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9-Methylisoguanine Hydrochloride Dihydrate: Crystal Structure and Charge Densities

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Isoguanine is a naturally occurring nucleobase. In order to investigate its tautomeric properties, 9-methylisoguanine(I).HCl.2H₂O, C₆H₈N₂O.HCl.2H₂O, was crystallized; space group $P2_1/c$, $a = 5.189$ (3), $b = 24.869$ (8), $c = 8.218$ (4) Å, $\beta = 101.70$ (6)°. The structure was determined by direct methods and refined to $R = 6.6\%$ based on 1527 X-ray structure amplitudes. 9-Methylisoguanine is protonated at N(3), in agreement with charge densities computed with CNDO/2. Two inversion-related molecules are linked by four hydrogen bonds N(1)H...O(2) and O(2)...H₂O...H–N(6) and the base pairs are stacked at a distance of 3.26 Å with the N(6) amino groups overlapping the imidazole rings of adjacent molecules.

Isoguanosine, originally isolated from croton seeds and referred to as crotonoside (Cherbuliez & Bernhard, 1952), was subsequently synthesized by Davoll (1951), and characterized as an isomer of guanosine and an analogue of adenosine, adenosin-2-one. The free base, isoguanine, is a constituent of butterfly wings (Purrrmann, 1940), and apparently of a cytokinin isolated from corn (Letham, 1973). Isoguanine has hitherto not been found in natural nucleic acids. Isoguanosine (Ravindranathan & Miles, 1965) and some methylated isoguanines (Kazimierczuk & Shugar,

1974) possess, in common with guanosine and its nucleotides, the property of forming aqueous gels which exhibit secondary structure *via* base-pair hydrogen bonding similar to that found in helical polynucleotides. A synthetic polynucleotide, polyisoguanilate, has been prepared and characterized as a multi-stranded helix (Golas, Fikus, Kazimierczuk & Shugar, 1976).

Characterization of the structures of isoguanosine gels, and of polyisoguanilate, requires information regarding the tautomerism of 9-substituted isoguanines.

A comprehensive study of such tautomerism of methylated isoguanines and isoguanosine itself, in both aqueous and non-aqueous media, has been reported (Sepiol, Kazimierczuk & Shugar, 1976). However, to date the only structural data available are for isoguanine sulphate (Subramanian & Marsh, 1971), in which H atoms are located at N(1), N(3) and N(7), and the base exhibits significant deviations from planarity. The rings are stacked at distances of 3.18 and 3.36 Å and are not linked by base-paired hydrogen bonds because the sulphate ion is the acceptor for most of the hydrogen bonds.

The present study is devoted to the X-ray structure of a formal analogue of isoguanosine, the model selected being 9-methylisoguanine, which provided suitable crystals.

Isoguanine is a formal purine analogue of isocytosine, so that these are complementary to each other and should be able to form, among others, complementary base pairs with three hydrogen bonds. Structural data in the solid state have been reported for isocytosine (Sharma & McConnell, 1965) and for 5,6-dihydroisocytidine (Kojić-Prodić, Ružić-Toroš & Cofou, 1976).

Experimental

The title compound was prepared as previously described (Kazimierczuk & Shugar, 1974), and crystallized from aqueous medium as 9-methylisoguanine hydrochloride dihydrate in the form of transparent needles. Crystallographic data obtained from photographic and four-circle diffractometer measurements are summarized in Table 1. Intensities were collected in the $2\theta/\omega$ scan mode with stationary background counts on both sides of each scan and Ni-filtered Cu $K\alpha$ radiation. Owing to the small size of the crystal, $0.3 \times 0.25 \times 0.15$ mm, data were corrected for Lorentz and polarization factors but not for absorption. Of the 1527 collected data, 1208 had $F_o > 3\sigma(F_o)$ and were considered as observed.

