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Molecular and Crystal Structure of 6-Azathymidine: Similarities with Azaribonucleosides

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(*Received* 12 *September* 1977; *accepted* 3 *November* 1977)

The crystal and molecular structure of the carcinostatic agent 6-azathymidine has been determined from X-ray counter data. The nucleoside crystallizes in the orthorhombic space group $P2_12_12_1$ with four molecules per unit cell of dimensions $a = 8{\cdot}169$ (4), $b = 7{\cdot}412$ (4), $c = 18{\cdot}287$ (9) Å. The structure was solved by direct methods and adjusted by full-matrix least-squares refinement to the 1014 data, yielding a final R factor of 4·3%. Similarly to other nucleosides with an aza group *ortho* to the glycosidic bond, 6-azathymidine occurs in an extreme *anti* conformation with O(1′)–C(1′)–N(1)–N(6) at 88°. The ribose conformation is C(2′)-*exo*, C(3′)-*endo* and the orientation of the C(5′)–O(5′) bond is *gauche*, *gauche*. This X-ray study suggests that the biochemical properties of 6-azathymidine can be explained similarly to those of 6-azauridine and its 5′-phosphate.

Introduction

Nucleosides with aza groups *ortho* to the glycosidic linkage were first discovered in sponges and later obtained synthetically (Suhadolnik, 1970). They are of biochemical interest owing to their pronounced cytostatic activity and have been tested for clinical use against human leukaemia (Skoda, 1963; Hernandez, Pinkel, Lee & Leone, 1969; Sorm & Veseley, 1961). In order to study structure–function relationships for this class of nucleosides, NMR studies were carried out for 6-azauridine (Hruska, 1973) and X-ray crystal structures were determined for this molecule (Schwalbe & Saenger, 1973), for 6-azauridine 5′-phosphate (Saenger & Suck, 1973), for 6-azacytidine (Singh & Hodgson, 1974*a*), for 8-azaadenosine (Singh & Hodgson, 1974*b*), and for formycin (Prusiner, Brennan & Sundaralingam, 1973; Prusiner & Sundaralingam, 1973). All these investigations yielded a consistent picture: while in normal nucleosides the preferred orientation about the glycosidic linkage is *anti* with the torsion angle O(1′)–C(1′)–N(1)–N(6) nearly *cis*-planar (~20°), in the azanucleosides this angle is about 90°, rendering the torsion angle C(2′)–C(1′)–N(1)–N(6) nearly *cis*-planar (extreme *anti* or high *anti* conformation). On the other hand, the preferred orientation about C(4′)–C(5′) is *gauche*, *gauche* for normal nucleosides but *gauche*, *trans* or *trans*, *gauche* in the aza series. Most striking is the *gauche*, *trans* conformation for 6-azauridine 5′-phosphate which is in contrast to the *gauche*, *gauche* observed thus far in all the 5′-ribonucleotides. This finding led to a proposal for the antimetabolic activity of 6-azauridine 5′-phosphate (Saenger & Suck, 1973; Saenger, Suck, Knappenberg & Dirkx, 1978).

The question arises as to whether the unusual conformational properties of the azanucleotides are limited to the ribo series or whether they are found in deoxyriboazanucleosides as well. In this contribution the X-ray analysis of 6-azathymidine is reported.

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Experimental

6-Azathymidine crystallizes from aqueous solution in the form of elongated prisms. Crystallographic data obtained from photographic and four-circle diffractometer measurements are summarized in Table 1. For X-ray intensity data collection, the diffractometer was operated in the $2\theta/\omega$ scan mode with stationary counts on both sides of each scan using Ni-filtered Cu $K\alpha$ radiation. In total, 1014 data were measured of which 865 were greater than $3\sigma_{F_o}$ and considered as observed. Owing to the small size of the crystal (0·30 × 0·25 × 0·15 mm) the data were corrected for geometrical factors but not for absorption.

