Table 6. (*a*) *Some distances and angles involved in hydrogen bonds and* (*b*) *the shortest potassium–oxygen distances*

(*a*)

Nitrogen	Oxygen	Hydrogen	N–O (Å)	(σ)	H–O (Å)	(σ)	∠N–H–O(°)	(σ)
N(1)	O(4) $(x+\frac{1}{2};\ \frac{1}{2}-y;\ z-\frac{1}{2})$	H(1)	3·038	(14)	2·28	(28)	148	(25)
N(1)	O(3) $(x;\ -y;\ z-\frac{1}{2})$	H(2)	3·000	(14)	2·06	(25)	138	(20)
N(2)	O(3) $(x;\ -y;\ z-\frac{1}{2})$	H(3)	2·962	(12)	2·08	(21)	142	(16)
N(3)	O(2) $(x;\ 1-y;\ z-\frac{1}{2})$	H(5)	3·031	(13)	2·13	(22)	156	(19)
N(4)	O(2) $(x;\ 1-y;\ z-\frac{1}{2})$	H(6)	2·909	(15)	2·12	(26)	148	(25)

(*b*)

	Oxygen	K–O (Å)	(σ)
K ———	O(1) $(x-\frac{1}{2};\ \frac{1}{2}-y;\ z-\frac{1}{2})$	2·684	(9)
	O(1) $(x-\frac{1}{2};\ y-\frac{1}{2};\ z)$	2·901	(8)
	O(2) $(x;\ y;\ z-1)$	2·764	(8)
	O(3) $(x;\ -y;\ z-\frac{1}{2})$	2·806	(12)
	O(4) $(x;\ y;\ z)$	2·700	(8)
	O(4) $(x;\ -y;\ z-\frac{1}{2})$	2·707	(8)

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References

Carlström, D. & Ringertz, H. (1965). *Acta Cryst.* **18**, 307.

Coppens, P., Leiserowitz, L. & Rabinovich, D. (1965). *Acta Cryst.* **18**, 1035.

Cruickshank, D. W. J. (1965). In *Computing Methods in Crystallography*. Oxford: Pergamon Press.

Glusker, J. P., Helm, D. van der, Love, W. E., Dornberg, M. L., Minkin, J. A., Johnson, C. K. & Patterson, A. L. (1965). *Acta Cryst.* **19**, 561.

Helm, D. van der, Glusker, J. P., Johnson, C. K., Minkin, J. A., Burow, N. E. & Patterson, A. L. (1968). *Acta Cryst.* B**24**, 578.

International Tables for X-ray Crystallography (1962). Vol. III. Birmingham: Kynoch Press.

Larsson, G. & Nahringbauer, I. (1968). *Acta Cryst.* B**24**, 666.

Okaya, Y. (1965). *Acta Cryst.* **19**, 879.

Ringertz, H. (1966). *Acta Cryst.* **20**, 932.

Acta Cryst. (1968). B**24**, 1692

A Reinvestigation of 8-Azaguanine Monohydrate

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The crystal structure of 8-azaguanine monohydrate, $C_4H_6N_6O_2$ has been refined by full-matrix least squares to an *R* index of 0·053. Standard deviations in the bond lengths involving only nonhydrogen atoms are in the range 0·0014–0·0018 Å and for C–H and N–H bonds about 0·02 Å. The corresponding standard deviations in angles are 0·1 and 1·0° respectively. The main difference from the previous investigation involves the water molecule which is found to be disordered. This gives rise to a hydrogen bonding scheme in which N(8) also participates. The ability of N(8) to form a hydrogen bond may be responsible for the anticarcinogenic action of 8-azaguanine.

Introduction

An X-ray crystallographic investigation of 8-azaguanine monohydrate was undertaken as part of a program in this laboratory to establish accurate molecular dimensions of purine and pyrimidine derivatives. The structure of this compound has already been reported (Macintyre, Singh & Werkema, 1965). The structure did not refine, however, to an *R* value less than 0·12 for the full set of data, presumably because the crystal used in that work was very small, 0·34 mm × 0·04 mm × 0·04 mm. In the present work a considerably larger crystal with dimensions 0·35 mm × 0·20 mm × 0·15 mm was used so that precision of the data, at least as far as counting statistics is concerned, is much improved. Furthermore, we have observed approximately twice the number of reflections used in the previous refine-

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ment. In the present work, precision of the parameters is improved by a factor of about 10, and certain structural details not previously clear are now evident.

