Acta Cryst.(1972). **B28**, 659

The Crystal and Molecular Structure of Lumazine Hydrate

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(*Received* 19 *April* 1971)

The crystal structure of dilumazine trihydrate, $2C_6H_4N_4O_2.3H_2O$, has been determined by direct methods from three-dimensional diffractometer data taken with Cu $K\alpha$ radiation. The crystals are monoclinic, space group $P2_1/c$, $a=14{\cdot}865$, $b=16{\cdot}691$, $c=6{\cdot}829$ Å and $\beta=109{\cdot}80°$. The atomic parameters were refined by full-matrix least squares to a final R of 0·043. The crystal structure is built up of almost coplanar, hydrogen-bonded dimers of lumazine. The lumazine molecules are nearly planar. The two oxygens attached to the pyrimidinoid ring are both of keto type. The π bond scheme consistent with the observed bond distances classifies the electrons of the π pyrazinoid ring as delocalized.

Introduction

Among the biologically important group of substances containing pteridine, only the crystal structure of pteridine itself (Hamor & Robertson, 1956) has been determined.

This group contains, for example, pteroylglutamic acid (folic acid), which is an essential nutrient for man, lower animals, insects and microorganisms (*cf.* Wagner & Folkers, 1964). Furthermore, derivatives of pteridine-2,4-dione (lumazine) have been shown to be intermediates in the biosynthesis of flavins (Malay & Plaut, 1956).

The present structure determination of dilumazine trihydrate (Fig. 1), is part of a research program of studies of pteridine derivatives undertaken at this Institute.

Experimental

A crystalline specimen of dilumazine trihydrate was obtained by slow evaporation of an aqueous solution of commercially available lumazine (Aldrich Chemical Co.). A suitable single crystal was selected and preliminary X-ray investigations of its properties, using Weissenberg and oscillation photographs revealed the Laue symmetry to be $2/m$. The systematic absences were consistent with space group $P2_1/c$. The selected prismatic crystal, of the dimensions $0{\cdot}22\times0{\cdot}22\times0{\cdot}29$ mm, was mounted on a goniometer head along the unique axis. Unit-cell dimensions were refined by means of least-squares fitting of the cell parameters to measurements of reflexion positions made on a single-crystal diffractometer. (Cu $K\alpha$: 1·54184Å) Crystal data are summarized in Table 1.

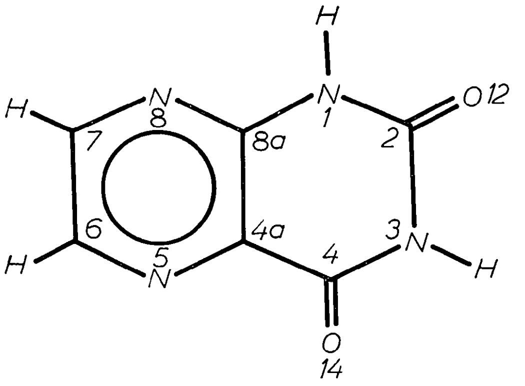

Fig. 1. Schematic drawing of lumazine (pteridine-2,4-dione).

Table 1. *Crystal data*

Composition of asymmetric unit	$2C_6H_4N_4O_2.3H_2O$
Space group	Monoclinic $P2_1/c$
Lattice constants	$a=14{\cdot}865$ (5) Å $b=16{\cdot}691$ (5) $c=\ 6{\cdot}829$ (5) $\beta=109{\cdot}80°$ (1)
Cell volume	1594·2 Å^3
Density (X-ray)	1·59 g.cm^{-3}

Molecules per unit cell $Z=4$
Linear absorption coefficient = 11·7 cm^{-1}

Three-dimensional single-crystal X-ray diffraction data were collected on a Siemens Automatic Single Crystal Diffractometer (Siemens AED), using graphite-monochromatized Cu $K\alpha$ radiation and a scintillation detector equipped with pulse height discriminator. The θ–2θ scan technique with a scan interval of 1·2° was used to collect all the 1689 independent reflexions with $\theta\leq51°$. For each reflexion the background intensity was estimated from intensity measurements at each end of the scan interval. The net intensities, I_{net}, and their estimated standard deviations, $\sigma(I_{\text{net}})$, based on counter statistics, were calculated and corrected for Lorentz, polarization and absorption ($\mu=11{\cdot}7$ cm^{-1}) effects. Only the 1543 most significant reflexions with $\sigma(I_{\text{net}})/I_{\text{net}}\leq0{\cdot}10$ were used in the subsequent calculations.

Structure determination and refinement

An approximate scale factor for the observed structure factors, and an overall temperature factor were calculated from a usual Wilson plot. Normalized structure factors, $|E|$, were calculated and rescaled to set the average of $|E|^2$ equal to 1. The distribution of the $|E|$'s obtained after rescaling is given in Table 2.

