

## The Crystal Structure of 8-Azaadenine Hydrochloride

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The crystal and molecular structure of 8-azaadenine hydrochloride,  $C_4H_5N_6Cl$ , has been determined from three-dimensional counter X-ray data. The purine derivative crystallizes in the monoclinic space group  $P2_1$ , with two molecules in a cell of dimensions  $a=7.728$  (4),  $b=7.177$  (4),  $c=6.122$  (4) Å,  $\beta=91.54$  (3)°. The structure has been refined by least-squares techniques using 513 independent data to a conventional  $R$  (on  $F$ ) of 0.038. The structure consists of disordered 8-azaadeninium cations which are hydrogen bonded to chloride anions. The disorder involves two orientations of the cation with C(4) and C(6) common to both, and one orientation is related to the other by an approximately 180° rotation around the C(4)–C(6) vector. Consequently, atoms C(2) and N(7) in one orientation occupy the sites of N(7) and C(2) respectively in the other orientation. An examination of the hydrogen bonding suggests that the cationic proton is on N(1). The apparent stability of 8-azaadenine to the acid conditions employed strongly suggests that the previously documented hydrolysis of this purine analogue on coordination to  $Cu^{2+}$  is primarily due to the interaction with the metal ion. The corresponding hydrobromide salt is isomorphous with the hydrochloride, and disorders in an analogous manner.

### Introduction

*ortho*-Azapurines, which are purines in which the C atom in the 8-position has been replaced by a N atom, have long been known to be potent chemotherapeutic agents (Skoda, 1963; Bennett & Montgomery, 1967; Bennett, Vail, Allan & Laster, 1973). For example, 8-azaguanine is noted for its antineoplastic value (Kidder & Dewey, 1949); 8-azaadenosine has also been studied for its antileukemic activity (Shealy & Clayton, 1973).

Chemical studies have shown that the substitution of a N atom into the 8-position of the purine alters the orientation of the purine ring relative to the sugar ring in the *ortho*-azapurine nucleosides (Koyama, Maeda, Umezawa & Iitaka, 1966; Prusiner, Brennan & Sundaralingam, 1973). It has been suggested that this change in orientation leads to the disruption of the hydrogen-bonding scheme of the nucleic acid into which the nucleoside is incorporated (Ward & Reich, 1969; Ward, Cerami, Reich, Acs & Altwerger, 1969). Studies have also been performed to determine the electronic effect of the substitution of the 8-position on the other positions of the ring, specifically on the resulting basicity of these positions. For example, N(7) has been found to be the most basic site of guanine since it is the site of protonation in the hydrochloride (Broomhead, 1951; Iball & Wilson, 1965) while N(3) and N(8) are the sites of protonation in 8-azaguanine hydrochloride and hydrobromide (Kozłowski, Singh & Hodgson, 1974, 1975). Crystallographic studies indicate that the site of protonation in adenine hydrochloride is N(1) (Kistenmacher & Shigematsu, 1974; Broomhead, 1948; Cochran, 1951), and Pullman &

Pullman (1963) have calculated that the preferred site of protonation in 8-azaadenine hydrochloride is N(1).

In addition to our interest in the site(s) of protonation of 8-azaadenine hydrochloride, we have also been concerned with the question of the stability of 8-azaadenine in acid solution. In a recent reaction of 8-azaadenine with  $Cu^{2+}$  in 0.36M HCl, we found that hydrolysis occurred at the C(2) position forming [(5-amino-4-carboxamidinium)-1,2,3-triazole]copper(II) monohydrate (Purnell, Shepherd & Hodgson, 1975). In order to determine if the hydrolysis was caused by the metal interaction or by the addition of the hydrochloric acid, we repeated the preparation in the absence of copper ions. We report here the results of that preparation.