The structure was solved with *MULTAN* (Germain, Main & Woolfson, 1971) and refined by full-matrix least squares with scattering factors from *International Tables for X-ray Crystallography* (1968). All

Table 1. *Crystal data*

9-Methylisoguanine hydrochloride ($C_6H_9N_5OCl$) + $2H_2O$

Monoclinic	Space group $P2_1/c$
a 5.189 (3) Å	M_r 952
b 24.869 (8)	$F(000)$ 500
c 8.218 (4)	V 1038.46 Å ³
β 101.70 (6)°	d_c 1.51 g cm ⁻³

the H atom positions were derived from difference syntheses. The refinement converged at $R = \sum ||F_o| - |F_c|| / \sum |F_o| = 6.6\%$ for all 1527 data.

Results and discussion

Final atomic coordinates are listed in Table 2.* Geometrical details are described in Figs. 3 to 5 and Tables 3 and 4; charge density calculations are presented in Figs. 2, 6 and 7.

* Lists of structure factors and anisotropic thermal parameters have been deposited with the British Library Lending Division as Supplementary Publication No. SUP 33450 (11 pp.). Copies may be obtained through The Executive Secretary, International Union of Crystallography, 5 Abbey Square, Chester CH1 2HU, England.

Table 2. *Fractional atomic coordinates ($\times 10^4$) with standard deviations in parentheses derived from the least-squares correlation matrix*

	x	y	z
Cl	1932 (2)	-1943 (0)	2948 (1)
N(1)	3065 (8)	187 (1)	-3352 (4)
C(2)	1361 (8)	618 (2)	-3499 (5)
O(2)	-690 (6)	626 (1)	-4542 (3)
N(3)	2034 (7)	1032 (1)	-2407 (4)
C(4)	4320 (8)	990 (2)	-1247 (5)
C(5)	6018 (8)	563 (2)	-1130 (5)
C(6)	5397 (8)	127 (2)	-2229 (5)
N(6)	6831 (7)	-300 (1)	-2241 (4)
N(7)	8140 (7)	642 (1)	210 (4)
C(8)	7603 (9)	1105 (2)	818 (5)
N(9)	5295 (7)	1338 (1)	-4 (4)
C(1')	4260 (9)	1864 (2)	294 (6)
W(1)	4393 (6)	1216 (1)	4131 (4)
W(2)	336 (7)	1964 (1)	3746 (4)
H(1C1')	4296	2901	4175
H(2C1')	5256	2969	6146
H(3C1')	2272	3174	5169
H(1N1)	2489	5080	990
H(1N6)	8521	5324	3650
H(2N6)	6373	5560	2264
H(1C8)	8866	3667	6942
H(1N3)	646	3667	2548

Table 3. *Bond lengths (Å) and angles (°) involving H atoms*

H(1)-C(1')	1.09	H(1)-C(1')-N(9)	105.6
H(2)-C(1')	0.89	H(2)-C(1')-N(9)	112.3
H(3)-C(1')	1.02	H(3)-C(2')-N(9)	107.4
H-N(1)	0.87	H-N(1)-C(6)	118.6
H-N(3)	1.03	H-N(1)-C(2)	114.0
H-C(8)	1.07	H-N(3)-C(2)	116.1
H(1)-N(6)	1.02	H-N(3)-C(4)	125.5
H(2)-N(6)	0.78	H-C(8)-N(7)	127.1
		H-C(8)-N(9)	118.4
		H(1)-N(6)-C(6)	116.9
		H(2)-N(6)-C(6)	124.9
		H(1)-N(6)-H(2)	117.0

Table 4. *Least-squares planes through the molecule and the base pair*

The plane equations are of the form $lx + my + nz + p = 0$ where x, y, z are along a, b, c^* . Atoms which define the plane are marked by an asterisk. The angle between the two molecular planes in the dimer is 0.01° . The overlapping (stacked) molecules are at (x, y, z) and at $(1 - x, 1 - y, 1 - z)$; the interplanar separation between overlapping molecules is 3.26 \AA .