Experimental

Crystal data

8-Azaguanine monohydrate, $C_4H_4N_6O.H_2O$,
M.W. 170·14.
Space group $P2_1/c$.
$Z=4$.
$D_m=1{\cdot}687\pm0{\cdot}008$ g.cm^{-3}; $D_x=1{\cdot}691$ g.cm^{-3}.
Unit-cell volume: 668·58 Å^3.
Cell dimensions:

$$a= 3{\cdot}5629(5)\ \text{Å}$$
$$b=11{\cdot}4404(9)\ \text{Å}$$
$$c=16{\cdot}4685(16)\ \text{Å}$$
$$\beta=95{\cdot}13(1)^\circ .$$

A sample of 8-azaguanine was obtained from Sigma Chemical Co., St. Louis, Mo. The compound dissolved readily in hot water, and crystallized on cooling as extremely long needles with cross section approximately 0·05 mm × 0·03 mm. However, thicker needles with a roughly hexagonal cross section were obtained by slow evaporation from a water–ethanol mixture kept in a narrow test tube. The cell parameters were determined from the 2θ settings for 16 reflections on the diffractometer (Mo $K\alpha$ radiation, $\lambda=0{\cdot}71069$ Å) by a least-squares treatment. The mean deviation from the computed 2θ values, $1/16\ \Sigma\ (2\theta_{\text{obs}}-2\theta_c)$ was 0·003°.

The crystal density was measured by flotation in a mixture of carbon tetrachloride and 1,1,2,2-tetrabromoethane.

The crystal selected for collecting data was mounted along **a***, which is approximately parallel to the needle axis. Integrated intensities were measured for 2952 unique reflections within a sphere of $\sin\theta/\lambda=0{\cdot}8$ using Mo $K\alpha$ radiation. Diffractometer data were collected as described elsewhere (Sletten, Sletten & Jensen, 1968).

Because of the small linear absorption coefficient of the crystal, 1·32 cm^{-1}, and the regular cross section of the needle, no absorption correction was made. The data were corrected for Lorentz–polarization effects, and, in addition, a correction for coincidence loss was applied (Sletten *et al.*, 1968). Of the 2952 reflections

