

The Molecular and Crystal Structure of Triuret

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Synthetic crystals of triuret (carbonyl diurea), $C_3H_6N_4O_3$, are monoclinic with eight molecules in a unit cell of dimensions

$$a = 7.209, b = 7.143, c = 24.836 \text{ \AA}, \beta = 119.83^\circ,$$

space group $C2/c$. The structure has been determined by two-dimensional Fourier methods and refined by three-dimensional least-squares computations. The triuret molecule is in the *trans* form. Each of the three urea fragments is perfectly planar but the three parts are twisted with respect to each other, resulting in a slightly distorted molecule. The molecules are stacked nearly parallel to the (100) plane, thus forming a layered structure. While the molecules within each layer are firmly connected by a network of hydrogen bonds, the consecutive layers are essentially held together only by van der Waals forces.

Introduction

Triuret (carbonyl diurea) is found in nature as an excretory product in the form of extremely small single crystals in the cytoplasm of certain species of free-living carnivorous amoebae. The crystals occur either as squared platelets which are highly birefringent or as 2–6 microns long truncated bipyramids, which exhibit no discernible birefringence. Many amoeba species contain a mixture of both types of crystals in their cytoplasm. Griffin (1960) was the first to demonstrate the identity of the birefringent crystals with synthetic triuret. Later on, Carlström & Max Møller (1961) showed that these crystals are monoclinic and that the 'isotropic' crystals consist of a tetragonal form of triuret. While all efforts to obtain the tetragonal form *in vitro* have been in vain the monoclinic form is easy to synthesize, even if earlier attempts to grow crystals large enough for single-crystal work have failed (Haworth & Mann, 1943).

The molecular and crystal structure of monoclinic triuret has now been investigated as part of our crystallographic program on nitrogen-containing excretory products in biological systems. The work was regarded as a contribution to the knowledge of compounds with terminal amide groups, but in addition it was hoped that the analysis would shed some light on the crystal structure of the tetragonal form which has a unit cell of a size and shape closely related to that of monoclinic triuret.

Experimental

Triuret was prepared according to Schittenhelm & Wiener (1909) by oxidation of uric acid with hydrogen peroxide in a hot alkaline solution. The product was purified by repeated recrystallization in distilled water. By slow cooling of a saturated solution, well-

formed single crystals of appreciable size could be obtained. They appeared as square plates (up to 1.3×1.3 mm) with a maximal thickness of 0.5 mm. Twinning frequent on (001). Perfect cleavage along (100). The crystallographic properties of triuret have earlier been examined in detail (Carlström & Max Møller, 1961) and it should only be mentioned that the crystals are biaxial negative and highly birefringent, $\gamma_D - \alpha_D = 0.280$. A redetermination of the unit-cell dimensions was now made by recording rotation photographs of a small single crystal in a 190 mm diameter precision powder camera. For calibration, the crystal was coated with silicon powder, $a_0 = 5.4306 \text{ \AA}$.

Crystal data

Triuret (carbonyl diurea), $C_3H_6N_4O_3$, mol.wt. 146.11.
Monoclinic,

$$a = 7.209 \pm 0.002, b = 7.143 \pm 0.002, \\ c = 24.836 \pm 0.003 \text{ \AA}; \beta = 119.83 \pm 0.01^\circ.$$

Volume of unit cell, 1109.49 \AA^3 .

Density, calculated (with $Z = 8$), 1.749 ± 0.002 g.cm^{-3} , measured, $d_4^{21} = 1.745 \pm 0.004$ g.cm^{-3} .

Total number of electrons per unit cell, $F(000) = 608$.

Absent spectra: $h0l$ when h and l is odd, hkl when $(h+k)$ is odd. The systematic absences indicated either the space group $C2/c$ or Cc . The lack of detectable piezoelectric activity favoured the former, and this space group was also confirmed by the structure analysis.