Moiety	Plane coefficients	Atom	Deviation from plane (\AA) [†]
(a) Purine base	$l = 0.6369$ $m = 0.4551$ $n = 0.6223$ $p = 5.4107$	N(1)*	0.025
		N(3)*	-0.018
		C(2)*	0.009
		C(4)*	-0.021
		C(5)*	-0.027
		C(6)*	-0.001
		N(7)*	-0.004
		C(8)*	0.018
		N(9)*	0.020
		C(1)	-0.041
		N(6)	0.004
O(2)	0.052		
(b) Base dimer	$l = 0.6087$ $m = 0.4322$ $n = 0.6653$ $p = -0.53741$	N(1)*	-+0.119
		N(3)*	-+0.100
		C(2)*	-+0.139
		C(4)*	-+0.037
		C(5)*	-+0.039
		C(6)*	-+0.080
		N(7)*	+ -0.058
		C(8)*	+ -0.117
		N(9)*	+ -0.075
		C(1)*	+ -0.038
		N(6)*	-+0.078
O(2)*	-+0.157		

[†] The first sign in the deviations of the base-pair atoms is for the atoms in one molecule, the second for the corresponding atoms in the molecule related by inversion.

(a) Site of protonation and molecular geometry

From a chemist's point of view, the neutral 9-methylisoguanine molecule with a proton attached to N(1) (Sepiol, Kazimierczuk & Shugar, 1976) should accept an additional proton at N(3) (Fig. 1) rather than at N(7) because then more mesomeric states can be formulated. Further, this site of protonation was also found in isoguanine sulphate (Subramanian & Marsh, 1971), but in this case N(7) also is protonated, whereas N(9) is free.

For 9-methylisoguanine hydrochloride, difference syntheses revealed that the site of protonation is at N(3), following the prediction based on Fig. 1. Quantum-chemical calculations performed by the CNDO/2 method (program QCPE 142) yielded the electronic charge distribution of 9-methylisoguanine displayed in Fig. 2, with the negative charge on N(3) greater than that on N(7), in agreement with the observation made above. The site of protonation is

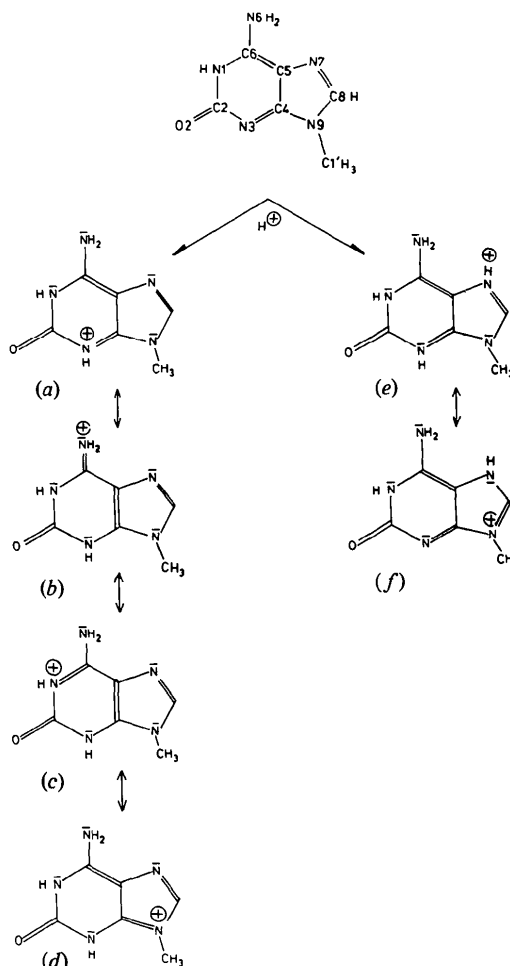


Fig. 1. The mesomeric structures of 9-methylisoguanine protonated at N(3) and at N(7).

further evidenced by some peculiarities in bond angle and length distributions (Fig. 3). When protonation occurs at N, the C-N-C angle generally increases by about 5° , an observation first described by Singh (1965) and corroborated by others (Saenger, 1970; Voet & Rich, 1970). In 9-methylisoguanine hydrochloride and isoguanine sulphate, C(2)-N(3)-C(4) is increased relative to the corresponding angle in guanine, 112.2° (Voet & Rich, 1970) but C(5)-N(7)-C(8) is comparable in 9-methylisoguanine (103.2°) and in guanine (103.8°) while it is 105.9° in isoguanine sulphate protonated at N(7). Further, the C-NH₂ distance in methylisoguanine hydrochloride, 1.297 \AA , is significantly shorter than the C-NH₂ distance in adenine, 1.34 , and in guanine, 1.335 \AA (Voet & Rich, 1970), and even shorter than in adenine protonated at N(1), 1.315 \AA (Saenger, 1970), suggesting preponderance of the mesomeric form depicted in Fig. 1(b). In addition, N(7)-C(8) and C(8)-N(9) are

