

## THE CRYSTAL STRUCTURE OF ANHYDROUS CYTOSINE

David L. Barker

### Abstract

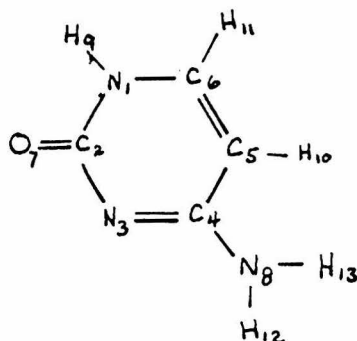
The crystal structure of anhydrous cytosine has been determined and refined by analysis of complete three-dimensional copper X-radiation data. The crystals are orthorhombic with space group  $P2_12_12_1$ ; the unit-cell dimensions are  $a = 13.041 \text{ \AA}$ ,  $b = 9.494 \text{ \AA}$ ,  $c = 3.815 \text{ \AA}$ . Refinement of the positional parameters for all 13 atoms and of the individual anisotropic temperature factors of the eight heavier atoms was by the method of least squares. For the 608 observed reflections of non-zero weight, the final R factor is 0.061.

The molecule is found to exist in the amino form with a hydrogen atom bonded to  $N_1$  and none to  $N_3$ . The amino nitrogen participates in two hydrogen bonds to carbonyl oxygens of other molecules. These bonds have relatively long lengths of 2.98 and 3.03  $\text{\AA}$ .  $N_1$  hydrogen bonds to  $N_3$  of a neighboring molecule at a distance of 2.84  $\text{\AA}$ .

### Introduction

This determination of the crystal structure of anhydrous cytosine (I) was undertaken as part of a program of research on the structure of compounds related to nucleic acids. Interest in this compound stems from the discovery (Hoogsteen, 1959) that

## (I) Cytosine



crystals composed of an equimolar mixture of l-methylthymine and 9-methyladenine exhibit hydrogen-bond pairing which differs from that proposed by Watson and Crick (1953) for DNA. If the Hoogsteen pairing were introduced into the DNA molecule, the distance between the sugar linkages would be reduced to about 9 Å from the Watson-Crick distance of about 11 Å, necessitating a similar pairing scheme for cytosine and guanine. Such a pairing is possible if there exists a tautomerism which places a proton on either N<sub>3</sub> of cytosine or on N<sub>7</sub> of guanine. Two tautomers of cytosine which could place a proton on N<sub>3</sub> involve, (1) no proton on N<sub>1</sub> or (2) the existence of the molecule in the imine form, with protons bonded to both N<sub>1</sub> and N<sub>3</sub>.

#### Experimental

Anhydrous crystals of cytosine were obtained from saturated methanol solutions allowed to evaporate at room temperature (20° C). The compound crystallizes as a monohydrate if small amounts of water are present in the solution. The structure of cytosine monohydrate has been reported by Jeffrey and Kinoshita (1963).

The anhydrous crystals are generally needles elongated about c. These orthorhombic crystals are stable for several months under atmospheric conditions.

Complete intensity data for Cu  $K\alpha$  radiation were collected by the multi-film equi-inclination angle Weissenberg technique. Of the two crystals used for this work, one was a thin needle from which layer lines 0 to 3 about c were recorded; the other was of approximately equal dimensions in the a and c directions from which layer lines 0 to 8 about b were recorded. The intensities were estimated visually and corrected for Lorentz and polarization factors; no absorption corrections were applied. Of the total of 649 independent reflections within the effective copper sphere ( $\sin \theta < 0.98$ ), 613 were observed. All reflections were put on a single scale by use of the 454 reflections which were measured about both the b and c axes.

The systematic absence of the odd reflections of  $h00$ ,  $0k0$ , and  $00l$  determined the space group as  $P2_12_12_1 (D_2^4)$ . The unit-cell dimensions, given in Table 1, were obtained from least-squares analysis of Straumanis-type rotation photographs about b and c. Twenty-one  $h0l$  and nineteen  $hk0$  reflections were indexed by comparison with the corresponding Weissenberg photographs. Their measured values of  $\sin^2 \theta$ , as determined from either Cu  $K\alpha_1$  ( $\lambda = 1.54050$ ) or Cu  $K\alpha_2$  ( $\lambda = 1.54434$ ), were used as input for the least-squares determination of the three cell parameters. The calculations were made by hand and also on the Burrough's 220

Table 1. Unit-cell dimensions  
of anhydrous cytosine

Orthorhombic; space group,  $P2_12_12_1$

$$\underline{a} = 13.041 \pm 0.002 \text{ \AA}$$

$$\underline{b} = 9.494 \pm 0.001 \text{ \AA}$$

$$\underline{c} = 3.815 \pm 0.001 \text{ \AA}$$

computer, using a program developed by Mr. Noel Jones. The density calculated on the basis of four molecules per unit cell is  $1.562 \text{ g.cm}^{-3}$ .