Table 2. *Statistical averages and distributions of normalized structure factors*

	Experimental	Theoretical (centric)
$\langle \lvert E \rvert \rangle$	0·72	0·798
$\langle \lvert E \rvert^2 - 1 \rangle$	1·14	0·968
$\langle \lvert E \rvert \rangle^2$	1·00	1·000
$\lvert E \rvert > 1$	24·3 %	32·0 %
$\lvert E \rvert > 2$	5·6	5·0
$\lvert E \rvert > 3$	1·8	0·3

The signs of the highest $|E|$ values were determined by solving the triple-product sign relationship among them (*cf.* Karle & Karle, 1966). The 250 highest $|E|$ values ($|E|$ greater than 1·30), were used to generate 958 triple relations with probabilities calculated to be greater than 0·91. Effectively, only 230 $|E|$ values were used, since 20 relatively small values did not enter into any triple relation. The high $|E|$ value of reflexions 002 and 004 (5·52 and 4·28 respectively) indicated the sign of 004 to be positive. The reflexion 002 entered into three different triple relations of $\sum_1$ type (Hauptman & Karle, 1953) all indicating negative sign.

With these two reflexions as a primary basis set, a computerized symbolic addition procedure (Norrestam, 1971) was undertaken to select a final basis set. To minimize the number of alternative solutions, *viz.* the number of unknown signs in the selected basis set, the following procedure was applied by the computer program. A new symbol was assigned, only when no more symbolic signs could be generated, to that reflexion which, at the current stage of the symbolic addition procedure, had received no phase assignment but had the largest frequency among the triple relations not used earlier. For the present structure four different symbols were needed to generate phase assignments for most of the reflexions.

Table 3. *Basis set used for solving the triple product sign relationships*

Indices	*E* value	Sign
002	5·52	−
004	4·28	+
1,14,0	5·07	+
−10,8,3	4·06	+
014	3·52	+
0,14,1	3·18	unknown (−)

Three of the symbol-defining reflexions could be assigned arbitrary signs, since they properly defined the

Table 4. *Observed and calculated structure factors*

Each group of three columns contains k, $10|F_o|$ and $10|F_c|$, and is headed by the values of h and l common to the group.

origin in this space group. The fourth of the symbol-defining reflexions remained unknown. Thus a final basis set consisting of five reflexions with known signs and one with unknown sign was obtained (Table 3). The corresponding two solutions of the triple product sign relationships were generated by running through the program twice more using only signs in the basis sets. Signs were generated for 228 of the possible 230 reflexions. At the completion of this structure determination, all the 228 signs determined from triple relations were found to be correct.

The $|E|$ map calculated from the solution for which the unknown 0,14,1 sign was set negative displayed the positions of all the 27 non-hydrogen atoms (Fig. 2) as the 27 highest peaks.

The structural model so obtained was refined by three cycles of full-matrix least-squares refinement, with isotropic temperature factors for all the 27 non-hydrogen atoms followed by three cycles with anisotropic temperature factors for all. A subsequent difference electron density map enabled the location of all the 14 hydrogen atoms. A final full-matrix least-squares refinement of all the structural parameters, with anisotropic temperature factors for the non-hydrogen atoms and isotropic ones for the hydrogens gave an R value ($R=\sum||F|_{\text{obs}}-|F|_{\text{calc}}|/\sum|F|_{\text{obs}}$) of 0·043 for the 1527 reflexions used. In this final refinement the strongest reflexion 002 was given zero weight, since it suffered severely from extinction. Zero weight was also given to 15 reflexions with $|F|_{\text{obs}}/|F|_{\text{calc}}$ less than 0·50 or greater than 2·00. The reflexions given zero weight in the final refinement are marked with an asterisk in Table 4, where $|F|_{\text{obs}}$ and $|F|_{\text{calc}}$ are listed. The R value for all the reflexions became 0·049.

Table 5. *Fractional atomic coordinates* ($\times 10^4$) *for the non-hydrogen atoms*

The estimated standard deviations are given in parentheses.

	x	y	z
N(1)	5155 (1)	2472 (1)	2048 (3)
C(2)	6063 (2)	2564 (1)	2047 (4)
N(3)	6580 (2)	1874 (1)	2142 (3)
C(4)	6286 (2)	1102 (2)	2321 (4)
C(4*a*)	5298 (2)	1042 (1)	2309 (4)
N(5)	4932 (1)	320 (1)	2423 (3)
C(6)	4037 (2)	302 (2)	2362 (4)
C(7)	3499 (2)	991 (2)	2172 (4)
N(8)	3846 (1)	1720 (1)	2062 (3)
C(8*a*)	4755 (2)	1740 (1)	2140 (3)
O(12)	6399 (1)	3230 (1)	1934 (3)
O(14)	6815 (1)	534 (1)	2456 (3)
N′(1)	−263 (1)	2957 (1)	2151 (3)
C′(2)	−1184 (2)	2857 (1)	2082 (4)
N′(3)	−1685 (2)	3541 (1)	2158 (3)
C′(4)	−1361 (2)	4320 (1)	2285 (4)
C′(4*a*)	−358 (2)	4389 (1)	2392 (3)
N′(5)	32 (1)	5124 (1)	2574 (3)
C′(6)	933 (2)	5156 (2)	2643 (4)
C′(7)	1431 (2)	4462 (2)	2515 (4)
N′(8)	1061 (1)	3733 (1)	2348 (3)
C′(8*a*)	151 (2)	3700 (1)	2297 (3)
O′(12)	−1544 (1)	2188 (1)	1977 (3)
O′(14)	−1863 (1)	4881 (1)	2354 (3)
O(*W*1)	4169 (1)	3802 (1)	2384 (3)
O(*W*2)	899 (2)	1663 (1)	2148 (3)
O(*W*3)	2430 (1)	2989 (1)	821 (4)