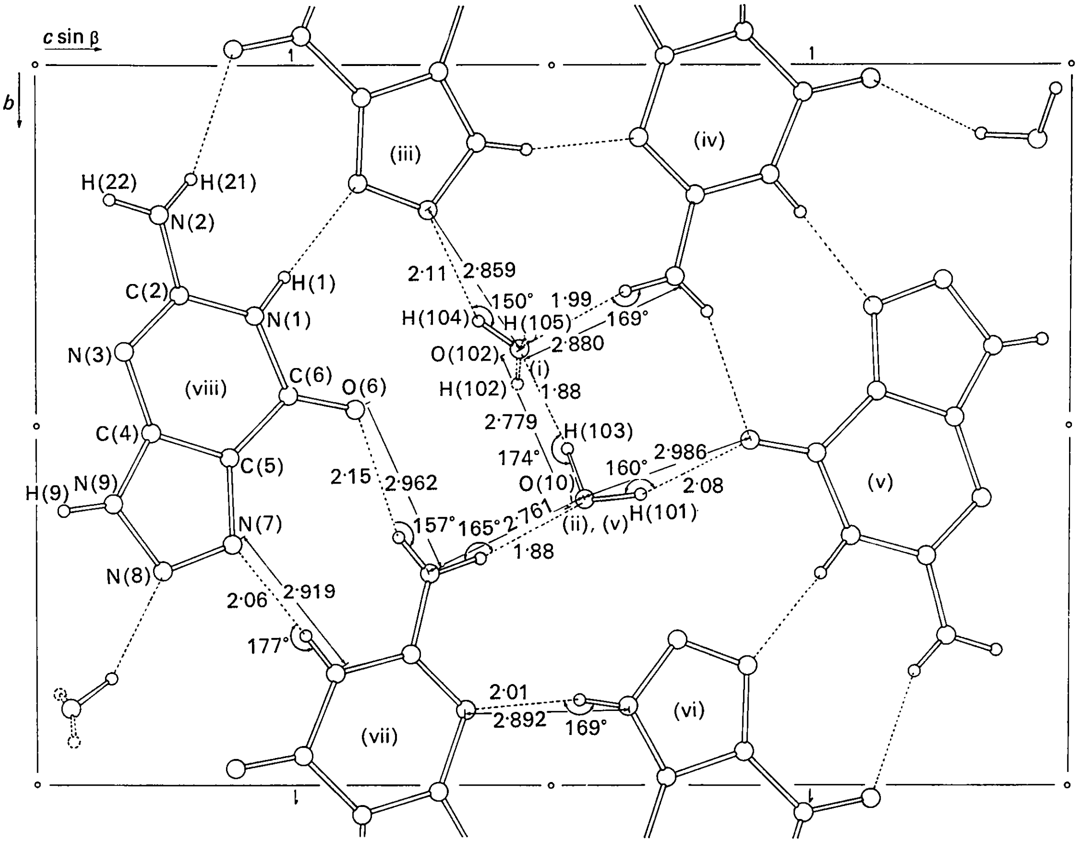

Fig. 1. The arrangement of the molecules in the unit cell viewed along the *a* axis. The figure in parentheses, assigned to each molecule, indicates the symmetry operation. (i) x,y,z; (ii) $2-x,\ 1-y,\ 1-z$; (iii) $2-x,\ y-\frac{1}{2},\ \frac{1}{2}-z$; (iv) $x,\ \frac{1}{2}-y,\ z+\frac{1}{2}$; (v) $1-x,\ 1-y,\ 1-z$; (vi) $x,\ \frac{2}{3}-y,\ z+\frac{1}{2}$; (vii) $2-x,\ y+\frac{1}{2},\ \frac{1}{2}-z$; (viii) $1+x,\ y,\ z$.

for which data were collected, 946 were less than $2\sigma_c$, where $\sigma_c=(N_{B1}+N_{\rm Pk}+N_{B2})^{1/2}$. These were given the value $2\sigma_c$ and were included in the refinement only if F_c was greater than the F_o corresponding to the cutoff limit. The error assigned to the net intensity of each reflection is taken as:

$$\sigma_I=[\sigma_c^2+(0\cdot01\sigma_c^2)^2]^{1/2},$$

and the standard deviation in the structure factors as

$$\sigma_F=[F^2+\sigma_I(\mathrm{Lp})^{-1}]^{1/2}-F.$$

Refinement of the structure

In the already published work (Macintyre *et al.*, 1965) the structure factor calculations were based only on the nonhydrogen atoms which were refined anisotropically in a block-diagonal least-squares procedure. The coordinates of the hydrogen atoms were determined from a difference synthesis.

By comparing our observed structure factors with those given by Macintyre, a scale factor was found by using the average ratio of 15 fairly strong reflections from the two sets of data. A structure factor calculation in which the hydrogen atoms were included gave an $R(=\Sigma\,||F_o|-|F_c||/\Sigma\,|F_o|)$ of 0·24 (B values of 3 Å^2 were assigned to the hydrogen atoms). In the first full-matrix least-squares cycle refining the nonhydrogen atoms, R decreased to 0·091. The function minimized was $\Sigma\,w(F_o-F_c)^2$ with weight $w=1/\sigma_F^2$. The maximum shift in coordinates was less than 0·04 Å, but the scale factor decreased by 11%, and the changes in thermal parameters were quite large. In the next cycle where hydrogen atoms were also refined R decreased to 0·061. At this stage all the atoms had reasonable thermal parameters, except the water oxygen for which B_{11} was 13·8 Å^2.

