[(U11a^*h^2 + U22b^*k^2 + U33c^*l^2 + 2U12a^*b^*hk + 2U13a^*c^*hl + 2U23b^*c^*kl)]$.

ATOM	X	Y	Z	U11	U22	U33	U12	U13	U23
S	.3299(1)	.5362(0)	.7324(1)	.0285(2)	.0257(1)	.0352(2)	-.0006(1)	.0003(1)	-.0103(1)
N1	.5317(2)	.4532(1)	.4850(3)	.0218(4)	.0276(4)	.0401(5)	-.0029(3)	.0091(4)	-.0095(3)
N3	.4280(2)	.3595(1)	.1253(4)	.0264(7)	.0283(14)	.0445(9)	.0004(8)	.0129(6)	-.0100(9)
N7	.0477(2)	.4339(1)	.2854(3)	.0226(7)	.0215(4)	.0383(6)	-.0014(4)	.0117(5)	-.0067(4)
N9	.1193(2)	.3481(1)	-.0074(3)	.0219(6)	.0180(4)	.0316(6)	.0014(4)	.0074(5)	.0047(4)
C2	.5519(2)	.3997(1)	.3037(3)	.0219(5)	.0310(5)	.0471(6)	.0010(5)	.0120(4)	.0129(5)
C4	.2676(2)	.3773(1)	.1435(4)	.0218(5)	.0175(5)	.0295(7)	.0010(4)	.0081(4)	.0034(5)
C5	.2274(2)	.4305(1)	.3243(4)	.0215(5)	.0168(5)	.0297(7)	-.0007(4)	.0092(5)	-.0022(5)
C6	.3687(2)	.4695(1)	.5000(3)	.0233(5)	.0182(4)	.0272(6)	.0016(4)	.0079(4)	.0027(4)
C8	-.0163(2)	.3845(1)	.0080(3)	.0207(4)	.0225(4)	.0304(5)	.0006(3)	.0009(4)	.0057(4)
C10	.5448(2)	.5736(1)	.0812(4)	.0308(5)	.0468(7)	.0505(9)	-.0106(5)	.0081(6)	.0239(6)
O4	.1324(2)	.3352(1)	.4237(3)	.0313(5)	.0279(4)	.0443(6)	.0046(5)	.0155(4)	.0097(4)
O1*	-.0946(2)	.2890(1)	-.3788(3)	.0280(5)	.0191(4)	.0311(5)	.0017(4)	.0202(4)	.0041(4)
O2*	.1985(2)	.1810(1)	-.3559(3)	.0377(5)	.0234(4)	.0405(5)	.0007(4)	.0201(4)	.0059(4)
O3*	-.0292(2)	.1056(1)	-.1067(4)	.0469(5)	.0182(5)	.0362(7)	.0003(4)	.0095(5)	.0064(5)
O5*	-.3367(2)	.2578(1)	-.0392(3)	.0350(5)	.0761(4)	.0408(5)	.0170(4)	.0102(4)	.0243(4)
C1*	.0848(2)	.2935(1)	-.2406(3)	.0260(5)	.0172(4)	.0247(5)	.0012(4)	.0054(4)	.0010(4)
C2*	.1466(2)	.2163(1)	-.1325(3)	.0246(5)	.0184(4)	.0246(5)	.0023(4)	.0048(4)	.0006(4)
C3*	-.0224(2)	.1032(1)	-.0674(3)	.0275(5)	.0193(4)	.0228(5)	.0010(4)	.0075(4)	.0014(4)
C4*	-.1611(2)	.2163(1)	-.3390(4)	.0256(6)	.0189(7)	.0226(7)	.0016(5)	.0005(5)	.0012(6)
C5*	-.3434(3)	.2223(2)	-.3017(6)	.0259(9)	.0350(11)	.0343(12)	-.0015(6)	.0066(8)	-.0049(10)