Table 6. *Anisotropic thermal parameters* ($\times 10^4$)

The estimated standard deviations are given in parentheses.
The temperature factor expression used is $\exp[-(h^2b_{11}+k^2b_{22}+l^2b_{33}+hkb_{12}+hlb_{13}+klb_{23})]$.

	b_{11}	b_{22}	b_{33}	b_{12}	b_{13}	b_{23}
N(1)	33 (1)	17 (1)	277 (6)	0 (2)	90 (4)	8 (3)
C(2)	33 (2)	22 (1)	206 (6)	−2 (2)	73 (4)	7 (4)
N(3)	33 (1)	26 (1)	290 (6)	4 (2)	96 (4)	13 (3)
C(4)	39 (2)	23 (1)	240 (7)	4 (2)	59 (5)	−6 (4)
C(4*a*)	36 (1)	20 (1)	206 (6)	−2 (2)	61 (4)	1 (4)
N(5)	46 (1)	24 (1)	277 (6)	−6 (2)	82 (4)	−4 (3)
C(6)	48 (2)	22 (1)	303 (8)	−19 (2)	101 (6)	1 (4)
C(7)	36 (2)	29 (1)	309 (8)	−8 (2)	102 (6)	−9 (4)
N(8)	36 (1)	26 (1)	275 (6)	−8 (2)	90 (4)	0 (3)
C(8*a*)	31 (1)	23 (1)	178 (6)	−2 (2)	53 (4)	0 (4)
O(12)	39 (1)	26 (1)	357 (6)	−4 (1)	115 (4)	16 (3)
O(14)	47 (1)	26 (1)	510 (7)	21 (2)	132 (4)	15 (3)
N′(1)	33 (1)	20 (1)	281 (6)	−2 (2)	78 (4)	−4 (3)
C′(2)	34 (1)	21 (1)	227 (7)	−4 (2)	70 (5)	5 (4)
N′(3)	34 (1)	26 (1)	304 (7)	1 (2)	94 (4)	−11 (3)
C′(4)	39 (1)	21 (1)	237 (7)	5 (2)	79 (5)	0 (4)
C′(4*a*)	39 (1)	21 (1)	180 (6)	−3 (2)	63 (5)	−5 (3)
N′(5)	47 (1)	27 (1)	232 (5)	−10 (2)	78 (4)	−3 (3)
C′(6)	49 (2)	27 (1)	249 (7)	−17 (2)	81 (5)	−7 (4)
C′(7)	35 (2)	32 (1)	299 (8)	−15 (2)	89 (5)	1 (4)
N′(8)	36 (1)	29 (1)	297 (6)	−10 (2)	93 (4)	11 (4)
C′(8*a*)	32 (2)	23 (1)	192 (6)	−5 (2)	60 (4)	7 (4)
O′(12)	40 (1)	24 (1)	406 (6)	−9 (1)	119 (4)	−19 (3)
O′(14)	51 (1)	28 (1)	456 (7)	15 (2)	137 (4)	−16 (3)
O(*W*1)	50 (1)	23 (1)	449 (7)	4 (2)	113 (5)	−18 (3)
O(*W*2)	69 (1)	29 (1)	377 (7)	17 (2)	131 (5)	27 (3)
O(*W*3)	46 (1)	38 (1)	536 (7)	17 (2)	150 (5)	35 (3)

In all the refinements Hughes's (1941) weighting scheme was used. The shifts in the parameters in the last cycle of refinement were all less than 0·1 of their estimated standard deviations. The atomic scattering factors used for carbon, nitrogen and oxygen were those given by Hanson, Herman, Lea & Skillman (1964), for hydrogen that given by Stewart, Davidson & Simpson (1965).

The asymmetric unit in the crystal structure contains two different lumazine molecules. To distinguish between these, the atomic labels used are primed for one of these molecules. The numbering of the atoms in the lumazine molecules is shown in Fig. 3. The three different water molecules in the asymmetric unit of the crystal structure are designated *W*1, *W*2 and *W*3 respectively.

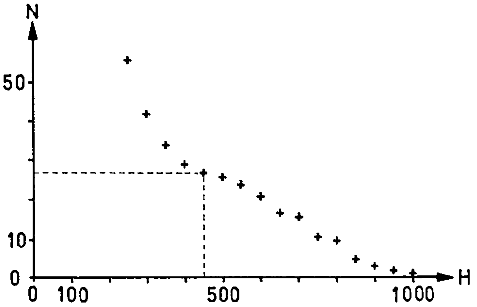

Fig. 2. Peak scan result obtained for the E map calculated with 228 phased E values. The peak heights, H, are normalized so that the highest peak has the height $H=999$. The number of peaks, N, higher than H is plotted against H. The dashed lines indicate the 27 highest peaks corresponding to the 27 non-hydrogens of the asymmetric unit.

In Table 5 the atomic coordinates for the non-hydrogen atoms together with their estimated standard deviations are given. The thermal parameters for these atoms are listed in Table 6. The parameters obtained for the hydrogens are given in Table 7. The atomic labels used are shown in Fig. 3.

