On Hydrogen Atom Positions in 3-Deazauracil

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The positions of the contentious and important hydrogen atoms in the modified nucleic acid base 3-deazauracil are determined from single crystal neutron diffraction data, with the fully characterised hydrogen bonding scheme emphasising the strength of this interaction in the structure.

The modified nucleoside 3-deazauridine exhibits powerful cytostatic properties,[1] believed to be associated with a very strong O···O hydrogen bond observed in the crystal structure.[2] The existence of this strong intermolecular hydrogen bond in the associated base, 3-deazauracil (2-hydroxypyrid-4-one, $C_5H_5NO_2$), was confirmed in an earlier X-ray study.[3] However, in neither X-ray study was the hydrogen atom involved in this strong O(4)···O(2) bond (with an O···O separation of some 2.55 Å) adequately located. Indeed, in the X-ray study of the title compound, it was not found possible to locate this hydrogen atom at all. Given the relevance of this hydrogen atom to both the hydrogen bonding scheme in this material and to the tautomeric form adopted by the molecule, neutron diffraction studies have been used to determine the hydrogen atom parameters.

The initial neutron diffraction work carried out on 3-deazauracil was peformed using powdered material, and structural information was extracted using constrained Rietveld refinement techniques.[4] The results of this study indicated that the hydrogen atom was indeed attached to oxygen O(4), but the refinement obtained was not of the highest quality owing to the problems inherent in the use of neutron powder diffraction with hydrogeneous materials. For this reason, once large single crystals of 3-deazauracil had been obtained, it was decided to perform a single crystal neutron diffraction study to elucidate further the details of the molecular geometry.

Large (*ca.* 3 × 3 × 2 mm^3) single crystals of the title compound were obtained from an aqueous solution (material purchased from Sigma Chemical Co.). Crystal quality and unit cell dimensions were determined by Weissenberg photographs, which confirmed the previous X-ray results. 3-Deazauracil crystallises in the orthorhombic space group $P2_12_12_1$, with $a = 8.638$, $b = 5.279$, $c = 11.220$ Å, $Z = 4$, $D_c = 1.44$ g cm^{-3}. No attempt was made to refine further these unit cell parameters, previously found in the X-ray study.

Data from one of these crystals were collected on the single crystal diffractometer SXD at the ISIS Spallation Neutron

Table 1 3-Deazauracil. Fractional coordinates and equivalent isotropic temperature factors for joint refinement and neutron only refinement [with X-ray parameters for comparison]

Atom	X/a	Y/b	Z/c	$U_{eq}/\times 10^{-3}$ Å^2
N(1)	0.3254(5)	0.3440(9)	0.7110(3)	27(1)
	0.3268(23)	0.3445(5)	0.7115(25)	10(4)
	[0.3255(3)	0.3429(5)	0.7102(2)	27(1)]
H(1)	0.413(6)	0.326(13)	0.755(5)	33(11)[a]
	0.418(6)	0.319(18)	0.754(7)	16(12)
C(2)	0.3238(5)	0.5448(10)	0.6358(4)	23(1)
	0.328(4)	0.533(6)	0.633(4)	17(8)
	[0.3245(3)	0.5441(5)	0.6344(2)	26(1)]
O(2)	0.4418(4)	0.6855(8)	0.6331(3)	35(1)
	0.4417(29)	0.690(1)	0.630(4)	21(9)
	[0.4417(3)	0.6865(5)	0.6315(2)	36(1)]
C(3)	0.1896(5)	0.5801(10)	0.5664(4)	23(1)
	0.1921(26)	0.5798(53)	0.5644(29)	5(6)
	[0.1899(3)	0.580(5)	0.5650(2)	24(1)]
H(3)	0.181(6)	0.736(14)	0.515(5)	42(12)[a]
	0.185(7)	0.749(17)	0.504(7)	25(15)
C(4)	0.0681(5)	0.415(7)	0.5760(4)	24(1)
	0.0702(33)	0.4151(67)	0.5775(32)	13(8)
	[0.0671(3)	0.4147(5)	0.5735(2)	26(1)]
O(4)	−0.0648(4)	0.4434(8)	0.5149(3)	35(1)
	−0.0610(33)	0.443(8)	0.514(4)	14(8)
	[−0.0638(2)	0.4432(5)	0.5143(2)	37(1)]
H(4)	−0.053(6)	0.627(13)	0.454(5)	120(25)[a]
	−0.058(6)	0.611(24)	0.456(8)	19(14)
C(5)	0.0753(6)	0.2083(11)	0.6533(5)	30(1)
	0.0735(34)	0.207(8)	0.652(4)	16(6)
	[0.0761(4)	0.2074(6)	0.6526(3)	32(1)]
H(5)	−0.022(6)	0.066(13)	0.663(5)	34(11)[a]
	−0.023(6)	0.065(20)	0.665(10)	26(13)
C(6)	0.2056(6)	0.1771(11)	0.7189(5)	31(1)
	0.2043(27)	0.178(7)	0.718(4)	34(9)
	[0.2055(4)	0.1774(6)	0.7184(2)	32(1)]
H(6)	0.217(6)	0.047(13)	0.766(5)	56(14)[a]
	0.219(6)	0.051(17)	0.768(7)	49(18)]

[a] Hydrogen atoms refined isotropically, U_{iso} quoted.

