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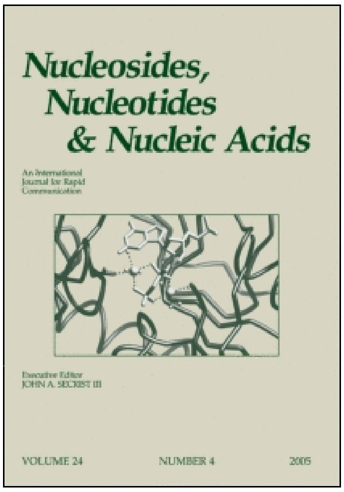

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HYDROGEN ATOM LOCATION IN THE MODIFIED NUCLEIC ACID BASE 3-DEAZAURACIL BY NEUTRON POWDER DIFFRACTION

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Abstract

The structure of 3-deazauracil [2-hydroxy-4-pyridone, $C_5H_5NO_2$], a modified nucleic acid base, has been studied by high resolution neutron powder diffraction. The accuracy of hydrogen atom parameters has been improved over an earlier single crystal X-ray experiment and the biologically important H4 atom has been located, having been unobserved in the X-ray work.

Introduction

Modified nucleosides and nucleic acid bases frequently demonstrate biological activity, often associated with inhibition of the replication process. This can be attributed to disruption of the normal course of DNA reproduction by the presence of such molecules either integral to the structure or competitively in solution with the normal monomeric materials. Since one of the major stabilising forces in DNA duplex structures is the base-pairing scheme (Adenine-Uracil, Guanine-Cytosine) within the hydrophobic core of the double helix, any disruption (mutation) of this scheme can have major effects on the properties of the polynucleotide.

The modified nucleoside 3-deazauridine does indeed show biological activity and in its crystal structure[1] there is a very strong O4...O2 hydrogen bond, believed to be significant in the manifestation of this activity. An earlier single crystal X-ray study of the title compound, 3-deazauracil[2] (Figure 1), confirmed that in the smaller fragment this strong intermolecular hydrogen bond still existed, this time with an O4...O2 separation of 2.55Å. For this

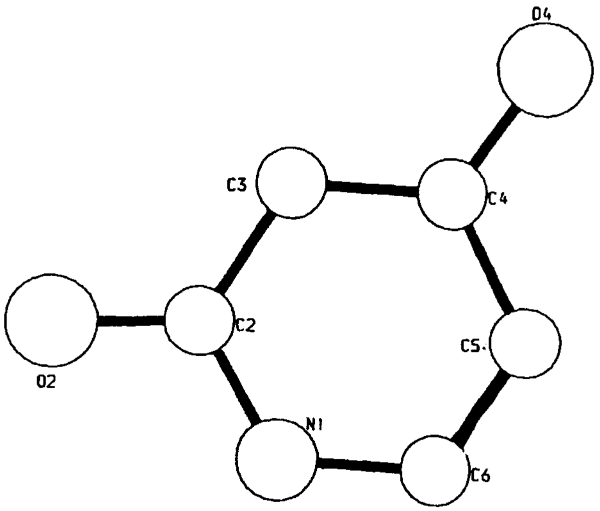


FIGURE 1 - View of the 3-deazauracil molecule, drawn using the plotting program PLUTO (Ref. 8). H atoms are attached to atoms N1, C3, O4, C5 and C6.

hydrogen bond to exist, the molecule must exist in the 4-enol form, rather than the 2,4-diol. Since 3-deazauracil is a small planar molecule, the potential for adopting an intercalating position within the DNA double helical structure obviously exists, and the strength of the hydrogen bonding interaction is of great significance for the ability of the molecule to exist in such a configuration. However, in neither X-ray study was the position of hydrogen H4 adequately determined, indeed in the case of 3-deazauracil H4 could not even be located in difference Fourier calculations on completion of refinement. Since the position of H4 has great relevance for both the detailed geometry of the hydrogen bonding scheme and for the tautomeric form adopted by the molecule in the crystal structure, it was decided to undertake a neutron diffraction study of the system. The relatively high scattering power of hydrogen for neutrons, as opposed to X-rays, makes this an obvious approach for hydrogen location.