A subsequent three-dimensional difference map, based only on contributions from the nonhydrogen atoms, clearly revealed the hydrogen atoms attached to the guanine part, the electron densities of the peaks ranging from 0·66 e.Å^{-3} to 0·83 e.Å^{-3}. The hydrogen atoms on the water molecule, however, could not be localized unambiguously, whereas in the earlier investigation two peaks of electron densities 1·0 and 0·9 e.Å^{-3} appeared in the difference map at proper distances from the oxygen atom. Instead, several smaller peaks of the order 0·3 to 0·4 e.Å^{-3} were observed around the oxygen atom, indicating disordered hydrogen atoms.

The hydrogen bonding scheme previously proposed (Macintyre *et al.*, 1965), involving a hydrogen bond between two water molecules related by a center of symmetry, implies disorder in the crystal or pseudosymmetry. The short oxygen–oxygen distance of 2·78 Å does, however, indicate the presence of a hydrogen bond. If the acentric space group $P2_1$ is taken instead of $P2_1/c$, such a hydrogen bond is compatible with an ordered structure. However, the Weissenberg and precession photographs gave no indications of the absence of the c-glide. It seems most reasonable, therefore, to assume a disordered structure, where the hydrogen bonds involving the water molecule may differ from one unit cell to the other. A model with disordered hydrogen atoms would also allow hydrogen bonds to form from the water molecule to the two potential hydrogen acceptors O(6) and N(8) (Fig. 1). The position

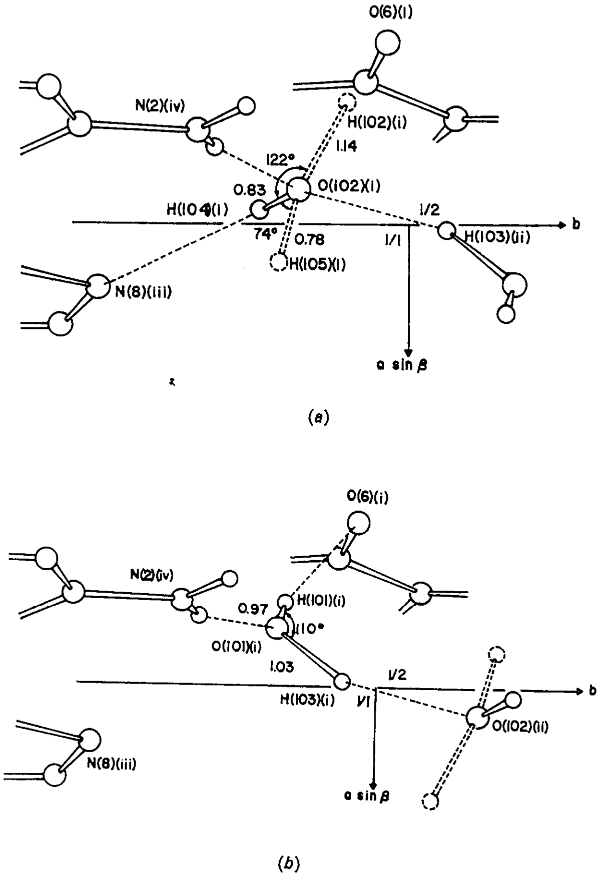

Fig. 2. (*a*) and (*b*). Two possible arrangements of hydrogen bonds formed by the water molecule.

Table 1. *Results of the least-squares refinement of the fractional hydrogen atoms*

	H(101)	H(102)	H(103)	H(104)	H(105)
B (Å^2)	9·1	10·5	4·4	5·3	0·2
Peak height (e.Å^{-3})	0·3	0·2	0·3	0·3	0·5
Distance to O(101) (Å)	0·97	0·87	1·03	1·01	1·09
Distance to O(102) (Å)	1·08	1·14	0·91	0·83	0·78

of the water oxygen atom will depend on which hydrogen bonds actually are formed. In Fig. 2(*a*), (*b*) are two possible arrangements of hydrogen bonds with the oxygen atom displaced in the x direction. Indeed, such a displacement is consistent with the large B_{11} value of the water oxygen.

