THE CRYSTAL AND MOLECULAR STRUCTURE OF 8-AZAGUANINE MONOHYDRATE

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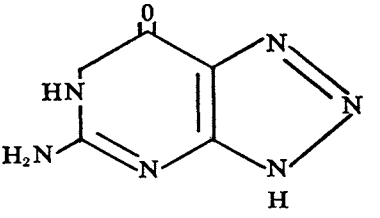
ABSTRACT The x-ray analysis of the crystal structure of 8-azaguanine monohydrate is reported. Bond lengths are given with standard deviations of 0.02 A. Bond angles are given with standard deviations of 1 to 2°. All the hydrogen atoms in the structure were found. The hydrogen atom in the imidazo ring is on N9. N8 was found to take no part in the hydrogen bonding pattern in the crystal, but all other possible hydrogen bonds are formed. A very short separation (3.25 A) was observed between the planes of successive 8-azaguanine molecules. This distance is characteristic of charge-transfer complexes. A mechanism for the cell poisoning action of 8-azaguanine is suggested. The mechanism is based upon the assumption that there is an interaction of the charge-transfer type between molecules of 8-azaguanine.

INTRODUCTION

Recent work in this laboratory has been devoted to x-ray analysis of the crystal structure of cell poisons which are incorporated into the nucleic acids of the host cells. The object of this work has been to determine whether these componuds show, in their crystal structures, any secondary structural features which might suggest an explanation of their biological properties.

One such cell poison is 8-azaguanine. It has the following structural formula.



It can be considered to be derived from guanine by substitution of a nitrogen atom for the CH group in position 8. (The atom numbering scheme used in this paper is shown in Fig. 1.)

Tracer studies with 8-azaguanine-2-^{14}C, in an *in vivo* system involving *Streptococcus faecalis,* have shown that 8-azaguanine is incorporated into the bacterial RNA (1). The cell poisoning action appears to be associated with such incorporation, since 8-azaguanine resistant strains of *S. faecalis* have been found to be unable to convert the 8-azaguanine to 8-azaguanylic acid (2). As a result of this defect the 8-azaguanine is not incorporated into the RNA of the resistant cells. These studies indicate that 8-azaguanine acts as a cell poison only after it is incorporated into the bacterial RNA.

It is conceivable that the cell poisoning action of 8-azaguanine is caused by a modification of the tertiary structure of the RNA molecules, resulting from some secondary structural features of 8-azaguanine. The x-ray analysis of the crystal structure of 8-azaguanine monohydrate was undertaken in order to determine whether 8-azaguanine showed any such secondary structural features in the crystal. The monohydrate was chosen since good crystals were easily prepared, and since in the absence of heavy atoms higher precision in the final results could be expected. It was not possible to prepare suitable crystals of the anhydrous 8-azaguanine.

Cell Data. Azaguanine monohydrate crystals grew as colorless needles on slowly cooling a warm, concentrated solution of 8-azaguanine in O.2N hydrobromic acid. One of the crystals was mounted to rotate about the needle axis. The cell dimensions and space group were determined using rotation, Weissenberg and precession photographs. These dimensions were checked later by carefully measuring 2θ angles for appropriate reflections on the G. E. single crystal orienter.

The data found are as follows:

Molecular formula: $C_4H_4ON_6 \cdot H_2O$
Formula weight: 156
Crystal system: monoclinic

$$a = 3.57\text{A} \pm 0.01\text{A}; \quad b = 11.41\text{A} \pm 0.02\text{A};$$

$$c = 16.53\text{A} \pm 0.02\text{A}; \quad \beta = 95.3^\circ \pm 0.1^\circ$$

Absent spectra: h0l with l odd; 0k0 with k odd
Space group: $P2_1/c$
Observed density: 1.70 gm/cc
Calculated density: 1.69 gm/cc
Number of formula units in the unit cell: 4.
F(000) = 352

Structure Analysis. The analysis was carried out using a full three-dimensional set of intensity data obtained using CASCADE,[1] our automatic signal crystal diffractometer (3). All of the independent reflections within the CuKα limiting sphere

[1] CASCADE, Colorado Automatic Single Crystal Analysis Diffraction Equipment.

were examined using MoKα radiation. Of these, 1143 were observed, representing 76 per cent of the total.

The crystal used in the collection of data had dimensions 0.34 × 0.04 × 0.04 mm. Absorption corrections were deemed unnecessary, because the linear absorption coefficient of the crystal for MoKα radiation is small, and because the dimensions of the crystal are small.

The data had a curious feature. Of the 1143 observed intensities there were only 349 which had structure amplitudes greater than 10 on an absolute scale. Since the errors in intensities measured by CASCADE are relatively higher for the weaker reflections, the average error in this set of intensities is relatively high.

The *a*-axis of the 8-azaguanine monohydrate crystal is very short. The authors believe it to be the shortest axis reported for any such organic compound. It was clear that in the projection of the structure down the *a*-axis all of the atoms would be completely resolved from one another, and it was decided to try to solve the structure in two dimensions first.

The Patterson function, $P(vw)$, showed a series of peaks along the *b*-axis corresponding to intramolecular vectors. The positions of these peaks suggested that the azaguanine molecules packed in the crystal with their long axis parallel to *b*. No further information was obtained from this Patterson projection.

A Bragg-Lipson structure factor graph was then drawn up for the plane 012. This plane has an observed intensity of almost zero. It was possible to arrange all the atoms in the molecule, using reasonable values for the bond lengths, in such a way that their individual contributions to the intensity of 012 almost exactly cancelled. The possible positions of the molecule on the graph were restricted by requiring that the long axis of the molecule be parallel to *b*. A trial structure obtained in this way gave a value of the residual R of 0.40, calculated over the 48 observed planes of class (Okl) with $\sin\theta < 0.5$.[2]

Fourier refinement of this structure reduced R to 0.17 over all the observed planes of class (Okl). All the atoms were clearly resolved at this point, and all interatomic distances were reasonable. The structure was then considered solved in two dimensions.