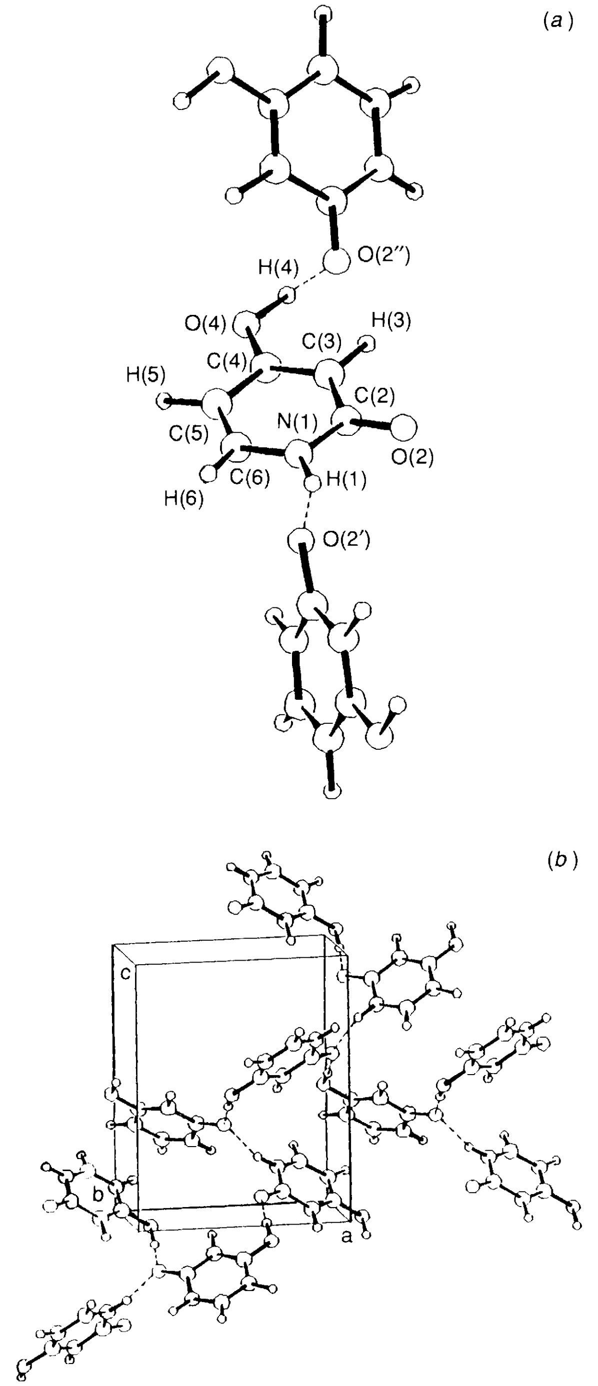

Fig. 1 (*a*) The structure of 3-deazauracil, showing atomic numbering and the detailed hydrogen bonding geometry determined from the neutron diffraction data. (*b*) A packing diagram indicating the molecular arrangement in the unit cell. Drawn using SCHAKAL.[8]

Table 2 3-Deazuracil. Bond lengths (Å) and angles (°) for joint X-ray and neutron and neutron only refinements (esds in parentheses) [with X-ray values for comparison]

	Joint	Neutron	X-Ray
N(1)–C(2)	1.362(6)	1.35(5)	[1.360(4)]
N(1)–C(6)	1.367(7)	1.38(4)	[1.359(4)]
C(2)–O(2)	1.266(6)	1.27(5)	[1.262(4)]
C(2)–C(3)	1.409(6)	1.42(5)	[1.412(3)]
C(3)–C(4)	1.375(7)	1.37(4)	[1.381(4)]
C(4)–O(4)	1.346(5)	1.36(6)	[1.319(3)]
C(4)–C(5)	1.400(7)	1.38(5)	[1.411(4)]
C(5)–C(6)	1.355(7)	1.36(5)	[1.348(4)]
N(1)–H(1)	0.91(6)	0.92(6)	[0.81]
C(3)–H(3)	1.01(8)	1.11(9)	
O(4)–H(4)	1.20(8)	1.10(13)	
C(5)–H(5)	1.14(7)	1.14(9)	
C(6)–H(6)	0.88(7)	0.90(10)	
H(1)–N(1)–C(2)	115(5)	114(6)	[113.7]
C(2)–N(1)–C(6)	122.8(4)	120.9(29)	[122.7(2)]
N(1)–C(2)–C(3)	117.1(5)	118.0(28)	[117.2(2)]
C(2)–C(3)–H(3)	118(4)	120(4)	
H(3)–C(3)–C(4)	121(4)	122(4)	
C(3)–C(4)–C(5)	121.1(5)	124(4)	[119.6(3)]
C(4)–O(4)–H(4)	108.1(28)	112(4)	
C(4)–C(5)–C(6)	118.0(5)	115.1(32)	[118.8(3)]
N(1)–C(6)–C(5)	120.9(5)	124(4)	[121.3(2)]
C(5)–C(6)–H(6)	118.0(32)	123(5)	
H(1)–N(1)–C(6)	122(5)	125(6)	[122.7]
N(1)–C(2)–O(2)	118.0(4)	120(4)	
O(2)–C(2)–C(3)	124.8(5)	122(4)	[124.3(2)]
C(2)–C(3)–C(4)	119.9(5)	117.9(30)	[120.4(2)]
C(3)–C(4)–O(4)	122.5(5)	121(4)	[123.4(2)]
O(4)–C(4)–C(5)	116.3(5)	114.9(34)	[116.9(3)]
C(4)–C(5)–H(5)	123(4)	126(6)	
H(5)–C(5)–C(6)	119(4)	119(6)	
N(1)–C(6)–H(6)	118.0(32)	113(5)	