#### Determination of the structure

A trial structure was developed from considerations of packing and hydrogen bonding, assuming that the molecule was in the amino form and noting that the short c axis prevented superposition of atoms in the (001) projection. This initial arrangement was confirmed by a Fourier projection on (001) with subsequent two-dimensional refinement by Fourier and least-squares methods. All refinement calculations were carried out on the Burrough's 220 computer, using a Fourier program developed by Dr. K. Hoogsteen and a structure-factor least-squares program developed by Dr. A. Hybl. The R factor fell gradually from an initial value of 0.50 to 0.13 at which point a projection difference Fourier was calculated in an attempt to locate the hydrogen atom positions. In addition to peaks in the expected positions near C<sub>5</sub>, C<sub>6</sub>, and N<sub>8</sub> (two peaks), the fifth hydrogen seemed clearly to be bonded to N<sub>1</sub>.

There was no indication of a proton bonded to  $N_3$ . Only one spurious peak of any consequence was present, this near  $N_3$  on the  $N_3-C_4$  bond. With these hydrogen positions included, four least-squares cycles together with adjustments in the estimated temperature factors reduced R to 0.089, completing the two-dimensional refinement. The final electron density projection onto (001) is shown in Fig. 1.

The z parameters were estimated by a geometrical construction utilizing the predicted dimensions of the molecule and its projection onto the (001) plane. These parameters were improved by a (010) Fourier projection. An initial weighting function of

$$\sqrt{w} = 1/(1 + F_0)$$

was chosen somewhat arbitrarily. Throughout the three-dimensional refinement, an external weight of one-half was applied to the 154 reflections which were observed on only one set of data. The first structure-factor calculation yielded an R factor of 0.27; this was reduced to 0.22 after three cycles of least-squares refinement, during which heavy-atom positional and anisotropic temperature factor parameter shifts were permitted, subject to arbitrary adjustment. The hydrogen atoms were kept stationary. At this point it was noted that the largest discrepancies between the observed and calculated structure factors was in the high-angle data. The weighting scheme was changed to  $\sqrt{w} = 0.1 + \sin \theta$ . Six least-squares cycles reduced R to 0.135 at which point the weighting scheme was changed back to the original function. Nine more

least-squares cycles resulted in an R value of 0.070. The hydrogen atom positional parameters were permitted to shift during the last three of these, when R had fallen to 0.085.

Another weighting function was then adopted which more truly represented the estimates of the reliabilities of the observed quantities  $F_o^2$ . This scheme, which increased the relative weight of the weak reflections, was

$$\sqrt{w} = 1/(1 + F_o^2).$$

Eight least-squares cycles with this weighting scheme completed the refinement. The final R factor for the 608 observed reflections of non-zero weight was 0.061. In the last cycle, no parameter shift exceeded one-fourth of its standard deviation. Unobserved reflections were not included in the R factor. These were included in the least-squares refinement only if the calculated structure factor exceeded the minimum observable value. The final atomic parameters and their standard deviations are given in Table 2.

#### Accuracy of the results

The standard deviations in the positional parameters (Table 2) were calculated from the sum of the residuals and the diagonal terms of the least-squares normal equations. The heavy atom positional parameters show an average standard deviation of about 0.002 Å, leading to a standard deviation in interatomic distance of about 0.003 Å, and a limit of error (chosen as three times the standard deviation) of 0.009 Å. The limit of error in a bond

Table 2. The final atomic parameters and their standard deviations.

The heavy atom values have been multiplied by  $10^4$ . The temperature factors are in the form  $T_i = \exp(-\alpha_i h^2 - \beta_i k^2 - \gamma_i l^2 - \delta_i hk - \epsilon_i hl - \eta_i kl)$ .

The hydrogen parameters have been multiplied by  $10^3$ . Each hydrogen was assigned an isotropic B value of 2.5.