### Experimental

Crystalline clusters of 8-azaadenine hydrochloride were grown from a 0.36M hydrochloric acid solution. One small diamond-shaped plate was removed from a cluster and used for data collection. The crystal was assigned to the monoclinic system on the basis of Weissenberg and precession photographs. The observed systematic absences of  $k=2n+1$  for  $0k0$  suggested that the space group was either  $P2_1/m$  or  $P2_1$ ; refinement of the structure revealed that the correct choice was the non-centrosymmetric space group  $P2_1$ . With the least-squares method of Busing & Levy (1967), lattice constants of  $a=7.728$  (4),  $b=7.177$  (4),  $c=6.122$  (4) Å and  $\beta=91.54$  (3)° were obtained. The calculated density is 1.689 g cm<sup>-3</sup> based on two formula units per unit cell. The observed density, obtained by flotation in iodomethane and dichloromethane, is 1.68 (2) g cm<sup>-3</sup>.

The diamond-shaped crystal plate with faces of (001), (00 $\bar{1}$ ), (010), (0 $\bar{1}$ 0), (130), and ( $\bar{1}$ 30) was mounted

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on a glass fiber and oriented parallel to the (001) face; the crystal dimensions found using these pairs of opposite faces were 0.065, 0.175 and 0.159 mm, respectively. Mosaicity tests using the narrow source, open counter  $\omega$ -scan technique indicated that the crystal was acceptable for data collection. Twelve reflections, centered at a take-off angle of  $1.0^\circ$  through a narrow slit, formed the basis for a least-squares refinement, and, with the logic of Busing & Levy (1967) in the PDP-8/L computer, the cell parameters and orientation matrix were refined.

Data were collected on an automatic four-circle Picker diffractometer using Mo  $K\alpha$  radiation and a graphite monochromator. The take-off angle used for collecting intensity data was  $1.2^\circ$ , which was the angle at which the peak intensity of a strong reflection was approximately 90% of the maximum value as a function of take-off angle. The counter aperture, positioned 32 cm from the crystal, was 5.0 mm high and 5.0 mm wide. With the  $\theta$ - $2\theta$  scan technique, data were collected at a scan rate of  $0.5^\circ \text{ min}^{-1}$ . To allow for both  $K\alpha_1$  and  $K\alpha_2$  radiations, the scan range was from  $0.7^\circ$  below the calculated  $K\alpha_1$  peak position to  $0.7^\circ$  above the calculated  $K\alpha_2$  peak position. After each scan, stationary-counter, stationary-crystal background counts of 20 s were taken at both ends of the scan.

A unique data set having  $2\theta (\text{Mo}) \leq 50^\circ$  was obtained, yielding 650 independent intensities. After every 75 reflections, the intensities of three standard reflections were measured; these standards showed only slight attenuation throughout the run.

Using the data processing method of Corfield, Døedens & Ibers (1967), intensities were assigned standard deviations according to the formula  $\sigma(I) = [C + 0.25(t_s/t_b)^2(B_H + B_L) + (pI)^2]^{1/2}$  after the data were corrected for background. A value of 0.03 was given  $p$ , the term in the expression used to prevent extremely high weight being given to a very intense reflection (Busing & Levy, 1957). The values of  $I$  and  $\sigma(I)$  were corrected for

Lorentz and polarization effects. Since the absorption coefficient was only  $5.0 \text{ cm}^{-1}$ , no correction for absorption was made. No correction for the decomposition mentioned above was applied. Of the 650 reflections, 513 were found to be greater than three times their estimated standard deviation,  $\sigma$ ; these 513 reflections were the only ones used in the refinement of the structure.