With the improving resolution of neutron powder diffractometers, structures such as the title compound, with a cell volume of some 500Å^3, should be very easy to study. The high resolution powder diffractometer (HRPD)[3] at the Spallation Neutron Source, ISIS, has a resolution approaching Δd/d of 5 × 10^{-4} allowing the study of materials with relatively large unit cells. The major drawback with the study of hydrogeneous materials such as 3-deazauracil arises because of the high incoherent neutron scattering cross section of the hydrogen atom, which leads to a very high background level in the diffraction profile. The high resolution of HRPD should reduce this problem by increasing the peak to background ratio sufficiently to make the peaks visible above this high background level. One further aspect of this study was the use of a fairly small amount of sample (<0.5 gm. of powder). In many branches of preparative chemistry and biochemistry yields are such as to mitigate against the production of large amounts (say 5-10 gm.) of good quality sample, and the demonstration of accurate refinement on small samples is crucial to the application of neutron powder diffraction techniques to this type of material. The use of such small samples is feasible because of both the resolution of HRPD and the high intensity of the ISIS source, reducing the need for prohibitively large counting times.

Experimental

The crystal data for 3-deazauracil from the X-ray study are shown in Table 1, along with the atomic coordinates.

Neutron diffraction profiles were collected from the title compound on HRPD in the time-of-flight regions 30-130 ms and 90-190 ms, at room temperature. With the sample at 95m from the moderator and the detectors in backscattering configuration ($2\theta_{av}$ = 168.329°) at 1.05m from the sample, these windows correspond to d-spacing ranges of 0.62-2.69Å and 1.86-3.93Å. As can be seen from Figure 2 which is the 90-190ms data as collected, the incoherent background level is extremely high, as expected.

Normalisation of both data sets was carried out using the data display and manipulation program GENIE[4] and the normalised data are shown in Figure 3. To avoid frame edge effects the extrema of the data were not used in the analysis, and in addition because of the extremely large number of overlapped reflections in the very lowest d-spacing data these were not used. The data range eventually used in the refinements was 55000-175000μs (d-spacing range of 1.14-3.62Å).

TABLE 1 - Data from the X-ray study of 3-Deazauracil (Ref. 1) (esd's in parentheses)

Cell Parameters

orthorhombic

$P2_12_12_1$

$a = 8.638(6)$Å

$b = 5.279(5)$Å

$c = 11.220(8)$Å

$Z = 4$

Atomic coordinates (fractional)

Atom	x	y	z	U_{eq} (Å^2)
N1	0.3255(3)	0.3429(5)	0.7102(2)	0.027(1)
C2	0.3245(3)	0.5441(5)	0.6344(2)	0.026(1)
O2	0.4417(3)	0.6865(5)	0.6315(2)	0.036(1)
C3	0.1899(3)	0.5811(5)	0.5650(2)	0.024(1)
C4	0.0671(3)	0.4147(5)	0.5735(2)	0.026(1)
O4	-0.0638(2)	0.4432(5)	0.5143(2)	0.037(1)
C5	0.0761(4)	0.2074(6)	0.6526(3)	0.032(1)
C6	0.2055(4)	0.1774(6)	0.7184(2)	0.032(1)

$$[\ U_{eq} = 1/3 \sum_i \sum_j U_{ij} a_i^* a_j^* \underline{a}_i \cdot \underline{a}_j\ ]$$