Table 7. *Fractional atomic coordinates* ($\times 10^3$) *and isotropic temperature factors for the hydrogen atoms*

The estimated standard deviations are given in parentheses.

	x	y	z	B
H(1)	485 (2)	288 (2)	199 (3)	2.2 (5) Å^2
H(3)	722 (2)	189 (2)	214 (4)	3·6 (6)
H(6)	377 (2)	−17 (2)	244 (4)	3·8 (6)
H(7)	288 (2)	99 (2)	206 (4)	3·7 (6)
H′(1)	10 (2)	247 (2)	219 (3)	2·6 (5)
H′(3)	234 (2)	345 (2)	212 (4)	4·0 (6)
H′(6)	131 (2)	565 (2)	207 (4)	5·0 (6)
H′(7)	201 (2)	454 (2)	259 (4)	4·2 (6)
H(*W*1*a*)	430 (2)	436 (2)	229 (5)	6·1 (8)
H(*W*1*b*)	347 (3)	379 (2)	179 (5)	6·5 (8)
H(*W*2*a*)	151 (4)	173 (3)	327 (9)	12·5 (18)
H(*W*2*b*)	70 (3)	105 (4)	213 (7)	11·2 (13)
H(*W*3*a*)	199 (3)	305 (2)	145 (7)	8·1 (11)
H(*W*3*b*)	286 (3)	260 (2)	122 (7)	7·8 (10)

In Tables 8, 9 and 10 the intramolecular bond distances and bond angles are listed. No corrections for

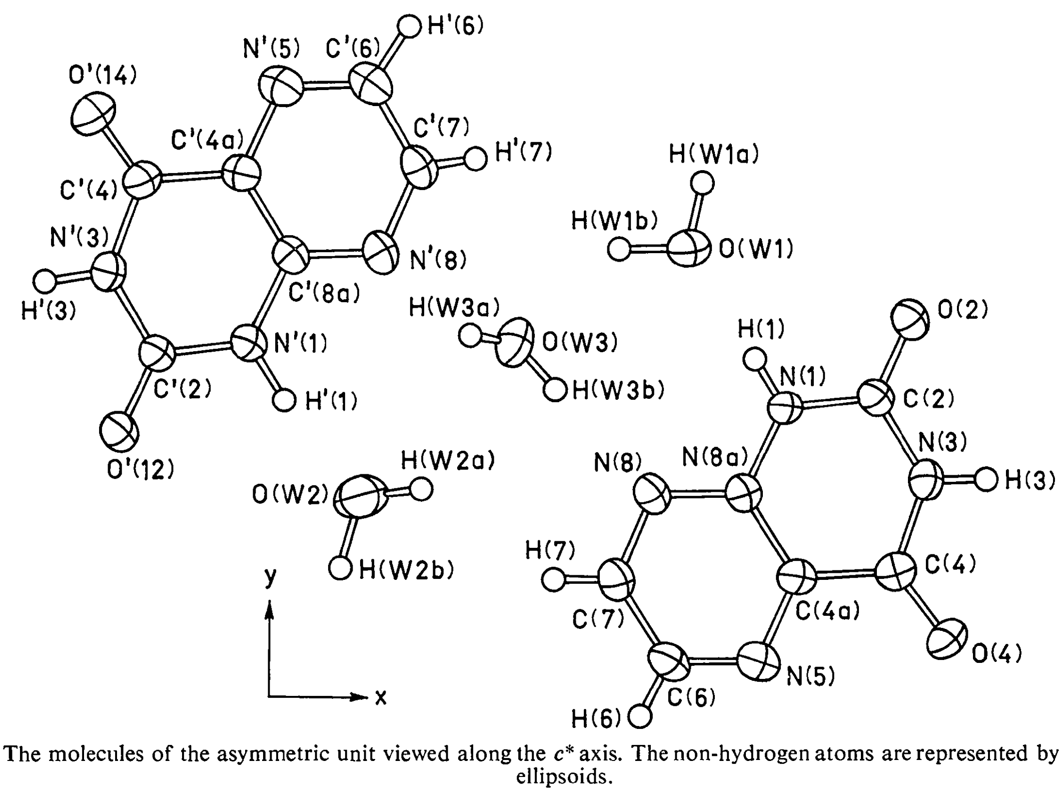

Fig. 3. The molecules of the asymmetric unit viewed along the c^* axis. The non-hydrogen atoms are represented by their thermal ellipsoids.

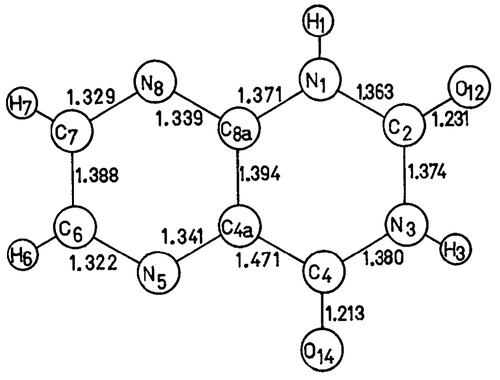

Fig. 4. The intramolecular distances between the non-hydrogen atoms averaged over the two different lumazine molecules in the asymmetric unit.

