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Canadian journal of Chemistry, 59, 2, 1981

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Crystal structures and conformations of 1-α-D-xylofuranosylcytosine and its protonated form (HCl salt)[1]

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Received July 24, 1980

This paper is dedicated to Prof. Raymond U. Lemieux on the occasion of his 60th birthday

MICHAEL L. POST, CAROL P. HUBER, GEORGE I. BIRNBAUM, and DAVID SHUGAR. Can. J. Chem. **59**, 238 (1981).

The structures of 1-α-D-xylofuranosylcytosine, $C_9H_{13}N_3O_5$ (**1**), and its hydrochloride salt, $C_9H_{13}N_3O_5{\cdot}HCl$ (**1**·HCl), have been determined by X-ray diffraction from diffractometer data, using direct method techniques. Both compounds crystallize in the orthorhombic system with $Z = 4$. Space group and cell parameters are, for **1**: $P2_12_12$, $a = 18.706$, $b = 8.127$, $c = 7.007$ Å; and for **1**·HCl: $P2_12_12_1$, $a = 16.800$, $b = 8.045$, $c = 8.897$ Å. Refinement by block-diagonal least-squares calculations gave a final R of 0.033 on 873 reflections and 0.034 on 914 reflections for **1** and **1**·HCl, respectively. The glycosyl torsion angles are in the *anti* domain, $\chi_{CN} = -25.1°$ (**1**) and $-28.6°$ (**1**·HCl), and the sugar puckers are nearly pure ${}^3_2T$ (**1**) and ${}^3E$ (**1**·HCl) forms. The C(4′)—C(5′) rotamer is *trans–gauche* in both cases. No intramolecular hydrogen bonding occurs in the xylofuranosyl rings. Lattice packing in the crystal structures occurs via intermolecular hydrogen bonding, with base stacking in pairs about one of the 2-fold axes for the neutral form, and with no base-stacking interactions for the protonated form. The biological implications of the structure and conformation of α-nucleosides are examined.

MICHAEL L. POST, CAROL P. HUBER, GEORGE I. BIRNBAUM et DAVID SHUGAR. Can. J. Chem. **59**, 238 (1981).

On a déterminé les structures de la 1-α-D-xylofurannosylcytosine $C_9H_{13}N_3O_5$ (**1**) et de son chlorhydrate par diffraction de rayons-X, en se basant sur des données obtenues par des méthodes directes. Les deux composés cristallisent dans le système orthorhombique avec $Z = 4$. Le groupe spatial et les dimensions de la maille sont pour le composé **1**: $P2_12_12$, $a = 18{,}706$, $b = 8{,}127$, $c = 7{,}007$ Å; et pour son chlorhydrate $P2_12_12_1$, $a = 16{,}800$, $b = 8{,}045$, $c = 8{,}897$ Å. On a affiné les structures par la méthode des moindres carrés (bloque diagonal) jusqu'à une valeur de $R = 0{,}033$ pour 873 réflexions et de 0,034 pour 914 réflexions respectivement pour le composé **1** et son chlorhydrate. Les angles de torsion glycosidiques sont dans le domaine *anti* $\chi_{CN} = -25{,}1°$ (**1**) et $-28{,}6°$ (**1**·HCl), et le plissement des cycles furannoses conduit à des formes presque parfaites ${}^3_2T$ (**1**) et ${}^3E$ (**1**·HCl). Dans les deux cas le rotamère C(4′)—C(5′) est *trans–gauche*. Il n'y a pas de liaison hydrogène intramoléculaire dans les cycles xylofurannosyles. L'organisation des molécules dans le cristal se fait par l'intermédiaire de liaisons hydrogène intermoléculaires; les bases s'entassent par paires le long de l'un des axes binaires de symétrie dans le cas de la forme neutre et sans interactions d'entassement des bases pour la forme protonée. On a examiné les implications biologiques de la structure et de la conformation des α-nucléosides.

[Traduit par le journal]

Introduction

Nucleotide residues hitherto found in natural nucleic acids occur exclusively in the β-anomeric configuration. There are, however, a number of examples of α-nucleosides and nucleotides, in the free state or as constituents of more complex molecules, such as the vitamin B_{12} group, in living cells (ref. 1, and references cited therein). Some α-nucleoside 5′-phosphates are substrates of 5′-nucleotidase (2, 3); α-5-formyluridine is sufficiently effective as a non-competitive inhibitor of *Escherichia coli* DNA-dependent RNA polymerase to permit the affinity labelling of the latter (4). Other α-nucleosides have also been found to be substrates for, and inhibitors of, a variety of enzymes (5, 6).

Increasingly widespread interest in the biological and physico-chemical properties of α-anomeric nucleosides and nucleotides derives largely from the unexpected findings that some are potent antimetabolites with antitumour and antiviral activities (7–11) which, in several instances, are comparable to, or even in excess of, those of the corresponding β-anomers (6, 10, 11). Undoubtedly the most striking illustration is α-2′-deoxy-6-thioguanosine, which is presently undergoing clinical trials (12). It is readily phosphorylated by kinase(s) in some tumour cells (13) and is incorporated into the terminal position of small oligodeoxynucleotides (14, 15), thus acting as a chain terminator. The foregoing has raised some questions as to the presumed gross structural dissimilarity between α- and β-anomeric nucleoside pairs, and led to the chemical synthesis of the two anomers of 3′-(hydroxymethyl)-branched 2′-deoxynucleosides of 6-thioguanine. *Both* of these, in agreement with expecta-

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0008-4042/81/020238-08$01.00/0

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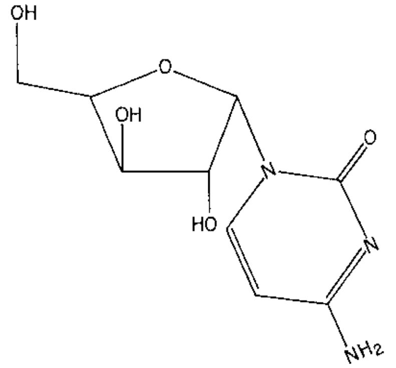
tions, were found to be not only *equally* effective as inhibitors of the growth of WI-L2 human lymphoblastoid cells, but to be even more effective in this regard than the parent α-2′-deoxy-6-thioguanosine (6).