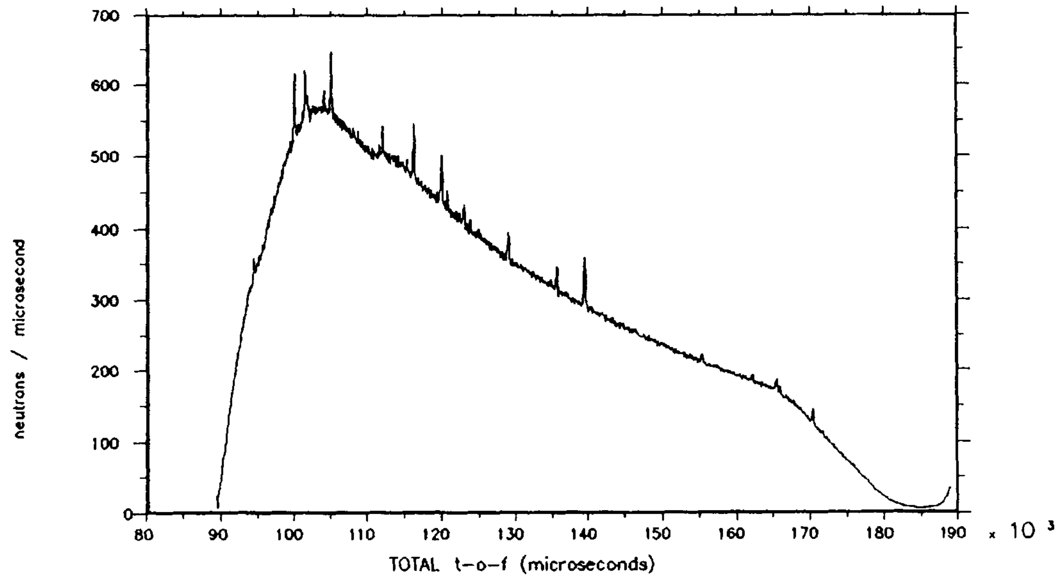


FIGURE 2 - Raw data as collected from HRPD in the time regime 90-190ms.