Table 1. *Crystallographic data for 6-azathymidine*

$C_9H_{13}N_3O_5$, $M_r = 243{\cdot}21$
Space group $P2_12_12_1$ (orthorhombic) from the systematic absences $h00$ for h odd, $0k0$ for k odd, and $00l$ for l odd

$a = 8{\cdot}169\ (4)$ Å	$D_c = 1{\cdot}57$ g cm^{-3}
$b = 7{\cdot}412\ (4)$	$D_o = 1{\cdot}58$
$c = 18{\cdot}287\ (9)$	$Z = 4$
$V = 1107{\cdot}3$ Å^3	$\mu = 6{\cdot}32$ cm^{-1}
λ(Cu $K\alpha$) = 1·54182 Å	m.p. 152–153 °C
$F(000) = 502$	
$R(=\Sigma\|\|F_o\| - \|F_c\|\|/\Sigma\|F_o\|) = 0{\cdot}043$	

Table 2. *Fractional atomic coordinates* (×10⁴)

	x	y	z
N(1)	7241 (3)	5669 (5)	5866 (2)
C(2)	8021 (5)	5805 (6)	5205 (2)
O(2)	9472 (3)	6191 (5)	5138 (1)
N(3)	7049 (4)	5463 (5)	4603 (2)
C(4)	5425 (5)	5026 (6)	4633 (2)
O(4)	4624 (4)	4804 (5)	4077 (1)
C(5)	4770 (5)	4850 (6)	5379 (2)
N(6)	5646 (4)	5162 (5)	5952 (2)
Me	3014 (5)	4309 (7)	5476 (3)
O(1′)	8891 (3)	4266 (4)	6765 (1)
C(1′)	8180 (5)	5914 (6)	6546 (2)
C(2′)	7120 (5)	6569 (6)	7177 (2)
C(3′)	6602 (5)	4798 (6)	7528 (2)
O(3′)	6247 (3)	4924 (4)	8295 (1)
C(4′)	8109 (5)	3606 (6)	7427 (2)
C(5′)	7778 (5)	1636 (7)	7357 (2)
O(5′)	6867 (3)	1180 (4)	6714 (1)
H(N3)	2457 (50)	409 (65)	890 (23)
H1(Me)	2189 (50)	−3990 (65)	960 (23)
H2(Me)	2802 (57)	4957 (59)	312 (23)
H3(Me)	2293 (50)	−2773 (65)	196 (23)
H(C1′)	4183 (57)	−1627 (59)	3609 (23)
H1(C2′)	2733 (50)	−2301 (62)	2445 (20)
H2(C2′)	1066 (52)	−2333 (59)	2952 (19)
H(C3′)	618 (47)	909 (62)	2785 (11)
H(O3′)	−391 (50)	−5059 (65)	3381 (23)
H(C4′)	3793 (57)	1321 (59)	2136 (23)
H1(C5′)	3857 (50)	3923 (62)	2618 (20)
H2(C5′)	2123 (57)	3462 (59)	2195 (23)
H(O5′)	2473 (50)	4009 (65)	3696 (23)

Table 3. *Selected torsion angles A–B–C–D within the 6-azathymidine molecule*

These angles are defined as zero if the bonds C–D are coplanar with bonds A–B looking down the B–C bonds, and they are defined as positive if the far bonds are rotated clockwise.

O(5′)–C(5′)–C(4′)–O(1′)	−53·9°
O(5′)–C(5′)–C(4′)–C(3′)	64·7
O(1′)–C(1′)–C(2′)–C(3′)	−29·8
C(1′)–C(2′)–C(3′)–C(4′)	35·2
C(2′)–C(3′)–C(4′)–O(1′)	−29·1
C(3′)–C(4′)–O(1′)–C(1′)	10·8
C(4′)–O(1′)–C(1′)–C(2′)	12·1
N(1)–C(1′)–C(2′)–C(3′)	91·7
N(1)–C(1′)–O(1′)–C(4′)	−110·9
C(1′)–C(2′)–C(3′)–C(2′)	152·8
C(5′)–C(4′)–C(3′)–C(2′)	−150·2
C(5′)–C(4′)–C(3′)–O(3′)	88·2
C(5′)–C(4′)–O(1′)–C(1′)	135·7
C(2′)–C(1′)–N(1)–N(6)	−31·6
O(1′)–C(1′)–N(1)–C(2)	−86·6
C(2′)–C(1′)–N(1)–C(2)	153·8
O(1′)–C(1′)–N(1)–N(6)	88·0

Table 4. *Least-squares planes through parts of 6-azathymidine and distances of some atoms from these planes*

The plane equations are of the form $Ax + By + Cz + D = 0$, where x, y and z are measured in Å along **a**, **b** and **c** respectively. The atoms defining the planes are marked by an asterisk. The angle between normals to planes (*a*) and (*c*) is 79·1°.