It has also been found that the α-anomers of at least two arabinosyl nucleosides exhibit antiviral activities comparable to those of the corresponding β-anomers (10). Since substrate and/or inhibitor properties of a given class of compounds are dependent, amongst other factors, on conformational parameters, it is of obvious interest to compare the conformations of the two anomeric forms of nucleosides. However, only very limited structural and conformational solid state data are at present available for α-nucleosides (see ref. 1 and references cited therein). The utility of such solid state data in the interpretation of solution conformations obtained by nmr spectroscopy has already been admirably illustrated in the case of ribo- and deoxyribonucleosides (16, 17). We report here on the solid state structure and conformation of α-xylofuranosylcytosine (**1**) and of its hydrochloride salt (**1**·HCl). The antimetabolic properties of some β-D-xylofuranosyl nucleosides have been well documented (18–21). It has been shown that 9-β-D-xylofuranosyladenine interferes with methylation of nuclear RNA (21) via inhibition of the synthesis of *S*-adenosyl-L-methionine (22). Insofar as we are aware, no attempts have yet been made to examine the possible antimetabolic properties of α-xylofuranosyl nucleosides, but their properties in some enzyme systems have received some attention as discussed below.



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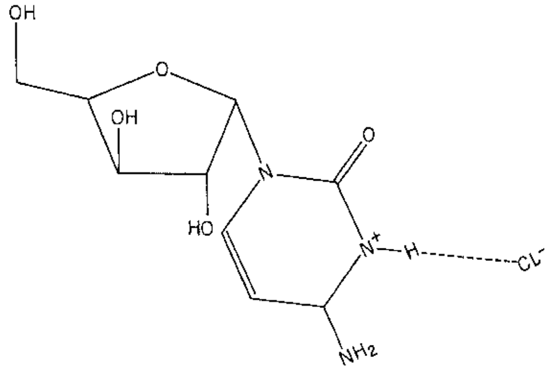

1·HCl

Experimental

Preparation of 1-α-D-Xylofuranosylcystosine (1) and 1·HCl

O^2,2′-anhydro-1-α-D-xylofuranosyluracil, obtained by the method of Holy (2), was heated under reflux with aqueous triethylamine (10% v/v) to produce 1-α-D-xylofuranosyluracil. The latter was then acetylated and treated with P_2S_5 in dioxane to give the 4-thio derivative (3) which, when aminated with methanolic ammonia in a sealed tube at 105°C, yielded **1**. Passage through a Dowex-50W (H^+) column followed by crystallization from anhydrous ethanol yielded pure **1**, mp 263–264°C (decomp.). The mother liquors from the crystallization were treated with excess HCl (2 *N*) and brought to dryness, to give the hydrochloride salt (**1**·HCl), which was then recrystallized several times from anhydrous ethanol to yield the pure product, mp 190–191°C (uncorr.).

X-ray Analysis of 1 and 1·HCl

Crystals of **1** and **1**·HCl were grown from anhydrous ethanol. Weissenberg and precession photographs were used to determine the space groups and preliminary cell constants. Accurate lattice parameters were obtained by least-squares treatment of the 2θ values of 19 and 14 (**1** and **1**·HCl, respectively) reflections centered on a diffractometer. Intensity data for both compounds were collected with Ni-filtered CuKα radiation (λ = 1.54178 Å) on a Picker diffractometer equipped with a scintillation detector and pulse height discriminator; θ–2θ scans were employed and background counts of 20 s were taken at each end of the scan range. Threshold levels for "unobserved" data were experimentally determined as the larger of 0.1 × total background *or* 50 counts (for **1**) and 48 counts (for **1**·HCl), and reflections with intensities lower than these values were excluded during structure refinement. Lorentz, polarization, and scaling factors (calculated with regard to frequently monitored check reflections) were applied to the raw data, but no corrections were made for absorption or secondary extinction. Crystal data and other pertinent details are as follows:

$C_9H_{13}N_3O_5$ (**1**) fw = 243.22
Orthorhombic, a = 18.706(4), b = 8.127(2), c = 7.007(1) Å, V = 1065.2 Å^3, Z = 4, ρ_{calcd} = 1.516, ρ_{meas} = 1.52 g cm^{-3} (20°C, CuKα Ni-filtered), μ = 10.8 cm^{-1}, crystal size 0.24 × 0.08 × 0.05 mm, $2\theta_{max}$ = 120°, scan speed 1° min^{-1} in 2θ, scan widths 1.7° for $2\theta \leq 90°$; 2.0° for $90° < 2\theta \leq 120°$, reflections observed/accessible = 873/947 (92%). Absences $h00$, $h = 2n + 1$; $0k0$, $k = 2n + 1$, space group $P2_12_12$.

$C_9H_{13}N_3O_5\cdot HCl$ (**1**·HCl) fw = 279.70
Orthorhombic, a = 16.800(3), b = 8.045(1), c = 8.897(1) Å, V = 1202.6 Å^3, Z = 4, ρ_{calcd} = 1.544, ρ_{meas} = 1.54 g cm^{-3} (20°C, CuKα Ni-filtered), μ = 30.4 cm^{-1}, crystal size 0.21 × 0.09 × 0.04 mm, $2\theta_{max}$ = 130°, scan speed 2° min^{-1} in 2θ, scan widths 1.4° for $2\theta \leq 80°$, 1.6° for $80 < 2\theta \leq 105°$, 1.8° for $105 < 2\theta \leq 130°$, reflections observed/accessible = 914/1209 (76%). Absences $h00$, $h = 2n + 1$; $0k0$, $k = 2n + 1$; $00l$, $l = 2n + 1$, space group $P2_12_12_1$.