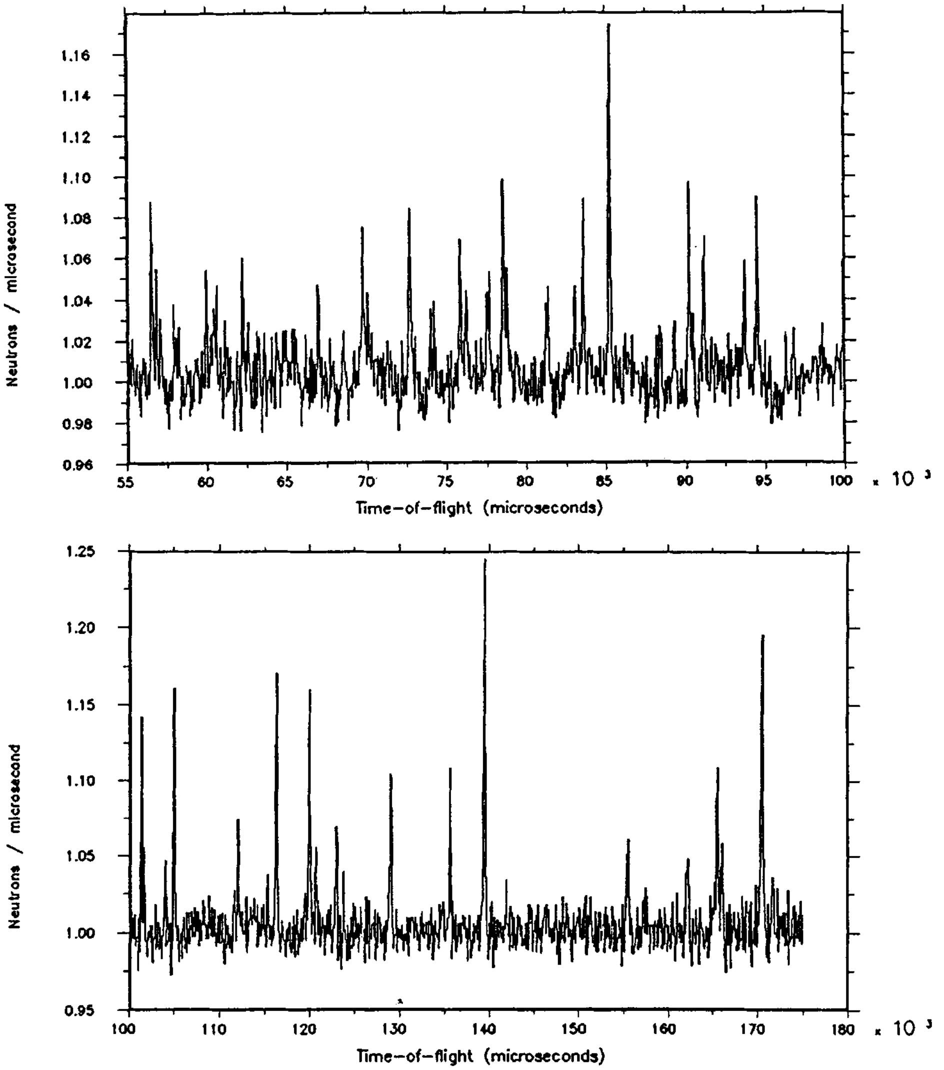


FIGURE 3 - Normalised data in the time regions (a) 55-100ms (b) 100-175ms.

Refinement of the data commenced using the program GDELSQ, which is a profile refinement program exploiting the Cambridge Crystallographic Subroutine Library (CCSL)[5], using a peak shape for each reflection consisting of a Gaussian convoluted with a double decaying exponential, to account for sample, instrument and source characteristics[6].

Initial refinements allowing the heavy atom coordinates to change were quite satisfactory, and reasonable profile fits were obtained. However, when the known hydrogen atoms were input at their X-ray positions the refinements became unstable. This is probably a consequence of the relatively poor quality of the data. To increase the number of data points available, one can introduce chemical constraints on certain of the known features in the structure. For example, the heavy-heavy atom bond lengths are known reasonably reliably from the X-ray study and these can be constrained to stay fairly close to these values in the neutron refinement. Constraining in this way is formally equivalent to increasing the number of observations available.

When these constraints were imposed the refinement was considerably stabilised, but in order to use the chemical information available to the fullest possible extent in this difficult problem, the bond length involving hydrogen atom H6 was also constrained to be close to a physically acceptable value - in one of the early refinements C6-H6 shrank to 0.628(8)Å which is obviously incorrect. Having imposed these constraints which are detailed in Table 2, the subsequent refinements were perfectly stable.

Using just the coordinates and hydrogen positions known from the X-ray study, the refinement converged as detailed in Table 3 (a) and (b), which show the agreement factors and bond parameters found. The introduction of H4 in a chemically sensible position close to 04 led to the refinement shown also in Table 3. Since there is some ambiguity as to exactly where this hydrogen atom is located, it was refined in an unconstrained manner, and converged to the position shown, bonded to 04 and forming a normal hydrogen bond with 02 with parameters as follows: 04--H4, 1.100(8)Å; 04...02', 2.703(6)Å, $\angle$ 04--H4...02', 148.2(9)°.

As a final check on the reproducability of this refinement, H4 was initially located close to 02 in the alternative possible configuration. Within a few cycles of least squares refinement H4 had refined back to the position where it was bonding to 04, thus confirming the earlier result.

TABLE 2 - Chemical constraints used in the neutron refinement (note these are weighted as $1/\sigma^2$)

Bond restrained	Bond length(Å)	σ(Bond length)(Å)
N1---C2	1.360	0.01
N1---C6	1.359	0.01
C2---O2	1.262	0.01
C2---C3	1.412	0.01
C3---C4	1.381	0.01
C4---O4	1.319	0.01
C4---C5	1.411	0.01
C5---C6	1.348	0.01
C6---H6	1.000	0.01

TABLE 3 - Comparison of the X-ray, neutron and neutron with H4 refinements

(a) Agreement factors (%)

	R_I	R_P	R_{wP}^{+}	R_E^{+}	χ^2 $(=R_{wP}/R_E)$
X-ray	6.5	-	-	-	-
Neutron	43.6	0.9	1.2/45.8	0.9/34.4	1.77
Neutron with H4	36.6	0.8	1.1/41.3	0.9/34.4	1.44
Neutron with H4 (restricted data range 100000-175000μs)	22.3	1.0	1.2/32.9	1.0/25.4	1.69

+ : The latter figure corresponds to the R-factors with the background subtracted.