The third coordinate was obtained as follows. The intensity of plane 102 was extremely large. $F_o(102)$ was almost 200. It was clear that the azaguanine molecule must lie on this plane, or very close to it. Using the *y* and *z* coordinates previously determined, imposing those restrictions on the position of the molecule required by the symmetry of the cell, and assuming the azaguanine molecule lay exactly on 102, *x* coordinates were calculated for all the atoms in the azaguanine molecule.

Phases were calculated for all the observed reflections (except the *Okl* reflections for which phases had already been determined) using these coordinates. These

[2] $R = (\Sigma \mid |F_o| - |F_c| \mid)/\Sigma|F_o|$

phases were only approximate, of course, since it was not possible to include the contribution of the water molecule at this point. A three-dimensional Fourier synthesis was then computed using as coefficients those observed structure factors whose phases were considered to be determined. This synthesis showed peaks corresponding to all the heavier atoms in the azaguanine molecule. A further peak was observed with the y and z coordinates of the water oxygen atom. This peak was assigned to the water oxygen atom, and so its x coordinate was determined from the first three-dimensional Fourier synthesis.

Further cycles of three-dimensional Fourier refinement eventually gave a structure from which phases could be calculated for most of the observed planes. This structure gave a value for R of 0.22 over all the observed planes. In the calculated structure factors, each atom was assigned an arbitrary Debye factor of 3.0 A.

Least squares refinement was carried out in the block diagonal approximation using a program written by P. K. Gantzel and K. N. Trueblood of University of California at Los Angeles and modified by R. Deverill of this laboratory. In the first cycle of refinement, the arbitrary isotropic temperature factors (3.0 A^2 for all atoms) and the atomic coordinates obtained from the Fourier synthesis were the input parameters. Atomic coordinates and individual anisotropic thermal parameters were refined simultaneously for all the non-hydrogen atoms in the structure. After eleven cycles of such refinement the structure converged with a value of 0.12 for R, calculated over all the observed planes.

The standard deviations of the atomic coordinates and anisotropic thermal parameters were then calculated using the usual procedure for least squares.

A three-dimensional Fourier difference synthesis was calculated using the final set of calculated structure factors. Peaks of appropriate height were observed at the expected positions of all six hydrogen atoms. These peaks were therefore identified as hydrogen atoms. There were no such peaks in the neighborhood of atoms N3, N7, N8, or O6.

A projection of the final structure down the a-axis is shown in Fig. 1.

The atomic coordinates of the non-hydrogen atoms and their estimated standard deviations (ESD) are listed in Table I. The anisotropic thermal parameters and their ESD are given in Table II. The atomic coordinates of the hydrogen atoms are given in Table III together with their electron densities as observed from the difference Fourier synthesis.

It was disappointing that the value of R did not converge to a value less than 0.12. While this value is acceptable, it does indicate that the agreement between observed and calculated structure factors is not as good as one might wish. The reason for the relatively high value for R is that the errors in the observed intensities are relatively high. This is because a large proportion of the intensities are quite small, and the errors in intensities measured by CASCADE are relatively higher for weak reflections.