The structures were solved by direct methods using the symbolic addition procedure with tangent refinement and a multisolution approach. In each case, all nonhydrogen atoms were located in *E*-maps calculated with 119 and 144 phased *E*'s ≥ 1.45

TABLE 1. Final atomic positional coordinates (×10⁴) for the non-H atoms, estimated standard deviations are in parentheses

	1			1·HCl		
Atom	*x/a*	*y/b*	*z/c*	*x/a*	*y/b*	*z/c*
N(1)	4746(1)	7156(3)	9701(3)	6655(2)	3420(4)	2654(3)
C(2)	5333(1)	8097(4)	9148(4)	6682(2)	3318(6)	4206(4)
O(2)	5440(1)	8352(3)	7425(3)	6146(2)	3827(5)	4992(3)
N(3)	5772(1)	8727(3)	10514(3)	7352(2)	2580(5)	4777(3)
C(4)	5639(1)	8417(4)	12357(4)	7948(2)	1918(5)	3950(4)
N(4)	6080(1)	9001(3)	13692(4)	8535(2)	1170(5)	4653(4)
C(5)	5034(2)	7461(4)	12938(4)	7895(2)	2056(5)	2379(4)
C(6)	4602(2)	6862(4)	11570(4)	7248(2)	2792(5)	1782(3)
C(1′)	4269(1)	6543(4)	8187(4)	5912(2)	4086(5)	2009(4)
O(1′)	3898(1)	5153(3)	8893(3)	6060(2)	4709(3)	561(3)
C(2′)	3693(1)	7784(4)	7560(4)	5275(2)	2714(5)	1855(4)
O(2′)	3426(1)	8708(3)	9123(3)	5622(1)	1127(3)	1692(3)
C(3′)	3105(2)	6616(4)	6885(4)	4867(2)	3181(4)	379(4)
O(3′)	3270(1)	5989(3)	5044(3)	4290(1)	4449(3)	605(3)
C(4′)	3153(2)	5240(4)	8348(4)	5561(2)	3852(5)	−539(4)
C(5′)	2945(1)	3525(4)	7682(4)	5364(2)	5112(5)	−1738(4)
O(5′)	2181(1)	3423(3)	7450(3)	4954(2)	4345(4)	−2946(3)
Cl^-	—	—	—	7842(1)	2242(2)	8097(1)

TABLE 2. Final hydrogen atom positional coordinates (×10³) and isotropic thermal parameters (Å² × 10²), estimated standard deviations are in parentheses

	1				1·HCl			
Atom	*x/a*	*y/b*	*z/c*	U_{iso}	*x/a*	*y/b*	*z/c*	U_{iso}
H(1)	598(2)	894(4)	1497(5)	65(10)	901(3)	74(6)	416(6)	81(16)
H(2)	645(2)	966(5)	1337(6)	62(11)	852(3)	104(7)	560(6)	79(16)
H(3)	—	—	—	—	736(4)	235(7)	574(7)	108(22)
H(5)	499(2)	707(4)	1430(4)	46(8)	833(2)	151(6)	183(5)	54(13)
H(6)	417(2)	616(6)	1181(6)	76(13)	712(2)	296(5)	77(4)	44(11)
H(1′)	459(1)	621(4)	700(4)	37(9)	575(2)	499(4)	265(4)	23(9)
H(2′)	387(1)	859(4)	656(4)	36(8)	490(2)	272(5)	281(4)	44(11)
H(O2′)	374(2)	962(6)	946(6)	92(15)	572(2)	75(5)	261(5)	60(13)
H(3′)	265(1)	713(4)	693(4)	26(8)	461(2)	222(5)	−2(4)	26(9)
H(O3′)	309(2)	684(7)	409(7)	118(18)	398(3)	393(6)	94(6)	77(16)
H(4′)	286(1)	552(4)	956(4)	31(8)	582(2)	285(5)	−93(5)	47(11)
H(5′1)	316(2)	338(5)	639(6)	70(12)	502(2)	587(5)	−128(4)	32(9)
H(5′2)	309(1)	269(3)	882(4)	25(7)	583(2)	583(5)	−207(5)	51(12)
H(O5′)	199(2)	351(6)	839(7)	96(15)	527(3)	402(8)	−356(6)	86(17)

and ≥ 1.50 for **1** and **1**·HCl, respectively. Refinement was carried out using block-diagonal least squares with the non-H atoms initially having isotropic, and then anisotropic, thermal motion. Hydrogen atoms were located in difference Fourier syntheses and their contribution was included in the refinement with isotropic thermal parameters. The function minimized throughout was $\Sigma w(|F_o| - |F_c|)^2$ with weights, *w*, in the form $\sqrt{w} = |F_o|/M$ for $|F_o| \leq M$; $\sqrt{w} = M/|F_o|$ for $|F_o| > M$ with $M = 12.4$ and 10.4 for **1** and **1**·HCl. These weighting schemes made the average values of $w(\Delta F^2)$ independent of $|F_o|$ and $\sin^2 \theta$. Convergence was attained at an *R* of 0.033 and 0.034 for the observed data (0.043 and 0.061 for all data)[3] and an R_w of 0.034 and 0.040,[4] the figures for **1** being given first. Scattering factors were taken from ref. 23 and ref. 24 (for H) and those for O and Cl^- were corrected for the real and imaginary components of anomalous dispersion (23). The absolute configuration was chosen with respect to the known D-xylofuranosyl moiety in each structure. Programs used were from the NRC crystallographic set (25) and most figures are based on ORTEP II (26) plots. The final atomic parameters are given in Tables 1 and 2.

Results and Discussion

Views of the two molecules, together with the atom numbering scheme, are exhibited in Fig. 2. Bond lengths and bond angles for each structure are listed in Table 3, and conformational parameters, together with those for the related ribofuranoside, α-cytidine (data from ref. 1), are summarized in Table 4.

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[3] Tables of observed and calculated structure factors, anisotropic thermal parameters, least-squares planes, and bond angles involving hydrogen atoms are available, at a nominal charge, from the Depository for Unpublished Data, CISTI, National Research Council of Canada, Ottawa, Ont., Canada K1A 0S2.

[4] $R_w = \Sigma\sqrt{w}(|F_o| - |F_c|)/\Sigma\sqrt{w}|F_o|$.

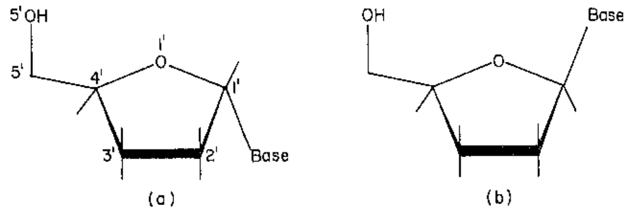

FIG. 1. The α (*a*) and β (*b*) anomeric configurations for a nucleoside.