A better model for the least-squares treatment would be one in which the oxygen atom is assigned to two sites, each with an occupancy of 0·5 and separated by 0·7 Å. The two half oxygen atoms were refined first isotropically, then anisotropically to $R=0{\cdot}058$. A difference map at this stage showed six peaks of electron density from 0·2 to 0·4 e.Å^{-3} at bonding distances from the oxygen sites. Hydrogen atoms, each with a weight of $\frac{1}{3}$, were placed in the six positions and refined isotropically. In the first least-squares cycle, R decreased to 0·055. For one of the hydrogen atoms, however, B had increased to 18 Å^2, and it was removed from the model. The remaining five hydrogen atoms with a weight of 0·4 were refined in two subsequent cycles together with all the other atoms and R decreased to 0·053. The magnitude of the shifts after the last refinement cycle was less than 1/10 of the standard deviation for the ring atoms and less than half the standard deviation for the rest of the atoms. At this stage the two half oxygen atoms oscillated in successive cycles about points separated by 0·34 Å with B_{11} values of 10·2 and 9·6 Å^2 respectively. The results of the refinement of the five fractional hydrogen atoms are given in Table 1. Only the three hydrogen atoms, H(101), H(103) and H(104), are pointing toward potential hydrogen acceptors [Fig. 2(*a*), (*b*)]. From the magnitudes of the O–H distances and the H–O–H angles, it cannot be decided unambiguously to which half oxygen the hydrogen atoms are attached.

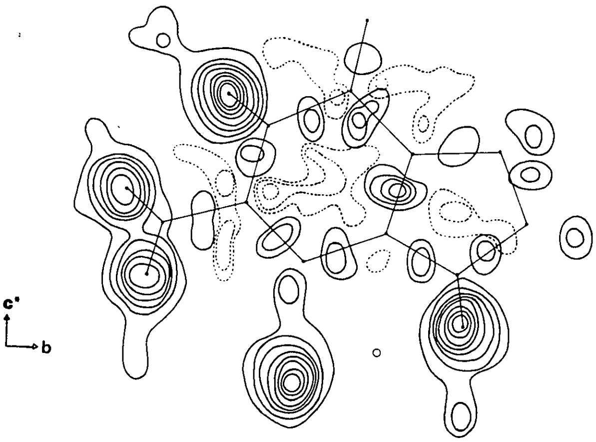

Fig. 3. Three-dimensional electron density difference map projected along [100]. Contours are at intervals of 0·1 e.Å^{-3} beginning at 0·1 e.Å^{-3}. Negative regions are shown by dashed contours.

In Fig. 3 a final difference map shows the hydrogen atoms attached to the 8-azaguanine molecule. It is interesting to notice the elongation of electron density from the hydrogen peaks toward the hydrogen acceptor. The pile-up of electron density in the middle of all the bonds has been observed in several earlier structure investigations and has been discussed elsewhere (Sletten *et al.*, 1968).

Molecular dimensions

The bond distances and angles calculated from the parameters in Table 2 are shown in Fig. 4. The esti-

Table 2. *Final coordinates and thermal parameters with the corresponding standard deviations in parentheses. Thermal parameters defined by the expression*:

$$T_i=\exp\left[-\tfrac{1}{4}(B_{11}h^2a^{*2}+B_{22}k^2b^{*2}+B_{33}l^2c^{*2}+2B_{12}hka^*b^*+2B_{13}hla^*c^*+2B_{23}klb^*c^*)\right]$$

