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Crystal and Molecular Structure of N^4-Aminocytosine

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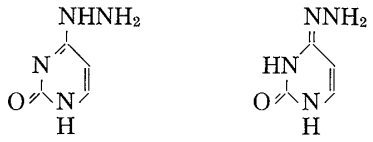
The crystal and molecular of N^4-aminocytosine was determined by X-ray diffraction. This compound belongs to the monoclinic space group $P2_1/a$ with four molecules in a unit cell of $a=7.950(2)$ Å; $b=11.156(2)$ Å; $c=6.054(3)$ Å; $\beta=94.59(2)°$; $V=535.2(3)$ Å^3. The final R value was 0.072 including hydrogen atoms. The present study established that this compound takes the C(4)-amino form rather than the imino form, and is consistent with the report (D.M. Brown, M.J.E. Hewlins and P. Schell, *J. Chem. Soc.* (*C*), **1968**, 1925 that 1-methyl-N^4-aminocytosine takes the amino form in solution.

Keywords——N^4-aminocytosine; crystal structure; molecular structure; tautomeric forms; X-ray diffraction

N^4-Aminocytosine is a compound of biological interest in view of its mutagenic activity and its incorporation as a residue in desoxyribonucleic acid (DNA) by the action of hydrazine.[2-5] Its structure has been suggested to be the amino form (**1**) rather than the imino form (**2**), based on ultraviolet absorption spectra in aqueous media.[6] Since such a structural assignment is ambiguous, we have determined its crystal structure by X-ray diffraction.



1 **2**

Fig. 1. Tautomeric Structures of N^4-Aminocytosine

Experimental Section

N^4-Aminocytosine was prepared by treatment of cytosine with a mixture of hydrazine, sodium bisulfite and sodium phosphate. Details of the reaction will be reported elsewhere. The material obtained was identical with an authentic sample[7] as judged from the ultraviolet absorption spectra (at pH 7 and pH 1) and by paper chromatography (solvent; ethanol–1 M ammonium acetate, pH 7.5, 7:3). Crystals were obtained by repeated recrystallization from aqueous solutions.

The intensity data for 2θ values within 80° were collected using a Rigaku four-circle diffractometer with graphite-monochromated Mo-Kα radiation by the ω-2θ scan method at a 2θ scan rate of 2°/min. The background was measured for 10 sec at each end of the scan. Those intensities exceeding three times the corresponding standard deviations were stored as observed; they were corrected for the Lorentz and polarization factors but not for the absorption and extinction factors. The atomic scattering factors for C, O and N were those given by Cromer and Mann,[8] and the factors for H, those given by Stewart *et al.*[9]

The structure of N^4-aminocytosine was determined by the symbolic addition method.[10] The distribution and statistics of $|E|$'s agreed well with the theoretical values calculated for the centrosymmetric case. The resulting E-map revealed the location of all nine non-hydrogen atoms. The oxygen and nitrogen atoms

1) Location: *a*) *Shirokane, Minato-ku, Tokyo 108, Japan*; *b*) *Tsushima, Okayama 700, Japan.*
2) B.C.F. Chu, D.M. Brown, and M.G. Burdon, *Mutation Res.*, **23**, 267 (1974).
3) R.F. Kimball, *Mutation Res.*, **39**, 111 (1977).
4) D.M. Brown, A.D. McNaught, and P. Schell, *Biochem. Biophys. Res. Comm.*, **24**, 967 (1966).
5) H. Hayatsu and A. Kitajo, "Progress in Genetic Toxicology," ed. by D. Scott, B.A. Bridges, and F.H. Sobels, Elsevier/North-Holland Biomedical Press, Amsterdam, 1977, pp. 285—292.
6) D.M. Brown, M.J.E. Hewlins, and P. Schell, *J. Chem. Soc.* (*C*), **1968**, 1925.
7) F. Lingens and H. Schneider-Bernlöhr, *Liebigs Ann. Chem.*, **686**, 134 (1965).
8) D. Cromer and J. Mann, *Acta Cryst.*, **A24**, 321 (1968).
9) R.F. Stewart, E.R. Davidson, and W.F. Sympson, *J. Phys. Chem.*, **42**, 3175 (1965).
10) I.L. Karle and J. Karle, *Acta Cryst.*, **A16**, 969 (1963); *idem*, *ibid.*, **A21**, 894 (1966).

were identified by structural considerations. Four cycles of least-squares refinement with isotropic temperature factors resulted in an R value of 0.13. Anisotropic thermal parameters were introduced for all non-hydrogen atoms, and the R value was reduced to 0.10. All hydrogen atoms were located on the difference map. Refinement with anisotropic temperature factors for non-hydrogen atoms and isotropic thermal factors for hydrogen atoms gave the final R value of 0.072 ($R=\sum||F_o|-|F_c||/\sum|F_o|$). The function minimized was $\sum w(|F_o|-|F_c|)^2$ with $w=1.0$ for all the reflections used.