Each structure exhibits the α-anomeric glycosyl linkage at C(1′) (see Fig. 1) with torsion angles, χ_{CN} (Table 5), of −25.1° and −28.6° for **1** and **1**·HCl, respectively, hence both in the *anti* domain. The glycosidic torsion angles are close to those reported for α-cytidine (1) and α-5-formyluridine (4), −28° and −22°, respectively, so that a χ_{CN} in this range appears reasonably typical for α-nucleosides with a 2′-hydroxyl *cis* to the aglycone.

Sugar Moieties

The pucker of the sugar rings is similar in the two structures: C(2′)*exo*—C(3′)*endo* in **1** and C(3′)*endo* in **1**·HCl. The former conformation is that observed in α-cytidine (1), and the latter in α-5-formyluridine (4), as well as in the α-nucleotide moiety of vitamin B_{12} (27). Furthermore, in the two crystallographically independent molecules of 9-α-D-arabinofuranosyladenine, the sugar moiety of one is C(2′)*exo*—C(3′)*endo*, and that of the other C(3′)*endo* (28). While it may be tempting to consider this narrow range of sugar conformations as preferred for nucleosides with the 2′-OH and the aglycone *cis* oriented, it should nonetheless be noted that a much broader range has been observed in the case of β-arabinofuranosyl nucleosides (29), as well as in other α-nucleosides (30).

This *cis* orientation of the 2′-OH and the aglycone results in a close approach between O(2′) and the ring N(1), which may be alleviated by various distortions. In **1** the $^{3}_{2}T$ pucker leads to the O(2′)—C(2′) and C(1′)—N(1) bonds being more staggered than in **1**·HCl, the difference in the torsion angle about C(1′)—C(2′) being 10° (Table 5). Furthermore, the N(1)—C(1′)—C(2′) bond angle is about 3° larger in **1** than in **1**·HCl. Consequently the N(1)···O(2′) contact in **1** is increased to 2.802 Å. On the other hand, the entire pyrimidine ring in **1**·HCl is displaced away from O(2′), as is clear from the large displacement (0.129 Å) of C(1′) from the ring plane. A similar, even larger, displacement (0.145 Å) was found in α-5-formyluridine (4). However, such distortion is not as effective in relieving the O(2)···N(1) interaction, this distance in **1**·HCl being a relatively short 2.674 Å. It follows that, in nucleosides in which the aglycone is *cis* to the O(2′), the sugar ring is unlikely to have ^{4}E or $_{4}E$ puckers, with the N(1)—C(1′)—C(2′)—O(2′) torsion angle approaching 0°, unless such a pucker is imposed by some structural features as, e.g. in the case of the cyclic O^2,2′-anhydro-1-α-D-xylofuranosyluracil (31).

In both **1** and **1**·HCl, the conformation about the C(4′)—C(5′) bond is *trans–gauche*. In the case of ribonucleosides, the most commonly encountered conformation of the exocyclic 5′-CH_2OH is *gauche–gauche*. It is consequently of interest that

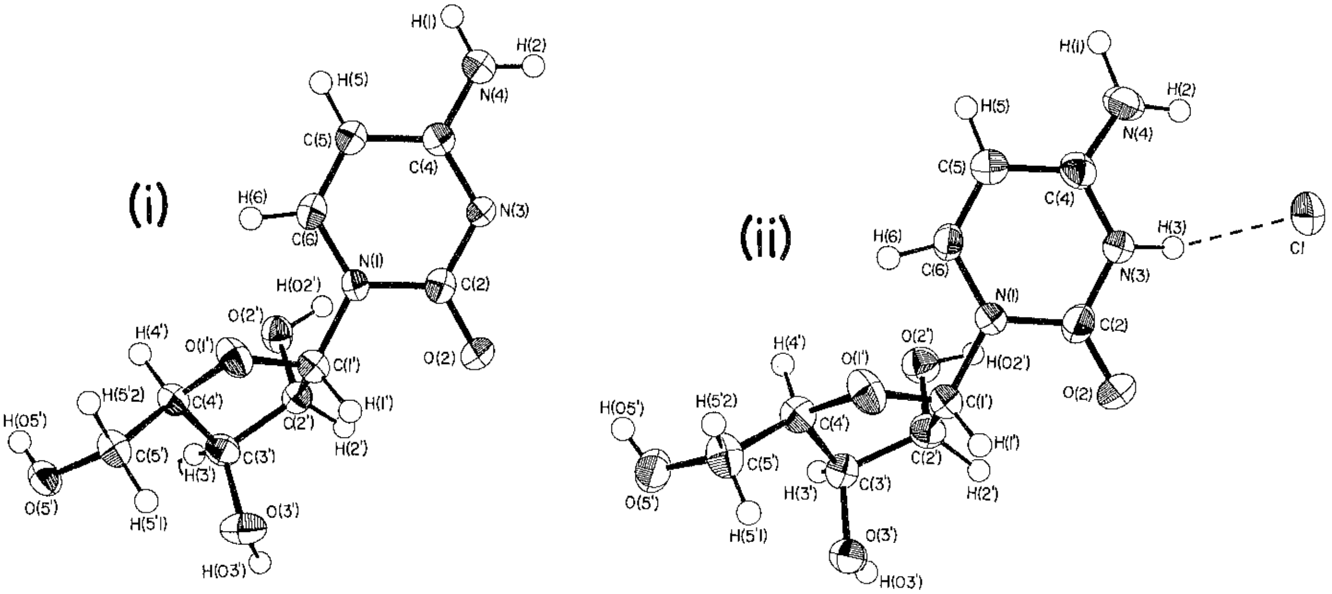

FIG. 2. Perspective views of (*i*) α-D-xylofuranosylcytosine (**1**), and (*ii*) α-D-xylofuranosylcytosine hydrochloride (**1**·HCl). Non-H atoms are represented by 50% probability thermal ellipsoids and H atoms by spheres of arbitrary radius.