Table 3 Hydrogen bonding scheme in 3-deazauracil[a]

	X-Ray/neutron	Neutron	
N(1)–H(1)···O(2)			
N(1)–H(1)	0.91(4) Å	0.92(7) Å	
H(1)···O(2′)[b]	1.93(4) Å	1.92(8) Å	
N(1)···O(2′)[b]	2.796(5) Å	2.79(4) Å	[2.807(4) Å][c]
N(1)···H(1)···O(2)[b]	158(4)°	157(8)°	
O(4)–H(4)···O(2)			
O(4)–H(4)	1.20(6) Å	1.10(13) Å	
H(4)···O(2″)[d]	1.40(6) Å	1.42(11) Å	
O(4)···O(2″)[d]	2.581(6) Å	2.52(8) Å	[2.550(4) Å][c]
O(4)–H(4)···O(2″)[d]	166(4)°	171(9)°	

[a] Esds in brackets. [b] O(2′) is in the molecule at ($1 - x$, $\frac{1}{2} + y$, $1\frac{1}{2} - z$). [c] X-Ray parameter. [d] O(2″) is in the molecule at ($-\frac{1}{2} + x$, $1\frac{1}{2} - y$, $1 - z$).

Source. For this experiment SXD, a time-of-flight Laue diffractometer, was operating in quasi-equatorial mode, with crystal rotation restricted to the vertical axis and a small area position-sensitive detector (PSD) installed. This PSD, of 80 × 80 mm^2 active area, and 5 mm pixel resolution, subtends some 20 × 20° at the sample. The crystal was mounted with *b* slightly off vertical, and data were collected in just over a quadrant of the equatorial plane (105° ω rotation, in 7° steps), using neutrons in the wavelength range 0.96–4.8 Å, recorded using the time-of-flight technique.

From a potential total of 124 reflections accessible to a maximum $\sin\theta/\lambda$ of 0.6 Å^{-1} in this geometry, compared with some 590 reflections in the whole octant to this limit, some 80 were regarded as observed [all with $I > 5\sigma(I)$] and used in the refinement. Intensities were extracted and reduced to $|F_h|$ values using standard procedures.[5] Initially refinement was carried out using the least-squares program SFLSQ, a member of the Cambridge Crystallographic MK3 suite of subroutines and programs.[6] Given the limited nature of the data collected, the 53 variables in a full isotropic refinement were refined using a blocked matrix approach. In addition, combined X-ray and neutron diffraction refinements were carried out in the GSAS program,[7] using the 80 neutron and 674 X-ray data. The latter refinements involved 94 refined parameters, including two scale factors, one of each of X-ray and neutron data, and anisotropic thermal parameters on all atoms except hydrogen, which were refined isotropically.

The final atomic parameters are shown in Table 1, with the refined structure shown in Fig. 1. The final agreement factor for the refinement was $R = 0.075$, $R_w = 0.087$. For the X-ray data alone, $R = 0.0702$, while for the neutron data $R = 0.10$. The refined bond lengths and angles are shown in Table 2, with values from the neutron only refinement added for comparison. The high esds on parameters involving hydrogen atoms reflect the limited neutron data set collected.

There is good agreement between the atomic parameters found in the X-ray study and in the more limited neutron single crystal study. In addition, the present refinements provide precise hydrogen atomic parameter information, which is inaccessible with X-rays. The refined hydrogen parameters clearly show H(4) to be bonded to O(4), with at most an only slightly elongated bond length of 1.20(8) Å. These results confirm the general conclusions obtained in the earlier neutron powder diffraction studies, but give more accurate and reliable atomic parameters.

The detailed hydrogen bonding scheme in 3-deazauracil obtained from the combined refinement, and from the neutron single crystal data alone is shown in Table 3.

This scheme completes the specification of the two hydrogen bonds in the 3-deazauracil structure. In particular, it is clear that the strong O···O hydrogen bond in the title compound is a normal, almost linear, hydrogen bond. These results also reaffirm that 3-deazauracil exists in the 4-enol form in the crystalline state, with hydrogen atoms attached to atoms N(1) and O(4), and fairly strong double bond character in the C(2)=O(2) bond. A full neutron diffraction structural refinement will be performed on this material once more complete neutron data are collected.

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