ATOM	X	Y	Z	B	ATOM	X	Y	Z	B
H2	.663(5)	.387(2)	.313(8)	3,3	H03*	.015(5)	.088(2)	.030(9)	3,6
H8	.126(5)	.371(2)	.018(8)	3,3	H05*	-.410(5)	.276(2)	-.023(8)	3,6
H10†	.542(5)	.607(2)	1.043(8)	4,9	H1*	.150(5)	.311(2)	-.358(7)	2,5
H10‡	.585(5)	.395(2)	.717(8)	4,9	H2*	.227(4)	.212(1)	.033(6)	2,5
H10§	.618(4)	.546(1)	.950(7)	4,9	H3*	-.043(4)	.201(1)	.008(6)	2,5
H11	.217(4)	.014(2)	.431(6)	3,7	H4*	-.174(4)	.191(1)	-.596(6)	2,5
H12	.075(4)	.011(2)	.521(6)	3,7	H5*1	-.423(6)	.245(3)	-.462(10)	2,5
H02*	.150(6)	.145(3)	-.377(11)	3,6	H5*2	-.400(6)	.176(3)	-.310(10)	2,5

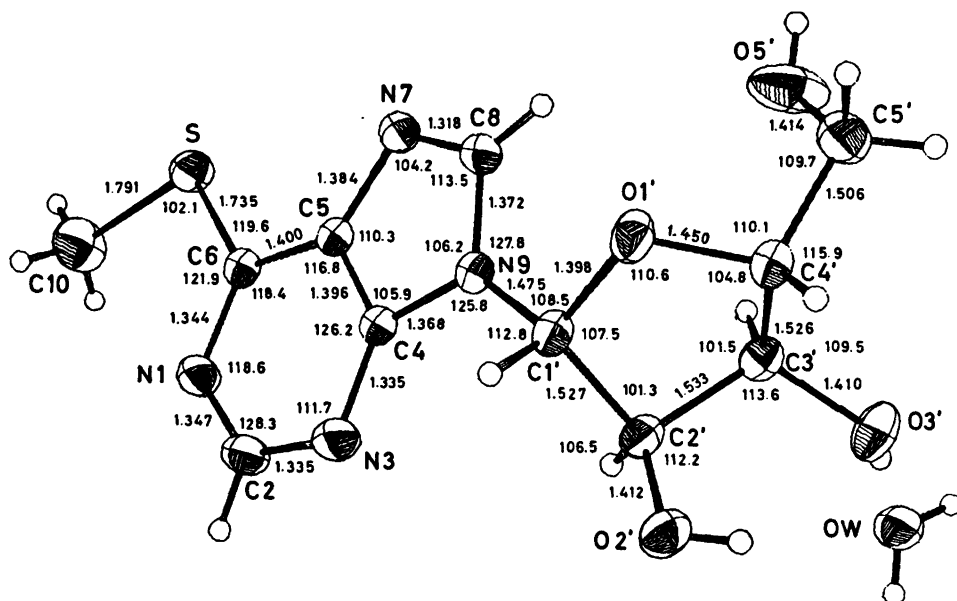


Fig. 1. Perspective view of the molecule as seen along c^* showing bond lengths (Å) and angles ($^\circ$). Non-hydrogen atoms are represented by thermal ellipsoids defined by the principal axes of thermal vibration and scaled to include 50% probability.

Table 1 lists the final parameters and their estimated standard deviations. Tables of observed and calculated structure factors are available from the authors.

DISCUSSION

Fig. 1 shows the conformation, thermal ellipsoids, and bond lengths and angles involving non-hydrogen atoms. Estimated standard deviations are 0.002 Å and 0.1 $^\circ$, respectively. C-H bond lengths were found in the range 0.78–1.01 Å, mean value 0.92 Å; for O-H the range is 0.72–0.86 Å, mean value 0.77 Å.

The 6-methylmercaptapurine moiety. The geometry of this part of the molecule is virtually

identical to that found for 6-methylmercaptapurine trihydrate.¹⁰ The purine part is planar with no atom situated more than 0.012 Å out of the least-squares plane through the 9 atoms. The sulfur atom is also close to the plane (0.015 Å) whereas the distance from the plane to C10 is 0.143 Å; the torsional angle N1–C6–S–C10 is 5.2 $^\circ$. The normal to the purine plane forms an angle of 46.5 $^\circ$ with the two-fold screw axis (b).

The glycosidic bond N9–C1' is of normal length, 1.475 Å; the C1' atom is displaced 0.112 Å from the purine plane. The dihedral angle C4–N9–C1'–O1' is –167.9 $^\circ$.