TABLE 3. Bond lengths (Å) and angles (deg), with estimated standard deviations in parentheses

Bond	Length 1	Length 1·HCl	Bond	Length 1	Length 1·HCl
N(1)—C(2)	1.393(4)	1.385(5)	C(4′)—C(5′)	1.520(4)	1.509(5)
C(2)—O(2)	1.241(3)	1.211(5)	C(5′)—O(5′)	1.441(3)	1.418(5)
C(2)—N(3)	1.361(4)	1.370(5)	N(4)—H(1)	0.92(4)	0.97(5)
N(3)—C(4)	1.339(4)	1.352(5)	N(4)—H(2)	0.90(4)	0.85(5)
C(4)—N(5)	1.334(4)	1.314(5)	N(3)—H(3)	—	0.88(6)
C(4)—C(5)	1.432(4)	1.405(5)	C(5)—H(5)	1.01(4)	0.98(4)
C(5)—C(6)	1.345(4)	1.347(5)	C(6)—H(6)	1.00(4)	0.93(4)
C(6)—N(1)	1.358(4)	1.360(4)	C(1′)—H(1′)	1.05(3)	0.97(3)
N(1)—C(1′)	1.473(4)	1.475(5)	C(2′)—H(2′)	1.02(3)	1.06(4)
C(1′)—C(2′)	1.540(4)	1.544(5)	O(2′)—H(O2′)	0.97(4)	0.88(5)
C(2′)—O(2′)	1.418(4)	1.411(5)	C(3′)—H(3′)	0.95(3)	0.96(4)
C(2′)—C(3′)	1.528(4)	1.528(5)	O(3′)—H(O3′)	1.02(4)	0.73(5)
C(3′)—O(3′)	1.421(4)	1.421(4)	C(4′)—H(4′)	1.03(3)	0.98(4)
C(3′)—C(4′)	1.520(4)	1.523(5)	C(5′)—H(5′1)	1.00(4)	0.94(4)
C(4′)—O(1′)	1.446(3)	1.460(5)	C(5′)—H(5′2)	1.08(3)	1.02(4)
O(1′)—C(1′)	1.416(4)	1.404(4)	O(5′)—H(O5′)	0.75(5)	0.81(5)

Bonds	Angle 1	Angle 1·HCl	Bonds	Angle 1	Angle 1·HCl
C(2)—N(1)—C(6)	121.4(2)	121.6(3)	N(1)—C(1′)—C(2′)	114.1(2)	111.2(3)
C(2)—N(1)—C(1′)	117.6(2)	115.9(3)	O(1′)—C(1′)—C(2′)	106.2(2)	107.3(3)
C(6)—N(1)—C(1′)	121.0(2)	122.2(3)	C(1′)—C(2′)—C(3′)	100.7(2)	102.2(3)
N(1)—C(2)—N(3)	119.1(2)	114.9(3)	C(1′)—C(2′)—O(2′)	111.9(2)	111.7(3)
N(1)—C(2)—O(2)	119.3(2)	122.2(3)	O(2′)—C(2′)—C(3′)	108.3(2)	108.6(3)
O(2)—C(2)—N(3)	121.6(3)	122.9(3)	C(2′)—C(3′)—C(4′)	101.9(2)	101.8(3)
C(2)—N(3)—C(4)	119.7(2)	125.3(3)	C(2′)—C(3′)—O(3′)	110.4(2)	111.1(3)
N(3)—C(4)—C(5)	121.5(3)	117.6(3)	O(3′)—C(3′)—C(4′)	109.6(2)	110.1(3)
N(3)—C(4)—N(4)	119.7(3)	118.5(3)	C(3′)—C(4′)—O(1′)	105.7(2)	104.3(3)
N(4)—C(4)—C(5)	118.9(3)	123.8(4)	C(4′)—O(1′)—C(1′)	110.0(2)	110.2(3)
C(4)—C(5)—C(6)	118.0(3)	118.5(4)	C(3′)—C(4′)—C(5′)	116.9(2)	116.7(3)
C(5)—C(6)—N(1)	120.3(3)	122.0(3)	O(1′)—C(4′)—C(5′)	106.4(2)	106.4(3)
N(1)—C(1′)—O(1′)	108.4(2)	109.7(3)	C(4′)—C(5′)—O(5′)	110.0(2)	110.5(3)

TABLE 4. Conformational features for 1, 1·HCl, and α-cytidine (α-C)[a]

Parameter	Value 1	Value 1·HCl	Value α-C[b]
Glycosyl torsion angle, χ_{CN}	−25.1	−28.6	−28.4
Pseudorotation angle, P	−1.9	18.1	−3.0
Sugar pucker	^3_2T	^3E	^3_2T
Maximum angle of pucker, τ_m	38.4	37.1	39.0
ϕ_{OO} ϕ_{OC}	$\left.\begin{matrix}169.3\\-72.9\end{matrix}\right\}$ tg	$\left.\begin{matrix}173.5\\-70.6\end{matrix}\right\}$ tg	$\left.\begin{matrix}-57.8\\59.4\end{matrix}\right\}$ gg

[a] All values expressed in deg.
[b] Data from ref. 1.

solution studies on a series of analogues of 9-β-D-xylofuranosyladenine demonstrated a relatively low (< 30%) population of the *gauche–gauche* form (32). Furthermore, in the solid state structure of 1-β-D-lyxofuranosyluracil in which both the 2′-OH and 3′-OH are "up", i.e. *cis* to the 5′-CH_2OH, the conformation of the latter is *gauche–trans*, while in solution the *gauche–gauche* populations for the neutral form and for the neutral and protonated forms of 1-β-D-lyxofuranosylcytosine are exceptionally low, from 10% to 20% (33). Similarly low *gauche–gauche* populations of the exocyclic 5′-CH_2OH have been reported for the solution conformations of the α- and β-xylofuranosides of oxidized and reduced nicotinamides (34). This effect has been ascribed to steric and electrostatic effects of the "up" 3′-OH, which is *cis*, and adjacent, to the 5′-CH_2OH group (33).