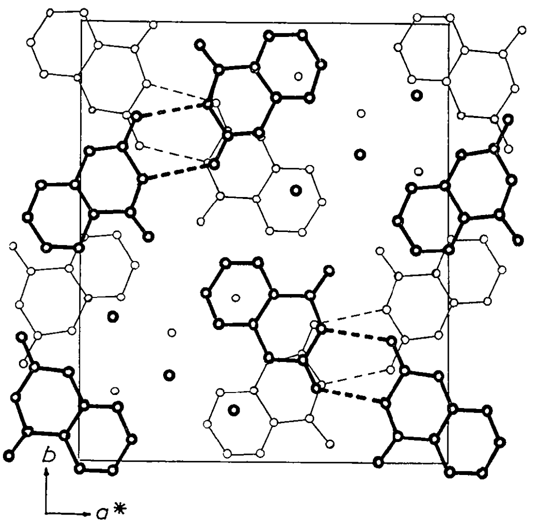

Fig. 5. Packing diagram of the crystal structure viewed along the *c* axis *i.e.* projected on the *a*b* plane. Molecules drawn with thicker lines lie at $z \simeq 0{\cdot}75$, while the others lie at $z \simeq 0{\cdot}25$.

thermal vibrations have been performed. The distances between the non-hydrogen atoms averaged over the two different lumazine molecules in the asymmetric unit are shown in Fig. 4.

Table 8. *Intramolecular bond distances within the two lumazine molecules*

The estimated standard deviations are given in parentheses.

N(1)—C(2)	1·359 (3) Å	N(1)–H(1)	0·81 (3) Å
N′(1)—C′(2)	1·365 (3)	N′(1)–H′(1)	0·97 (3)
C(2)—N(3)	1·374 (3)	N(3)–H(3)	0·96 (3)
C′(2)—N′(3)	1·374 (3)	N′(3)–H′(3)	0·97 (3)
N(3)—C(4)	1·380 (3)	C(6)—H(6)	0·89 (3)
N′(3)—C′(4)	1·379 (3)	C′(6)–H′(6)	0·98 (3)
C(4)—C(4*a*)	1·470 (3)	C(7)—H(7)	0·90 (3)
C′(4)—C′(4*a*)	1·472 (3)	C′(7)–H′(7)	0·86 (3)
C(4*a*)–N(5)	1·335 (3)		
C′(4*a*)-N′(5)	1·346 (3)		
N(5)—C(6)	1·318 (4)		
N′(5)—C′(6)	1·325 (4)		
C(6)—C(7)	1·382 (4)		
C′(6)—C′(7)	1·394 (4)		
C(7)—N(8)	1·333 (3)		
C′(7)—N′(8)	1·325 (3)		
N(8)—C(8*a*)	1·335 (3)		
N′(8)—C′(8*a*)	1·342 (3)		
C(8*a*)–N(1)	1·369 (3)		
C′(8*a*)-N′(1)	1·373 (3)		
C(8*a*)–C(4*a*)	1·399 (3)		
C′(8*a*)-C′(4*a*)	1·390 (3)		
C(2)—O(12)	1·232 (3)		
C′(2)—O′(12)	1·229 (3)		
C(4)—O(14)	1·215 (3)		
C′(4)—O′(14)	1·209 (3)		

Discussion

As shown in Fig. 5, the crystal structure is built up by an almost planar arrangement of hydrogen bonded molecules perpendicular to the *c* axis. Each of these molecular planes consists of hydrogen bonded dimers of the two different, not symmetry related, lumazine molecules. The lumazine dimers are held together in

Table 9. *Intramolecular bond angles within the two lumazine molecules*

Only bond angles involving non-hydrogen atoms are given.
The estimated standard deviations are given within parentheses.

C(8*a*) -N(1)-C(2)	123·2 (2)°	C(4)—C(4*a*)-N(5)	119·1 (2)°
C′(8*a*)-N′(1)-C′(2)	122·2 (2)	C(4)—C(4*a*)-C(8*a*)	119·4 (2)
N(1)—C(2)—N(3)	116·4 (2)	C(8*a*)-C(4*a*)-N(5)	121·4 (2)
N(1)—C(2)—O(12)	121·8 (2)	C′(4)—C′(4*a*)-N′(5)	118·3 (2)
O(12)-C(2)—N(3)	121·8 (2)	C′(4)—C′(4*a*)-C′(8*a*)	119·4 (2)
N′(1)—C′(2)-N′(3)	116·6 (2)	C′(8*a*)-C′(4*a*)-N′(5)	122·3 (2)
N′(1)—C′(2)-O′(12)	121·7 (2)	C(4*a*)-N(5)—C(6)	116·5 (2)
O′(12)-C′(2)-N′(3)	121·8 (2)	C′(4*a*)-N′(5)—C′(6)	116·0 (2)
C(2)— N(3)-C(4)	126·8 (2)	N(5)—C(6)—C(7)	122·0 (3)
C′(2)—N′(3)-C′(4)	127·2 (2)	N′(5)—C′(6)—C′(7)	121·1 (3)
N(3)—C(4)—C(4*a*)	114·1 (2)	C(6)—C(7)—N(8)	122·8 (3)
N(3)—C(4)—O(14)	121·3 (2)	C′(6)—C′(7)—N′(8)	123·8 (3)
O(14)-C(4)—C(4*a*)	124·6 (2)	C(7)—N(8)—C(8*a*)	115·3 (2)
N′(3)—C′(4)-C′(4*a*)	113·8 (2)	C′(7)—N′(8)—C′(8*a*)	115·1 (2)
N′(3)—C′(4)-O′(14)	121·7 (2)	N(8)—C(8*a*)-N(1)	118·0 (2)
O′(14)-C′(4)-C′(4*a*)	124·5 (2)	N(8)—C(8*a*)-C(4*a*)	122·0 (2)
		C(4*a*)-C(8*a*)-N(1)	120·0 (2)
		N′(8)—C′(8*a*)-N′(1)	117·4 (2)
		N′(8)—C′(8*a*)-C′(4*a*)	121·7 (2)
		C′(4*a*)-C′(8*a*)-N′(1)	120·8 (2)