Atom	x ( $\sigma_x$ )	y ( $\sigma_y$ )	z ( $\sigma_z$ )	$\alpha$ ( $\sigma_\alpha$ )	$\beta$ ( $\sigma_\beta$ )	$\gamma$ ( $\sigma_\gamma$ )	$\delta$ ( $\sigma_\delta$ )	$\epsilon$ ( $\sigma_\epsilon$ )	$\eta$ ( $\sigma_\eta$ )
N <sub>1</sub>	225(1)	292(2)	4402(6)	31(1)	50(2)	520(15)	-4(2)	3(7)	-4(10)
C <sub>2</sub>	-163(2)	1559(2)	3272(6)	30(1)	55(3)	422(18)	1(3)	27(6)	-13(11)
N <sub>3</sub>	401(1)	2745(2)	3875(5)	27(1)	45(2)	504(14)	6(2)	6(6)	1(10)
C <sub>4</sub>	1310(2)	2647(3)	5480(7)	28(1)	66(3)	441(16)	0(2)	8(7)	0(11)
C <sub>5</sub>	1707(2)	1335(3)	6675(8)	31(1)	77(3)	533(19)	15(3)	-41(8)	28(13)
C <sub>6</sub>	1138(2)	181(3)	6085(7)	39(1)	60(3)	476(19)	20(3)	18(9)	7(12)
O <sub>7</sub>	-993(1)	1593(2)	1717(6)	27(1)	69(2)	679(16)	-2(2)	-58(5)	13(10)
N <sub>8</sub>	1844(2)	3823(2)	5978(7)	35(1)	63(2)	747(20)	-18(3)	-76(9)	-3(12)
H <sub>9</sub>	-10(2)	-50(4)	392(10)						
H <sub>10</sub>	226(3)	126(4)	797(12)						
H <sub>11</sub>	133(3)	-76(4)	711(11)						
H <sub>12</sub>	160(3)	466(4)	549(13)						
H <sub>13</sub>	247(3)	384(4)	669(10)						

angle is about  $0.6^\circ$ . For bonds involving hydrogen atoms, the limits of error are about  $0.12 \text{ \AA}$  in bond length and  $6^\circ$  in bond angle.

The standard deviations of the temperature-factor parameters of the heavy atoms were calculated from the diagonal coefficients of the inverse matrices of the normal equations and include interactions among the six temperature parameters of the same atom. Since the temperature factors include the effects of extinction, absorption, scaling, and spot shape, the meaning of the calculated standard deviations is difficult to assess. However, because the crystals were of regular shape and without pronounced cleavage, a single isotropic temperature parameter should be sufficient to correct for these systematic experimental errors, permitting the application of the reported uncertainties to the relative values of the anisotropic vibrational amplitudes discussed below.

### Discussion of the structure

#### (i) Bond distances and angles

The bond distances and angles calculated from the parameters of Table 2 are shown in Fig. 2 and listed in Table 3. The results of two best plane calculations, using the method of least squares (Schomaker, et al., 1959), are given in Table 4. Plane (A) was calculated on the basis of equal weight for the six ring atoms and zero weight for the rest. Plane (B) results from equal weight for all the heavy atoms and zero weight for the hydrogens.

Table 3. Bond distances and angles

The estimated standard deviations are approximately 0.003 Å and 0.2° for the heavy atoms and 0.04 Å and 2° where hydrogens are involved.

N <sub>1</sub> -C <sub>2</sub>	1.374 Å	C <sub>6</sub> -N <sub>1</sub> -C <sub>2</sub>	122.7°
C <sub>2</sub> -N <sub>3</sub>	1.364	N <sub>1</sub> -C <sub>2</sub> -N <sub>3</sub>	118.1
N <sub>3</sub> -C <sub>4</sub>	1.337	C <sub>2</sub> -N <sub>3</sub> -C <sub>4</sub>	119.9
C <sub>4</sub> -C <sub>5</sub>	1.424	N <sub>3</sub> -C <sub>4</sub> -C <sub>5</sub>	122.0
C <sub>5</sub> -C <sub>6</sub>	1.342	C <sub>4</sub> -C <sub>5</sub> -C <sub>6</sub>	117.3
C <sub>6</sub> -N <sub>1</sub>	1.357	C <sub>5</sub> -C <sub>6</sub> -N <sub>1</sub>	120.1
C <sub>2</sub> -O <sub>7</sub>	1.234	N <sub>1</sub> -C <sub>2</sub> -O <sub>7</sub>	119.8
C <sub>4</sub> -N <sub>8</sub>	1.330	N <sub>3</sub> -C <sub>2</sub> -O <sub>7</sub>	122.2
N <sub>1</sub> -H <sub>9</sub>	0.88	N <sub>3</sub> -C <sub>4</sub> -N <sub>8</sub>	118.2
C <sub>5</sub> -H <sub>10</sub>	0.87	C <sub>5</sub> -C <sub>4</sub> -N <sub>8</sub>	119.9
C <sub>6</sub> -H <sub>11</sub>	1.01	C <sub>2</sub> -N <sub>1</sub> -H <sub>9</sub>	120
N <sub>8</sub> -H <sub>12</sub>	0.87	C <sub>6</sub> -N <sub>1</sub> -H <sub>9</sub>	117
N <sub>8</sub> -H <sub>13</sub>	0.86	C <sub>4</sub> -C <sub>5</sub> -H <sub>10</sub>	123
		C <sub>6</sub> -C <sub>5</sub> -H <sub>10</sub>	119
		N <sub>1</sub> -C <sub>6</sub> -H <sub>11</sub>	118
		C <sub>5</sub> -C <sub>6</sub> -H <sub>11</sub>	122
		C <sub>4</sub> -N <sub>8</sub> -H <sub>12</sub>	123
		C <sub>4</sub> -N <sub>8</sub> -H <sub>13</sub>	124
		H <sub>12</sub> -N <sub>8</sub> -H <sub>13</sub>	114