### Solution and refinement

The structure was solved by direct methods (Hauptman & Karle, 1953), with the multiple solution program *MULTAN* (Main, Woolfson & Germain, 1971). The first solution chosen revealed that the structure was disordered, and gave the locations of the heavy atoms of the disordered azaadenine ring and the chloride ion. Least-squares refinement on  $F$  was performed, the function minimized being  $\sum w(|F_o| - |F_c|)^2$ ; the weights,  $w$ , were taken as  $4F_o^2/\sigma^2(F_o)^2$ . In all calculations of  $F_c$ , the atomic scattering factors for N were taken from Cromer & Waber (1965), those for C from Ibers (1962), those for Cl from Hanson, Herman, Lea & Skillman (1964), and those of H from Stewart, Davidson & Simpson (1965). A scattering factor was obtained for the two atoms which are assigned as C atoms in one of the disordered rings and N atoms in the other ring from averaging the above-mentioned C and N tables. The effects of the anomalous dispersion of Cl were included in the calculation of  $F_c$  (Ibers & Hamilton, 1964), the values of  $\Delta f'$  and  $\Delta f''$  being taken from the tabulations of Cromer & Liberman (1970). Least-squares refinement of each disordered fragment led to the same  $R$  value (see below) (0.18), and so a 50/50 disorder was deduced; in subsequent calculations, the occupancies of the disordered atoms were therefore set equal to 0.5.

Preliminary attempts at full-matrix refinement of the disordered structure failed, the refinement oscillating wildly; this is presumably due to the paucity of data and the high correlations between parameters. Consequently, refinements were performed in blocks, with one cycle of least-squares refinement on the positional and thermal parameters of ring *A* of the disordered model, holding the parameters of the 8-azaadenine *B* constant, one cycle performed on 8-azaadenine *B*, holding the parameters of 8-azaadenine *A* constant, and one cycle performed on both 8-azaadenine *A* and 8-azaadenine *B* (see Fig. 1). The Cl atom and the four shared positions were included in all cycles. Isotropic refinement of the disordered model gave  $R_1 (= \sum |F_o| - |F_c| / \sum |F_o|)$  0.100 and  $R_2 \{ = [\sum w(|F_o| - |F_c|)^2 / \sum w|F_o|^2]^{1/2} \}$  0.110. Anisotropic refinement of the structure gave a final  $R_1$  of 0.038 and  $R_2$  of 0.042. Attempts to refine the structure in the centrosymmetric space group  $P2_1/m$  were unsuccessful. No H atoms could be located in the final difference Fourier map. Examination of the final values of  $|F_o|$  and  $|F_c|$  suggested that no correction for secondary extinction was necessary. In the final cycle of least-squares refinement, several thermal

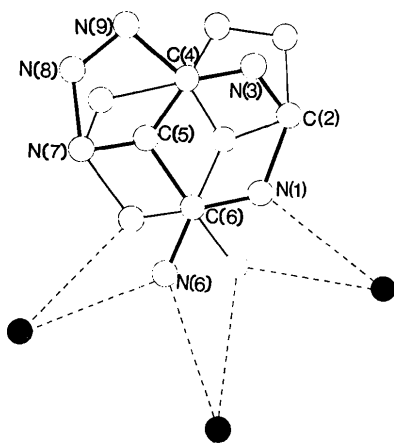


Fig. 1. View of the disordered molecule. The wide-lined cation is *A* and the narrow-lined cation *B*. The atoms in cation *A* are labeled. The shaded spheres are three symmetry-related chloride ions which are hydrogen bonded to the cations.

parameters experienced a shift of as much as 1.8 times their estimated standard deviation; since some of the  $U_{ij}$ 's are non-positive definite (see Table 2), this failure to converge is insignificant and is presumably due to the approximate nature of the model and the dearth of data. The final difference Fourier map was essentially free of electron density, the highest peak being  $0.31 \text{ e } \text{Å}^{-3}$ .

Tables 1 and 2 contain the positional and thermal parameters from the last cycle of least-squares refinement, along with their standard deviations as estimated from the inverse matrix.\* As in Fig. 1, *A* and *B* refer to the different 8-azaadenine rings in the disordered model, *A* being the ring joined by thick lines and *B* the ring joined by thin lines. The programs used in this analysis are documented elsewhere (Singh & Hodgson, 1974).

\* A list of structure factors has been deposited with the British Library Lending Division as Supplementary Publication No. SUP 31458 (5 pp.). Copies may be obtained through The Executive Secretary, International Union of Crystallography, 13 White Friars, Chester CH1 1NZ, England.