one molecular plane by hydrogen bonds involving the water molecules.

Table 10. *Intramolecular bond distances and bond angles within the three water molecules*

The estimated standard deviations are given in parentheses.

O(*W*1)-H(*W*1*a*)	0·96 (4) Å
O(*W*1)-H(*W*1*b*)	0·98 (4)
O(*W*2)-H(*W*2*a*)	0·98 (6)
O(*W*2)-H(*W*2*b*)	1·06 (6)
O(*W*3)-H(*W*3*a*)	0·90 (4)
O(*W*3)-H(*W*3*b*)	0·89 (4)
H(*W*1*a*)-O(*W*1)-H(*W*1*b*)	102 (3)°
H(*W*2*a*)-O(*W*2)-H(*W*2*b*)	107 (4)
H(*W*3*a*)-O(*W*3)-H(*W*3*b*)	120 (4)

The hydrogen bond scheme, shown in Fig. 6, connects the molecules located in the planes $z = 0{\cdot}25$ and 0·75. The only remarkable deviation from the sheet structure is the position of one of the three water molecules, *W*3. This water molecule deviates by about 0·8 Å from the molecular planes and is involved in the only hydrogen bond [O(*W*2)-H···O(*W*3)] formed between two adjacent molecular planes.

A compilation of the hydrogen bond distances and angles is given in Table 11. In Table 12 all intermolecular distances less than 3·2 Å are listed.

As mentioned above the two different lumazine molecules of the asymmetric unit form hydrogen bonded dimers. The two hydrogen bonds responsible for the dimerization occur between the nitrogen atom N(3) of one molecule and the keto oxygen O(12) of another. The nitrogen–oxygen distances in these bonds, 2·878 and 2·847 Å respectively, together with the corresponding hydrogen–oxygen distances, 1·94 and 1·88 Å, indicate normal N–H···O hydrogen bonds (Hamilton & Ibers, 1968).

The hydrogens on the other nitrogen atoms N(1) [and N′(1)] of each lumazine molecule, are involved in hydrogen bonds to the water molecules *W*1 and *W*2, giving nitrogen–oxygen distances of 2·712 and 2·765 Å and corresponding hydrogen–oxygen distances of 1·91 and 1·80 Å.

The four hydrogens of the water molecules *W*1 and *W*2 participate in two hydrogen bonds to the nitrogen atoms N(5) and N′(5) of each of the lumazine molecules and in two hydrogen bonds to the third water molecule *W*3. The two hydrogens of the remaining water molecule *W*3 form hydrogen bonds to the nitrogens N(8) and N′(8) of the lumazine molecules.

Thus, each of the three water molecules acts as a hydrogen donor in two hydrogen bonds. Each of the two water molecules *W*1 and *W*2 is acceptor in one hydrogen bond, while *W*3 is an acceptor in two hydrogen bonds.

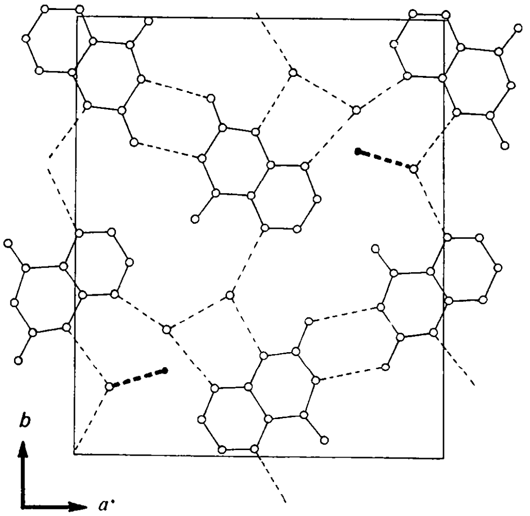

Fig. 6. Schematic drawing of the hydrogen bonds between molecules at $z \simeq 0{\cdot}25$. The thick dashed lines indicate the hydrogen bonds in the **c** direction between the water molecules *W*2 and *W*3.

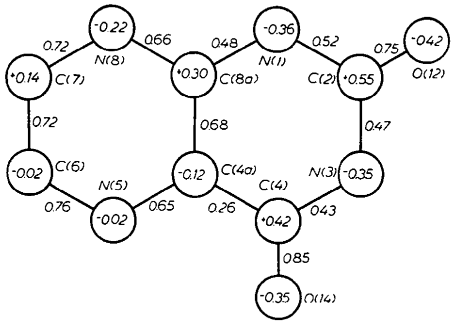

Fig. 7. Atomic charges (within the circles) from CNDO calculations and estimated π-bond orders.