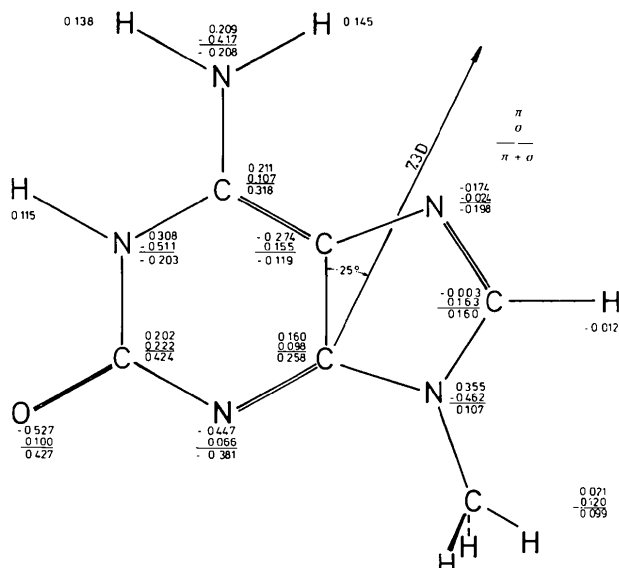


Fig. 2. Electronic structure and dipole moment of the neutral form of 9-methylisoguanine.

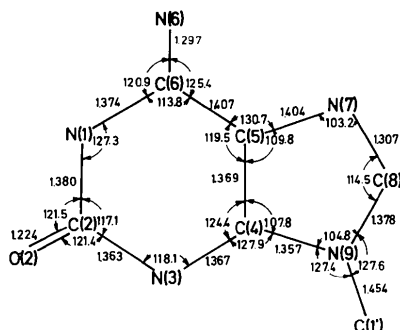


Fig. 3. Bond angles and distances in 9-methylisoguanine protonated at N(3). Average c.s.d.'s are 0.8° and 0.006 Å.

quite different in 9-methylisoguanine hydrochloride, 1.307 and 1.378 Å, while they are about equal in N(7)-protonated isoguanine, 1.340 and 1.334 Å, and would be predicted to be equal if 9-methylisoguanine were protonated at N(7) (Fig. 1e,f). As observed for isoguanine sulphate, the heterocyclic ring in 9-methylisoguanine hydrochloride is significantly non-planar, with N(1), C(2), C(8) on one side of the least-squares plane and C(4), C(5) on the other, Table 4. The methyl group is oriented such that one C—H bond is *cis*-planar to N(9)—C(8), *i.e.* the other two H atoms envelope the proton attached to N(3).

(b) Hydrogen-bonding scheme

Of the two potential hydrogen-bond acceptors, O(2) and N(7), only O(2) is involved in hydrogen bonding. This bonding is across an inversion centre, giving rise to two identical N(1)—H...O(2) interactions. Further, O(2) is bonded to the water molecule *W*(1), being also in contact with the amino group N(6) of the base-pair partner, with water molecule *W*(2) and with the Cl⁻ ion. The latter is hydrogen-bonded to the N(3)—H group and forms two additional bonds with symmetry-related molecules *W*(2). The water H atoms could not be located and the assignment of hydrogen bonds with water as donor is based on distance criteria.