(b) Bond lengths (Å)
(esd's in parentheses)

Bond	X-ray	Neutron	Neutron (+H4)
N1---C2	1.360(4)	1.394(4)	1.382(4)
N1---C6	1.359(4)	1.380(5)	1.362(4)
C2---O2	1.262(4)	1.313(5)	1.313(4)
C2---C3	1.412(3)	1.441(5)	1.404(4)
C3---C4	1.381(4)	1.400(5)	1.413(5)
C4---O4	1.319(3)	1.362(5)	1.366(5)
C4---C5	1.411(4)	1.463(5)	1.456(5)
C5---C6	1.348(4)	1.378(5)	1.382(5)
N1---H1	0.81(4)	1.234(7)	1.098(7)
C3---H3		1.222(7)	1.023(7)
C5---H5		1.080(8)	1.025(7)
C6---H6		1.037(8)	1.001(7)
O4---H4			1.100(8)

TABLE 4 - Final parameters from neutron refinement including H4

Cell dimensions :

a = 8.64264(6), b = 5.32765(3) , c = 11.23056(5)Å

Atomic coordinates :

	x	y	z
N1	0.3339(4)	0.3265(6)	0.7037(3)
H1	0.4082(8)	0.3134(13)	0.7642(6)
C2	0.3236(5)	0.5394(6)	0.6370(3)
02	0.4406(6)	0.6868(8)	0.6317(4)
C3	0.1956(4)	0.5927(7)	0.5631(4)
H3	0.2094(9)	0.7256(14)	0.4985(6)
C4	0.0821(5)	0.4206(7)	0.5822(3)
04	-0.0504(5)	0.4192(8)	0.5235(4)
H4	-0.0651(9)	0.5909(12)	0.4359(7)
C5	0.0639(5)	0.2068(8)	0.6558(3)
H5	-0.0121(8)	0.0712(13)	0.6446(6)
C6	0.2019(4)	0.1836(7)	0.7105(3)
H6	0.2227(8)	0.0572(12)	0.7743(5)

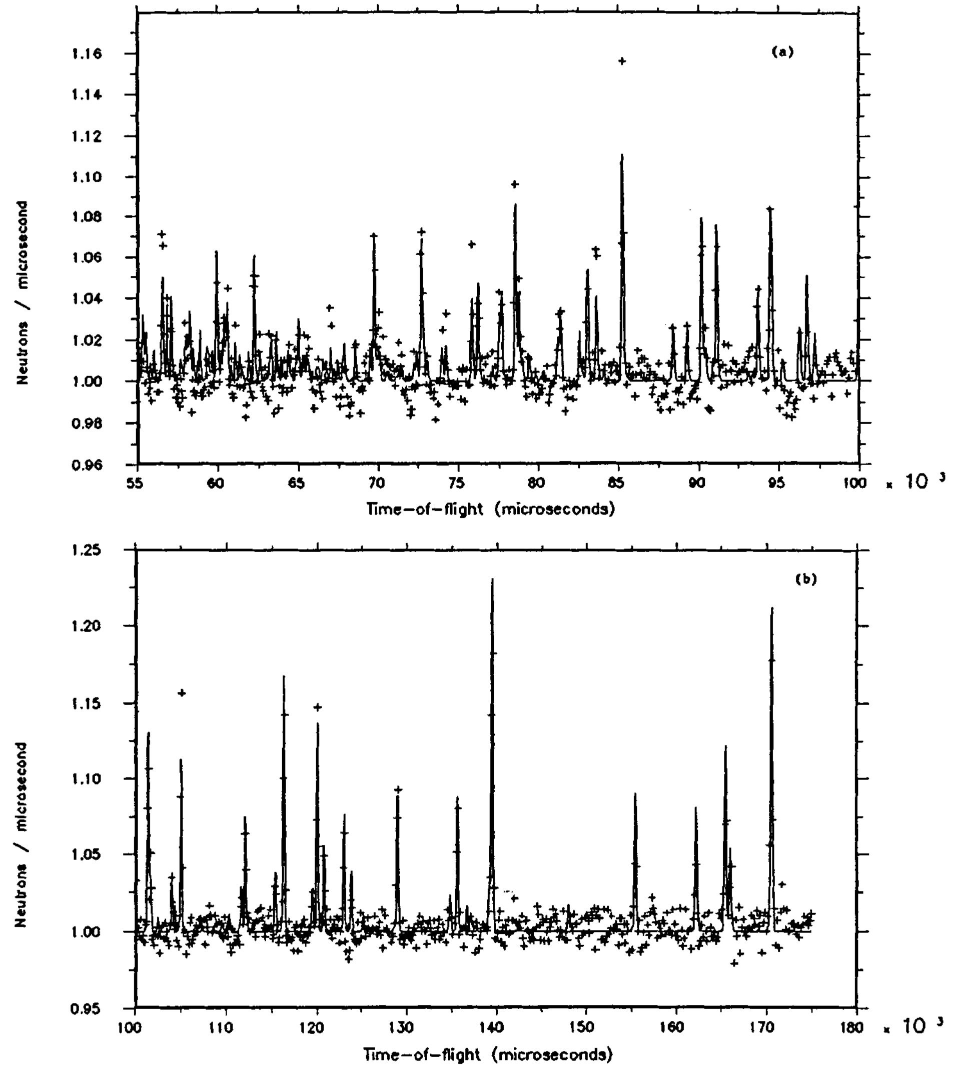
Overall Temperature Factor refined U = 0.020(1)$Å^2$