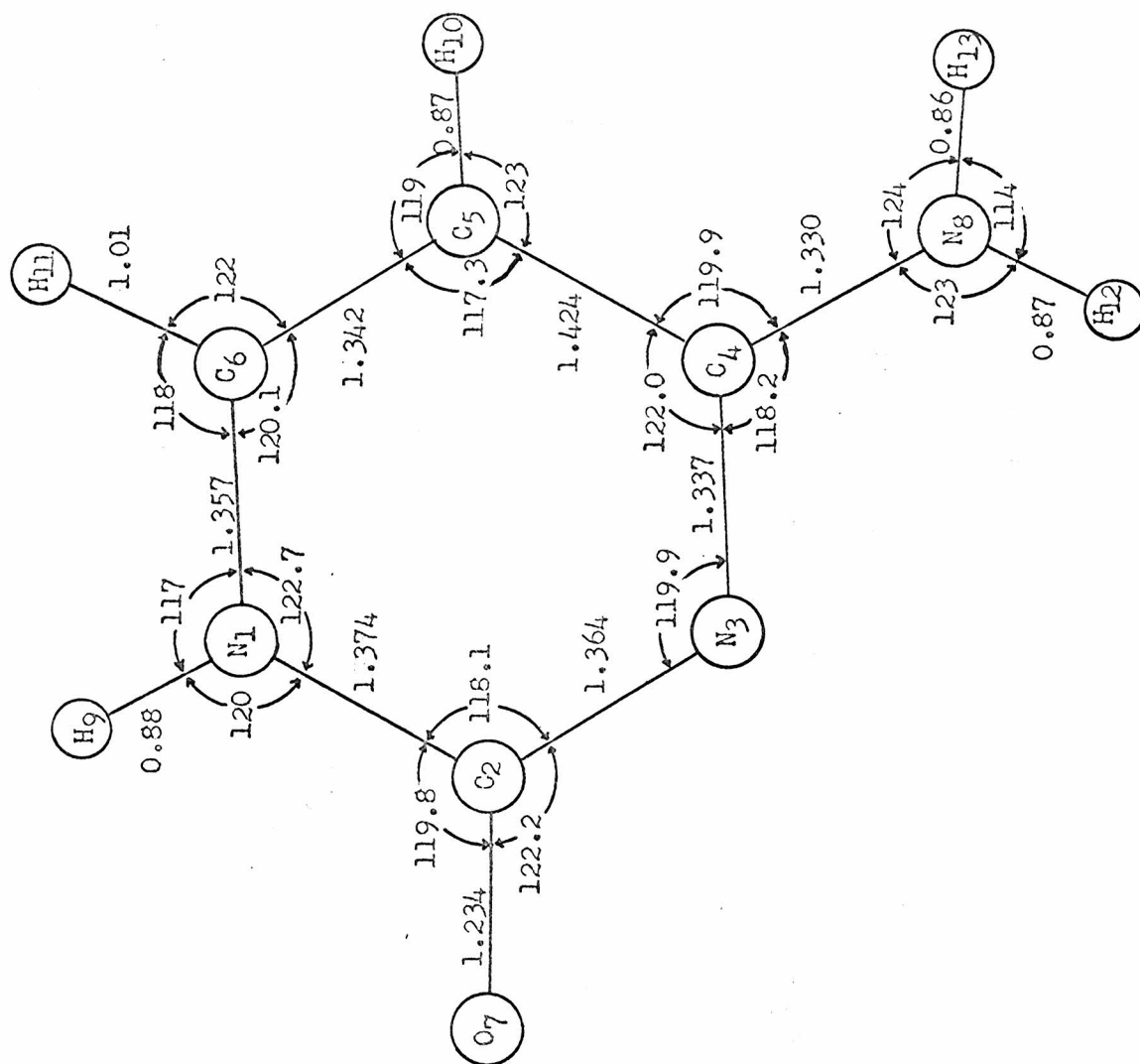
Figure 2. Bond distances and angles

Table 4. Deviations from the least-squares planes

Plane (A), ring atoms:

$$0.4568 X - 0.1257 Y - 0.8806 Z = - 1.3839$$

Plane (B), all heavy atoms:

