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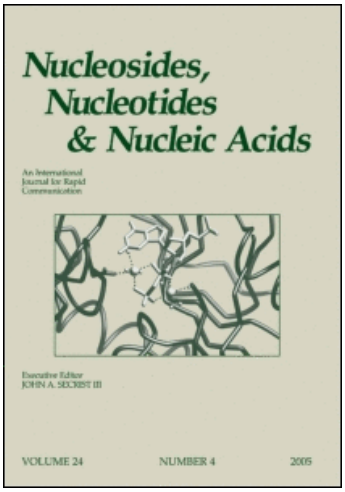

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The Structure of 5-Cyclohexyl-2'-Deoxyuridine as Studied by X-Ray Crystallography and NMR Spectroscopy

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THE STRUCTURE OF 5-CYCLOHEXYL-2'-DEOXYURIDINE AS STUDIED BY X-RAY CRYSTALLOGRAPHY AND NMR SPECTROSCOPY

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ABSTRACT: 5-Cyclohexyl-2'-deoxyuridine (I) is an example of a 5-substituted pyrimidine 2'-deoxynucleoside which exhibits no antiviral activity and which is not a substrate for either cellular or viral (herpes) kinases. Despite the fact that a cursory inspection of NMR spectra of the compound, taken in DMSO-d_6 solution, suggested that the compound had a normal conformation, we here show that in the crystal and in aqueous solution (analysed by 2D NMR techniques), the conformation of this nucleoside has a *syn*-glycosidic and C4'-exo ($_4$E) sugar pucker conformation.

INTRODUCTION

Many 5-substituted 2'-deoxyuridines, which act as analogues of thymidine, have been shown to exhibit antiviral properties, usually by inhibiting enzymatic functions or acting as substrates in different stages of viral nucleic acid synthesis.[1] A series of new 5-alkyl(cycloalkyl)-β-2'-deoxyuridines has been synthesized recently and tested for antiviral activity with the aim of rationalizing the structure-antiviral activity relationship, especially with regard to the size and character of the substituent in the position-5 of the pyrimidine ring [SCHEME 1].[2] Compounds II and III are known to exhibit antiviral properties and are also known to exhibit an *anti*-glycosidic conformation and C2'-endo (S) pucker of the sugar ring.

As a part of this series, 5-cyclohexyl-β-2'-deoxyuridine (I) was synthesized. This did not manifest any significant activity against a range of herpesviruses, influenza virus

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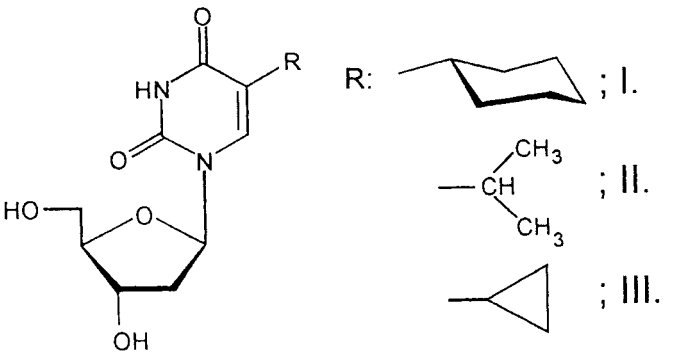

SCHEME 1.

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and HIV-1. The ^{1}H and ^{13}C NMR spectra of this compound obtained in DMSO-d_6 provided values for the chemical shifts which were very close to those of all the other members of the series, which would seem to indicate that within that series of 5-alkyl(cycloalkyl)-β–2′-deoxyuridines, all compounds have predominantly an *anti*-glycosidic conformation and C2′-endo (S) pucker of the sugar ring in DMSO-d_6 solution.[2] Any differences in antiviral activity within the series would therefore be attributed to the size and character of the substituent in position-5, rather than to differences in the overall shape of the molecule and/or the conformational character of the sugar moiety. Because the cyclohexyl group mimics both an extended (isopropyl, cyclopropyl) and a reduced structural pattern of some other substituents (adamantyl) within the series, the structure of 5-cyclohexyl-2′-deoxyuridine (I) was chosen for study in detail by X-ray crystallography and NMR spectroscopy. In this paper, we show that 5-cyclohexyl-2′-deoxyuridine has an unusual glycosidic and sugar conformation both in the crystal and in aqueous solution.

RESULTS AND DISCUSSION

Crystal Structure of Compound I

Selected crystal data and structure refinement parameters are given under EXPERIMENTAL. An ORTEP drawing[15] of the crystal structure of the title compound

I showing the numbering system used and a stereoscopic view of its packing arrangement are presented in FIGURES 1 and 2, respectively. Atomic coordinates and equivalent isotropic displacement parameters are given in TABLE 1.

