# Structure of 1-β-D-xylofuranosyluracil in the crystal and in solution

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The molecular and crystal structure of 1-β-D-xylofuranosyluracil hydrate was established by X-ray diffraction analysis. The mutual arrangement of the xylofuranose fragment and the nucleic base corresponds to the anti conformation. The furanose ring adopts a C-envelope conformation. The structure of the nucleoside in a DMSO-$d_6$ solution has been determined by $^1$H NMR spectroscopy.

**Key words:** molecular and crystal structure, X-ray diffraction analysis, conformation, 1-β-D-xylofuranosyluracil; $^1$H NMR spectroscopy.

Studies of the structure—biological activity relationship of nucleosides involve the determination of the conformational characteristics of these compounds.[1–5] It was established that nucleosides with the restricted conformational flexibility of the hydrocarbon residue are most promising antiviral pharmaceuticals. However, attempts to formulate rigorous physicochemical criteria for conformations of hydrocarbon fragments have been unsuccessful.[1]

The use of X-ray diffraction analysis for establishing spatial structures of nucleosides in crystals also allows one to elucidate the structure—property relationship.

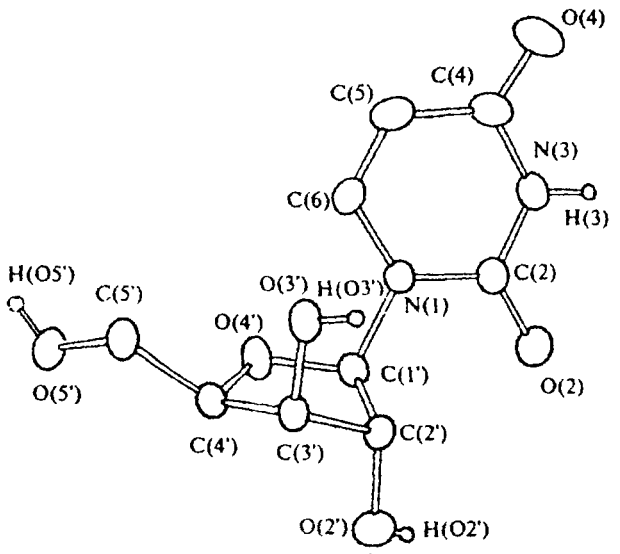


**Fig. 1.** Overall view of the 1-β-D-xylofuranosyluracil molecule in the crystal.

Previously,[2] it has been demonstrated that in the solid state the known anti-HIV compounds, with one exception, contain the sugar ring in the C(3')-*exo* conformation. X-ray structural data on many biologically active nucleosides, for example, 3'-azido-3'-desoxythymidine,[6] 3'-amino-3'-desoxythymidine,[7] 3'-fluoro-3'-desoxythymidine,[8] 2',3'-didesoxynucleosides,[9,10] 5'-chloro-5-desoxy-β-D-arabinofuranosylcytosine,[11] *etc.*, are available in the literature.

In this work, we report the results of studies of the structure of 1-β-D-xylofuranosyluracil hydrate (XyloU) in the crystal and in solution.[12] It is known[13,14] that nucleosides of this series exhibit high antiviral and antitumor activity.

The crystal structure of XyloU is shown in Fig. 1. The bond lengths in the furanose ring and the pyrimidine fragment (Table 1) are consistent with the expected values.[8]

It was established that the uracil fragment is planar, and the xylofuranose fragment adopts a C-envelope conformation with the C(3') atom deviating from the C(1')—C(2')—C(4')—O(4') plane (planar within 0.04 Å) by 0.355(3) Å. The phase angle of pseudorotation P = 180°, the maximum amplitude of pseudorotation $\varphi_{max}$ = 36.0°, and the angle between the planes of these rings is 102° (Table 2).