In using the multiple-film technique, equi-inclination integrating Weissenberg photographs were taken with Cu $K\alpha$ radiation about the b axis from the zero to the sixth layer, and about the c axis from the zero to the twenty-first layer. All intensities were recorded in a microdensitometer, the range being about 10,000

to 1. The same crystal, measuring $0.12 \times 0.12 \times 0.18$ mm was used for both the b -axis and the c -axis photographs. In addition, the intensities of the 43 strongest reflexions were measured in a General Electric goniostat equipped with a scintillation counter. All intensities were corrected for Lorentz and polarization factors but no absorption correction was applied. The corrected intensities were put on a single scale by correlating the various layers, the absolute scale being established later by correlation with the calculated structure factors. 1063 independent reflexions were observed, representing 86.4% of the possible number observable with the experimental conditions used.

Structure analysis and refinement

The optical properties of triuret, the perfect cleavage along (100), and the outstanding intensity of the 200 reflexion, all suggested that the triuret molecules were flat, forming layers essentially parallel to the (100) plane. From infrared absorption spectra which indicated the presence of hydrogen bonds, it was further assumed that the molecules within each layer would be held together by such bonds involving a maximal number of hydrogen atoms. Taking these assumptions, as well as packing requirements and probable bond lengths and bond angles, as a basis, several trial structures of the a -axis projection were designed and tested in an optical diffractometer constructed according to Wyckoff, Bear, Morgan & Carlström (1957). One of the configurations soon proved to be much superior to all the others and was accordingly chosen for the further structure determination.

The first electron density projection along the a axis, based on signs calculated from the y and z coordinates of the trial structure, was quite encouraging as it showed well-resolved peaks of all non-hydrogen atoms. After six cycles of two-dimensional Fourier refinement, including structure factor calculations, carried out on the digital computer WEGEMATIC 1000, a fair agreement was obtained with the observed amplitudes. The value of $R(0kl)$ after six cycles was thus 0.183 with the use of a common isotropic temperature factor, $B=1.66 \text{ \AA}^2$, and atomic scattering factors for C, O and N calculated according to Vand, Eiland & Pepinsky (1957). The electron density projection along the a axis at this stage (Fig. 1) showed not only that the triuret molecule had a *trans* form but also that the molecule was not as flat as initially presumed. Some of the projected bond lengths and bond angles differed markedly from those expected, indicating that part of the molecule was inclined to the (100) plane. With the use of the average bond lengths and bond angles of different biuret compounds as standards (Cavalca, Nardelli & Fava, 1960; Hughes, Yakel & Freeman, 1961; Freeman, Smith & Taylor, 1961; Nardelli, Fava & Giraldi, 1963) the x coordinates of triuret were calculated from the a -axis projection. However, the severe superposition of peaks in the electron density maps of the b -axis projection made the Fourier refinement difficult and the value of $R(h0l)$ could not be brought much below 0.50. In this situation it was decided to try a three-dimensional least-squares refinement on the fast digital computer FACIT EDB for which an SFLS-program was available. The calculation involved a block-diagonal approximation and the weighting scheme