Table 11. *Distances and angles of hydrogen bonds*

The estimated standard deviations are given in parentheses.

Hydrogen bond	Hydrogen bond angle	Distance	
N(1)—H(1)···O(*W*1)	169 (2)°	H(1)···O(*W*1)	1·91 (3) Å
N′(1)—H′(1)···O(*W*2)	172 (2)	H′(1)···O(*W*2)	1·80 (3)
N(3)—H(3)···O′(12)	167 (2)	H(3)···O′(12)	1·94 (3)
O(*W*1)-H(*W*1*a*)···N(5)	158 (3)	H(*W*1*a*)···N(5)	1·93 (4)
N′(3)—H′(3)···O(12)	177 (2)	H′(3)···O(12)	1·88 (3)
O(*W*2)-H(*W*1*b*)···N′(5)	157 (4)	H(*W*1*b*)···N′(5)	1·95 (6)
O(*W*1)-H(*W*1*b*)···O(*W*3)	139 (3)	H(*W*1*b*)···O(*W*3)	1·98 (4)
O(*W*2)-H(*W*1*a*)···O(*W*3)	162 (5)	H(*W*1*a*)···O(*W*3)	1·87 (6)
O(*W*3)-H(*W*3*a*)···N′(8)	151 (4)	H(*W*3*a*)···N′(8)	2·04 (4)
O(*W*3)-H(*W*3*b*)···N(8)	179 (4)	H(*W*3*b*)···N(8)	2·01 (4)

Table 12. *Intermolecular distances less than* 3·2 Å *between nonhydrogen atoms*

Estimated standard deviations are given in parentheses.
Code for symmetry related atoms

Superscript	Atom at		
None	x,	y,	z
i	$1+x$,	y,	z
ii	$-1+x$,	y,	z
iii	$1-x$,	$-\frac{1}{2}+y$,	$\frac{1}{2}-z$
iv	$-x$,	$\frac{1}{2}+y$,	$\frac{1}{2}-z$
v	$-x$,	$-\frac{1}{2}+y$,	$\frac{1}{2}-z$
vi	$1-x$,	$\frac{1}{2}+y$,	$\frac{1}{2}-z$
vii	x,	$\frac{1}{2}-y$,	$-\frac{1}{2}+z$
viii	x,	$\frac{1}{2}-y$,	$\frac{1}{2}+z$

N(1)—O(*W*1)	2·712 (3) Å
N′(1)—O(*W*2)	2·765 (3)
N(3)—O′(12$^{\mathrm{i}}$)	2·878 (3)
N′(3)--O(12$^{\mathrm{ii}}$)	2·847 (3)
N(5)—O(*W*1$^{\mathrm{iii}}$)	2·847 (3)
N′(5)—O(*W*2$^{\mathrm{iv}}$)	2·954 (3)
C(7)—O′(14$^{\mathrm{v}}$)	3·159 (3)
C′(7)—O(14$^{\mathrm{vi}}$)	3·156 (3)
N(8)—O(*W*3)	2·902 (3)
N′(8)—O(*W*3)	2·865 (3)
C(8*a*)—O(*W*1$^{\mathrm{vii}}$)	3·195 (3)
C′(8*a*)—O(*W*2$^{\mathrm{viii}}$)	3·175 (4)
O(*W*1)–O(*W*3)	2·792 (3)
O(*W*2)–O(*W*3$^{\mathrm{viii}}$)	2·819 (3)

In the lumazine molecules all the nitrogens, but only one of the two keto oxygens, O(12), are involved in hydrogen bonds. The other keto oxygen, O(14), is involved in the shortest intermolecular contact (3·16 Å), other than hydrogen bonding, found in the structure. This contact is formed between O(14) and the carbon atom C(7). There are two more fairly short contact distances (3·195 and 3·175 Å) in the structure, from the water oxygens *W*1 and *W*2 to the carbon atoms C(8*a*) and C′(8*a*) respectively. As described above the two water oxygens participate as acceptors in only one hydrogen bond each. Thus, these oxygens are well suited to form electrostatically favorable short contacts with positively charged atoms.

To investigate these short contacts from a simplified electrostatic point of view, semi-empirical molecular orbital calculations of the CNDO type (Pople, Santry & Segal, 1965) were carried out for the lumazine molecule. The computer program used in these calculations was kindly put to our disposal by Rolf Manne (Quantum Chemistry Group, Uppsala). From the computed atomic charges (Fig. 7) it is seen that the largest positive atomic charges, except for those of the carbon atoms of the keto groups, are calculated for the carbon atoms C(8*a*) and C(7). Since short intermolecular contacts are formed between these two carbons and different negatively charged oxygen atoms, it is probable that the crystal structure is stabilized not only by hydrogen bonds and conventional van der Waals forces, but also by an electrostatically favourable molecular packing.