Selected bond distances, angles, torsion angles and pseudorotation parameters are given in TABLE 2 and are compared with those of 5-isopropyl-2'-deoxyuridine (II)[3] and thymidine.[10] As can be seen from FIGURE 1 and TABLE 2, the title compound I has a *syn*-glycosidic conformation[4] with a torsion angle χ = 64.9°, compared to the *anti*-glycosidic conformation found in II and in thymidine. The 2'-deoxyribose moiety of the title compound I is found in an unusual C4'-exo ($_4$E) puckered conformation with a pseudorotation phase angle[5] P = 62.8° and maximum puckering amplitude ν_m = 31.7°.The same moiety in both compound II and thymidine is found in the C2'-endo (South-type, S) puckered conformation (P = 166.6° and 187.5°).[3,5,6]

The C4'-exo ($_4$E) sugar-ring pucker is further confirmed by the arrangement of the atoms O4'-C1'-C2'-C3', which are coplanar to within ±0.023Å (r.m.s deviation 0.018 Å) and C4' is displaced from the mean plane of these four atoms by 0.445Å. The conformation about the C4'-C5'(γ) bond is *trans*(t) with γ[O5'-C5'-C4'-C3'] = -174°, as distinct from the *gauche-gauche*(g_+) conformation in the isopropyl analogue II with γ[O5'-C5'-C4'-C3'] = 53.0°. The value of the torsion angle C8-C7-C5-C6 = 30.7° in the title compound I (TABLE 2) implies that the cyclohexyl group has an orientation towards the pyrimidine ring[7] just between synperiplanar and +synclinal, compared to the unequivocally synperiplanar conformation of the isopropyl group in compound II [C8-C7-C5-C6 = 12.8°]. The atoms of the pyrimidine ring [N1,C2,N3,C4,C5,C6] in I are coplanar to within ±0.009Å (r.m.s.deviation 0.007Å) and the anomeric carbon C1' is displaced from the mean plane of the atoms in pyrimidine ring by 0.105Å. In the crystal, the molecules form intermolecular hydrogen bonds, O3'-H.....O2, O5'-H.....O4, and N3-H.....O3'. The H.....O distances are, respectively, 1.95, 2.12, and 2.20Å, and the corresponding O/N-H.....O angles are 135, 141 and 178°.

As proved by the above data, the overall crystal structure of the title compound I is completely different from that of the 5-isopropyl-analogue II and thymidine. Hence, the question has to be answered, as to whether this is due to packing forces and steric

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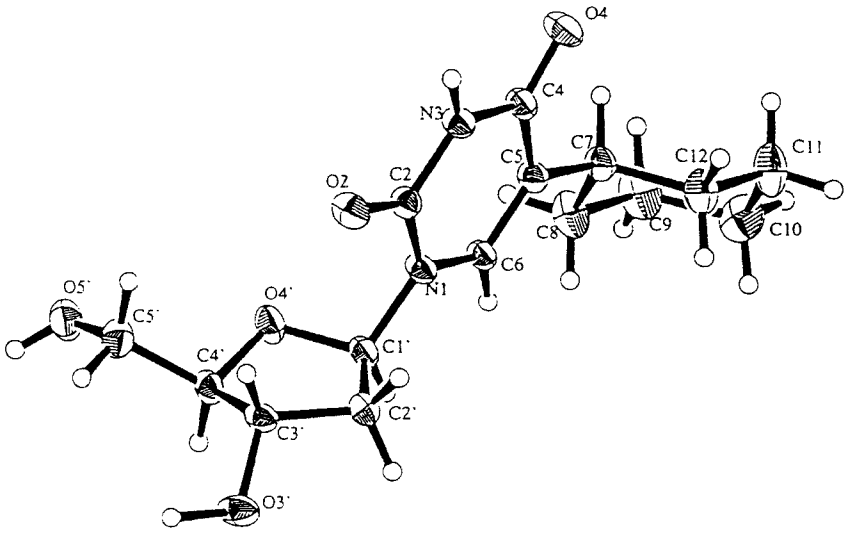

FIGURE 1. Perspective view of the molecule showing the atoms as 30 % probability elipsoids