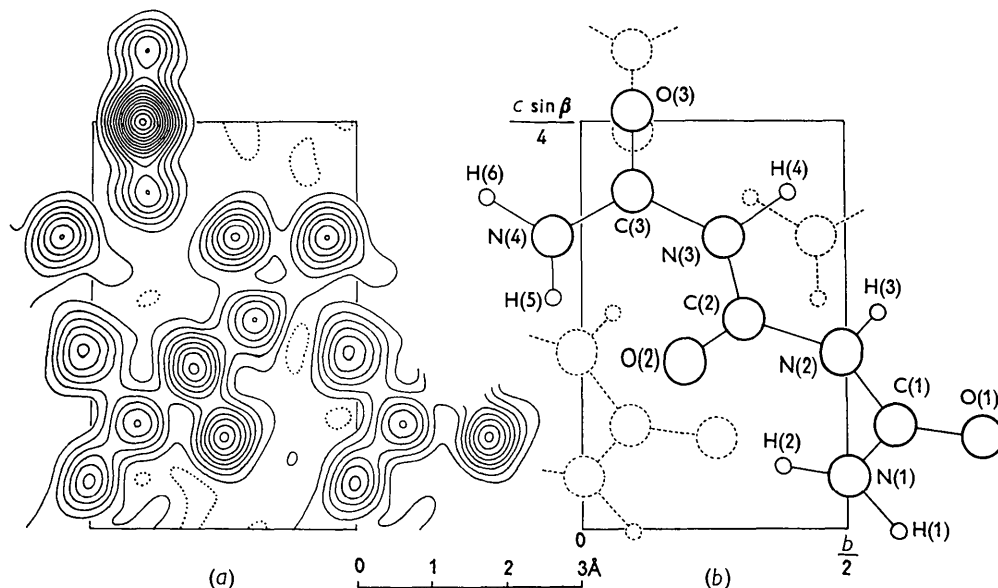


Fig. 1. (a) Preliminary electron density projection along the a axis. Contours at equal and arbitrary intervals (of about 1 e. \AA^{-3}). The zero contour is dotted. (b) Same projection showing the convention adopted for the labelling of the atoms in the triuret molecule.

was that of Cruickshank, Pilling, Bujosa, Lovell & Truter (1961). Only isotropic individual temperature factors could be treated and the f curves were interpolated from the mean atomic scattering factors given in *International Tables for X-ray Crystallography* (1962). After eight refinement cycles, R for observed reflexions alone had dropped from 0.534 to 0.164. At this stage the outstandingly strong 200 reflexion was excluded, as its observed intensity apparently was reduced by extinction, and the 168 accidentally absent reflexions were introduced. They were assigned an $F_o = F_{o(\min)}/2 = 1.8$. A difference Fourier synthesis now calculated indicated the position of five out of the six hydrogen atoms. When the hydrogen atoms were introduced at their expected positions the R index dropped to 0.143. After three additional cycles the shifts in the coordinates became insignificantly small and the final figures for the R index were 0.137 for all reflexions and 0.126 with non-observed reflexions omitted. A further improvement of the R index could probably have been gained by applying anisotropic temperature parameters. Observed reflexion amplitudes and final calculated structure factors are given in Table 1.

Results and discussion

The positional parameters and the individual temperature factors of the non-hydrogen atoms with corresponding standard deviations are shown in Table 2. The bond lengths and angles are shown in Table 3 and Fig. 2. The estimated standard deviation for bond lengths is 0.009 Å for C–O bonds and 0.010 Å for C–N bonds, and 0.5°–0.6° for bond angles. Examination of the bond distances and angles reveals certain interesting features. The two $\text{NH}_2\text{--CO--NH}$ parts have bond lengths quite normal for amide groups; the mean C–NH₂-distance, 1.322 Å, indicates

Table 2. *Positional parameters of non-hydrogen atoms in fractional coordinates $\times 10^4$ and isotropic thermal parameters (Å^2)*

Atom	x/a	y/b	z/c	B
C(1)	2186 ± 6	5906 ± 6	633 ± 2	1.43 ± 0.06
C(2)	2461 ± 6	3086 ± 5	1266 ± 2	1.36 ± 0.05
C(3)	2443 ± 6	988 ± 6	2059 ± 2	1.47 ± 0.06
O(1)	1633 ± 5	7563 ± 5	560 ± 2	2.32 ± 0.05
O(2)	3062 ± 5	1963 ± 5	1015 ± 1	1.96 ± 0.05
O(3)	2498 ± 5	993 ± 5	2565 ± 2	2.25 ± 0.05
N(1)	2988 ± 6	5027 ± 6	329 ± 2	2.02 ± 0.06
N(2)	1918 ± 5	4898 ± 5	1077 ± 2	1.84 ± 0.05
N(3)	2222 ± 5	2706 ± 6	1770 ± 2	1.76 ± 0.05
N(4)	2553 ± 6	-568 ± 6	1785 ± 2	2.04 ± 0.06

