6-AZAURIDINE: CRYSTAL STRUCTURE AND CONFORMATION OF THE ANTILEUKEMIA DRUG

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Received May 11, 1971

SUMMARY

The cytostatic nucleoside analog 6-azauridine has been investigated by single-crystal X-ray diffraction. The pseudo-face-centered structure exhibits an extensive branched network of hydrogen bonds connecting ribose moieties and pairwise stacked bases. The packing resembles that in 4-thiouridine, but the glycosidic bond conformation is anti. The riboses are puckered with C(3') out of the best four-atom plane.

The nucleoside analog ribo-6-azauridine (AzUR), NSC-32074 (figure 1) has been recommended for further clinical trial in the chemotherapy of human leukemia (1, 2). Its cytostatic effect (3) appears to depend on highly specific behaviour towards three enzymes involved in uridine metabolism: in the Ehrlich ascites system AzUR is not destroyed by uridine phosphorylase (4), which would cleave uridine; it is converted to the active metabolite through phosphorylation by uridine kinase at about 80 % the rate of uridine (5); and as the 5'-phosphate it strongly inhibits orotidylic acid decarboxylase (6, 7), thereby blocking the de novo synthesis of uridylic acid.

The structure of AzUR is obviously of interest in view of this activity.

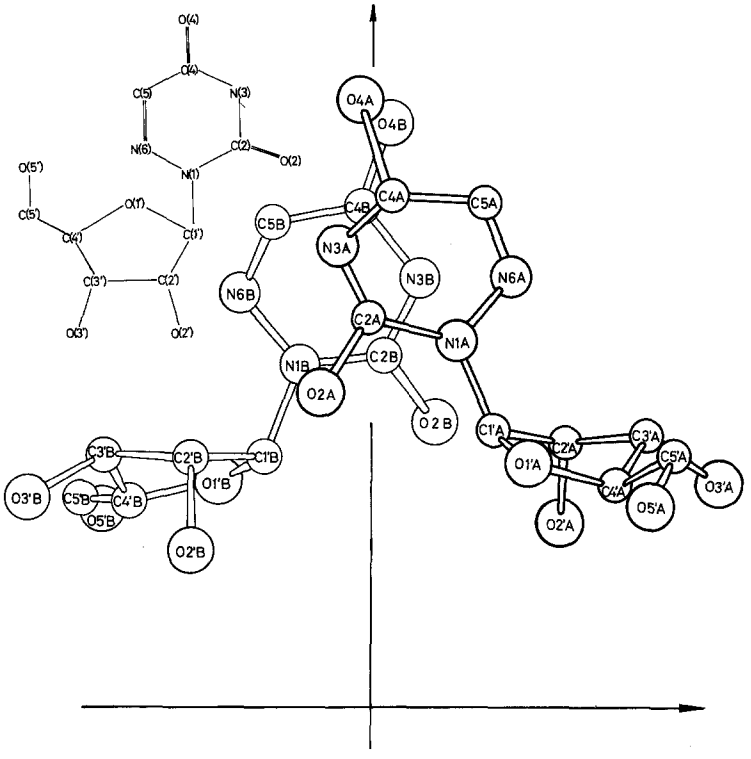

Figure 1. Chemical formula of AzUR (insert) and projection of one asymmetric unit along the c-axis to show the base overlap.

Crystals of AzUR grew from water as brittle jagged colorless laths in the orthorhombic space group $P2_12_12_1$ with a = 20.230, b = 7.709, c = 12.863 Å, observed flotation density 1.62 g cm^{-3}, and calculated density 1.63 g cm^{-3} for 8 molecules per cell. Thus two independent molecules with 34 nonhydrogen atoms had to be determined in the asymmetric unit. A small well-formed lath of longest dimension 0.15 mm (greates absorption correction 1.3 % to F) was examined with MoKα radiation (= 0.70926 Å) on a 4-circle diffractometer for cell dimension as well as intensity data. By the ω-2θ scan technique 1629 reflections were collected up to $2\theta = 46^{\circ}$, corrected

for geometrical factors but not for absorption, and assigned (8) errors of counting σ. The 1246 reflections with $F > 3\sigma$ were considered observed.