Results and Discussion

The crystal was monoclinic, having space group $P2_1/a$ with four molecules in a unit cell of $a=7.950$ (2) Å; $b=11.156$ (2) A; $c=6.054$ (3) Å; $\beta=94.59$ (2)°; V=535.2 (3) Å^3 (the numbers in parentheses are the standard deviations). The calculated and observed densities were 1.560 g/cm^3 and 1.57 g/cm^3, respectively.

The atomic parameters are presented in Table I along with the standard deviations. Selected torsion angles are given in Table II. The bond lengths and angles are listed in Table

Table I.

(i) Fractional Atomic Coordinates ($\times 10^4$) and Anisotropic Temperature Factors[a] for Non-hydrogen Atoms

Atom	x	y	z	U_{11}	U_{22}	U_{33}	U_{12}	U_{13}	U_{23}
N (1)	810(3)[b]	3784(2)	8498(4)	447(14)	259(11)	408(13)	−22(9)	197(11)	−16(9)
C (2)	1498(4)	4724(2)	7435(4)	402(15)	282(12)	384(14)	−25(10)	128(12)	−14(10)
N (3)	2400(3)	4527(2)	5668(4)	438(13)	237(10)	354(11)	−22(9)	148(10)	−18(8)
C (4)	2562(4)	3396(2)	5000(4)	337(13)	283(12)	313(11)	−5(10)	73(9)	−11(9)
N (4)	3357(3)	3179(2)	3193(4)	511(15)	309(12)	410(12)	19(11)	145(11)	−50(10)
C (5)	1938(4)	2390(2)	6146(5)	446(16)	246(12)	430(14)	−5(11)	126(12)	−14(11)
C (6)	1069(4)	2629(2)	7898(5)	404(16)	266(12)	451(15)	−39(11)	92(13)	15(11)
N (7)	4136(4)	4075(2)	1962(5)	543(17)	410(14)	400(14)	−32(13)	189(12)	−19(11)
O (2)	1288(3)	5759(2)	8173(4)	755(15)	222(9)	586(13)	−34(9)	392(12)	−43(8)

(ii) Atomic Coordinates ($\times 10^3$) and Isotropic Temperature Factors ($\times 10^2$) for Hydrogen Atoms

Atom	x	y	z	U
H (11)	22(4)	394(2)	954(4)	2(1)
H (41)	362(4)	247(3)	283(5)	5(1)
H (51)	219(5)	160(3)	568(5)	6(1)
H (61)	57(4)	200(3)	899(5)	4(1)
H (71)	340(5)	463(4)	156(6)	1(1)
H (72)	503(5)	440(3)	288(5)	7(1)

a) The anisotropic temperature factors are expressed in the following form: $\exp[-2\pi^2(U_{11}h^2a^{*2}+U_{22}k^2b^{*2}+U_{33}l^2c^{*2}+2U_{12}hka^*b^*+2U_{13}hla^*c^*+2U_{23}klb^*c^*)]$.
b) Standard deviations are given in parentheses.

Table II. Selected Torsion Angles (°)[a]

C (5)-C (4)-N (4)-N (7)	4.1
C (4)-N (4)-N (7)-H (71)	−55.7
C (4)-N (4)-N (7)-H (72)	64.8
H (41)-N (4)-N (7)-H (71)	136.6
H (41)-N (4)-N (7)-H (72)	−103.0

a) Torsion angle A(i)–A(j)–A(k)–A(l) is viewed down A(j)-A(k) with a clockwise rotation of A(i) to A(l) taken to be positive.

Table III. Bond Lengths and Bond Angles

	Bond lengths (Å)			Bond angles (°)	
	N^4-Aminocytosine	Cytosine[a]		N^4-Aminocytosine	Cytosine[a]
N(1)–C(2)	1.368(4)[b]	1.371	N(1)–C(2)–N(3)	120.3(2)	119.6
C(2)–N(3)	1.352(4)	1.350	C(2)–N(3)–C(4)	117.6(2)	119.1
N(3)–C(4)	1.335(3)	1.341	N(3)–C(4)–C(5)	123.4(3)	121.7
C(4)–C(5)	1.429(4)	1.425	C(4)–C(5)–C(6)	116.7(2)	117.5
C(5)–C(6)	1.338(4)	1.333	C(5)–C(6)–N(1)	120.1(3)	120.5
C(6)–N(1)	1.358(4)	1.353	C(6)–N(1)–C(2)	121.7(3)	121.5
C(4)–N(4)	1.329(4)	1.326	N(1)–C(2)–O(2)	117.9(2)	118.5
C(2)–O(2)	1.254(3)	1.251	N(3)–C(2)–O(2)	121.7(2)	121.9
N(4)–N(7)	1.418(4)	—	N(3)–C(4)–N(4)	119.0(2)	118.1
			C(5)–C(4)–N(4)	117.6(2)	120.2
			C(4)–N(4)–N(7)	124.1(2)	—

a) Data taken from ref. 11.
b) Standard deviations are shown in parentheses.