The ribose ring has the C3' *endo* conformation with the C3' atom situated 0.58 Å from

Table 2. Hydrogen bond distances (Å) and angles ($^\circ$).

D	H	A	D–A	H–A	>D–H...A
OW	HW1	N1 ($1-x, -\frac{1}{2}+y, 1-z$)	2.971	2.21	172
OW	HW2	N7 ($-x, -\frac{1}{2}+y, 1-z$)	2.912	2.06	168
O2'	HO2'	OW ($x, y, -1+z$)	2.844	2.20	145
O3'	HO3'	OW (x, y, z)	2.845	2.08	177
O5'	HO5'	N3 ($1-x, y, z$)	2.881	2.21	157

the plane through C1', C2', C4', and O1'. The deviations of the atoms defining the plane are as follows: C1' (-0.041 Å), C2' (0.024 Å), C4' (-0.026 Å), O1' (0.032 Å). The configuration of the sugar ring is described by the following torsional angles about the ring bonds: 7.1° for (O1'-C1'), -28.2° for (C1'-C2'), 36.9° for (C2'-C3'), -34.0° for (C3'-C4'), and 17.3° for (C4'-O1'). The corresponding values for the phase angle of pseudorotation P and the amplitude of pucker τ_m ¹¹ are 7.9 and 37.3°, respectively. The conformational angles of the side chain are (O1'-C4'-C5'-O5') = -67.7° and (C3'-C4'-C5'-O5') = 51.1°. The configuration of the ribose moiety is thus the same as the one found for cytidine.¹² Bond lengths and angles are found to be quite normal; the C1'-O1' bond is shorter than O1'-C4' by 0.05 Å, the three ring C-C distances are equal to 1.529 Å within twice their standard deviation and the three hydroxy C-O bonds 1.412 Å. The C-C-C, C-C-O and C-O-C angles are 101.4, 106.2, and 110.6°, respectively, as compared to 101.7, 105.8, and 109.3° given as average values for five-membered sugar rings.¹³

Molecular packing. All hydroxyl hydrogen atoms are engaged in hydrogen bonding; dimensions are given in Table 2. 6-Methylmercaptapurine riboside molecules are linked through hydrogen bonds from O5' in one molecule to N3 in another; chains along the *a*-axis are thus formed. The other hydrogen bonds all involve water molecules which are bonded approximately tetrahedrally to nitrogen and oxygen atoms of four different 6MeMPR molecules. The hydrogen bonds involving N1, N3 and N7 are situated approximately in the purine plane. No particularly short contacts are found to the sulfur atom, and apart from the hydrogen bonds mentioned the intermolecular contacts are of the van der Waals type.

It is of interest to compare crystallographic directions with the corresponding directions obtained from magnetic resonance (ESR/ENDOR) experiments. Pugh and Alexander report the angle between the normal to the purine plane and the *b*-axis from several independent measurements of magnetic interactions to be in the range 44-48°,⁴ close to the value 46.5° given above. The same authors also show that the direction cosines for either the C2-H or the C8-H bond in the *a*bc* coordinate

system are (0.97, 0.23, 0.05). In the present work the corresponding numbers for C8-H are found to be (0.96, 0.27, 0.09), given in the same reference frame. The C8-H direction is thus within the standard deviation (3°) the same as that determined from the ENDOR experiments, and the combined data demonstrate that the radical formation on irradiation is due to hydrogen addition to the C2 position.

Sagstuen and Alexander report that the C6-S bond direction in a radical formed by hydrogen abstraction from the methyl group is less than 0.8° from that in the undamaged molecule.⁵ Furthermore, one of the methyl carbon-proton directions is approximately 12° from the direction of the in-plane methyl carbon-proton bond in the undamaged molecule. This angle corresponds to a rotation about the S-C10 bond to increase the conjugation of the unpaired electron with the π -molecular orbital of the purine moiety.

The above results indicate that even if large amounts of energy are deposited on crystals of this highly conjugated purine molecule, it is not reasonable to expect any large changes in molecular geometry to occur, even in areas in close proximity to the ultimate center of radiation damage. This is in agreement with other magnetic resonance experiments on irradiated purine and pyrimidine compounds (Ref. 14 and references cited therein).

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