The intensity distribution is strikingly nonuniform: entire reciprocal lattice rows with h + l odd are weak. A Wilson plot (9) shows wide scatter although the temperature factor is a reasonable 3.0 Å^2, and the squared normalized structure factors (10) average to $E^2_{hkl} = 1.27$ with h + l even but 0.71 with h + l odd. This pseudo-A-centering holds strongly at low resolution but fails at high: below $2\theta = 32^\circ$ there is not one $E_{hkl} > 1.50$ with h + l odd, but at $2\theta = 39^\circ$ comes $E_{067} = 2.85$, the third strongest in the whole set. Thus a direct phase determination must rely on a small set of phases poorly coupled to the rest to break the pseudo-centering. The multiple-solutions (11) procedure that finally succeeded included three such phases in the starting set of six. Immediate tangent formula (10) extension and refinement with Zechmeister's program (12) produced one E map that revealed 32 nonhydrogen atoms based on just 148 phases. After routine structure completion and full-matrix least squares refinement with isotropic thermal parameters the R factor $= \Sigma \| F_o | - | F_c \| \, / \Sigma | F_o |$ has reached 0.103 for 1246 observations.

At this stage of refinement the conformational features and the packing of the 6-azauridine molecules within the crystal lattice became noteworthy:
The ribose conformation (figure 1) in both AzUR molecules

A and B is best described as the envelope form C(3')-endo with atom C (3') displaced from the almost coplanar atoms C(4'), O(1'), C(1'), C(2') and on the same side as C(5'). Due to this pucker the dihedral angle between the C(2')-O(2') and C(3')-O(3') bonds is -39° and -35°, values common in C(3')-nucleosides. The position of the O(5') hydroxyl group with respect to the ribose moiety is defined by the two dihedral angles ϕ_{oo}, O(5')-C(5')-C(4')-O(1') and ϕ_{oc}, O(5')-C(5')-C(4')-C(3') (13). The usual gauche, gauche angles with O(5') located "above" the ribose ring should be even more favoured in AzUR since an intramolecular O(5')-H...N(6) hydrogen bond could be formed. However, the angles ϕ_{oo}, ϕ_{oc} are gauche, trans for molecules A and B. The reason for this unexpected conformation might be the highly symmetric branched intermolecular hydrogen bonding system in the crystal structure (fig. 2) which involves O(5').

The conformation about the glycosidic C(1')-N(1) bond is anti (16) as in all so far investigated pyrimidine nucleosides in crystalline state except 4-thiouridine (15), i.e. the dihedral angles C(2')-C(1')-N(1)-N(6), -37° and -40° for molecules A and B resp., are both in the range 0 to -180° (15).

The packing of the molecules within the crystal structure is determined by zones of hydrophobic and hydrophilic character (figure 2) and bears a striking similarity to the packing of the 4-thiouridine (15) molecules. In both structures the hydrophobic zones are formed by the heterocycles, which are stacked along a crystallographic axis in such a way that two heterocycles are related to each other