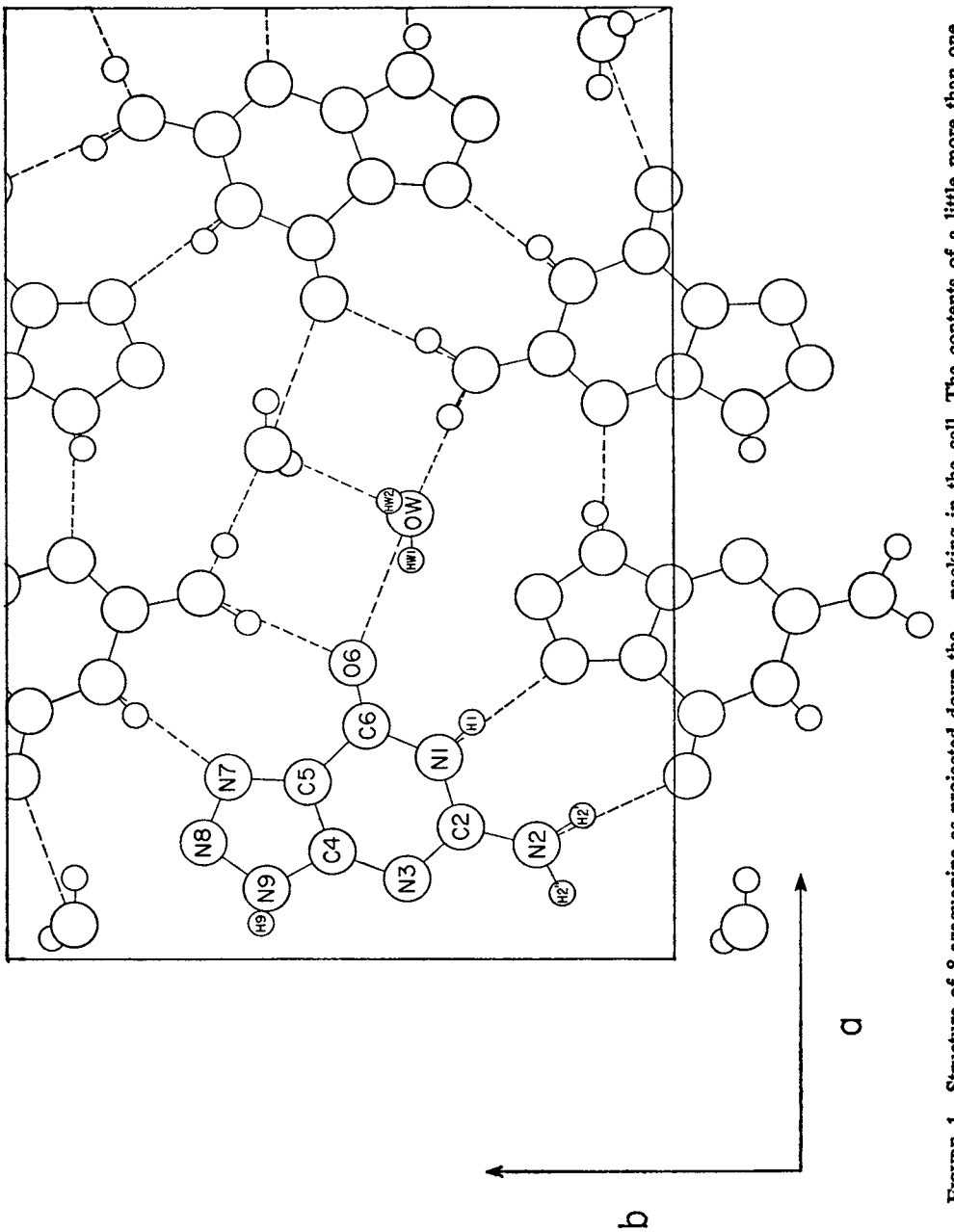


FIGURE 1 Structure of 8-azaguanine as projected down the *a* crystallographic axis. The diagram shows the molecular packing in the cell. The contents of a little more than one cell are shown. The dashed lines represent hydrogen bonds.

TABLE I

ATOMIC COORDINATES FOR THE NON-HYDROGEN ATOMS IN 8-AZAGUANINE MONOHYDRATE AND THEIR ESTIMATED STANDARD DEVIATIONS (σ)

All data are given in fractions of the cell edges $x\ 10^4$

	x	$\sigma(x)$	y	$\sigma(y)$	z	$\sigma(z)$
C2	7155	22	3190	8	1362	4
C4	7959	19	5086	7	1113	4
C5	6797	22	5458	8	1849	4
C6	5733	22	4596	7	2424	4
N1	5977	20	3486	7	2121	4
N2	7237	23	2059	7	1187	4
N3	8220	18	3976	6	838	3
N7	6890	21	6629	6	1885	4
N8	8136	22	7024	6	1214	4
N9	8746	19	6081	6	729	4
O6	4583	19	4787	6	3092	3
OW	8691	32	3944	8	4678	5

TABLE II

ANISOTROPIC THERMAL PARAMETERS FOR THE NON-HYDROGEN ATOMS IN 8-AZAGUANINE MONOHYDRATE, AND THEIR ESTIMATED STANDARD DEVIATIONS (σ)

The b_{ij} are defined by the expression $T = \exp -(h^2b_{11} + k^2b_{22} + l^2b_{33} + hkb_{12} + hlb_{13} + klb_{23})$ which is the factor applied to the scattering factor of an atom to correct for thermal vibration of the atom. All quantities are given $x\ 10^5$.

	b_{11}	$\sigma(b_{11})$	b_{22}	$\sigma(b_{22})$	b_{33}	$\sigma(b_{33})$	b_{12}	$\sigma(b_{12})$	b_{13}	$\sigma(b_{13})$	b_{23}	$\sigma(b_{23})$
C2	5910	640	691	74	162	24	−163	360	462	200	−44	70
C4	4460	540	643	66	116	20	79	320	333	170	8	63
C5	5680	620	728	72	121	22	325	340	347	190	11	67
C6	6100	690	575	67	202	26	848	340	682	210	20	68
N1	7500	650	706	65	155	21	−228	320	952	190	20	59
N2	10700	810	517	58	279	28	−327	360	1240	240	−101	66
N3	6390	550	526	54	144	20	−266	290	410	160	7	55
N7	8200	680	596	60	233	25	−139	330	1030	210	92	64
N8	9390	730	507	56	239	25	−3	330	729	210	37	62
N9	7130	590	574	59	184	21	524	320	830	180	16	60
O6	11400	670	729	57	178	19	261	320	1640	180	16	55
OW	28200	1600	865	82	349	31	1150	590	1390	370	113	83

Another refinement of the structure was carried out using only those 349 structure factors for which $|F_o| > 10$. This refinement converged to a structure with a value for R of 0.08, calculated over these 349 structure factors. This was a much more satisfactory value. However, the atomic coordinates in this structure were all within one standard deviation of those given in Table I. Therefore the data in

TABLE III

COORDINATES OF THE HYDROGEN ATOMS IN 8-AZAGUANINE MONOHYDRATE, AND THE ELECTRON DENSITIES OF THE ATOMIC CENTERS (ρ)

The atomic coordinates are given as fractions of the cell edges $x\ 10^3$. No standard deviation were calculated for these data.

Atom	x	y	z	$\rho(xyz)$
HW1	856	392	418	0.9
HW2	623	423	481	1.0
H1	525	300	248	0.9
H2′	726	136	150	0.9
H2″	835	168	68	0.8
H9	937	617	34	0.6

Table I are considered reliable despite the relatively high R value associated with them.

DISCUSSION

Bond Lengths and Angles. The bond lengths and angles found are listed in Tables IV and V respectively, together with their estimated standard deviations. Individual standard deviations for the bonds involving hydrogen atoms are not given. These are quite large and are all of the order of 0.15 A.

One bond length that is noteworthy is C2-N2, between the nitrogen atom of the amino group and the C2 atom of the pyrimidine ring. This is quite short (1.324 A) and is, in fact, equal to the shortest carbon-nitrogen bond in the pyrimidine ring (1.327 A). A similar short $\rangle C\text{-}NH_2$ bond has been observed in cytidine (4), adenylic acid (5), and adenine (6). By constrast in *p*-aminophenol (7), the length is given as 1.47 A. There seems to be a rather large shift of electron density from the amino nitrogen atom in the direction of the ring. This shift has the effect of strengthening the bond between the amino nitrogen atom and the C2 atom of the ring. It also has the effect of making the amino nitrogen atom electrically positive relative to the ring, and so making that nitrogen atom less basic. This no doubt is the explanation of the somewhat surprising fact that the azaguanine monohydrate was crystallized from 0.2N HBr solution.