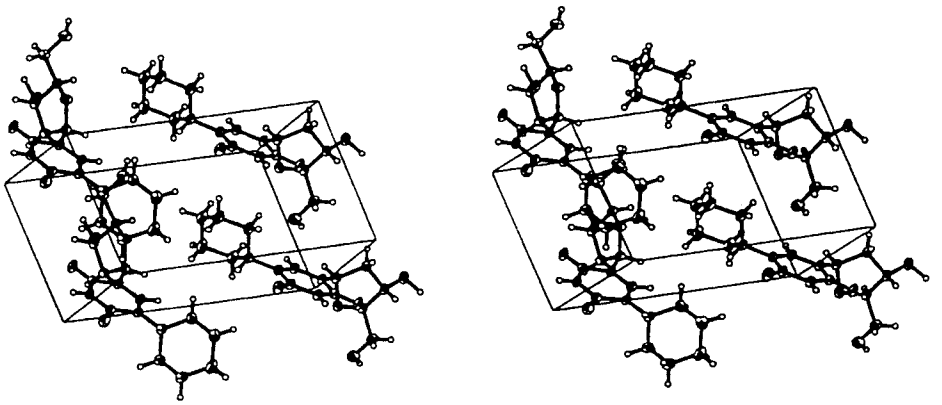

FIGURE 2. Stereoscopic view of the packing arrangement

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TABLE 1 Atomic coordinates (x 10^4) and equivalent isotropic displacement parameters ($Å^2$ x 10^3) for I. U(eq) is defined as one third of the trace of the orthogonalized Uij tensor.

	x	y	z	U(eq)
O(2)	3323(4)	3282(3)	703(2)	49(1)
O(4)	367(4)	7178(3)	2236(2)	53(1)
O(3')	3455(4)	-2202(3)	252(2)	53(1)
O(4')	3979(3)	701(3)	2299(2)	46(1)
O(5')	7939(4)	-460(4)	2875(2)	57(1)
N(1)	1493(4)	2524(3)	1909(2)	35(1)
N(3)	1803(4)	5188(4)	1489(2)	37(1)
C(2)	2282(4)	3625(4)	1322(2)	36(1)
C(4)	653(5)	5730(4)	2174(3)	37(1)
C(5)	-101(4)	4519(4)	2789(2)	35(1)
C(6)	361(5)	2976(4)	2617(3)	35(1)
C(7)	-1355(5)	4986(4)	3563(3)	40(1)
C(8)	-1208(6)	3849(6)	4509(3)	57(1)
C(9)	-2445(7)	4464(7)	5292(3)	68(1)
C(10)	-4479(7)	4689(7)	4761(4)	70(1)
C(11)	-4621(6)	5785(7)	3816(3)	67(1)
C(12)	-3410(5)	5165(6)	3041(3)	54(1)
C(1')	2013(4)	839(4)	1869(3)	36(1)
C(2')	1691(5)	70(4)	777(3)	39(1)
C(3')	3630(5)	-614(4)	670(3)	37(1)
C(4')	4762(4)	-585(4)	1794(2)	38(1)
C(5')	6875(5)	-314(5)	1843(3)	50(1)

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demands of the crystal lattice or whether it is energetically the most predominant structure which prevails in solution as well. Therefore, the title compound I was further studied in aqueous solution by means of ^{1}H NMR spectroscopy.

^{1}H NMR Study of Compound I

The proton chemical shift values and coupling constants of compound I obtained in D_2O are given in TABLES 3 and 4 and are compared with the values for 5-cyclopropyl-2'-deoxyuridine (III), which were obtained under the same conditions.[8]

A direct assignment of the multiplets due to the individual protons from their chemical shift values and their multiplicity is fairly straightforward except for those of protons H2'and H2''[8] The chemical shift values of the protons H2'', H3', H4', H5', H5''and H6 for the two compounds are the same within the range ±0.01ppm. However, the chemical shift values of the protons H1' and H2' for compound I are slightly higher than the

TABLE 2 Selected bond lengths, bond angles and torsion angles of the title compound I compared with those of compound II and thymidine