Table 1. *Positional parameters* ( $\times 10^4$ )

	<i>x</i>	<i>y</i>	<i>z</i>
Cl	4065 (2)	2500	1386 (2)
C(6) <i>A, B</i>	2926 (7)	7473 (29)	4214 (8)
C(4) <i>A, B</i>	1236 (7)	7668 (23)	7458 (9)
C(2) <i>A, N(7)B</i>	1834 (22)	10313 (15)	6220 (21)
N(7) <i>A, C(2)B</i>	2056 (26)	4687 (44)	6042 (29)
C(5) <i>A</i>	1884 (36)	6413 (43)	6032 (48)
N(6) <i>A</i>	3762 (22)	6530 (19)	2658 (24)
N(9) <i>A</i>	366 (35)	6214 (30)	8846 (35)
N(1) <i>A</i>	2903 (24)	9317 (24)	4430 (22)
N(8) <i>A</i>	774 (30)	4684 (29)	7832 (43)
N(3) <i>A</i>	1318 (29)	9461 (31)	7682 (28)
N(6) <i>B</i>	3687 (27)	8477 (32)	2539 (34)
C(5) <i>B</i>	2120 (30)	8477 (31)	5865 (27)
N(9) <i>B</i>	492 (26)	8647 (30)	8637 (26)
N(1) <i>B</i>	3078 (34)	5783 (28)	4290 (45)
N(8) <i>B</i>	643 (33)	10448 (29)	8147 (32)
N(3) <i>B</i>	1354 (39)	5346 (66)	7211 (51)

Table 2. *Thermal parameters* ( $U_{ij}$  in  $\text{Å}^2$ )

The form of the anisotropic thermal ellipsoid is

$$\exp [-2\pi^2(U_{11}h^2a^{*2} + U_{22}k^2b^{*2} + U_{33}l^2c^{*2} + 2U_{12}hka^*b^* + 2U_{13}hla^*c^* + 2U_{23}klb^*c^*) \times 10^{-3}].$$

	$U_{11}$	$U_{22}$	$U_{33}$	$U_{12}$	$U_{13}$	$U_{23}$
Cl	55 (1)	67 (1)	43 (1)	-4 (3)	17 (1)	3 (2)
C(6) <i>A, B</i>	24 (3)	54 (5)	26 (3)	-8 (9)	0 (2)	6 (7)
C(4) <i>A, B</i>	23 (3)	22 (4)	26 (3)	-9 (6)	3 (3)	22 (7)
C(2) <i>A, N(7)B</i>	68 (10)	4 (4)	43 (6)	-18 (5)	0 (5)	5 (5)
N(7) <i>A, C(2)B</i>	58 (9)	174 (17)	57 (9)	-9 (11)	13 (7)	-4 (10)
C(5) <i>A</i>	17 (11)	56 (18)	70 (16)	-3 (11)	14 (10)	-22 (17)
N(6) <i>A</i>	48 (13)	27 (10)	29 (7)	-20 (7)	16 (8)	26 (7)
N(9) <i>A</i>	60 (14)	22 (10)	73 (13)	-27 (9)	-2 (10)	21 (9)
N(1) <i>A</i>	37 (8)	6 (8)	22 (7)	0 (7)	36 (6)	11 (7)
N(8) <i>A</i>	13 (11)	47 (15)	27 (9)	-12 (8)	22 (7)	-8 (9)
N(3) <i>A</i>	25 (11)	28 (9)	15 (6)	-4 (9)	16 (6)	5 (9)
N(6) <i>B</i>	66 (15)	117 (44)	46 (9)	-36 (14)	14 (9)	52 (16)
C(5) <i>B</i>	32 (12)	0 (6)	14 (7)	9 (8)	2 (7)	-13 (7)
N(9) <i>B</i>	28 (12)	56 (12)	8 (5)	11 (8)	22 (7)	0 (7)
N(1) <i>B</i>	49 (15)	36 (13)	110 (19)	-4 (11)	-50 (13)	32 (14)
N(8) <i>B</i>	53 (14)	31 (14)	32 (9)	-14 (8)	19 (8)	-24 (8)
N(3) <i>B</i>	23 (15)	142 (36)	154 (39)	-18 (17)	19 (16)	119 (34)