(c) Packing of molecules

In the stereoview (Fig. 4) the packing of molecules in the cell is described. It is evident that two zones dominate, one with hydrophobic character located in the *ac* plane at *b* = 0 and $\frac{1}{2}$ and built up of 9-methylisoguanine molecules while the other zone containing

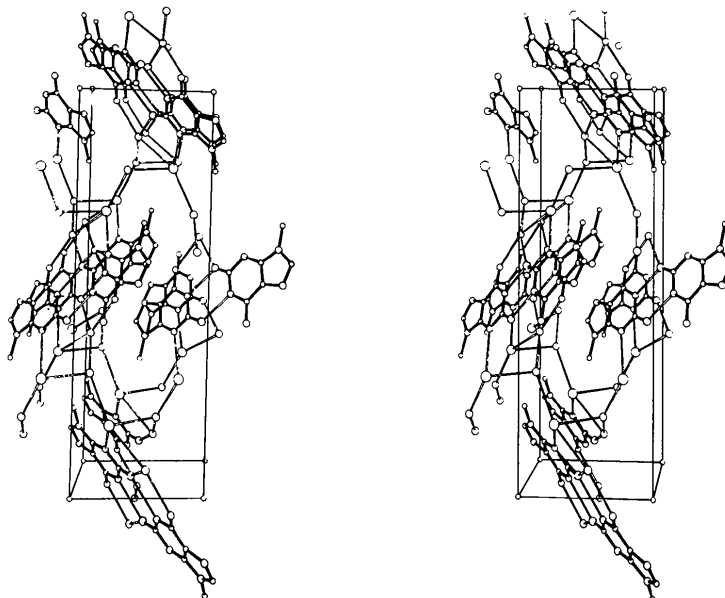


Fig. 4. Stereodiagram showing packing of molecules in the unit cell viewed along *a**.

water molecules and Cl^- ions displays hydrophilic character. Bases related by inversion centres at $0, \frac{1}{2}, 0$ and at $0, 0, \frac{1}{2}$ are connected by hydrogen bonds $\text{N}(1) \cdots \text{H} \cdots \text{O}(2)$ while bases related by centres at $0, 0, 0$ and at $\frac{1}{2}, \frac{1}{2}, \frac{1}{2}$ are stacked at an average distance of 3.26 \AA (Fig. 5). This stacking distance is an average of the two distances, 3.18 and 3.36 \AA , observed in isoguanine sulphate and significantly shorter than the 3.4 \AA usually observed. Similar stacking patterns with the amino group over the imidazole ring of the adjacent molecule are often observed in crystal structures of purine nucleosides (Bugg, Thomas, Sundaralingam & Rao, 1971).

(d) *Electronic structure of 9-methylisoguanine hydrochloride*

The following discussion will be divided into two parts. First, we treat the 9-methylisoguanine cation, and subsequently the ion pair consisting of this cation and the Cl^- anion.

The charge distribution in neutral 9-methylisoguanine was calculated by the CNDO/2 method and is described in Fig. 2 while Fig. 6 gives the differences

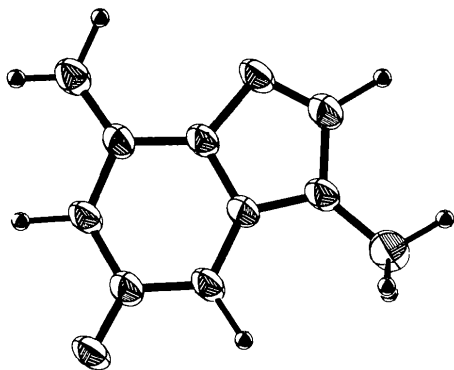


Fig. 5. ORTEP plot of 9-methylisoguanine cation. Thermal ellipsoids are drawn at 50% probability (Johnson, 1965).

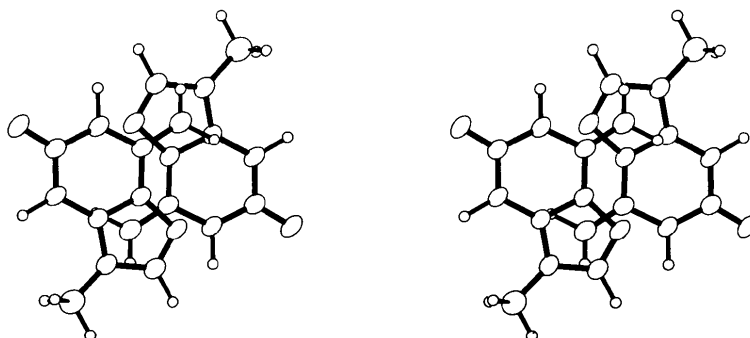


Fig. 5 (cont.). Stereoplot of stacked bases.