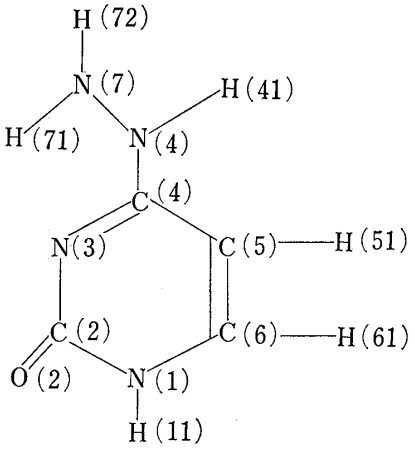


Fig. 2. The Atomic Numbering Scheme

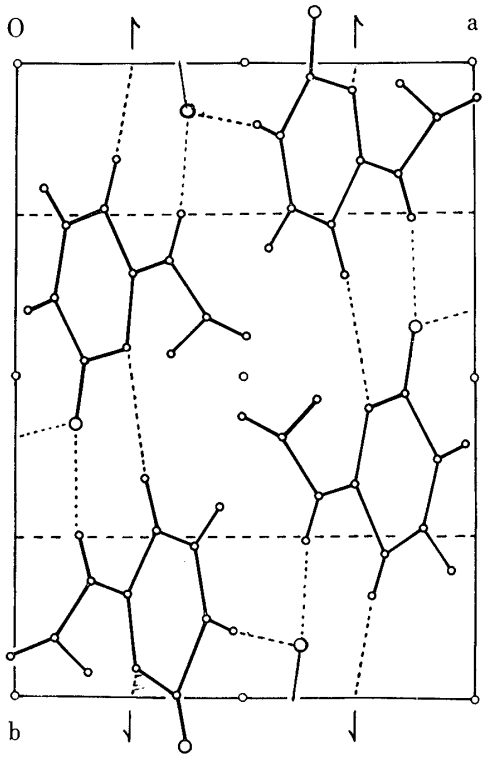


Fig. 3. Crystal Structure viewed along the c-Axis
The hydrogen bonds are shown by dotted lines.

III, together with those of cytosine[11] for comparison. The dimensions of N^4-aminocytosine are very close to those of cytosine. The C(4)–N(4) bond length found here, 1.329 Å, is very much longer than that reported for the imino form compound, 1,5-dimethyl-N^4-hydroxycytosine, 1.288 Å,[12] while the length of the N(3)–C(4) bond, 1.335 Å, is markedly less than that in 1,5-dimethyl-N^4-hydroxycytosine, 1.387 Å. The bond angles are quite similar to those of cytosine. On the other hand, the angles of C(2)–N(3)–C(4), 117 .6°, and N(3)–C(4)–C(5), 123.4°, differ significantly from the corresponding angles of 1,5-dimethyl-N^4-hydroxycytosine,[12] 125.7° and 116.2°, respectively. The difference Fourier map clearly showed the presence of a hydrogen atom bonded to N(4) but not to N(3). These findings indicate clearly that N^4-aminocytosine takes the amino form (**1**).

11) R.J. McClure and B.M. Craven, *Acta Cryst.*, **B29**, 1234 (1973).
12) D. Shugar, C.P. Huber, and G.I. Birnbaum, *Biochim. Biophys. Acta*, **447**, 274 (1976).

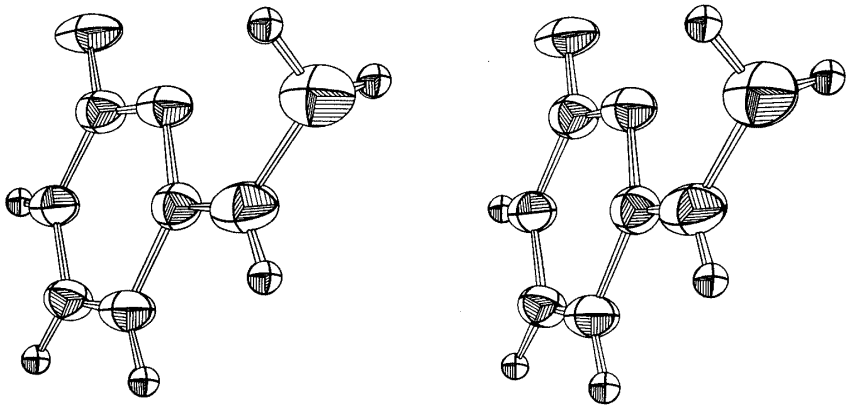


Fig. 4. Stereoscopic View of the Molecule

Thermal ellipsoids are drawn at the 50% probability level.

The crystal structure projected along the c-axis is shown in Fig. 3. Intermolecular hydrogen bonds are formed between the nitrogen atom N(4) and a neighboring carbonyl oxygen O(2), between C(5) and N(3), and between O(2) and N(1). The lengths of these bonds are H(41)–O(2) 2.01 Å, H(51)–N(3) 2.48 Å, and O(2)–H(11) 1.93 Å.

The fact that N^4-aminocytosine takes the amino form in its crystalline state suggests that it also takes the amino form in solution. This view is consistent with the spectroscopic studies of Brown *et al.*[6] who suggested that 1-methyl-N^4-aminocytosine takes the amino form in aqueous solutions.

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