	I	II[3]	Thymidine[10]
	Bond Lengths (Å)		
C1'-C2'	1.531(5)	1.524(4)	1.517(7)
C2'-C3'	1.522(4)	1.502(6)	1.523(7)
C3'-C4'	1.532(5)	1.537(4)	1.529(7)
C1'-O4'	1.416(4)	1.420(3)	1.434(5)
C4'-O4'	1.421(4)	1.426(5)	1.460(5)
C1'-N1	1.461(4)	1.438(4)	1.480(6)
	Angles(°)		
C1'C2'C3'	104.5(3)	103.1(2)	102.7(4)
C2'C3'C4'	104.0(2)	102.9(3)	102.1(3)
C3'C4'O4'	105.3(2)	106.5(3)	104.4(3)
C4'O4'C1'	108.8(2)	110.6(2)	110.1(3)
O4'C1'C2'	107.7(2)	105.6(2)	106.5(3)
O4'C1'N1	107.7(3)	108.3(3)	108.2(4)
	Torsion angles(°)		
C4'O4'C1'C2' (ν_0)	-22.7(3)	-17.8	-7.0(3)
O4'C1'C2'C3' (ν_1)	3.9(4)	31.5	27.8(3)
C1'C2'C3'C4' (ν_2)	14.5(3)	-32.2	-36.9(4)
C2'C3'C4'O4' (ν_3)	-28.2(3)	22.6	33.2(4)
C3'C4'O4'C1' (ν_4)	32.1(3)	-3.0	-16.7(4)
C5'C4'C3'O3' (δ)	92.0(4)	145.3	-
O5'C5'C4'C3' (γ)	-173.6(3)	53.0	165.3(3)
O4'C1'N1C2 (χ)	64.9(4)	-	-139.4(3)
O4'C1'N1C6	-108.9(3)	64.4	-
C8C7C5C6	30.7(5)	12.8	-
	Pseudorotation parameters(°)		
Phase angle (P)	62.8	166.6	187.5
Puckering amplitude (ν_m)	31.5	33.1	37.8

TABLE 3. ^{1}H NMR chemical shift values in D_2O (δ, ppm).

Comp	H1′	H2′	H2″	H3'	H4′	H5′	H5″	H6
I	6.30	2.42	2.35	4.48	4.04	3.85	3.77	7.60
III[a]	6.27	2.38	2.35	4.47	4.02	3.85	3.78	7.61

[a]: Lit.[8]

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TABLE 4. ^{1}H NMR coupling constants in D_2O (*J*, Hz).

Comp	$J_{1',2'}$	$J_{1',2''}$	$J_{2',3'}$	$J_{2'',3'}$	$J_{3',4'}$	$J_{4',5'}$	$J_{4',5''}$	$J_{6,CH}$	$J_{2',2''}$	$J_{5',5''}$
I	6.5	6.5	6.5	4.9	4.3	3.4	4.3	0.9	-14.3	-12.5
III[a]	6.8	6.4	6.7	4.6	4.1	3.2	4.3	1.1	-14.5	-12.5

[a]: Lit.[8]

corresponding values for compound III (+0.03ppm for H1' and +0.04ppm for H2').The chemical shift values of the protons H2' and H2'' in III are very close to each other (2.38 and 2.35ppm) so that a very complex multiplet is created.

^{13}C NMR chemical shift values of the title compound I were assigned to the corresponding carbon atoms on the basis of a 2D inverse-detected CH correlation experiment. The assignment was consistent with the published ^{13}C NMR data on uracils[16] and 2'-deoxyuridines[17] and confirmed the identity and structure of I. The chemical shift values of I are given in TABLE 5 together with the values for compound II, which were recorded under the same conditions. The chemical shift values of the pyrimidine carbons in both I and II are the same within the range of ±0.3ppm. Small differences are attributed to the different electron-donor effect of the cyclohexyl-group in I, compared with that of the isopropyl-group in II. The chemical shift values of the sugar carbons in I and II are nearly identical (±0.1ppm) except for C2'. In the title compound I, this carbon is 0.2ppm to higher frequency compared with compound II. The shift to higher frequency of both C2' and H2' (see above) in I compared with II can be related to deshielding of these two atoms by the C2-carbonyl group as a result of the *syn*-glycosidic conformation in I.

One dimensional ^{1}H nOe difference spectroscopy was recently successfully used for a conformational analysis of the whole range of base- and/or sugar-modified nucleosides with respect to their *syn-anti* conformer equilibrium.[9] In both thymidine[9] and 4'-thio-thymidine[6] with a predominant *anti*-glycosidic conformation and C2'-endo (S) sugar pucker, a strong nOe contact was observed between H6 and H2' which was twice as intense as the nOe contact between H6 and H1', or that between H6 and H3'. A small, but significant nOe was also observed between H6 and H5',5'' in both cases. We

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TABLE 5. ^{13}C NMR chemical shift values in D_2O (δ, ppm).

Comp	C2	C4	C5	C6	C1′	C2′	C3′	C4′	C5′	CR5[a]
I	154.0	168.5	123.6	138.9	88.1	41.6	73.0	89.3	63.5	[b]
II	153.9	168.1	124.1	138.4	88.0	41.3	73.0	89.2	63.6	[b]

[a]: CR5 = R in SCHEME 1. [b]: See EXPERIMENTAL

have applied 2D NMR techniques for the same purpose. Phase-sensitive NOESY and ROESY experiments were performed on compound I and the observed interactions are summarized in TABLES 6 and 7 respectively.