	x	y	z	B_{11} or B	B_{22}	B_{33}	B_{12}	B_{13}	B_{23}
C(2)	0·71468 (33)	0·31877 (10)	0·13671 (7)	2·44 (5)	2·22 (4)	1·91 (4)	−0·01 (3)	0·67 (3)	0·07 (3)
C(4)	0·79541 (30)	0·50817 (10)	0·11140 (6)	1·94 (4)	2·09 (4)	1·54 (3)	0·06 (3)	0·38 (3)	0·17 (3)
C(5)	0·68053 (33)	0·54490 (10)	0·18526 (6)	2·34 (4)	2·14 (4)	1·67 (4)	0·12 (4)	0·52 (3)	−0·02 (3)
C(6)	0·57041 (32)	0·46023 (11)	0·24246 (7)	2·24 (5)	2·43 (5)	1·78 (4)	0·15 (4)	0·49 (3)	0·15 (3)
N(1)	0·59966 (29)	0·34814 (9)	0·21181 (6)	2·89 (4)	2·13 (4)	1·73 (3)	−0·06 (3)	0·90 (3)	0·32 (3)
N(2)	0·71997 (38)	0·20644 (10)	0·11835 (8)	5·28 (7)	2·06 (4)	2·81 (5)	−0·49 (4)	1·87 (4)	−0·01 (4)
N(3)	0·82170 (27)	0·39760 (9)	0·08375 (5)	2·67 (4)	2·01 (4)	1·69 (3)	−0·05 (3)	0·76 (3)	0·05 (3)
N(7)	0·69257 (32)	0·66375 (9)	0·18877 (6)	3·41 (5)	2·28 (4)	2·27 (4)	0·21 (4)	1·03 (3)	0·01 (3)
N(8)	0·81011 (33)	0·70210 (9)	0·12097 (6)	3·86 (5)	2·14 (4)	2·50 (4)	0·14 (4)	1·12 (4)	0·07 (3)
N(9)	0·87436 (29)	0·60854 (9)	0·07326 (6)	2·98 (4)	2·08 (4)	1·81 (3)	0·06 (3)	0·86 (3)	0·19 (3)
O(6)	0·45672 (28)	0·47802 (8)	0·30936 (5)	4·37 (5)	3·13 (4)	2·02 (3)	0·34 (3)	1·59 (3)	0·14 (3)
O(101)	0·8186 (43)	0·3941 (9)	0·4693 (5)	10·18 (63)	6·04 (35)	2·76 (19)	0·31 (31)	−0·11 (25)	1·20 (18)
O(102)	0·9103 (44)	0·3941 (7)	0·4670 (6)	9·59 (51)	2·90 (20)	5·45 (29)	2·07 (24)	3·16 (30)	−0·27 (16)
H(1)	0·5224 (44)	0·2922 (15)	0·2411 (10)	3·62 (35)					
H(21)	0·6416 (52)	0·1530 (17)	0·1489 (11)	4·84 (42)					
H(22)	0·7806 (43)	0·1844 (14)	0·0687 (10)	3·45 (33)					
H(9)	0·9659 (42)	0·6173 (14)	0·0246 (10)	3·39 (33)					
H(101)	0·7200 (230)	0·4047 (67)	0·4128 (52)	9·1 (22)					
H(102)	0·6308 (254)	0·4426 (80)	0·4654 (60)	10·5 (30)					
H(103)	0·9887 (182)	0·4640 (45)	0·4879 (31)	4·4 (1·2)					
H(104)	0·9710 (169)	0·3560 (50)	0·4277 (38)	5·3 (1·3)					
H(105)	1·1202 (87)	0·3761 (30)	0·4777 (21)	0·2 (6)					

Table 3. *Observed and calculated structure factors multiplied by* 10

Reflections with observed intensity less than $2\sigma_c$ are marked with < signs

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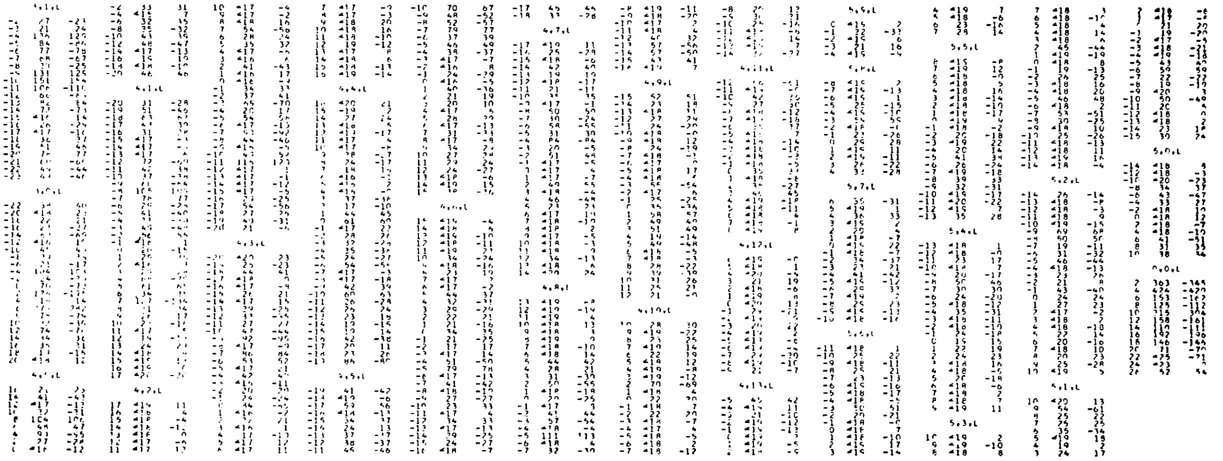
Table 3 (*cont.*)