The hydroxyl groups at positions 2' and 3' are in axial positions, and the $CH_2OH$ group is in an equatorial position. The mutual orientation of the furanose ring and the base in the molecule corresponds to the anti conformation of the nucleoside with an O(4')—C(1')—N(1)—C(2) torsion angle of −169.9° (Fig. 2). The projection along the N(1)—C(1) bond in the crystal is shown below. The conformational flexibility of the molecule with respect to the glycoside bond is somewhat

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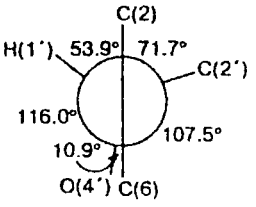
**Table 1.** Bond lengths ($d$) in the structure of 1-β-D-xylofuranosyluracil

| Bond | $d$/Å | Bond | $d$/Å |
|---|---|---|---|
| O(2)—C(2) | 1.217(5) | N(1)—C(1′) | 1.493(5) |
| O(4)—C(4) | 1.238(5) | N(3)—C(2) | 1.365(5) |
| O(4′)—C(1′) | 1.398(5) | N(3)—C(4) | 1.370(6) |
| O(4′)—C(4′) | 1.450(5) | C(4)—C(5) | 1.432(6) |
| O(3′)—C(3′) | 1.418(5) | C(5)—C(6) | 1.339(6) |
| O(5′)—C(5′) | 1.421(5) | C(1′)—C(2′) | 1.534(6) |
| O(2′)—C(2′) | 1.407(5) | C(2′)—C(3′) | 1.524(5) |
| N(1)—C(2) | 1.378(5) | C(3′)—C(4′) | 1.528(5) |
| N(1)—C(6) | 1.366(5) | C(4′)—C(5′) | 1.499(5) |

**Table 2.** Bond angles (ω) in the structure of 1-β-D-xylofuranosyluracil

| Angle | ω/deg | Angle | ω/deg |
|---|---|---|---|
| C(1′)—O(4′)—C(4′) | 110.4(3) | O(4′)—C(1′)—N(1) | 109.1(3) |
| C(2)—N(1)—C(6) | 121.1(4) | O(4′)—C(1′)—C(2′) | 107.1(3) |
| C(2)—N(1)—C(1′) | 115.8(3) | N(1)—C(1′)—C(2′) | 112.1(3) |
| C(6)—N(1)—C(1′) | 123.1(3) | O(2′)—C(2′)—C(1′) | 110.9(3) |
| C(2)—N(3)—C(4) | 126.0(4) | O(2′)—C(2′)—C(3′) | 107.0(3) |
| O(2)—C(2)—N(1) | 121.0(4) | C(1′)—C(2′)—C(3′) | 102.3(3) |
| O(2)—C(2)—N(3) | 123.2(4) | O(3′)—C(3′)—C(2′) | 111.9(3) |
| N(1)—C(2)—N(3) | 115.8(4) | O(3′)—C(3′)—C(4′) | 108.4(3) |
| O(4)—C(4)—N(3) | 120.0(4) | C(2′)—C(3′)—C(4′) | 101.2(3) |
| O(4)—C(4)—C(5) | 124.9(4) | O(4′)—C(4′)—C(3′) | 105.0(3) |
| N(3)—C(4)—C(5) | 115.1(4) | O(4)—C(4′)—C(5′) | 110.2(3) |
| C(4)—C(5)—C(6) | 119.6(4) | C(3′)—C(4′)—C(5′) | 114.9(3) |
| N(1)—C(6)—C(5) | 122.1(4) | O(5′)—C(5′)—C(4) | 108.8(3) |

hindered due to the 1,3-axial-axial arrangement of the substituents at the C(1′) and C(3′) atoms in the furanose ring. The substituents at the C(2′) and C(4′) atoms are in axial and equatorial positions, respectively. The C(5′)—O bond has a *trans*, *gauche* (tg) conformation.




In the structure of XyloU, strong O—H...O and N—H...O hydrogen bonds occur. It should be noted that the water molecule of crystallization acts both as the donor and as the acceptor of the amine proton.

The conformational characteristics of the nucleoside were studied in a DMSO-$d_6$ solution. The $^1$H NMR spectral parameters of the protons of the xylofuranose fragment of the molecule were refined by calculation procedures using the iterative PANIC program available for the software of the Bruker-AM300 spectrometer (the Aspect-3000 computer).

The protons of the methylene, three methine, and three hydroxyl groups form a nine-spin ABCDEFGHI system. First for simplicity, this system may be considered as a six-spin system without the hydrogen atoms of the methylene group and without the proton of the 5′-hydroxyl group. This is possible because the above-mentioned protons interact only with the H(4′) atom.