### Description of the structure

The structure consists of disordered 8-azaadeninium cations hydrogen bonded to chloride ions. Fig. 1 shows a view of the disordered cations along with three symmetry-related chloride ions to which they are hydrogen bonded. The intramolecular distances and angles in the two 8-azaadenine rings and the average values of the two rings are all normal (Ringertz, 1972), considering their large standard deviations and the problems associated with the disorder of the rings. Their precision is so limited, however, that individual derived values are not listed here.

Attempts to locate the hydrogen atom(s) on the triazole portion of the purine through intermolecular and intramolecular hydrogen-bonding interactions were unsuccessful. The positions of the chloride ions, however, indicate that hydrogen bonds exist with N(6) and possibly with N(1). Here, N(6) of 8-azaadenine *A* interacts with two chloride ions at distances of 3.11 and 3.01 Å, and N(6) of 8-azaadenine *B* interacts with two chloride ions at distances of 3.08 and 2.99 Å. These distances are less than the sum of the van der Waals radii of the two atoms involved (Pauling, 1960). N(1) of 8-azaadenine *A* is 3.096 Å from a chloride ion, and N(1) of 8-azaadenine *B* is 3.060 Å from a chloride ion; therefore, there is a strong possibility that a hydrogen bond exists and, consequently, that the additional H atom due to the cationic nature of the purine is on N(1). If this is the case, the basicity of 8-azaadenine is comparable to that of adenine, which also is protonated at N(1) in the hydrochloride (Kistenmacher & Shigematsu, 1973; Broomhead, 1948; Cochran, 1951); it is, moreover, consistent with the quantum mechanical calculations of Pullman & Pullman (1963), which show that N(1) is the most basic atom in 8-azaadenine.

Neither of the 8-azapurine rings is planar, but both can be viewed as consisting of two approximately planar rings. Once again, the molecular dimensions of

this structure are not accurate and so the evaluation of planarity is only approximate. In 8-azaadenine *A*, the triazole ring is nearly planar with no atom deviating by more than 0.07 Å from the best five-atom least-squares plane; the pyrimidine ring is also approximately planar, with no atom deviating from the best six-atom least-squares plane by more than 0.09 Å. The angle between the two planes is 4.02°. In cation 8-azaadenine *B*, the triazole ring is once again planar with no deviation from the best five-membered least-squares plane greater than 0.01 Å; the pyrimidine ring is also planar with no deviation greater than 0.06 Å from the best six-membered least-squares plane. Here, the angle between the two planes is 2.69°. The angle between the best nine-membered least-squares planes of the *A* and *B* rings is 3.17°.

Although H atoms were not definitely located, the results of this study are very important in relation to our study of the interaction of Cu<sup>2+</sup> with 8-azaadenine (Purnell, Shepherd & Hodgson, 1975). It appears that the hydrolysis of the azapurine is indeed assisted by the coordination to the metal at the N(8) position, since the present study indicates that 8-azaadenine is stable under the same conditions in the absence of the metal ion. This interaction could be of chemotherapeutic value, if, when incorporated into the nucleic acid, 8-azaadenosine reacts with Cu<sup>2+</sup> ions in a similar manner. This hydrolysis could increase the antineoplastic potency of the drug through the use of 8-azapurine-metal couples, since the ring opening would presumably lead to a non-functional nucleic acid unit.

The corresponding hydrobromide salt, prepared in an analogous manner using HBr, is isomorphous with the hydrochloride. Least-squares refinement of the hydrobromide structure demonstrates that this system is disordered in a similar way to that described above for the hydrochloride salt.

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