The only other structure analysis of an 8-aza-purine ring system is that of xanthazole monohydrate (8). The bond lengths given for xanthazole monohydrate are equal to those found in 8-azaguanine monohydrate to within experimental error.

The crystal structure of guanine hydrochloride dihydrate has been reported recently (9). The bond lengths in the pyrimidine ring, and the C6-O6 and C2-N2 bonds, are equal to those found in azaguanine to within experimental error. How-

TABLE IV

INTRAMOLECULAR BOND LENGTHS IN 8-AZAGUANINE MONOHYDRATE AND THEIR STANDARD DEVIATIONS (σ)

No standard deviations are given for the bonds formed by hydrogen atoms; these are estimated to be of the order of 0.15 A. All quantities given in A.

Bond	Length (r)	$\sigma(r)$
N1–C2	1.401	0.019
C2–N2	1.324	0.019
C2–N3	1.327	0.017
N3–C4	1.351	0.016
C4–C5	1.389	0.018
C5–C6	1.444	0.019
C6–O6	1.232	0.017
C6–N1	1.368	0.018
C4–N9	1.343	0.017
N9–N8	1.372	0.017
N8–N7	1.313	0.017
N7–C5	1.338	0.018
N1–H1	0.86	
N2–H2′	0.95	
N2–H2″	1.05	
N9–H9	0.71	
OW–HW1	0.82	
OW–HW2	0.98	

ever, in guanine the bonds N7-C5 and C4-N9 were found to be 1.378 A and 1.397 A respectively. These bonds are then about 0.04 A and 0.06 A larger than the corresponding bonds in 8-azaguanine (Table IV). The standard deviations in the guanine bond lengths are said to be not greater than 0.006 A. Thus these differences should be regarded as significant.

06 is a keto oxygen. This is demonstrated by the C6-06 bond length (1.23 A) and the fact that N1 is found to have a hydrogen atom attached to it.

Planarity of the Ring and the Interplanar Distance. The equation of the best plane, passing through the non-hydrogen atoms of the azaguanine molecule, was found to be: $0.9102X - 0.0506Y + 0.4110Z = 2.886$. X, Y, and Z are the coordinates, expressed in A, of a point on the plane with respect to an orthogonal system of axes. The transformation of the coordinates of the point with respect to the crystal axes (xyz) to the orthogonal axes is given by the relations: $X = ax + cz \cos \beta$; $Y = by$; $Z = cz \sin \beta$.

Table VI gives the deviations of the atoms of the 8-azaguanine molecule from this plane. It is evident that all of the non-hydrogen atoms of the 8-azaguanine mole-

TABLE V

INTRAMOLECULAR BOND ANGLES IN 8-AZAGUANINE AND THEIR STANDARD DEVIATIONS (σ)

All quantities are given in degrees.

	Angle	σ
N1 C2 N3	123.3	1.2
N1 C2 N2	116.5	1.2
N2 C2 N3	120.2	1.3
C2 N3 C4	112.5	1.1
N3 C4 C5	128.1	1.2
N3 C4 N9	127.6	1.2
C4 C5 C6	119.1	1.2
N7 C5 C6	131.2	1.3
C5 C6 N1	111.1	1.2
C5 C6 O6	126.8	1.2
O6 C6 N1	122.0	1.2
C6 N1 C2	125.9	1.2
C4 C5 N7	109.7	1.2
C5 N7 N8	108.3	1.2
N7 N8 N9	108.0	1.1
N8 N9 C4	109.7	1.1
N9 C4 C5	104.3	1.1

TABLE VI

DISTANCES IN 8-AZAGUANINE MONOHYDRATE FROM THE MEAN PLANE THROUGH THE 8-AZAGUANINE MOLECULE

The estimated standard deviation of position [$\sigma(r)$] of the atoms is also listed. All quantities are given in A ($\times 10^3$).

Atom	Displacement of atoms from mean plane	$\sigma(r)$
N1	−5	13
C2	−12	14
N3	7	11
C4	6	12
C5	2	14
C6	15	13
N7	−16	12
N8	6	12
N9	−2	12
O6	−11	11
N2	−14	13
H1	−21	80 (est.)
H9	−14	80 (est.)
H2′	204	80 (est.)
H2″	97	80 (est.)
OW	295	17

cule are accurately coplanar. The hydrogen atoms on N1 and on N9 are also in that plane.

The amino group hydrogen atoms (H2′ and H2″) appear to be displaced from the plane, but little significance can be attached to these distances in view of the rather high standard deviations in the positions of the hydrogen atoms.

The azaguanine molecules are stacked on top of one another up the *a*-axis. The perpendicular distance between successive molecular planes is 3.245 A (σ = 0.01 A).

The distance between the molecular planes is shorter than the van der Waals approach normally found between the planes of aromatic molecules. For example, in graphite the interplanar spacing is 3.40 A.

It is believed that the short interplanar spacing in 8-azaguanine represents something more than a van der Waals interaction.

It might be argued that the van der Waals distance between sheets of nitrogen atoms would be less than that between sheets of carbon atoms, in a comparable state of hybridization, owing to the smaller size of the nitrogen atom. If this were the explanation for the short interplanar spacing in 8-azaguanine, the molecules would have to be arranged in the crystal in such a way that the carbon atoms on one molecule would not overlap the carbon atoms on molecules above or below. In fact, carbon atoms on adjacent molecules are permitted a rather high degree of interaction with each other as is evident from Fig. 2.

A projection of a molecule on to the plane of the molecule below is shown in Fig. 2. The molecules do not overlap directly, one being displaced somewhat relative to the other.