Table 3. *Bond lengths and bond angles not including hydrogen atoms*

Bond	Length	Angle	
C(1)–O(1)	1.233 Å	O(1)–C(1)–N(1)	125.1°
C(2)–O(2)	1.220	O(1)–C(1)–N(2)	116.5
C(3)–O(3)	1.236	N(1)–C(1)–N(2)	118.4
C(1)–N(1)	1.318	C(1)–N(2)–C(2)	128.7
C(1)–N(2)	1.408	O(2)–C(2)–N(2)	124.2
C(2)–N(2)	1.365	O(2)–C(2)–N(3)	124.9
C(2)–N(3)	1.373	N(2)–C(2)–N(3)	110.9
C(3)–N(3)	1.390	C(2)–N(3)–C(3)	127.7
C(3)–N(4)	1.326	O(3)–C(3)–N(3)	117.4
		O(3)–C(3)–N(4)	123.0
		N(3)–C(3)–N(4)	119.7

a large amount of double bond character while the mean C–NH-distance, 1.399 Å, is closer to that of a single bond. The mean length of the two C–O bonds, 1.235 Å, is appreciably longer than the standard C–O double bond showing that the C–O bonds only have about 50% double-bond character. The marked resonance in these two terminal parts of the molecule suggests that they should be perfectly planar. The central urea fragment, --NH--CO--NH-- , on the other hand, exhibits a relatively small double-bond character of the C–N bonds (mean length 1.369 Å) and the

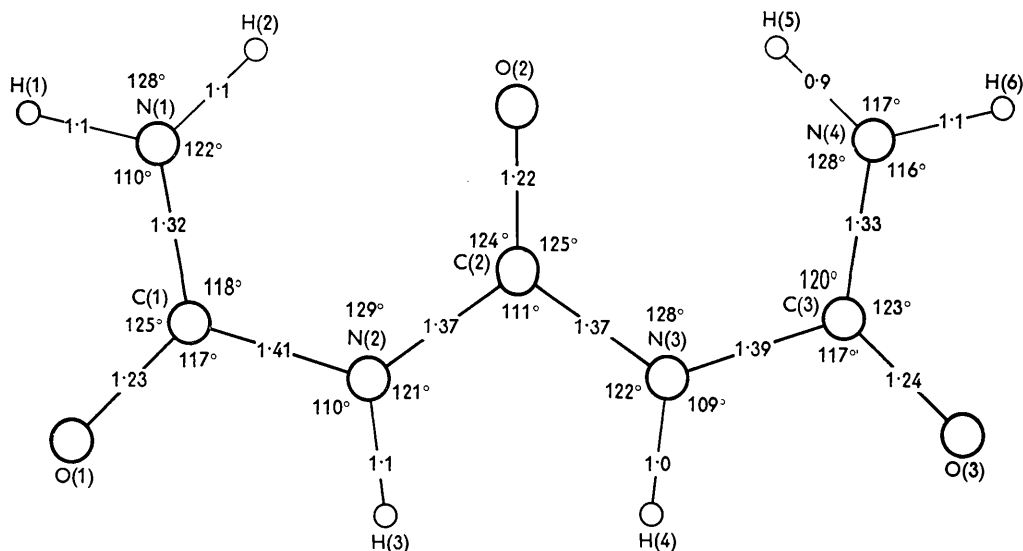


Fig. 2. Schematic diagram showing bond lengths and bond angles.

C–O bond (1.220) is predominantly double-bond in character. The resonance in this part of the molecule is thus less pronounced than in the terminal parts but still large enough to secure planarity.

Planarity of the molecule

An analysis of the planarity of the three parts; N(1)C(1)O(1)N(2) (I), N(2)C(2)O(2)N(3) (II) and N(3)C(3)O(3)N(4) (III) was carried out by calculating the best plane through the non-hydrogen atoms of each part from the orthogonal axes x' , y and z' according to Blow (1960). Equal weight was given to all atoms. The three mean planes are defined by the equations;

$$\begin{aligned} \text{(I)} \quad & 0.6570x' + 0.2636y + 0.7063z' = 2.5958, \\ \text{(II)} \quad & 0.7412x' + 0.2801y + 0.6100z' = 2.4347 \text{ and} \\ \text{(III)} \quad & 0.8803x' + 0.0893y + 0.4660z' = 1.4361. \end{aligned}$$