$$0.4595 X - 0.1298 Y - 0.8787 Z = - 1.3793$$

Atom	Deviation from Plane (A)	Deviation from Plane (B)
N <sub>1</sub>	0.004 Å	0.002 Å
C <sub>2</sub>	0.001	-0.007
N <sub>3</sub>	-0.007	-0.018
C <sub>4</sub>	0.007	0.001
C <sub>5</sub>	-0.001	0.000
C <sub>6</sub>	-0.004	-0.001
O <sub>7</sub>	0.025	0.013
N <sub>8</sub>	0.018	0.010
H <sub>9</sub>	0.07	0.07
H <sub>10</sub>	-0.10	-0.09
H <sub>11</sub>	-0.12	-0.11
H <sub>12</sub>	-0.06	-0.07
H <sub>13</sub>	0.15	0.14

The non-planarity of the ring implied by Plane (A) seems of doubtful significance. The carbonyl oxygen and amino nitrogen, however, are evidently displaced slightly from the plane of the ring atoms by approximately the same amount and in the same direction.

A comparison of bond distances in anhydrous cytosine, cytosine monohydrate (Jeffrey and Kinoshita, 1963), and cytosine-5-acetic acid (Marsh, Bierstedt, and Eichhorn, 1962) is shown in Table 5.

Table 5. Comparison of bond distances in anhydrous cytosine, cytosine monohydrate, and cytosine-5-acetic acid

	Anhydrous	Cytosine	Cytosine-5-
Bond	Cytosine	Monohydrate	Acetic Acid
	( $\sigma=0.003$ )	( $\sigma=0.004$ )	( $\sigma=0.006$ )
N <sub>1</sub> -C <sub>2</sub>	1.374 Å	1.376 Å	1.351 Å
C <sub>2</sub> -N <sub>3</sub>	1.364	1.354	1.366
N <sub>3</sub> -C <sub>4</sub>	1.337	1.351	1.353
C <sub>4</sub> -C <sub>5</sub>	1.424	1.432	1.427
C <sub>5</sub> -C <sub>6</sub>	1.342	1.348	1.363
C <sub>6</sub> -N <sub>1</sub>	1.357	1.361	1.365
C <sub>2</sub> -O <sub>7</sub>	1.234	1.260	1.250
C <sub>4</sub> -N <sub>8</sub>	1.330	1.332	1.323

It does not seem possible to note any significant differences in the dimensions of these molecules. The difference between

the lengths of any two corresponding bonds does not exceed the sum of the limits of error estimated for the two molecules involved, with the lone exception of the difference between the carbonyl bond distances in anhydrous cytosine and cytosine monohydrate. Here the difference in bond lengths is 0.026 Å while the sum of the estimated limits of error is 0.021 Å. It is almost a certainty that this difference will also fall within the limits of error when corrections have been made for librations implied by the temperature parameters of anhydrous cytosine (Cruickshank, 1956). When this correction is made for cytosine-5-acetic acid, the carbonyl bond length is increased from 1.250 to 1.264 Å, and the amino bond ( $C_4-N_9$ ) is increased from 1.323 to 1.334 Å.

Seven canonical structures for the pyrimidine ring of cytosine are shown in Fig. 3. The observed bond distances can be explained reasonably well by assuming the percentage contributions to the resonance hybrid as indicated in the figure. The calculated distances shown in the final drawing of Fig. 3 were obtained from bond numbers, from Table 7-9 of Pauling (1960) (for the C--C distances, bond numbers 1.3 and 1.7), and from two curves drawn through points representing observed C--N and C--O bond distances. Table 6 gives the values chosen for these points. It may be desirable to note that the carbonyl bond distance shows the worst agreement, again indicating the need for consideration of corrections implied by the temperature motion.

Figure 3. Canonical structures for the cytosine molecule and the predicted bond distances.