mated standard deviations in bond lengths and angles involving nonhydrogen atoms are in the range 0·0014–0·0018 Å and 0·09–0·12° respectively. The standard deviations in bond lengths and angles involving full hydrogen atoms are 0·02 Å and 1·0°, and the corresponding values involving fractional hydrogen atoms are 0·1 Å and 7°.

By comparing the structure of 8-azaguanine with the structures of other purine derivatives which have been determined accurately, some conclusions about the effect of substituents on the purine skeleton can be drawn. The changes in the geometry from one derivative to the other are closely related to the positions of the protons; *e.g.* in purine (Watson, Sweet & Marsh, 1965) and 6-mercaptopurine monohydrate (Sletten *et al.*, 1968), where the hydrogen is attached to N(7) and not to N(9), the imidazole rings have nearly identical dimensions. It will be noticed that the angle at N(7) is greater than the corresponding angle at N(9) while angle C(4)–C(5)–N(7) is smaller than angle C(5)–C(4)–N(9). In 8-azaguanine where the proton has shifted to N(9), the relationship between the angles is reversed. In guanine hydrochloride dihydrate (Iball & Wilson, 1965) where both N(7) and N(9) carry hydrogen atoms, the imidazole ring is nearly symmetrical. In 8-azaxanthine monohydrate (Nowacki & Bürki, 1955) the angles at N(7) and N(9) do not differ significantly, in agreement with the assumption that the hydrogen is attached to N(8).

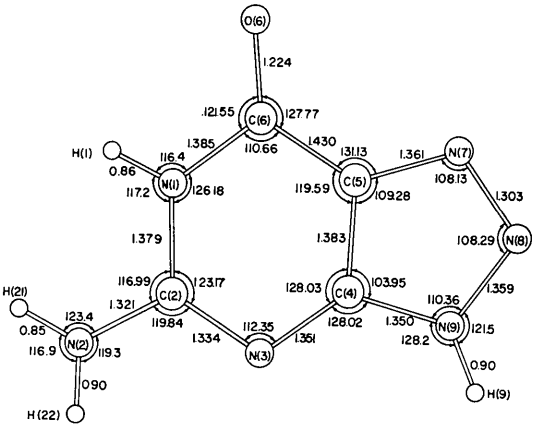


Fig. 4. Bond distances and angles in the azaguanine molecule.

For 8-azaguanine Pullman has shown that the lactam form is more stable than the lactim form, although the extra stabilization due to resonance energy favors the latter (Pullman & Pullman, 1963). The shift of the proton from N(7) to N(9) compared to purine and 6-mercaptopurine is not easily explained, because several factors govern this tautomerism. The overall effect of an electron withdrawing oxygen atom on C(6) and an electron releasing amino group on C(2) is to leave N(7) relatively more positive than N(9), and accordingly N(7) is less likely to be protonated. In the crystalline state, however, the requirement of a tight hydrogen bonding scheme is probably the major factor in determining the position of the proton.