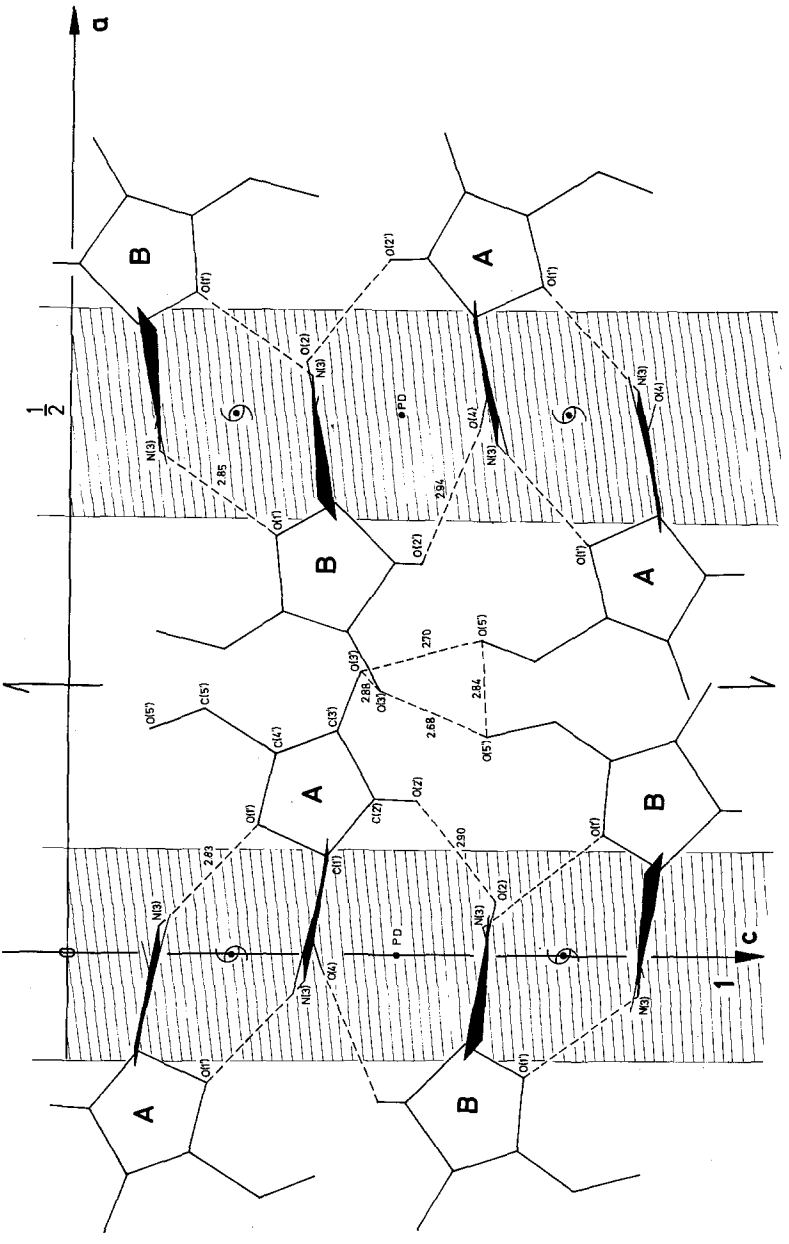

Figure.2. Projection of the structure along the b-axis. The heterocycles are filled in. Hydrogen bonds were estimated from short intermolecular contacts and are drawn as dashed lines with distances in Å. The hydrophilic zones are indicated.

by a diad in 4-thiouridine or a pseudo-diad (PD between molecules A and B in figure 2) in AzUR. Such a pair of nucleosides whose heterocycles are partly overlapped (figure 1) is related to another pair of nucleosides by a twofold screw axis parallel to b in a = o, c = $\frac{1}{4}$ (fig. 2). It is remarkable that in the structure of AzUR the screw -related heterocycles are arranged such that the C(4)-O(4) and C(2)-O(2) "dipole" bonds of different molecules are close together (3.3 Å) and antiparallel. In 4-thiouridine the heterocycle C(2)-O(2) and C(4)-S bonds pack differently due to the syn conformation of the nucleoside.

The hydrophilic region in AzUR is formed by the ribose residues which are hydrogen bonded to each other through hydroxyl groups O(3') and O(5') and to the heterocycle O(2), N(3) and O(4) atoms through atoms O(1') and O(2') in an intricate, yet pseudo-symmetrical manner (fig. 2). The hydrogen bonds linking N(3) and O(1') are quite unusual since the ribose O(1') oxygen atom to our knowledge has only been involved in hydrogen bonding in the crystal structure of ethyl-1-thio-α-glucofuranoside (16). The reason for this unusual hydrogen bond in the AzUR structure might be the relatively high acidity of the N(3)-proton indicated by the pK value of 6.7.

In 4-thiouridine the hydrophilic region is also formed by the ribose residues, but water of hydration adds to the hydrogen bonding scheme.

The pseudo-symmetry in the hydrogen bonding scheme of AzUR is due to the pseudo A face centering of the crystal structure mentioned earlier. From figure 2 it is clear that only

slight changes of the molecular packing could yield a face-centered structure as in 4-thiouridine.

ACKNOWLEDGEMENTS

We thank Prof. F. Cramer for generous support, Dr. B. Krebs for helpful discussions, and Miss U. Wittenberg for skillful technical assistance.

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