The crystal structure observed is similar to that found in charge-transfer complexes between aromatic molecules (10). The interplanar separation of 3.25 A is of the order found in such complexes. The relative displacement of the molecules shown in Fig. 2, is frequently observed in charge-transfer complexes. It has been shown that, in some cases, bonding can be achieved only when such displacement occurs (11, 12).

One property of the crystal, however, is not in accord with the charge-transfer

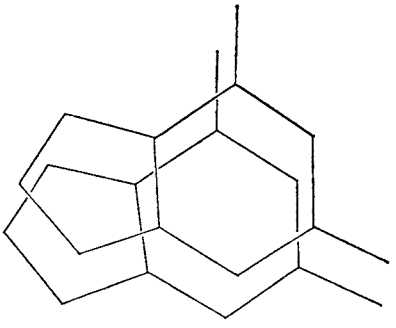


FIGURE 2 Diagram shows a projection of one molecule of 8-azaguanine on to the molecule below. The direction of the projection is normal to the molecular planes. The molecular plane coincides approximately with the crystallographic plane (102).

complex interpretation. The charge-transfer absorption spectrum normally occurs in the visible regions; the crystals of 8-azaguanine monohydrate are colorless. In discussing charge-transfer selfcomplexes Mulliken has pointed out that their charge-transfer absorption will occur at shorter wavelengths than is the case with hetero-complexes (13). It is possible that it may occur in the ultraviolet region. In such a case the crystal could be colorless.

Charge-transfer selfcomplexes—those in which donor and acceptor molecules are the same—are uncommon, although one other structure studied in this laboratory has been interpreted as a charge-transfer selfcomplex (dipotassium squarate monohydrate) (14).

Proposed Mechanism for the Biological Activity. Whatever the explanation, the approach of 3.25 A between the planes of 8-azaguanine molecules is a definite indication of a bonding interaction between the molecules and is of some biological interest.

It is known that 8-azaguanine acts as a cell poison only after incorporation into the RNA of the cell.

The following hypothesis is offered to describe the poisoning action of 8-azaguanine. Suppose that an 8-azaguanine molecule, after incorporation into messenger RNA, forms a complex with another 8-azaguanine molecule, in the nucleic acid, or with one of the four natural bases. This complex would be of the type found in the crystals of 8-azaguanine monohydrate.

The formation of such a complex would cause the RNA molecule to form a closed loop as shown in Fig. 3*b*. If such a loop should be formed, a possible mechanism for the biological action of 8-azaguanine suggests itself. Fig. 3*a* illustrates schematically a series of ribosomes in position on a molecule of messenger RNA during protein synthesis. Protein synthesis is considered to involve movement of a ribosome from one end of the messenger RNA molecule to the other (15).

An obstruction on the RNA molecule, which prevents free movement of the ribosome down that RNA molecule, will interfere with protein synthesis. Such an obstruction would be provided by the 8-azaguanine complex (Fig. 3*b*). The ribosomes would not be able to proceed along the RNA molecule beyond the complex

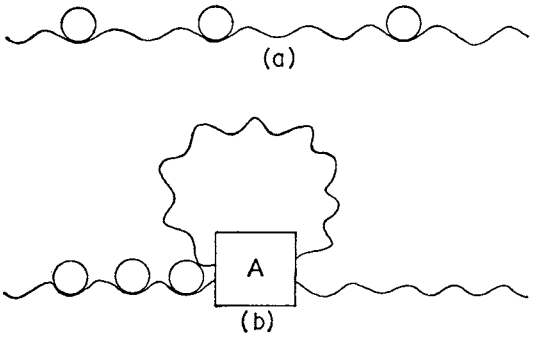


FIGURE 3 Highly schematic representation of two polysomes. (*a*) Normal polysome with RNA molecule and three ribosomes represented. (*b*) Polysome in which RNA is self-complexed at A through 8-azaguanine. The complex at *A* is supposed to prevent the movement of ribosomes past *A*. In both (*a*) and (*b*) the ribosomes are considered to be moving from left to right.

and the only polypeptides produced would be of comparatively low molecular weight. Under such circumstances the cell would lose all or part of its enzyme system and cease to be a viable unit.

It must be emphasized that this mechanism is only a suggestion. There is nothing in the x-ray analysis to verify it. However, it should be possible to put the suggestion on to a firmer basis by electron microscopy of the polysomes in 8-azaguanine inactivated cells.

Hydrogen Bonding. The hydrogen bonding pattern observed in 8-azaguanine monohydrate is shown in Fig. 1. The lengths of the hydrogen bonds are given in Table VII. Some of the relevant angles are given in Table VIII.

All but one of the hydrogen bonds are between molecules at roughly the same x-level. The exception is the bond between the water molecules. Thus the hydrogen bonds help to hold together sheets of molecules. Such a sheet is connected to the

TABLE VII

LENGTHS OF HYDROGEN BONDS IN 8-AZAGUANINE MONOHYDRATE AND THEIR STANDARD DEVIATIONS (σ)

All quantities are given in A. The full line in the bond designation indicates a σ bond between hydrogen and the heavier atom.

Bond	Bond length (r)	$\sigma(r)$
N1–H - - - - - - N7	2.926	0.017
N9–H - - - - - - N3	2.904	0.016
OW–H - - - - - - OW	2.759	0.024
OW–H - - - - - - O6	3.027	0.020
N2–H - - - - - - O6	2.952	0.017
N2–H - - - - - - OW	2.839	0.021

TABLE VIII

HYDROGEN BOND ANGLES IN 8-AZAGUANINE MONOHYDRATE AND THEIR STANDARD DEVIATIONS

All quantities are given in degrees. The vertex atom is the proton donor in each case. $\overline{\text{OW}}$ represents the water oxygen atom related by the center of symmetry to OW, and hydrogen bonded to OW.