in π , σ and total charges if protonation occurs at $\text{N}(3)$. In general, the transfer of π and σ electrons to the site of protonation, $\text{N}(3)$, occurs mainly from the exocyclic substituents $\text{O}(2)$ and $\text{N}(6)$ and each atomic centre becomes more positive, the ratios of σ and π contributions being different in each case. On protonation, $\text{N}(6)$ and $\text{N}(9)$ become more positive by $\sim 0.1 e$, as predicted by forms (b) and (d) in Fig. 1 and the local π -electronic structures of $\text{N}(1)$ and $\text{N}(3)$ become almost the same, 1.705 and $1.720 e$ (Fig. 6).

If we include the Cl^- ion in our calculations in the relative position derived from the X-ray analysis, the electronic structure of the 9-methylisoguanine cation changes as displayed in Fig. 7. These changes are caused by the electrostatic repulsion between Cl^- ($\sim -0.9 e$) and the electron cloud of the cation, and they are smaller compared with those when the neutral 9-methylisoguanine molecule becomes protonated. The electrostatic repulsion dominates at short distances from Cl^- , *i.e.* $\text{N}(3)$ and $\text{C}(4)$ lose negative charges of 0.05 and $0.04 e$, which are distributed over the other atomic centres, Fig. 7.

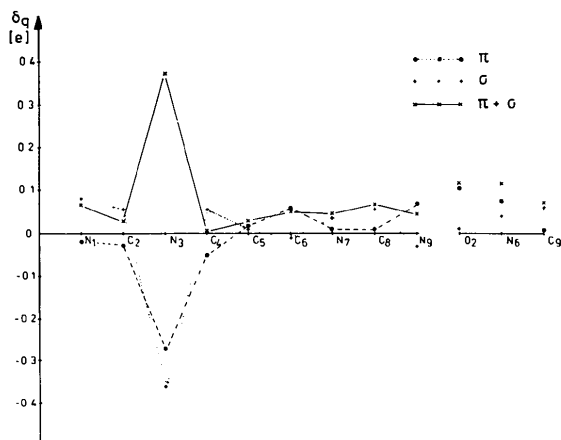


Fig. 6. Changes in electronic structure of 9-methylisoguanine after protonation at $\text{N}(3)$. The total change of the charge at $\text{N}(3)$ is $\delta\pi + \delta\sigma + 1$ [$\text{N}(3)$ is the site of protonation].

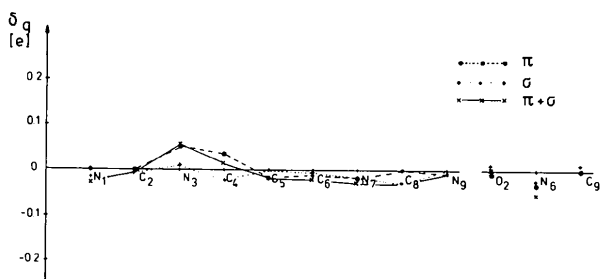


Fig. 7. Changes in electronic structure of the 9-methylisoguanine cation after ion-pair formation with Cl^- .

9-Methylisoguanine hydrochloride possesses a dipole moment of 19.9 D and therefore must interact strongly with other molecules. In the crystal structure, two 9-methylisoguanine hydrochloride ion pairs are related by an inversion centre to form a base pair such that the dipole moments cancel each other.

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On the Disorder in the Crystal Structure of *trans*-Tetrachlorostilbene

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The crystal structure of *trans*-tetrachlorostilbene [Norrestam, Hovmöller, Palm, Göthe & Wachtmeister (1977), *Acta Cryst.* **B33**, 370–376] has been reinvestigated by means of a completely disordered model in which each atom is distributed equally over two positions. This model, refined to an *R* value of 5.3%, does not violate the stacking of molecules.

Introduction

Recently, Norrestam, Hovmöller, Palm, Göthe & Wachtmeister (1977) reported the crystal structures of

the *cis* and *trans* isomers of tetrachlorostilbene [1,2-bis(4-chlorophenyl)-1,2-dichloroethylene]. The *trans* isomer is centrosymmetric and crystallizes with two molecules in the space group $P2_1/c$. The authors

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