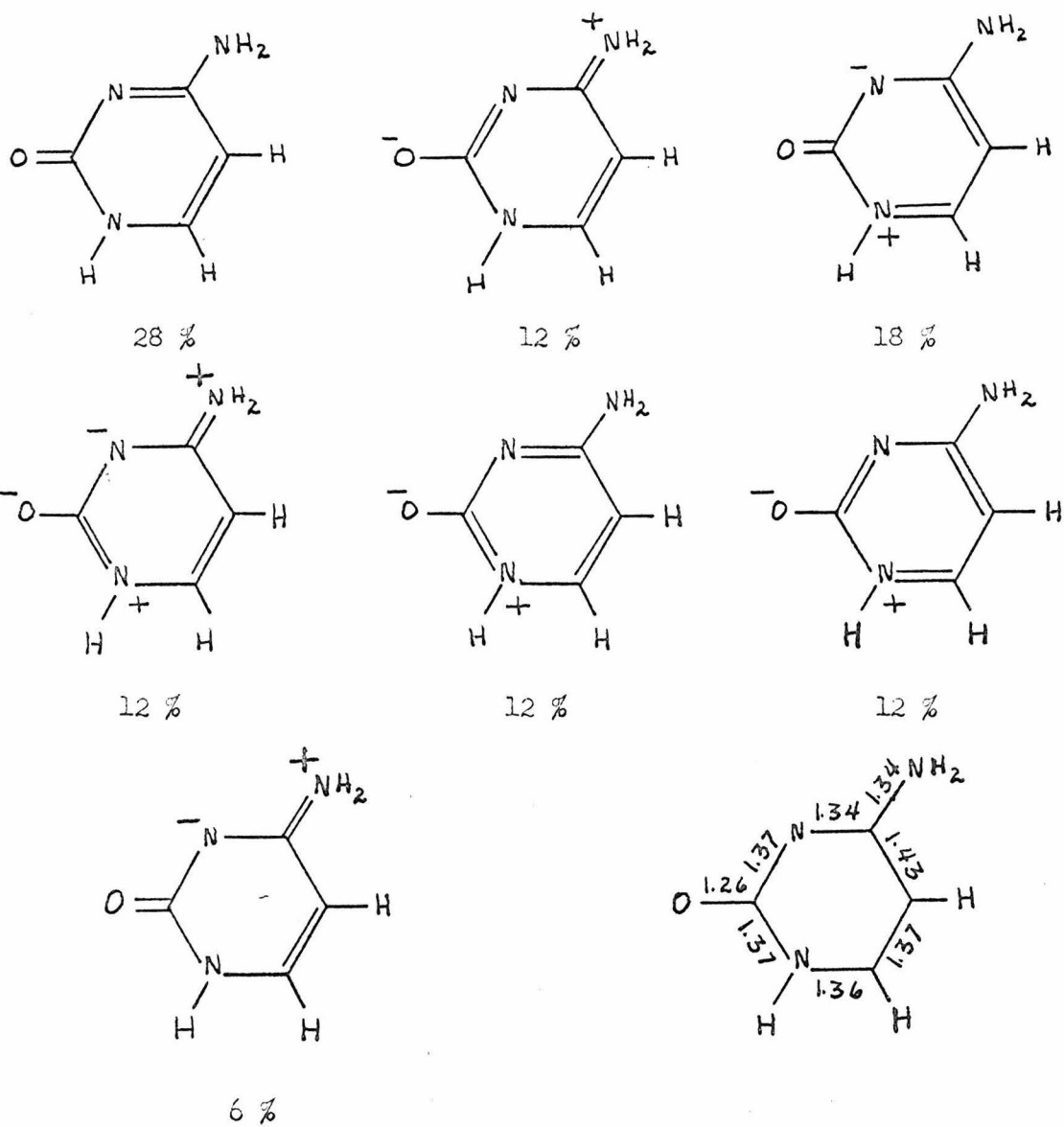


Table 6. Values of C--N and C--O  
distances for bond numbers n

<u>n</u>	C--N	References	C--O	References
1.0	1.48 Å	(a), (b), (d)	1.41 Å	(a), (d)
1.5	1.31	(a), (c)	1.26	(a), (b)
2.0	1.24	(a), (d)	1.21	(a), (d)

(a) Hahn, 1956. (b) Marsh, 1958. (c) Wheatley, 1955.

(d) Pauling, 1960; Table 7-5.

(ii) The hydrogen atom positions

As was described previously, the hydrogen positions originally determined by a difference Fourier projection on (001) were refined during the three dimensional refinement after the R factor had dropped to 0.085. No difficulty was encountered in this refinement which would have indicated a spurious result. In order to further check the proton positions, electron density maps were calculated in the best plane defined by the heavy atoms (Plane B), using a Burrough's 220 computer program developed by Mr. D. Duchamp. The molecular plane Fourier employing the complete refined data is shown in Fig. 4. A molecular plane difference Fourier, for which the phases were determined by the heavy atoms alone, is shown in Fig. 5. In each figure, the crosses are the atomic positions, determined by the least-squares refinement, projected normally onto Plane B. The results clearly demonstrate that in the anhydrous form, cytosine exists in the amino form with a proton bonded to N<sub>1</sub>.

Thus there is no indication of a tautomerism, such as is found in 50 % of the molecules of cytosine-5-acetic acid (Marsh, Bierstedt, and Eichhorn, 1962), which would permit  $N_3$  to be a proton donor. As has been reported in numerous other X-ray diffraction investigations, the bond distances involving the hydrogen atoms (Table 3) are on the average about 0.1 to 0.2 Å shorter than the accepted values for the internuclear separations.