Coefficients of plane equation		Distances (Å) of atoms from plane	
(*a*) Pyrimidine base			
$A =$	−0.2604	*N(1)	0·019
$B =$	0·9649	*C(2)	−0·092
$C =$	−0·0327	*N(3)	−0·011
$D =$	−2·1437	*C(4)	0·020
		*C(5)	−0·011
		*N(6)	−0·008
		O(2)	−0·037
		O(4)	0·065
		Me	−0·030
(*b*) Three-atom sugar plane			
$A =$	0·6671	*C(1′)	0·000
$B =$	0·4987	*O(1′)	0·000
$C =$	0·5534	*C(4′)	0·000
$D =$	−13·2682	C(2′)	0·305
		C(3′)	−0·278
		C(5′)	−0·979
		O(3′)	0·351
(*c*) Best four-atoms sugar plane			
$A =$	0·5623	*C(1′)	0·0360
$B =$	0·5497	*O(1′)	−0·0615
$C =$	0·6178	*C(4′)	0·0582
$D =$	13·5261	C(2′)	0·5289
		*C(3′)	−0·034
		C(5′)	−0·9756
		O(3′)	0·7306

Table 5. *Geometrical data for hydrogen bonds*

A (in *x,y,z*)	*D*	*A*···*D*	H···*A*	H–*D*	∠H–*D*···*A*
O(4)	O(5′)[$-\frac{1}{2}+x, \frac{1}{2}-y, 1-z$]	2·77 Å	1·98 Å	0·91 Å	11°
O(5′)	O(3′)[$1-x, -\frac{1}{2}+y, \frac{3}{2}-z$]	2·71	2·03	0·91	6
O(3′)	N(3)[$\frac{3}{2}-x, 1-y, \frac{1}{2}+z$]	2·78	1·80	0·73	4

Solution and refinement of the structure

The crystal structure of 6-azathymidine was solved by direct methods using the program *MULTAN* (Germain, Main & Woolfson, 1971). A Fourier synthesis based on the most consistent phase set obtained with the 200 *E*'s greater than 1·4 revealed 13 non-hydrogen atoms in chemically meaningful positions. The remaining atoms, including H atoms, were determined from difference Fourier maps computed after several cycles of isotropic and then anisotropic full-matrix least-squares refinement with scattering factors taken from *International Tables for X-ray Crystallography* (1974). The refinement converged at $R = \Sigma||F_o| - |F_c||/\Sigma|F_o| = 4{\cdot}3\%$ for all data with the average shifts in atomic parameters less than $\frac{1}{4}$ of the standard deviations.*

Results

Atomic coordinates are presented in Table 2, some selected dihedral angles in the molecule in Table 3, least-squares planes in Table 4 and geometrical data for hydrogen bonds in Table 5.

Fig. 1 is a sketch of 6-azathymidine showing the numbering convention, with all non-hydrogen bond distances and bond angles indicated. Fig. 2 shows a thermal-ellipsoids plot of the 6-azathymidine molecule and Fig. 3 presents a stereoview of the packing pattern of 6-azathymidine.

Discussion

Geometrical data of the heterocycle

As described earlier for 6-azauridine (Schwalbe & Saenger, 1973), and 6-azauracil and 6-azacytidine (Singh & Hodgson, 1974*a*,*b*), the geometrical data not involving N(6) are comparable with those observed in the natural analogues. In 6-azathymidine, the bond C(5)–N(6) is shorter by 0·053 Å than that in thymidine (Young, Tollin & Wilson, 1969) but C(4)–C(5) is longer by 0·019 Å, a feature also observed for 6-azauridine. Further, as in the other 6-azanucleosides, the angle C(5)–N(6)–N(1) is decreased by 5·1° in 6-azathymidine relative to that in thymidine but the internal angles with N(1) and C(5) as vertex are increased by 2·7 and 5·2°.