None of the atoms deviates more than 0.005 Å from the appropriate plane and the three parts of the molecule are thus perfectly planar within the limits of error. However, the planes are inclined to each other as was noticed in the initial Fourier refinement. The angle between the normals to the best planes through (I) and (II) is 7.4°, that between (II) and (III) is 16.0°. The molecule is slightly twisted and parts (I) and (III) are twisted in opposite directions with respect to part (II). Such a distortion of the molecule has also been observed in biuret hydrate where the angle between the two NH₂–CO–NH–fragments is 5.6° (Hughes *et al.*, 1961), and in other biuret compounds where it amounts to 5.0°–5.2° (Nardelli *et al.*, 1963). The mean plane through the non-hydrogen atoms of the entire triuret molecule is defined by the equation: $0.7642x' + 0.1937y + 0.6152z' = 2.2687$, which gives a distance of 3.45 Å between planes through molecules in different layers. The deviations of the individual atoms from the best plane through the molecule are given in Table 4. The distortion of the triuret molecule is probably due to

Table 4. *Perpendicular distances of atoms from mean plane*

C(1) –0.0060 Å	O(1) –0.1091 Å	N(1) +0.1985 Å
C(2) –0.0032 Å	O(2) +0.0771 Å	N(2) –0.1238 Å
C(3) –0.0009 Å	O(3) +0.2230 Å	N(3) +0.0046 Å
		N(4) –0.2604 Å

the repulsion of the two nitrogen atoms, N(1) and N(4), from the central oxygen atom, O(2), but the hydrogen bonding system may also play a role in the twist of the molecule. The small double bond character of the four C–NH bonds makes the observed distortion possible, resulting in abnormally large C–NH–C angles (128°) and an abnormally small NH–C–NH angle (111°). Apart from the twist, the molecule is rather symmetrical in that corresponding bond lengths and angles are closely similar in the two halves (Fig. 2).

Hydrogen bonding system

The positional parameters of the hydrogen atoms are given in Table 5. Although the accuracy in the hydrogen positions is rather low

$$(\sigma(x) = \sigma(y) = \sigma(z) \simeq 0.08 \text{ \AA}),$$

the positions obtained seem to be in agreement with ordinarily accepted bond lengths, angles and packing requirements. The hydrogen atoms lie essentially in the plane of the molecule, and the N–H bond lengths and angles are indicated in Fig. 2.

Table 5. *Positional parameters of hydrogen atoms in fractional coordinates* $\times 10^3$

Atom	x/a	y/b	z/c
H(1)	318 ± 12	602 ± 11	11 ± 4
H(2)	349 ± 12	355 ± 11	42 ± 4
H(3)	149 ± 11	582 ± 11	132 ± 3
H(4)	222 ± 11	376 ± 10	206 ± 4
H(5)	254 ± 11	–67 ± 11	141 ± 3
H(6)	268 ± 12	–189 ± 12	203 ± 4

Table 6. *Hydrogen bonds and short contacts*

Atoms	Distance (Å)	
N(1)–O(2)	2.76	intramolecular
N(4)–O(2)	2.79	
N(1)–O(1)	$(\frac{1}{2} - x, \frac{3}{2} - y, \bar{z})$	intermolecular (molecules of same layer)
N(3)–O(3)	$(\frac{1}{2} - x, \frac{1}{2} + y, \frac{1}{2} - z)$	
N(4)–O(3)	$(\frac{1}{2} - x, y - \frac{1}{2}, \frac{1}{2} - z)$	
N(4)–O(1)	$(x, y - 1, z)$	
N(1)–O(1)	$(\frac{1}{2} + x, y - \frac{1}{2}, z)$	intermolecular (molecules of adjacent layers)
N(2)–O(2)	$(x - \frac{1}{2}, \frac{1}{2} + y, z)$	

Five of the six hydrogen atoms are involved in hydrogen bonds and the actual N–O distances are listed in Table 6. The hydrogen bonding system is pictured in Fig. 3 which is an a -axis projection of the structure. As can be seen, there are two strong intramolecular hydrogen bonds, N(1)–O(2) and N(4)–O(2), of nearly the same lengths (2.77 and 2.79 Å respectively). This arrangement is in complete agreement with the situation found in biuret hydrate and biuret compounds having a *trans* form, where the intramolecular hydrogen bond has a length of 2.73–2.76 Å (Cavalca *et al.*, 1960; Hughes *et al.*, 1961).