Atoms	Angle	σ
OW N2 C2	124.6	1.0
C2 N2 O6	140.2	1.0
N2 OW $\overline{\text{OW}}$	93.1	0.7
O6 OW $\overline{\text{OW}}$	105.1	0.6
N7 N1 C2	112.9	0.9
N8 N9 N3	121.0	0.8

ones above and below by the interaction between successive azaguanine molecules and by the hydrogen bond between the water molecules.

Fig. 1 shows the position of the hydrogen atoms associated with each of the hydrogen bonds. As is usually the case, the hydrogen atoms are displaced slightly from the line of the bond.

The most remarkable feature of the hydrogen bonding system is that N8 forms no hydrogen bond. It is natural to assume that hydrogen bonding to N8 is an important element in the biological action of 8-azaguanine. The absence of such a bond in the crystal does not mean that the 8-azaguanine molecule never forms such a bond. However, experience with other hydrogen bonding systems in crystals would lead one to expect all atoms to take part in the hydrogen bonding which possibly can. Thus it would seem that the electron distribution on the 8-azaguanine ring, in the crystal at any rate, is such as to leave N8 relatively positive, and so less likely to form hydrogen bonds than N7 or N9.

The length of the hydrogen bond between the water molecules, 2.75 A, is in the range most commonly found for O-H---O bonds (16). Likewise there is nothing remarkable about the lengths of the bonds N1-H---N7 (2.93 A) and N9-H-----N3 (2.90 A).

The bond OW-H-----O6 (3.03 A) is at the long end of the range for such bonds. However an analogous bond, from a water oxygen atom to a keto oxygen atom, has been found in Rochelle salt with length 3.07 A (16). Several other examples are given of such bonds with lengths close to 3 A (16). Thus the OW-H---O6 bond is not unique in being relatively long.

Pimentel and McClellan (16) find that the bond lengths of N-H---O hydrogen bonds, formed by amide nitrogens, are most commonly found to lie in the range 2.8 to 3.0 A. On the other hand, if the nitrogen atom is an amino nitrogen, the N-H---O bond lengths are most commonly in the range 3.0 to 3.2 A. In 8-azaguanine, N2 is formally an amino nitrogen atom. However, as was pointed out above, the C2-N2 bond is quite short, indicating a drift of electrons into the C-N bond. Thus, in fact, N2 can be expected to behave more like an amide nitrogen atom. The hydrogen bonds formed by N2 are both shorter than 3.0 A, in general agreement with this reasoning.

CONCLUSION

The purpose of this x-ray analysis was to determine whether the 8-azaguanine molecule demonstrated any structural properties in the crystals of the monohydrate which might shed light on the mechanism of the cell poisoning action. While such an analysis could not be expected to elucidate the mechanism, it was hoped that the results might indicate avenues of approach which might be explored in other ways.

The outstanding findings in the x-ray analysis were, (*a*) the discovery of the very

close approach between the planes of the azaguanine molecules, and (*b*) the absence of a hydrogen bond to N8.

If indeed N8 does not form hydrogen bonds in the biological system, it becomes easier to understand why 8-azaguanine is incorporated into RNA so readily. If N8 were to form hydrogen bonds in the biological system, these bonds would be likely to interfere in the RNA synthesis and so inhibit the RNA synthesis. It would appear then that the cell poisoning by 8-azaguanine should involve some property of the molecule which operates only after incorporation. It is proposed, therefore, that the interaction between 8-azaguanine molecules, indicated by the close approach of 3.25 A as found in this analysis, is a more logical structural explanation of the biological activity. It is easier to see how such an interaction can inhibit the transfer of information by RNA than to see how it can affect the incorporation of 8-azaguanine into RNA.

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APPENDIX

OBSERVED STRUCTURE FACTORS FOR 8-AZAGUANINE (× 10).