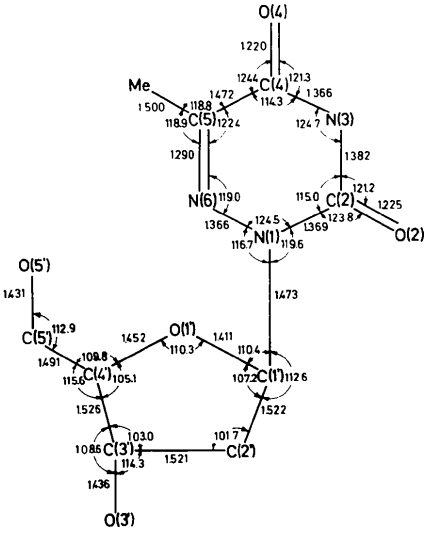

Fig. 1. A schematic sketch of the 6-azathymidine molecule showing the numbering convention with all non-hydrogen bond distances (Å) and bond angles (°) indicated.

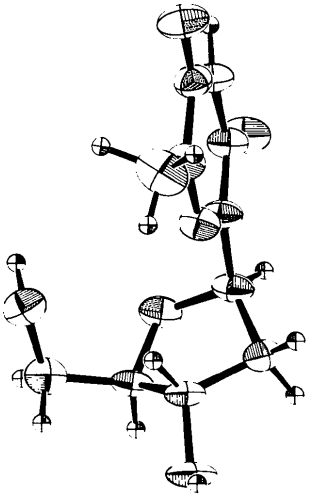

Fig. 2. A view down **b** of the 6-azathymidine molecule with 50% thermal ellipsoids drawn (Johnson, 1965).

* Lists of structure factors and anisotropic thermal parameters have been deposited with the British Library Lending Division as Supplementary Publication No. SUP 33210 (8 pp.). Copies may be obtained through The Executive Secretary, International Union of Crystallography, 13 White Friars, Chester CH1 1NZ, England.

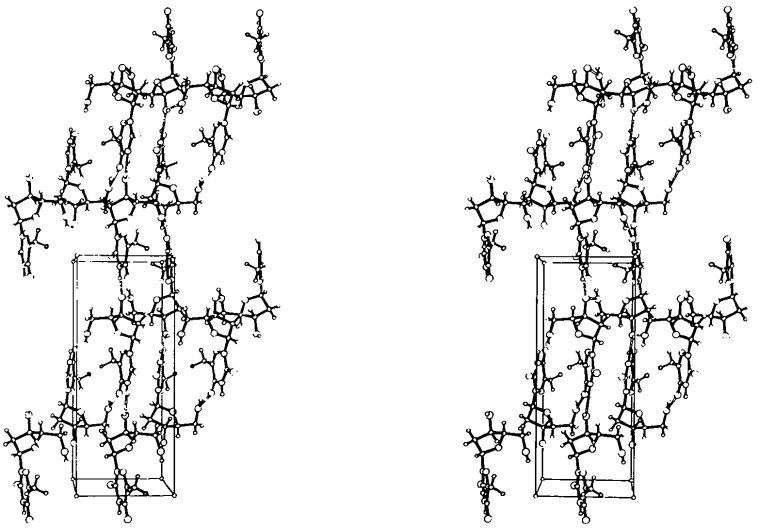

Fig. 3. Stereoview of the packing pattern (down **a**) and the hydrogen-bonding scheme of the 6-azathymidine structure.

Ribose geometry and conformation of the molecule

The ribose displays a rather regular C(2′)-*exo*, C(3′)-*endo* half-chair conformation, similar to that found for 6-azauridine 5′-phosphate. All the bond lengths agree within 0·15 Å with average values for C(3′)-*endo* nucleosides (Saenger & Eckstein, 1970), indicating that on an energetical basis these two conformations are comparable (Saenger, 1973).

Similarly to other nucleosides with an aza group *ortho* to the glycosidic linkage, the 6-azathymidine molecule displays an extreme *anti* conformation with the torsion angle O(1′)–C(1′)–N(1)–N(6) at 88·04° [or O(1′)–C(1′)–N(1)–C(2) at −86·6°]. The orientation about the C(4′)–C(5′) bond is *gauche*, *gauche* (Table 4), like that found for 6-azacytidine, but unlike 6-azauridine, its 5′-phosphate and thymidine.