(iii) The intermolecular environment

Fig. 6 shows a drawing of the unit cell of anhydrous cytosine viewed down the  $c$  axis. Each molecule participates in six hydrogen bonds, donating the three available protons, and accepting two hydrogen bonds to the carbonyl oxygen and one to  $N_3$ . The three-dimensional network of hydrogen bonds is characterized by a spiral up the two-fold screw axis of alternating  $N_8-H \cdots O_7$  and  $O_7 \cdots H-N_8^+$  linkages. The pair of molecules joined by two hydrogen bonds,  $N_1-H \cdots N_3$  and  $O_7 \cdots H-N_8$ , have a dihedral angle of  $15^\circ$  between the planes of their heavy atoms.

The hydrogen-bond lengths and angles are given in Table 7. The  $N_1 \cdots N_3$  distance, 2.84 Å, is somewhat shorter than the average  $N-H \cdots N$  value reported by Fuller (1959) in his compilation of hydrogen-bond distances. For two ring nitrogens this is not surprising, for the canonical structures discussed above indicate that  $N_1$  has approximately 40 % positive character while  $N_3$  has about the same amount of negative charge. In view of this argument, however, the  $N \cdots O$  distances, 2.98 and 3.03 Å must be viewed as surprisingly

long, for N<sub>8</sub> and O<sub>7</sub> also exhibit positive and negative character, respectively.

Table 7. Hydrogen-bond distances and angles

N <sub>1</sub> ····N <sub>3</sub>	2.843 Å	N <sub>1</sub> -H <sub>9</sub> ··N <sub>3</sub>	155°
N <sub>8</sub> ····O <sub>7</sub>	3.034	N <sub>8</sub> -H <sub>12</sub> ··O <sub>7</sub>	170
N <sub>8</sub> ····O <sub>7</sub>	2.980	N <sub>8</sub> -H <sub>13</sub> ··O <sub>7</sub>	168

(iv) The temperature factors

The magnitudes and direction cosines of the principal axes of thermal motion, as derived from the anisotropic temperature parameters of Table 2, are listed in Table 8.

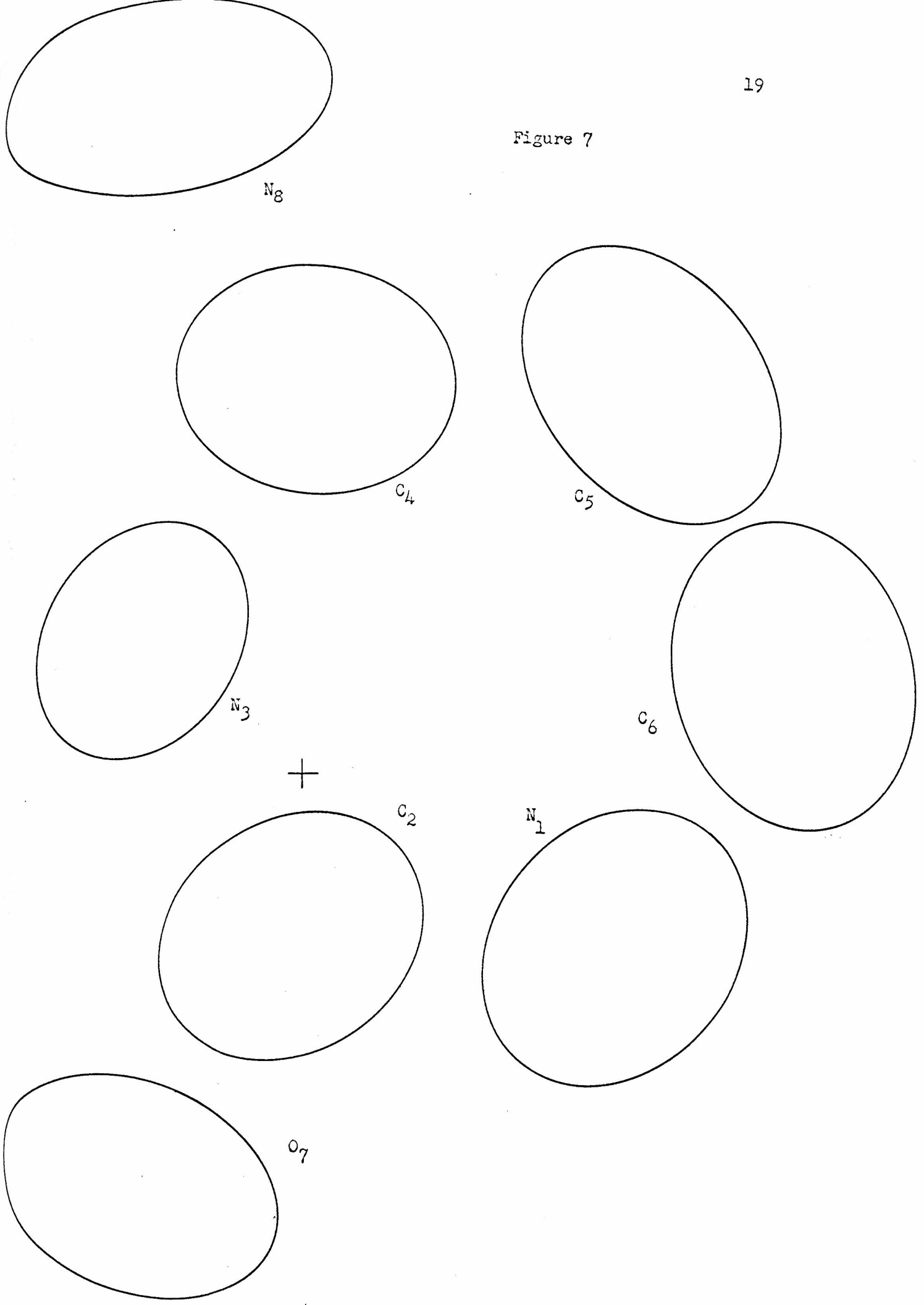
For almost all the atoms, the principal motion is approximately in the direction of the c axis. The greatest principal vibrations are those of the carbonyl oxygen and the amino nitrogen, which are within 10° of normal to the plane of the ring atoms, the displacement from normality being primarily toward parallelism with the c axis.