Planarity of the molecule

The least-squares plane calculated for the atoms in the purine ring, has the equation

$$0{\cdot}9116X-0{\cdot}0502Y+0{\cdot}4080Z=0{\cdot}2882$$

in orthogonal Ångstrom space (**a**, **b**, **c***). Of the nonhydrogen atoms only N(2) and O(6) are displaced significantly from the plane. The two hydrogen atoms on the amino group are both displaced by 0·087 Å to the same side of the plane as N(2). This deviation is probably caused by the strong hydrogen bonds in which they are involved. The hydrogen atoms on N(1) and N(9) are also displaced from the plane toward the atoms to which they are hydrogen bonded.

Packing of the molecules

The 8-azaguanine molecules pack on top of one another as in crystals of other purine and pyrimidine compounds. This tendency of 'stacking', which is also found for certain purine derivatives in solution (Broom, Schweizer & Ts'o, 1967), is supposed to be mainly due to London dispersion forces (Hanlon, 1966). The interplanar distance in 8-azaguanine is fairly short (3·25 Å) and Macintyre has interpreted this as due to formation of a charge-transfer self-complex, responsible for the anticarcinogenic activity of the compound. However, in light of the fact that most natural occurring derivatives stack together in a similar way, a more probable explanation of the anticarcinogenic action of 8-azaguanine involves the ability of N(8) to participate in hydrogen bonding.

The molecules are held together in a tight network of hydrogen bonds as shown in Fig. 1. In one configuration the water molecule, O(101) (ii), provides protons to form hydrogen bonds to O(6) (ii) and to the other water molecule, O(102) (i), which in turn provides a proton for a hydrogen bond to N(8) (iii). The other proton on O(102) does not participate in any hydrogen bond and seems to alternate betweent the two positions H(102) and H(105) [Fig. 2(*b*)]. It is interesting to note that the disordered water molecule permits the maximum number of hydrogen bonds to form.

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References

Broom, A. D., Schweizer, M. P. & Ts'o, P. O. P. (1967). *J. Amer. Chem. Soc.* **89**, 3612.
Hanlon, S. (1966). *Biochem. Biophys. Res. Comm.* **23**, 861.
Iball, J. & Wilson, H. R. (1965). *Proc. Roy. Soc.* A**288**, 418.
Macintyre, W. M., Singh, P. & Werkema, M. S. (1965). *Biophys. J.* **5**, 697.
Nowacki, W. & Bürki, H. (1955). *Z. Kristallogr.* **106**, 339.
Pullman, B. & Pullman, A. (1963). *Quantum Biochemistry*, p. 205. New York: Int. Science Publisher.
Sletten, E., Sletten, J. & Jensen, L. H. (1968). To be published.
Watson, D. G., Sweet, R. M. & Marsh, R. E. (1965). *Acta Cryst.* **19**, 573.

Acta Cryst. (1968). B**24**, 1698

Crystal Structure of 2-Mercapto-6-methyl-purine Monohydrate*

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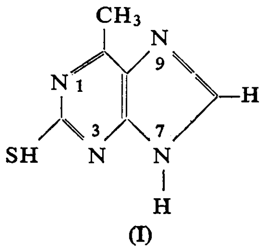
$C_6H_4N_4S.H_2O$ crystallizes in space group $P2_1/a$ with $a=8{\cdot}72$, $b=27{\cdot}60$, $c=4{\cdot}26$ Å, $\beta=93{\cdot}75$, $Z=4$. The three nearest neighbours of the oxygen atom are N(7), N(9) and S distant 2·81, 2·81 and 3·26 Å respectively and belonging to three different purine molecules, and the intermolecular distance N(7)···S is 3·37 Å. The structure determination is not accurate enough to indicate which of the two atoms N(7) or N(9) is protonated or to draw definite conclusions regarding the hydrogen bonding system.

The application of a computer program using direct methods to the solution of the crystal structure of a purine derivative was briefly reported earlier (Cochran, Srinivasan & Tollin, 1963). The compound, which had the chemical formual $C_6H_6N_4S$, was found to be 2-mercapto-6-methyl purine (I) as revealed by the projection on which the direct method was applied.



(I)

Although the projection was well resolved, the positions of hydrogen atoms were not located and consequently no conclusions could be drawn as to whether

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