**Table 3.** Parameters of the $^1$H NMR spectrum of 1-β-D-xylofuranosyluracil

| Atom | δ $^1$H ($J$/Hz) |
|---|---|
| H(5′) (A) | 3.70 ($J_{H(2')H(3')}$ = 0.5—0.7) |
| H(5″) (B) | 3.82 ($J_{H(3')H(4')}$ = 3.5) |
| H(3′) (C) | 3.90 ($J_{H(3')OH(2')}$ = 3.5) |
| H(2′) (D) | 3.95 ($J_{H(1')H(2')}$ = 0.8—1.12) |
| H(4′) (E) | 4.08 ($J_{H(2')OH(3')}$ = 4.2) |
| OH(5′) (F) | 4.78 ($J_{H(4')H(5')}$ = 5.05) |
| OH(2′) (G) | 5.45 ($J_{H(4')H(5'')}$ = 5) |
| H(1′) (H) | 5.65 ($J_{H(5')H(5'')}$ = 12) |
| OH(3′) (I) | 5.78 ($J_{H(5')OH(5')}$ = $= J_{H(5'')OH(5')}$ = 5.6) |

The second simplified system is a seven-spin system without the H(1′) atom and the proton of the hydroxyl substituent at the C(2′) atom. Initially, trial parameters, which have been determined by the double resonance method, were used in the calculations, and then the nine-spin system was treated as a whole. The parameters determined are given in Table 3. The pseudorotation in the sugar fragment of the nucleoside can be described using the population coefficients of the conformers of the S and N forms.[15]

It is known[16,17] that the sugar ring in 9-β-D-xylofuranosyladenine (XyloA) and in its *O*-methyl derivatives exists mainly as the *N* conformer. The fraction of the latter depends only slightly on the pH of the solution and on the solvent ($D_2O$ or DMSO-$d_6$).

Based on the above-mentioned X-ray structural data ($P_N$ = 0°, $\varphi_N$ = 36°) and assuming that $\varphi_N = \varphi_S$ and $P_S$ = 180°, we estimated the ratio between these forms in solution. According to the data on the spin-spin coupling constants $J_{1'2'}$ = 1.12 Hz; $J_{2'3'}$ = 0.7 Hz, and $J_{3'4'}$ = 3.5 Hz (the accuracy is ±0.05 Hz), the conformer of the N type predominates at room temperature. The $J_{1'2'}$ value first decreases and then increases to 1.7 Hz as the temperature increases (Fig. 2). The mole fraction of the conformers of the N type is 0.98, 1, and 0.90 at 30, 50, and 70 °C, respectively.

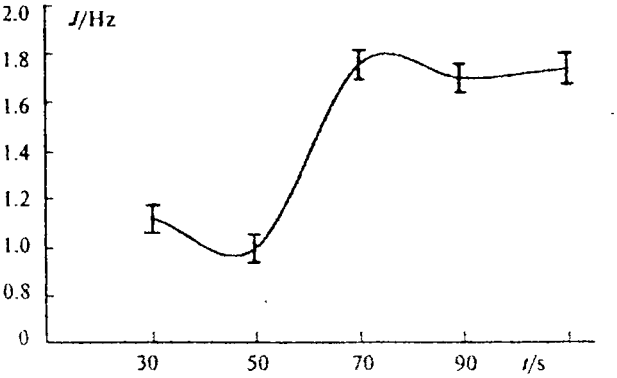



**Fig. 2.** Temperature dependence of the spin-spin coupling constant ($J_{1'2'}$) of β-D-xylofuranosyluracil.

**Table 4.** Coordinates of nonhydrogen atoms in the structure of 1-β-D-xylofuranosyluracil and equivalent isotropic temperature factors ($B_{eq}$)