TABLE 5. Some torsional angles

Bonds	Torsional angle (deg) 1	Torsional angle (deg) 1·HCl
C(6)—N(1)—C(1′)—O(1′):χ_{CN}	−25.1	−28.6
O(1′)—C(4′)—C(5′)—O(5′):ϕ_{OO}	169.3	173.5
C(3′)—C(4′)—C(5′)—O(5′):ϕ_{OC}	−72.9	−70.6
O(1′)—C(1′)—C(2′)—C(3′)	−32.8	−22.9
C(1′)—C(2′)—C(3′)—C(4′)	38.4	35.3
C(2′)—C(3′)—C(4′)—O(1′)	−31.8	−36.0
C(3′)—C(4′)—O(1′)—C(1′)	11.5	22.7
C(4′)—O(1′)—C(1′)—C(2′)	13.8	0.3
N(1)—C(1′)—C(2′)—O(2′)	−37.4	−26.9

Effects of Base Protonation

In the case of **1**·HCl, protonation occurs at the ring N(3), as might have been anticipated from previous X-ray studies (35), as well as from nmr solution data (36). Values of bond lengths in this portion of the cytosine ring are closely similar to those found in other protonated 1-substituted cytosines (35, 37–40). Thus, relative to the parent **1**, bonds C(2)—O(2) and C(4)—N(4) are shorter, and C(2)—N(3) and N(3)—C(4) slightly longer. This is accompanied by a decrease in bond angle at N(3)—C(4)—C(5) and N(1)—C(2)—N(3) of about 4°, with a similar increase at C(2)—N(3)—C(4). All of these changes are compatible with delocalization of the positive charge between the ring N(3) and the exocyclic N(4) (38, 39), as also observed in solution by ^{15}N nmr spectroscopy (36). In the neutral form, **1**, the heterocyclic ring atoms are planar within the limits of error, whereas in **1**·HCl N(3) is significantly displaced (0.012 Å) towards the chloride ion to which it is hydrogen bonded. The deviations of most of the hydrogen bond donor/acceptor atoms in the cytosine ring are in a direction such that a closer approach between H-bonded atom pairs occurs.

Hydrogen Bonding

All the hydrogen bonding in both structures is intermolecular (Table 6). In contrast to O^2,2′-1-α-D-xylofuranosyluracil, where *intra*molecular hydrogen bonding is observed between O(3′) and O(5′), viz. O(3′)—H···O(5′), in the solid state (31), no such intramolecular bonds are present in either **1** or **1**·HCl, where both the *trans–gauche* orientation at C(4′)—C(5′) and the C(3′)*endo* sugar pucker preclude its formation on purely geometrical grounds. Furthermore, for 9-β-D-xylofuranosyladenine in solution, where side chain and sugar ring conformations are not subject to crystal packing forces, no such hydrogen bonding occurs to any appreciable extent, if at all, for the neutral and protonated forms, or even in strongly alkaline medium where the sugar hydroxyl(s) ionize (32).

Crystal Packing

The modes of lattice packing are illustrated in Fig. 3, in which the hydrogen bonding interactions are also indicated. For the protonated form there is no overlap of bases in the solid state, the translational symmetry elements (see Fig. 3) ensuring, in this instance, the absence of close approaches of base atoms to symmetry-related molecules.

The situation is quite different for the neutral form, with mean base plane separations of 3.37 and 3.36 Å, these distances being between planes symmetrically related by the two unique 2-fold axes at ($\frac{1}{2}$, 1) and ($\frac{1}{2}$, $\frac{1}{2}$), respectively, in the *ab* plane. The result is a base-stacking type of interaction (41) which is appreciable and occurs through the crystal lattice along a direction parallel to the *b*-axis. This is similar to the stacking pattern referred to as type III by Motherwell *et al.* (42), a pattern which is not the energetically preferred one and which is observed only infrequently. The perpendicularly projected overlap of bases amounts to approximately 30% in the case of the bases related by the ($\frac{1}{2}$, 1) 2-fold axes, and approximately 0% for the ($\frac{1}{2}$, $\frac{1}{2}$) related bases. Consequently, extended base stacking is not predominant in the solid state. The

TABLE 6. Hydrogen bond distances (in Å) and angles (in deg)

Compound	*D* *A*	*A* at	Distance H···*A*	Distance *D*···*A*	Angle *D*—H···*A*
1	O(2′)—H···N(3)	$1-x, 2-y, z$	1.79	2.747	169
	O(5′)—H···O(2)	$\frac{1}{2}-x, -\frac{1}{2}+y, 2-z$	1.92	2.667	177
	O(3′)—H···O(5′)	$\frac{1}{2}-x, \frac{1}{2}+y, 1-z$	1.75	2.771	176
	N(4)—H(1)···O(2)	$x, y, 1+z$	2.06	2.925	158
	N(4)—H(2)···O(5′)	$\frac{1}{2}+x, 1\frac{1}{2}-y, 2-z$	2.15	3.043	169
1·HCl	N(3)—H···Cl$^-$	x, y, z	2.25	3.079	158
	O(3′)—H···Cl$^-$	$-\frac{1}{2}+x, \frac{1}{2}-y, 1-z$	2.30	3.018	169
	O(2′)—H···O(3′)	$1-x, -\frac{1}{2}+y, \frac{1}{2}-z$	1.90	2.761	163
	O(5′)—H···O(2)	$x, y, -1+z$	1.96	2.748	165
	N(4)—H(1)···O(5′)	$\frac{1}{2}+x, \frac{1}{2}-y, \bar{z}$	1.93	2.856	160
	N(4)—H(2)···O(2′)	$1\frac{1}{2}-x, \bar{y}, \frac{1}{2}+z$	2.46	2.952	118