The well-developed intermolecular hydrogen bonding system of triuret runs essentially parallel to the plane of the molecules. There are thus three bonds, N(1)–O(1) = 2.92 Å, N(3)–O(3) = 2.82 Å and N(4)–O(3) = 2.95 Å, which form an almost perfect network (dashed lines in Fig. 3) connecting molecules belonging to the same layer. Furthermore, there is a short contact, N(4)–O(1) = 3.08 Å (dotted line in Fig. 3) which may indicate the presence of a weak hydrogen bond. In addition to these four bonds, running more or less parallel to the (100) plane, there are two short distances between nitrogen atoms in one molecular layer and oxygen atoms in adjacent layers (dotted lines in Fig. 3). However, it is very

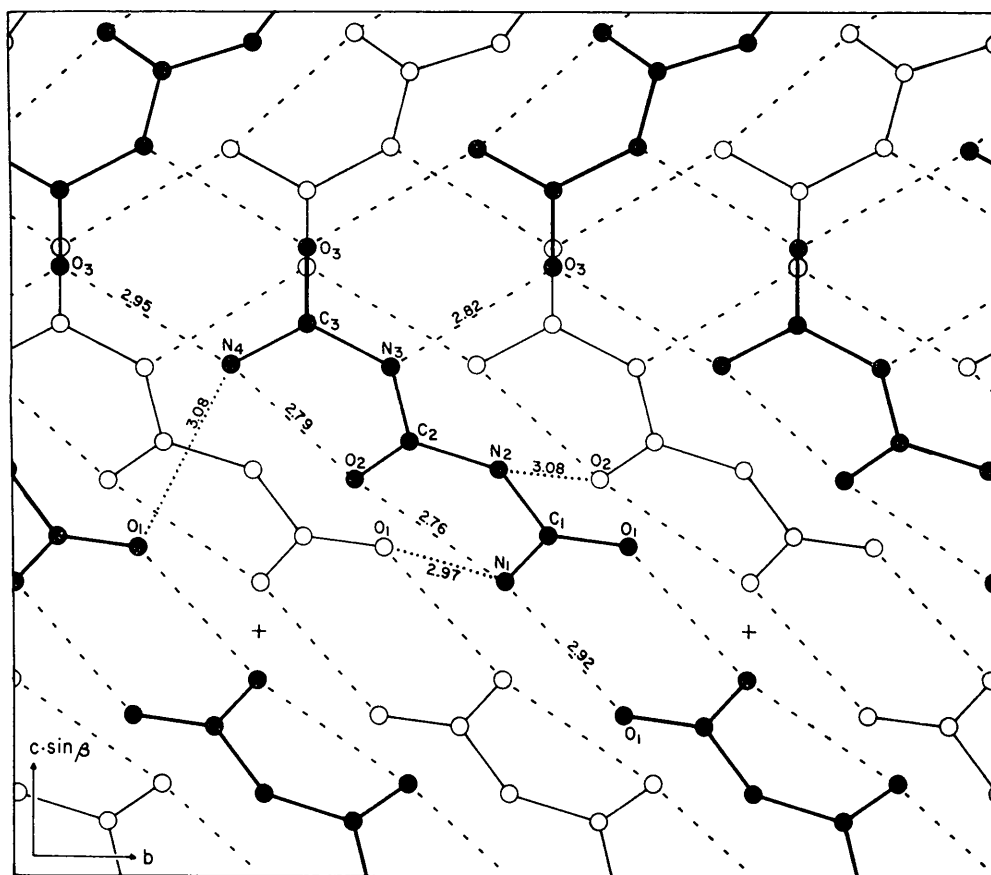


Fig. 3. Arrangement of molecules in part of a projection along the a axis. Origin at +. Filled circles indicate atoms in molecules belonging to the same layer, open circles refer to atoms in molecules of adjacent layers. Hydrogen bonding system indicated by dashed lines and short intermolecular contacts by dotted lines. Distances in Å.