| Atom | *x* | *y* | *z* | $B/Å^2$ |
|---|---|---|---|---|
| O(2) | −0.0861(3) | 0.63 | 0.7567(1) | 3.20(3) |
| O(4) | −0.2498(3) | 1.1745(4) | 0.9605(1) | 3.71(4) |
| O(4′) | 0.4438(2) | 0.9441(3) | 0.7206(1) | 2.09(3) |
| O(3′) | 0.1433(2) | 1.2598(3) | 0.6143(1) | 2.04(3) |
| O(5′) | 0.7750(2) | 1.2414(3) | 0.6832(1) | 2.42(3) |
| O(2′) | 0.2146(2) | 0.6842(3) | 0.5518(1) | 2.36(3) |
| O(7)* | 0.5261(3) | 0.5827(4) | 0.9040(1) | 3.72(4) |
| N(1) | 0.1325(3) | 0.9270(3) | 0.7863(1) | 1.75(3) |
| N(3) | −0.1569(3) | 0.9001(3) | 0.8645(1) | 2.16(3) |
| C(2) | −0.0410(3) | 0.8096(4) | 0.8003(2) | 1.96(3) |
| C(4) | −0.1239(3) | 1.1015(4) | 0.9098(2) | 2.27(4) |
| C(5) | 0.0621(4) | 1.2116(4) | 0.8943(2) | 2.58(4) |
| C(6) | 0.1822(3) | 1.1214(4) | 0.8345(2) | 2.13(4) |
| C(1′) | 0.2575(3) | 0.8299(3) | 0.7150(2) | 1.68(3) |
| C(2′) | 0.1513(3) | 0.8534(3) | 0.6109(2) | 1.60(3) |
| C(3′) | 0.2404(3) | 1.0710(3) | 0.5789(1) | 1.58(3) |
| C(4′) | 0.4600(3) | 1.0582(4) | 0.6306(1) | 1.66(3) |
| C(5′) | 0.5640(3) | 1.2787(4) | 0.6501(2) | 2.08(4) |

* The oxygen atom of the water molecule of crystallization.

The conformational behavior of XyloU can be explained by the existence of the O(3′)—H...O(5′)—H intramolecular hydrogen bond resulting in the stabilization of the S conformer at ~20 °C. The flexibility of the five-membered ring increases as the temperature increases, which leads to an increase in the fraction of the S conformer and to an increase in the constant $J_{1'2'}$.

The conformation of the 5′-$CH_2OH$ group was estimated according to the work reported previously.[18] Unlike XyloA, for which the population of the *gauche-gauche* (gg) rotamer decreases to 15% as the fraction of the N conformer increases (0.8),[16] in the case of XyloU when the N form predominates subtantially (0.98), the fraction of the *gauche-gauche* rotamer is approximately twice as large (0.295) as that observed in the case of XyloA.

## Experimental

The $^1H$ NMR spectra (300.13 MHz) were recorded on a Bruker AM-300 spectrometer. The resolution of the one-dimensional proton spectra was improved using the Lorentz—Guass transformation of the free induction decay (FID) (LB = −0.8; GB = 0.3) and by adding 32 K zero points to 32 K FID.

The $^1H$ shifts were referenced to $Me_4Si$ as the internal standard; DMSO-$d_6$ was used as a solvent. The spectra were calculated using the PANIC program available for the Bruker Software (Version 1987).

Crystals of 1-β-D-xylofuranosyluracil hydrate were prepared by slow crystallization from an aqueous solution upon evaporation of the solvent in air. The crystals are colorless with dimensions of no more than 3 mm.

**X-ray diffraction study of the compound.** Crystals are monoclinic, at 20 °C: $a$ = 6.576(1) Å, $b$ = 6.009(3) Å, $c$ = 13.974(4) Å, β = 97.66(2)°, $V$ = 547.3(7) Å$^3$, $d_{calc}$ = 1.591 g cm$^{-3}$, $Z$ = 2, space group $P2_1$, $C_9H_{12}N_2O_6 \cdot H_2O$.

The unit cell parameters and intensities of 1802 independent reflections were measured on an automated Enraf-Nonius CAD-4 diffractometer (Mo-Kα radiation, graphite monochromator, ω/2θ scanning technique, 2θ ≤ 60°) at room temperature. The structure was solved by the direct method using the MULTAN program to reveal all nonhydrogen atoms and refined by the full-matrix least-squares method with anisotropic thermal parameters for nonhydrogen atoms using 1324 reflections with $I > 3\sigma(I)$. The final value of the $R$ factor was as follows: $R$ = 0.029 ($R_w$ = 0.029). The coordinates of nonhydrogen atoms are given in Table 4.

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