The final profile fit is shown in Figure 4 and the final refined atomic parameters in Table 4.

Discussion

The results presented above represent the completion of the definition of the hydrogen bonding scheme in 3-deazauracil and confirms the suggestion that H4 is unambiguously bonded to 04 and is involved in a very strong (2.7Å) hydrogen bond with 02.

In spite of the relatively poor statistics of the data collected and the intrinsic difficulties of studying hydrogeneous materials by neutron powder diffraction, the results from the refinement are rather encouraging. The sharpness of the diffraction peaks result in exceptionally accurate cell parameters as shown in Table 4. From these data, one can be certain that the assignment of H4 to 04 is correct. The strength of neutron studies on materials such as these can be seen in the accuracy to which the hydrogen atoms have been located. In



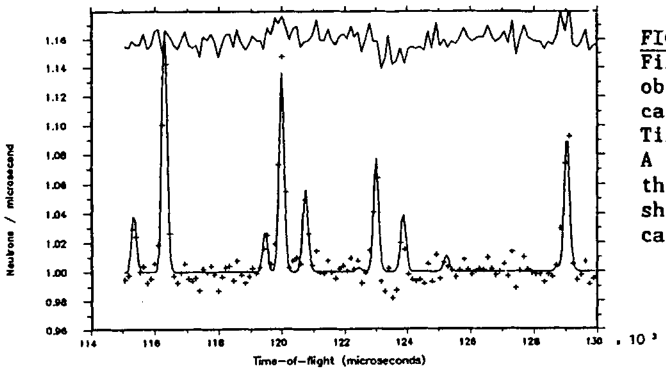


FIGURE 4 -
Final profile fits showing observed (crosses) and calculated (line) profiles. Time regions as above.
A more detailed plot of the 115-130ms region is shown, with the (observed-calculated) profile added.

particular there is an order of magnitude improvement in the accuracy of determination of the bond parameters involving hydrogen atoms in the neutron as opposed to the X-ray study (Table 3).

This significant improvement in the precise definition of the hydrogen bonding scheme is an indication of what is possible even from powder samples when a high resolution neutron diffractometer is used. The possibilities of using such instruments in structural determination, as opposed to refinement as here, have been widely discussed recently[7] and if this potential is realised the chemical or biochemical crystallographer will have a very powerful tool with which to tackle structural problems of materials for which single crystals are unobtainable. The fact that a normal hydrogeneous sample as opposed to a deuterated material was used is also significant. With deuterium in place of hydrogen the problem of incoherent background is substantially reduced and the deuterium, like hydrogen, is located more easily with neutrons than with X-rays. Deuteration, however, can often be difficult and expensive and the demonstration of these results with normal hydrogen in the structure is important.

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