In Table 13 the intramolecular distances, averaged over the two different lumazine molecules in the asymmetric unit, are compared with the distances evaluated from self-consistent field molecular orbital calculations of the Pariser–Parr–Pople type. The molecular orbitals have been evaluated by means of a computer program kindly put at our disposal by Marianne Sundbom (Institute of Theoretical Physics, University of Stockholm). The agreement between predicted and observed distances is encouraging; the largest differences, 0·026 Å, occur for the C(8a)–N(1) and C(4)–O(14) bonds. However, the calculated distances are on the average 0·009 Å longer than the observed ones. This difference is at least partly ascribable to the fact that the observed distances in the present study were calculated as the separations between the maxima of the distributions of atoms undergoing thermal motions rather than the separations between true atomic positions, *i.e.* the calculated distances have not been corrected for effects of thermal vibration. Such corrections will always lead to slightly increased bond distances (Busing & Levy, 1964) and thus to a better accordance between predicted and observed distances in the present study.

Table 13. *Comparison of the observed intramolecular distances in lumazine with those evaluated from molecular orbital calculations of the Pariser–Parr–Pople type*

	Observed	Calculated
N(1)—C(2)	1·363 Å	1·375 Å
C(2)—N(3)	1·374	1·376
N(3)—C(4)	1·380	1·373
C(4)—C(4*a*)	1·471	1·465
C(4*a*)–N(5)	1·341	1·342
N(5)—C(6)	1·322	1·334
C(6)—C(7)	1·388	1·403
C(7)—N(8)	1·329	1·335
N(8)—C(8*a*)	1·339	1·344
C(8*a*)–N(1)	1·371	1·397
C(8*a*)–C(4*a*)	1·394	1·410
C(2)—O(12)	1·231	1·245
C(4)—O(14)	1·212	1·238

To visualize the π-bond scheme consistent with the observed intramolecular distances in the crystal structure of dilumazine trihydrate, approximate π-bond orders were estimated by means of the linear π-bond order–bond length correlation functions suggested by Roos & Skancke (1967) and Fischer-Hjalmars & Sundbom (1968). The obtained bond orders are shown in Fig. 7. The π electrons of the pyrazinoid ring are largely delocalized giving estimated π-bond orders (ranging from 0·65 to 0·76) of the same magnitude as in *e.g.* benzene, in accordance with the naive bond scheme shown in Fig. 1. In the pyrimidinoid ring the estimated π-bond orders are generally smaller, with the lowest value, 0·26, obtained for the C(4)–C(4*a*) bond. The two oxygens O(12) and O(14) are obviously both of keto type.

Least-squares planes calculated for the two different lumazine molecules of the asymmetric unit show that these are nearly planar, though not provable so according to the χ^2 test. Table 14 gives the deviations of individual atoms, from the four least-squares planes fitted to the different six atoms forming the pyrazinoid and pyrimidinoid rings of the two different lumazine molecules. The lumazine molecule having the primed atomic labels is somewhat more nearly planar than the other lumazine molecule. The angle between the normals to the planes through the pyrazinoid and pyrimidinoid rings is 1·1° for the molecule having primed atomic labels, while it is 1·7° for the other molecule.

Table 14. *Deviations of the atoms from least-squares planes*

The planes are of the form $AX+BY+CZ=D$, where X, Y and Z are in Å relative to the axes a^*, b and c. The atoms indicated with asterisks were omitted from the calculations of the least-squares planes.

Deviation from plane I		Deviation from plane II	
N(1)	0·008 Å	N′(1)	−0·003 Å
C(2)	0·008	C′(2)	0·003
N(3)	−0·018	N′(3)	0·004
C(4)	0·010	C′(4)	−0·009
C(4*a*)	0·006	C′(4*a*)	0·009
C(8*a*)	−0·015	C′(8*a*)	−0·003
*O(12)	0·010	*O′(12)	0·020
*O(14)	0·024	*O′(14)	0·000
*H(1)	0·02	*H′(1)	0·06
*H(3)	−0·03	*H′(3)	0·01

Deviation from plane III		Deviation from plane IV	
C(4*a*)	−0·003 Å	C′(4*a*)	−0·005 Å
C(8*a*)	0·004	C′(8*a*)	0·006
N(5)	−0·001	N′(5)	0·000
C(6)	0·004	C′(6)	0·005
C(7)	−0·003	C′(7)	−0·004
N(8)	0·000	N′(8)	−0·001
*H(6)	0·01	*H′(6)	0·13
*H(7)	−0·04	*H′(7)	0·01

Plane	*A*	*B*	*C*	*D*	E.s.d. of the six atoms from the plane
I	0·3310	0·0705	0·9410	1·543	0·016 Å
II	0·3100	−0·0620	0·9487	1·103	0·008
III	0·3042	0·0757	0·9496	1·353	0·004
IV	0·3132	−0·0798	0·9463	0·980	0·006

All the calculations were performed on the IBM 1800 and IBM 360/75 computers.

The authors are indebted to Professor Peder Kierkegaard for his active and stimulating interest in this work and for all the facilities placed at their disposal. The authors also wish to thank Drs Marianne Sundbom and Björn Roos for helpful discussions. Thanks are also due to Dr Don Koenig for his correction of the English of this paper. This investigation has been performed with financial support from the Tri-Centennial Fund of the Bank of Sweden and from the Swedish Natural Science Research Council. A research fellowship from the Stiftelsen Bengt Lundqvists Minne to one of us (RN) is gratefully acknowledged.

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