Packing arrangement

In nucleosides, O(1′) does not usually accept hydrogen bonds (Sundaralingam, 1968). This is also true for 6-azathymidine, while all the other 6-azapyrimidine and 8-azapurine nucleosides are involved in such interactions. It has been proposed that this feature of azanucleoside crystal structures is not due to increased electronegativity residing on O(1′) but is rather a steric effect: O(1′) is more exposed if a nucleoside assumes the extreme *anti* conformation (Saenger *et al.*, 1978).

The segregation of hydrophobic and hydrophilic zones so frequently found in nucleoside crystals (Saenger & Suck, 1973) is also observed for 6-azathymidine (Fig. 3). The hydrophobic and hydrophilic structural elements are endless pillars of stacked bases and riboses, extending by the action of diads parallel to **b**. The molecules are linked by a network of hydrogen bonds involving both heterocycles and riboses (see Table 5). A packing pattern similar to that in this crystal structure also exists in crystals of 6-azauridine and 6-azacytidine.

Conclusions

The fact that 6-azathymidine occurs in an extreme *anti* conformation similar to that in all the other nucleosides with aza groups *ortho* to the glycosidic linkage suggests that the nature of the sugar moiety has no influence on this particular feature. It is to be expected, therefore, that the biochemical properties of 6-azathymidine can be explained on the same basis as described earlier for 6-azauridine (Schwalbe & Saenger, 1973) and its 5′-phosphate (Saenger & Suck, 1973; Saenger *et al.*, 1978).

AB gratefully acknowledges the receipt of a fellowship from the Deutsche Forschungsgemeinschaft. The computations were carried out with the Univac 1108 computer of the Gesellschaft für Wissenschaftliche Datenverarbeitung, Göttingen.

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Acta Cryst. (1978). B**34**, 1298–1304

Bile Pigment Structures. II. The Crystal Structure of Mesobilirubin IXα–Bis(chloroform)

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(*Received* 1 *October* 1977; *accepted* 5 *November* 1977)

Mesobilirubin IXα (mesobilirubin) crystallizes with two molecules of chloroform in space group $P\bar{1}$ with a = 12·146 (14), b = 15·093 (14), c = 11·815 (14) Å, α = 103·56 (10), β = 105·58 (11), γ = 78·10 (9)°, Z = 2. The structure was refined to R = 0·112 for 2790 reflexions. The chromophore takes up a 'ridge-tile' conformation with an angle of 104·0° between local planar *syn-Z* configurated pyrromethenone units. This conformation is stabilized by two sets of three intramolecular hydrogen bonds involving the carboxylic acid function, the two pyrrole-imino H atoms and the terminal lactam system. The diffraction data allow an unequivocal assignment of the lactam formulation. The bond-length distribution suggests that there is relatively limited delocalization over the local pyrromethenone systems. The molecules are stacked with their pyrromethenone systems parallel to one another, thereby giving rise to channels in the crystal lattice in which the two chloroform molecules occur.

Introduction

The orange-yellow bile pigment bilirubin IXα (hereafter bilirubin) is the first isolable product of the oxidative breakdown of haem in mammals. It is excreted as its conjugated water-soluble diglucuronide salt with the bile into the duodenum, but hydrolysis in the intestinal tract regenerates free bilirubin, which is reduced by intestinal bacteria to yield urobilinoid chromogens as the final products. A complex mixture of bile pigments is excreted in the faeces of healthy mammals (Fig. 1). The most common source of bilirubin is ox gallstones, in which it occurs as the pure Ca salt. The yellow skin pigmentation observed under pathological conditions (jaundice) is the result of an increased concentration of bilirubin in the gall; it is then retained, in particular, in the elastin-rich tissues and also excreted in the urine.

Although the constitution of bilirubin is well established (Fischer, Plieninger & Weissbarth, 1941), stereochemical ambiguities have remained. The significant possible structural variables for the bilirubin molecule may be summarized as:

(1) *Z* or *E* configuration at the methine bridges.

(2) Conformational preference at the methine bridges (*i.e. syn* or *anti* forms, degree of twisting of the interplanar angles between pyrrole rings).

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