L	K	H=0	1	2	3	4
0	0	3520	270	316	67	U
0	1	A	145	90		U
0	2	277	177	133	90	U
0	3	A	U	64	U	53
0	4	223	252	28	U	40
0	5	A	115	47	U	
0	6	331	259	50	38	
0	7	A	115	33	39	
0	8	U	83	64	64	
0	9	A	87	29	44	
0	10	174	170	81	77	
0	11	A	23	30	30	
0	12	63	U	25		
0	13	A	23	34		
0	14	77	27			
1	0	A	A	A	A	
1	1	452	651	205	38	
1	2	87	43	259	22	
1	3	209	131	26	U	
1	4	305	105	U	31	27
1	5	U	71	74	U	37
1	6	117	U	104	56	
1	7	51	58	58	21	
1	8	142	159	67	49	
1	9	U	U	120	68	
1	10	26	U	60	22	
1	11	60	43	U		
1	12	U	U	23		
1	13	U	26			
1	14	30	23			
2	0	402	1843	124	167	28
2	1	U	268	78	91	U
2	2	170	U	54	36	33
2	3	329	86	29	28	22
2	4	447	46	67	23	U
2	5	214	72	34	U	23
2	6	472	242	80	34	
2	7	74	53	28	40	
2	8	152	31	20	29	
2	9	104	57	32	U	
2	10	254	108	81	40	
2	11	33	U			
2	12		30			
2	13		U			
2	14		40			
3	0	A	A	A	A	A
3	1	531	165	439	99	U
3	2	183	103	64	65	U
3	3	593	164	67	U	U
3	4	199	199	26	U	31
3	5	27	196	45	40	
3	6	320	118	U	56	
3	7	38	23	42	36	
3	8	74	55	71	49	
3	9	130	81	40	67	
3	10	91	U	U		
3	11		75	30		
3	12		U			
3	13		U			
3	14		29			
4	0	468	465	791	U	80
4	1	80	36	141	40	58
4	2	437	81	49	U	23
4	3	32	246	27	U	27
4	4	254	76	U	U	U
4	5	276	154	40	U	U
4	6	133	235	118	26	23
4	7	193	59	58	67	
4	8	96	84	26	U	
4	9	49	108	58	U	
4	10	225	164	43	32	
4	11	U	83	U		
4	12	U	60	32		
4	13	25	20			
5	0	A	A	A	A	A
5	1	380	75	40	217	59
5	2	420	U	U	74	26
5	3	383	253	86	23	25
5	4	58	101	U	U	U
5	5	119	131	159	43	20
5	6	146	136	50	26	
5	7	U	U	20	27	
5	8	U	U	24	23	
5	9	227	93	64	U	
5	10	64	84	20	21	
5	11	137		58		
5	12	U				
5	13	51				
6	0	179	396	223	285	38
6	1	313	73	70	110	94
6	2	23	99	23	29	
6	3	240	U	73	25	
6	4	131	82	U	U	
6	5	U	U	92	U	
6	6	199	28	91	42	
6	7	U	187	41	53	
6	8	105	U	48	24	
6	9	43	38	73	39	
6	10	116	217	94		
6	11	55	25	50		
6	12	40				
6	13	44				
7	0	A	A	A	A	A
7	1	322	90	U	U	116
7	2	20	139	U	U	43
7	3	266	235	103	42	33
7	4	94	23	U	21	
7	5	350	32	91	64	
7	6	57	53	U	U	
7	7	45	U	U	U	
7	8	166	35	39	27	
7	9	42	159	25	41	
7	10	86	42	43		
7	11	35	94			
7	12	56	U			
7	13		36			
8	0	143	259	143	79	105
8	1	228	134	81	34	42
8	2	338	U	U	U	
8	3	241	94	37	39	
8	4	289	198	53	U	
8	5	54	37	79	47	
8	6	36	144	U	47	
8	7	107	58	60	U	
8	8	129	74	31	30	
8	9	U	26	U	36	
8	10	106	81	136		
8	11	U	U	31		
8	12	57	35			
8	13		25			
9	0	A	A	A	A	
9	1	167	144	48	U	
9	2	193	53	35	45	
9	3	42	84	77	39	
9	4	40	65	U	U	
9	5	122	150	34	53	
9	6	U	45	26	26	
9	7	121	63	U		
9	8	59	U	U		
9	9	U	23	83		
9	10	59	49	U		
9	11	23	26	52		
9	12	21	40			
9	13	39				
10	0	353	94	132	45	
10	1	150	70	38	33	
10	2	83	176	33	U	
10	3	23	33	U	20	
10	4	63	197	97	36	
10	5	U	U	30	43	
10	6	137	76	57	23	
10	7	88	35	59	23	
10	8	52	82	30		
10	9	79	U	U		
10	10	U	53	23		
10	11	U	U			
10	12	61	32			
11	0	A	A	A	A	
11	1	64	171	92	37	
11	2	43	76	48	U	
11	3	64	U	37	20	
11	4	100	U	36	U	
11	5	188	U	138	84	
11	6	235	43	U	23	
11	7	23	105	47		
11	8	U	80	31		
11	9	33		U		
11	10			22		
12	0	175	139	U	69	
12	1	178	98	U	U	
12	2	177	66	84	78	
12	3	127	60	32	U	
12	4	79	U	81	41	
12	5	36	33	U	21	
12	6	177	84	64	29	
12	7	213	81	29		
12	8	51	26			
12	9	84	53			
12	10		U			
12	11		23			
13	0	A	A	A	A	
13	1	132	32	78	42	
13	2	U	57	28	22	
13	3	31	29	39	39	
13	4	112	U	U	U	
13	5	158	165	63	60	
13	6	U	133	44		
13	7	39	21	61		
13	8	119	U	59		

(*Appendix—continued*)

OBSERVED STRUCTURE FACTORS FOR 8-AZAGUANINE (× 10)
(continued)

L	K	H=0	1	2	3
13	9	117	26		
13	10	46			
14	0	115	82	73	35
14	1	53	61	44	U
14	2	148	138	U	U
14	3	82	36	22	23
14	4	163	22	U	
14	5	74	23	25	
14	6	42	120	40	
14	7	64	148	29	
14	8	U	29		
14	9	46	60		
14	10	32	24		
15	0	A	A	A	
15	1	U	38	U	
15	2	176	23	47	
15	3	177	53	48	
15	4	101	57	23	
15	5	76	112	77	
15	6	34	53	74	
15	7	U	36		
15	8	U	29		
15	9	U	71		
15	10	36			
16	0	191	50	36	
16	1	47	66	U	
16	2	39	123	72	
16	3	U	75	U	
16	4	146	164	30	
16	5	60	40	22	
16	6	95	36	70	
16	7	26	26		
16	8	71			
17	0	A	A	A	
17	1	60	38	26	
17	2	27	31	U	
17	3	95	98	U	
17	4	37	81	43	
17	5	30			
17	6	71			
18	0	137	130	35	
18	1	77	22	32	
18	2	66	45		
18	3	92	77		
18	4	81	88		
18	5	32	22		
18	6	26	43		
19	0	A	A		
19	1	43	U		
19	2	86	43		
19	3	53	71		
19	4	U	U		
19	5	U	U		
19	6	61	U		
20	0	67	85		
20	1	22	32		
20	2	40	23		
20	3	U			
20	4	43			