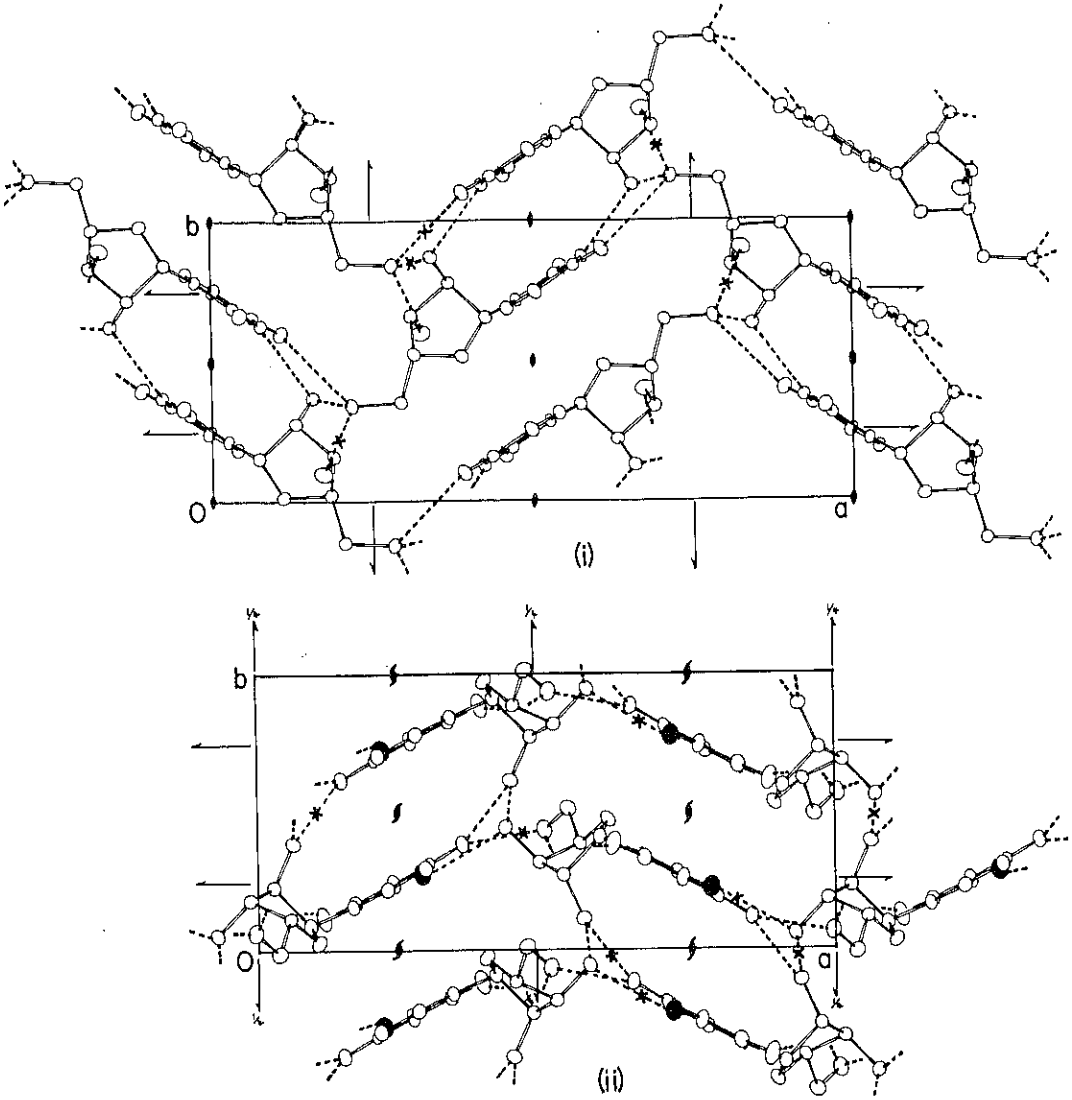

FIG. 3. Packing diagrams for (*i*) α-D-xylofuranosylcytosine (**1**) and (*ii*) α-D-xylofuranosylcytosine hydrochloride (**1**·HCl). In each case the view direction is parallel to the *c*-axis. Broken lines represent H-bond interactions with a cross to indicate that one of the molecules involved is related to that shown by a cell translation along *c*. In (*ii*), the chloride ion is shaded.

position of N(3), which is close to the centre of its "paired" base, when viewed in projection, is closely similar to the arrangement in the solid state structure of α-cytidine (1). Since protonation of N(3) usually precludes base stacking (38), this position may possibly be preferred for certain stacking modes (41), and is one which cannot be maintained on protonation, owing to the proximity of positive charges. It should, nonetheless, be noted that base stacking has been observed in the solid state structures of cytosine hydrochloride (38) and 1-methyl-N^4- hydroxycytosine hydrochloride (43).

Furthermore, in a study on the structure of the (1-methylcytosine)–(1-methylcytosine·H^+) complex, the two molecules were found to form asymmetrically hydrogen-bonded dimers, which were stacked in such a manner that molecular overlap was between charged and neutral bases (40). The mean stacking distance was 3.22 Å. The resulting base stacking mode, which is of obvious significance in relation to the solution conformation of protonated poly(rC), was considered to be favoured by overlapping of protonated with neutral species. It is, consequently, of interest to note that in the crystal structure of 1-methyl-N^4-hydroxycytosine·HCl, base overlapping is at least as extensive as in the foregoing complex, with an interplanar distance of about 3.25 Å (43); furthermore, the protonated N(3) atoms of the stacked molecules are even closer to each other in the latter structure than the protonated and non-protonated N(3) atoms in the (1-methylcytosine)–(1-methylcytosine·H^+) structure.

Biological Aspects

In the absence of data regarding antimetabolic activities of α-xylofuranosyl nucleosides, it is perhaps premature to discuss the conformational features of the present structures which may be of significance in various enzymatic systems. Model building studies do not reveal structural analogies between the α- and β-anomers of xylofuranosylcytosine as in the case of the anomers of 2′- deoxy-6-thioguanosine (6). However, some data of Bennett and Hill (44) are relevant to the foregoing. These authors examined the substrate properties towards adenosine kinase of a variety of anomeric nucleosides of adenine and 8-azaadenine in which the sugar moieties included arabinofuranose, xylofuranose, and lyxofuranose. Amongst these 9-β-D-xylofuranosyl-8-azaadenine and 9-α-L-lyxofuranosyladenine were good substrates. In general, it appeared that substrate activity required the presence of a 2′-hydroxyl *trans* to the aglycone, and a considerable degree of freedom about the glycosidic bond; with these conditions, it was irrelevant whether the 3′-hydroxyl or the exocyclic carbinol group are *cis* or *trans* relative to the aglycone. From these criteria it is obvious that the α-anomer of xylofuranosyladenine should not be a substrate, but this analogue was apparently not available. In view of the known importance of intracellular conversion of nucleoside analogues to their 5′-phosphates, which are the active analogues, it will obviously be of interest to examine the substrate properties towards pyrimidine nucleoside kinase(s) of the title compound of this study, the more so in that the α-anomers of a number of ribo-, deoxyribo-, and arabino-furanosyl nucleosides have been established as potent antimetabolites.