If the ellipsoids implied by the principal axes of vibration are intersected with the plane of the heavy atoms, the resulting ellipses seem to imply a center of molecular oscillation near C<sub>2</sub> (Fig. 7). This apparent oscillatory motion, as well as the large normal librations of the carbonyl and amino groups indicates the need for corrections to the bond distances (Cruickshank, 1956). The calculation of these corrections is presently in progress.

Table 8. The magnitudes  $B_i$  and direction cosines  $q_i$   
relative to the crystallographic axes of  
the principal axes of the temperature  
factor ellipsoids

Atom	Axis $i$	$B_i$	$q_i^1$	$q_i^2$	$q_i^3$
$N_1$	1	3.03	0.033	-0.024	0.999
	2	2.17	0.966	-0.257	-0.038
	3	1.80	0.258	0.966	0.015
$C_2$	1	2.59	0.413	-0.115	0.903
	2	2.00	0.488	0.866	-0.113
	3	1.85	0.769	-0.487	-0.413
$N_3$	1	2.94	0.061	0.010	0.998
	2	1.94	0.890	0.453	-0.059
	3	1.55	-0.453	0.891	0.019
$C_4$	1	2.57	0.111	-0.015	0.994
	2	2.39	-0.008	0.999	0.016
	3	1.89	0.994	0.009	0.111
$C_5$	1	3.26	-0.271	0.177	0.946
	2	2.94	0.423	0.905	-0.048
	3	1.75	0.865	-0.387	0.320
$C_6$	1	3.06	0.741	0.419	0.524
	2	2.66	0.421	0.318	-0.850
	3	1.85	-0.523	0.850	0.059
$O_7$	1	4.10	-0.245	0.008	0.970
	2	2.50	-0.060	0.998	-0.024
	3	1.68	0.968	0.064	0.244
$N_8$	1	4.61	0.326	-0.051	-0.944
	2	2.62	0.586	-0.772	0.245
	3	1.76	0.742	0.633	0.222

Figure 7



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Legends for Figures

- Figure 1. The electron density projected onto (001). Contours are at intervals of  $2 \text{ e}/\text{\AA}^2$  beginning with  $1 \text{ e}/\text{\AA}^2$ .
- Figure 2. Bond distances and angles.
- Figure 3. Canonical structures for the cytosine molecule and the predicted bond distances.
- Figure 4. The electron density in the best plane of the heavy atoms. Contours are at intervals of  $2 \text{ e}/\text{\AA}^3$  beginning with  $1 \text{ e}/\text{\AA}^3$ .
- Figure 5. The final difference map in the plane of the heavy atoms, in which the hydrogen contributions were omitted from the  $F_c$ 's. Contours are at intervals of  $0.1 \text{ e}/\text{\AA}^3$  beginning with  $0.2 \text{ e}/\text{\AA}^3$ .
- Figure 6. The structure viewed down the  $\underline{c}$  axis. The dashed lines represent hydrogen bonds.
- Figure 7. The ellipses formed from the intersection of the plane of the heavy atoms with the ellipsoids implied by the principal axes of vibration. The cross locates an apparent center of oscillation of the molecule.