L	K	1	2	3	4
-1	0	A	A	A	A
-1	1	457	127	37	
-1	2	462	73	81	68
-1	3	260	77	29	41
-1	4	35	U	94	U
-1	5	26	U	55	28
-1	6	190	153	33	33
-1	7	64	U	23	
-1	8	57	84	U	
-1	9	139	132	55	
-1	10	94	29	28	
-1	11	U	81		
-1	12	40			
-2	0	366	102	25	32
-2	1	77	U	23	44
-2	2	387	117	40	34
-2	3	168	43	110	39
-2	4	126	41	130	61
-2	5	115	31	U	U
-2	6	115	91	28	U
-2	7	30	U	U	29
-2	8	98	85	39	
-2	9	30	44	34	
-2	10	88	89	30	
-2	11	U	29	42	
-2	12	U	36		
-2	13	27			
-3	0	A	A	A	A
-3	1	425	119	26	48
-3	2	177	143	U	U
-3	3	228	148	U	U
-3	4	U	143	U	U
-3	5	84	161	31	21
-3	6	222	90	57	70
-3	7	U	35	U	
-3	8	77	36	U	
-3	9	262	92	39	
-3	10	43			
-3	11	118			
-3	12	20			
-3	13	33			
-4	0	U	30	93	U
-4	1	135	33	126	31
-4	2	91	149	74	30
-4	3	43	137	36	57
-4	4	50	172	44	33
-4	5	64	41	U	U
-4	6	124	27	44	54
-4	7	U	U	61	60
-4	8	88	86	72	
-4	9	34	35	U	
-4	10	84	64	28	
-4	11	50	67		
-4	12	43	42		
-4	13	36			
-5	0	A	A	A	A
-5	1	347	72	87	41
-5	2	134	65	98	62
-5	3	334	47	U	41

L	K	1	2	3	4
-5	4	206	29	50	26
-5	5	394	90	24	48
-5	6	95	43	36	59
-5	7	21	U	U	
-5	8	103	36	69	
-5	9	101	U	49	
-5	10	55	65	50	
-5	11	33			
-5	12				
-6	0	U	308	U	36
-6	1	146	236	74	27
-6	2	301	134	43	U
-6	3	239	75	52	57
-6	4	227	38	95	73
-6	5	78	26	51	30
-6	6	22	74	89	33
-6	7	62	105	124	
-6	8	68	66		
-6	9	29	29		
-6	10	96	33		
-6	11	43	U		
-6	12	53	26		
-7	0	A	A	A	A
-7	1	53	U	64	69
-7	2	81	98	98	82
-7	3	74	26	U	67
-7	4	U	98	46	U
-7	5	177	U	62	61
-7	6	U	104	80	41
-7	7	64	29	36	
-7	8	40	101	139	
-7	9	U	31		
-7	10	60			
-7	11	U			
-7	12	U			
-7	13	32			
-8	0	530	51	34	33
-8	1	212	139	21	98
-8	2	161	93	20	44
-8	3	32	U	91	U
-8	4	30	196	104	29
-8	5	U	60	54	40
-8	6	99	147	U	
-8	7	201	217	46	
-8	8	101	33	40	
-8	9	46	28		
-8	10	U	20		
-8	11	32			
-8	12	20			
-9	0	A	A	A	A
-9	1	U	88	120	53
-9	2	139	115	156	U
-9	3	64	53	135	88
-9	4	136	77	30	33
-9	5	80	77	107	46
-9	6	238	80	74	
-9	7	28	41	24	
-9	8	72	167	25	
-9	9	29	40		

OBSERVED STRUCTURE FACTORS FOR 8-AZAGUANINE (× 10) (*concluded*)

L	K	1	2	3	4	L	K	1	2	3
-9	10		34			-15	0	A	A	A
-10	0	116	32	48	36	-15	1	102	92	
-10	1	253	U	143	38	-15	2	30	153	
-10	2	200	29	54	48	-15	3	156	U	30
-10	3	95	121	23	38	-15	4	43	34	U
-10	4	205	189	36	62	-15	5	84	71	43
-10	5	59	105	97		-15	6	61	96	
-10	6	215	58	U		-15	7	U	23	
-10	7	240	U	54		-15	8	U		
-10	8	36	67	36		-15	9	U		
-10	9	66	48	36		-15	10	77		
-10	10	34	40			-16	0	127	77	25
-10	11	21	51			-16	1	100	46	25
-11	0	A	A	A	A	-16	2	68	55	U
-11	1	124	156	100	33	-16	3	108	46	U
-11	2	37	256	U	112	-16	4	76	72	32
-11	3	74	197	143	41	-16	5	81	43	
-11	4	105	69	26		-16	6	26	54	
-11	5	107	119	81		-16	7	U	20	
-11	6	45	53	34		-16	8	45	63	
-11	7	44	U	U		-17	0	A	A	
-11	8	209	29	31		-17	1	67		
-11	9	82	U			-17	2	115	32	
-11	10	57	U			-17	3	U	U	
-11	11	45	50			-17	4	U	U	
-12	0	51	167	U	49	-17	5	45	35	
-12	1	U	132	62		-17	6	105	29	
-12	2	88	40	22		-17	7	68	53	
-12	3	110	66	70		-18	0	86	27	
-12	4	237	54	63		-18	1	U	49	
-12	5	121	114	98		-18	2	60	U	
-12	6	65	47	36		-18	3	20	U	
-12	7	50	43	29		-18	4	72	29	
-12	8	74	57	21		-18	5	23	27	
-12	9	46	43			-18	6	68		
-12	10	24	26			-18	7	37		
-13	0	A	A	A		-19	0	A	A	
-13	1	95	122	77		-19	1	U	29	
-13	2	292	25	153		-19	2	19	U	
-13	3	236	174	23		-19	3	U	46	
-13	4	96	22	26		-19	4	U	37	
-13	5	118	118	60		-19	5	U		
-13	6	30	41	32		-19	6	26		
-13	7	U	U			-20	0	32		
-13	8	U	U			-20	1	37		
-13	9	U	27			-20	2	U		
-13	10	U				-20	3	U		
-13	11	30				-20	4	24		
-14	0	225	55	U		-21	0	A		
-14	1	77	94	65		-21	1	28		
-14	2	U	36	36						
-14	3	76	104	39						
-14	4	104	51	38						
-14	5	87	109	48						
-14	6	61	U	40						
-14	7	65	59							
-14	8	59								
-14	9	23								
-14	10	30								