Acknowledgments

We are indebted to Dr. J. Giziewicz for synthesis of the title compounds. One of us (M.L.P.) is grateful to the National Research Council of Canada for the award of a Research Associateship (1975–1978) during the course of this investigation. The support of the Polish National Cancer Research Program (PR-6/1701, to D.S.) is also acknowledged.

1. M. L. Post, G. I. Birnbaum, C. P. Huber, and D. Shugar. Biochim. Biophys. Acta, **479**, 133 (1977).
2. A. Holy. Coll. Czech. Chem. Commun. **38**, 100 (1973).
3. T. Kulikowski and D. Shugar. J. Med. Chem. **17**, 269 (1974).
4. V. W. Armstrong, H. Sternbach, and F. Eckstein. Biochemistry, **15**, 2086 (1976).
5. A. J. Grant and L. M. Lerner. J. Med. Chem. **22**, 1016 (1979).
6. E. M. Acton, R. N. Goerner, H. S. Uh, K. J. Ryan, D. W. Henry, C. E. Cass, and G. A. LePage. J. Med. Chem. **22**, 518 (1979).
7. C. W. Smith, R. W. Sidwell, R. K. Robins, and R. L. Tolman. J. Med. Chem. **15**, 883 (1972).
8. D. Shugar. FEBS Lett. **40** (Suppl.), S48 (1974).
9. L. L. Bennett, W. M. Shannon, P. W. Allan, and G. Arnett. Ann. N.Y. Acad. Sci. **255**, 342 (1975).
10. L. L. Bennett, J. A. Montgomery, R. W. Brockman, and Y. F. Shealy. Adv. Enz. Regul. **16**, 255 (1978).
11. P. B. Sehgal and I. Tamm. Biochem. Pharmacol. **27**, 2475 (1978).
12. DCT Bulletin, Cancer Therapy Evaluation Program, N.C.I., No. 3 (1979).
13. A. Peery and G. A. LePage. Cancer Res. **29**, 617 (1969).
14. Y. Nakai and G. A. LePage. Cancer Res. **32**, 2445 (1972).
15. T. Tamaoki and G. A. LePage. Cancer Res. **35**, 1015 (1975).
16. M. Sundaralingam. Ann. N.Y. Acad. Sci. **255**, 3 (1975).
17. C. Altona and M. Sundaralingam. J. Am. Chem. Soc. **95**, 2333 (1973).
18. P. Roy-Burman. Recent Results Cancer Res. **25**, 1 (1970).
19. J. F. Henderson, C. M. Smith, F. F. Snyder, and G. Zombor. Ann. N.Y. Acad. Sci. **255**, 489 (1975).
20. G. A. LePage and S. Naik. Ann. N.Y. Acad. Sci. **255**, 481 (1975).
21. R. I. Glazer and A. L. Peale. Biochem. Biophys. Res. Commun. **81**, 521 (1978).
22. R. I. Glazer and A. L. Peale. Cancer Lett. **6**, 193 (1979).
23. J. A. Ibers and W. C. Hamilton (*Editors*). International tables for X-ray crystallography. Vol. IV. Kynoch Press, Birmingham. 1974.
24. R. F. Stewart, E. R. Davidson, and W. T. Simpson. J. Chem. Phys. **42**, 3175 (1965).
25. F. R. Ahmed, S. R. Hall, M. E. Pippy, and C. P. Huber. J. Appl. Crystallogr. **6**, 309 (1973).
26. C. K. Johnson. ORTEP Report ORNL-3794. 2nd revision. Oak Ridge, TN. 1970.
27. P. G. Lenhert. Proc. R. Soc. Ser. A, **303**, 45 (1968).
28. S. J. Cline and D. J. Hodgson. Biochim. Biophys. Acta, **563**, 540 (1979).
29. G. I. Birnbaum, E. Darzynkiewicz, and D. Shugar. J. Am. Chem. Soc. **97**, 5904 (1975).
30. M. Sundaralingam. J. Am. Chem. Soc. **93**, 6644 (1971).
31. G. I. Birnbaum, J. Giziewicz, C. P. Huber, and D. Shugar. J. Am. Chem. Soc. **98**, 4640 (1976).
32. I. Ekiel, E. Darzynkiewicz, L. Dudycz, and D. Shugar. Biochemistry, **17**, 1530 (1978).
33. I. Ekiel, E. Darzynkiewicz, G. I. Birnbaum, and D. Shugar. J. Am. Chem. Soc. **101**, 4724 (1979).
34. N. J. Oppenheimer. J. Carbohydr. Nucleosides Nucleotides, **5**, 251 (1978).
35. J. J. Guy, L. R. Nassimbeni, G. M. Sheldrick, and R. Taylor. Acta Crystallogr. Sect. B, **32**, 2909 (1976).
36. V. Markowski, G. R. Sullivan, and J. D. Roberts. J. Am. Chem. Soc. **99**, 714 (1977).
37. J. S. Sherfinski and R. E. Marsh. Acta Crystallogr. Sect. B, **29**, 192 (1973).
38. N. S. Mandel. Acta Crystallogr. Sect. B, **33**, 1079 (1977).
39. M. A. Viswamitra, B. S. Reddy, G. H.-Y. Lin, and M. Sundaralingam. J. Am. Chem. Soc. **93**, 4565 (1971).
40. T. J. Kistenmacher, M. Rossi, and L. Marzilli. Biopolymers, **17**, 2581 (1978).
41. C. E. Bugg, J. M. Thomas, M. Sundaralingam, and S. T. Rao. Biopolymers, **10**, 175 (1971).
42. W. D. S. Motherwell, L. Riva di Sanseverino, and O. Kennard. J. Mol. Biol. **80**, 405 (1973).
43. G. I. Birnbaum, T. Kulikowski, and D. Shugar. Can. J. Biochem. **57**, 308 (1979).
44. L. L. Bennett and D. L. Hill. Mol. Pharmacol. **11**, 803 (1975).