doubtful if these short distances, $N(1)-O(1)=2.97$ Å and $N(2)-O(2)=3.08$ Å, really can be regarded as hydrogen bonds. Not only are the angles involved quite unfavourable but the actual H-O distances, 2.27 Å and 2.34 Å, are also almost as long as the ordinary van der Waals hydrogen-oxygen contact distance. In any case, these two 'bonds' must be very weak and the different layers of triuret molecules are primarily held together by van der Waals forces. This feature explains the presence of the perfect cleavage of triuret crystals along (100). All other intermolecular distances are consistent with ordinary packing requirements.

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A Refinement of the Crystal Structure of Cytidine

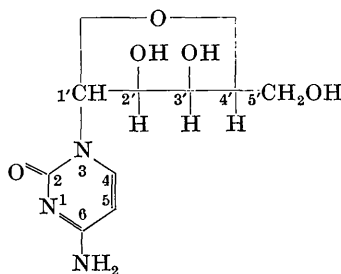
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The crystal structure of cytidine has been refined by least-squares calculations to a final R index of 5.6%. Three-dimensional photographic data were used. The essential features of the original structure are confirmed, but the standard deviations in the bond lengths are reduced to about 0.006 Å and in the bond angles to less than 0.5°. The distances and angles in the cytosine part of the molecule are closely similar to those found in cytosine monohydrate. The two angles at each external bond to pyrimidine appear to differ significantly. The N -glycosidic linkage is found to be 1.497 Å. The two bonds to the furanose ring oxygen atom differ in length by 0.03 Å. The existence of an intramolecular C-H...O interaction at 3.23 Å is confirmed.

The crystal structure of cytidine, $C_9H_{13}O_5N_3$, was determined by one of us fifteen years ago from three electron density projections refined to $R=0.17-0.19$ (Furberg, 1950). Pauling & Corey (1956), not considering this structure analysis reasonably reliable, deduced bond lengths and angles in the cytosine part of cytidine by considering the crystal structures of a number of other pyrimidines, mainly heavy atom derivatives. This work was later extended by Spencer (1959). Recently, an accurate crystal structure analysis



Cytidine

of cytosine hydrate has been published (Jeffrey & Kinoshita, 1963), and precise information on the ribose residue has been obtained through three-dimensional analysis of calcium thymidylate (Trueblood, Horn & Luzzati, 1961) and adenosine 5-phosphate (Kraut & Jensen, 1963). However, cytidine is still the only unsubstituted nucleoside the structure of which has been solved. It contains no heavy atoms and its aromatic ring is not protonated. We have therefore refined the structure in order to obtain more exact information on this part of the nucleic acids.

Experimental

The unit-cell dimensions were redetermined by least-squares calculations based on measurements of 28 lines registered on a Guinier camera calibrated against KCl. The following values were found:

$$a = 13.991 \pm 0.002, \quad b = 14.786 \pm 0.002, \\ c = 5.116 \pm 0.001 \text{ \AA}.$$

The uncertainties indicated are estimated standard deviations. The space group is $P2_12_12_1$ and there are four molecules in the unit cell.

Integrated equi-inclination Weissenberg photographs $hk0-hk2$, $h0l-hsl$ and $0kl$ were taken. Of the total of about 1450 unique reflexions within the $Cu K\alpha$ limit, 1195 were recorded. Of these 165 were too weak to measure. The intensities of each of the four equivalent reflexions were measured on a Hilger photometer and the mean value taken. The weakest reflexions were estimated visually. Crystals with cross-sections approximately 0.1×0.1 mm were used and no correction for absorption was applied. The various levels were placed on an approximately common absolute scale by means of calculated structure factors based on the two-dimensionally refined structure.

Refinement of the structure

The coordinates of the original structure were first refined by a number of difference syntheses in the a and c projections, including finally the thirteen hydrogen atoms. A common isotropic temperature factor was applied, and the values 0.12 and 0.10 were obtained for the R index. These coordinates were