The NOESY experiment revealed strong nOe interactions of a similar intensity between H6 and H1′ as well as between H6 and H2′,2″. No nOe interaction was observed between H6 and H3′ or between H6 and H5′,5″. The ROESY experiment also revealed strong rOe interactions of a similar intensity between H6 and H1′ as well as between H6 and H2′,2″. In addition, a less intense, but significant rOe interaction was observed between H6 and H3′, but again, no interaction was found between H6 and H5′,5″. There were no rOe interactions observed between protons of the cyclohexyl group and protons of the sugar moiety. The summarized results of the NOESY and ROESY experiments, when compared with the published data on thymidine[9] and 4′-thiothymidine,[6] clearly indicate a predominantly *syn*-glycosidic conformation in compound I in D_2O.

In conclusion, the overall conformation found in 5-cyclohexyl-β-2′-deoxyuridine (I) in the solid state was established to be a *syn*-glycosidic orientation with an C4′-exo ($_4E$) sugar-pucker. The cyclohexyl group has a synperiplanar/+synclinal orientation towards the plane of the pyrimidine ring. In aqueous solution, the same overall conformation of compound I seems to predominate as well as can be deduced from the NOESY and ROESY studies. This result is not apparent solely from an examination of the chemical shift and coupling constants data because these values are very similar for compound I (*syn*-glycosidic orientation) and compound III (*anti*-glycosidic orientation). Therefore, the lack of antiviral activity of compound I could well be attributed to both the unusual glycosidic and sugar conformation, as well as to the size of the cyclohexyl

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TABLE 6: NOESY spectrum of the compound I, (D_2O, +31°C): Summary of the interactions.

	H1′	H2′,2″	H3′	H4′	H5′,5″	H6
H6	+	+				
H1′		+				+
H4′					+	

+: indicates the observation of positive nOe cross-peaks between the appropriate nuclei

TABLE 7. ROESY spectrum of the compound I, (D_2O, +31°C): Summary of the interactions.

	H1′	H2′,2″	H3′	H4′	H5′,5″	H6
H6	+	+	+			
H1′		+		+		+
H2′,2″	+		+	+		+
H3′		+			+	+
H4′	+	+				+
H5′,5″			+			

+: indicates the observation of positive rOe cross-peaks between the appropriate nuclei

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group. This combination of factors is likely to prevent the compound being a substrate for either the cellular or herpesvirus-encoded thymidine kinase.[12]

EXPERIMENTAL

Crystal data: $C_{15}H_{22}N_2O_5$, M_r=310.35, monoclinic, space group $P2_1$, a=7.114(2), b=8.365(2), c=12.922(4)Å, β=100.10(2)°, U=757.1Å^3, Z=2, D_c=1.361gcm^{-3}, μ(MoKα)=0.102mm^{-1}.

Cell dimensions and intensity data were measured with MoKα from a crystal of size 0.18x0.18x0.07mm mounted on a Rigaku R-AXIS II area detector diffractometer. A total of 4908 reflections (θ_{max}=25°, I>σ(I)) were collected of which 2570 were independent (Friedel pairs not merged). The data were measured from one orientation of

the crystal, resulting in an 87% complete data set. There were no problems with overloaded spots. The structure was determined by direct methods[13] and refined[14] by least-squares using anisotropic thermal parameters for the non-hydrogen atoms.Although all hydrogen atoms could be located from a difference map, it was decided to place hydrogen atoms in calculated positions. Those linked to the oxygen atoms O3′ and O5′ were located from the difference map. Hydrogen atom coordinates were not refined. The final R value is 0.0552 and the residual electron density is within the range -0.24 to +0.31eÅ^{-3}.

NMR spectra were recorded on a Bruker AMX 400 spectrometer. This instrument operates at 400.13 MHz for ^{1}H and 100.62 MHz for ^{13}C. All 2D experiments were recorded with the sample non-spinning. NMR data processing was carried out on a Bruker ASPECTstation 1 off-line processing facility with standard XWINNMR software (version 1.0). The spectra were run in D_2O at +31°C and are referenced internally with the sodium salt of 3-(trimethylsilyl)-1-propane sulphonic acid (DSS). ^{13}C spectra were recorded using the PENDANT[19] sequence with ^{1}H-decoupling and using a value of 145 Hz for the $^{1}J_{CH}$ coupling constant. The chemical shift values of the carbons in cyclohexyl group (I, CR5) are 37.7 (CH), 34.6 and 34.5 (2xCH_2), 28.8 (2xCH_2), 28.3ppm (CH_2) and those of isopropyl group (II, CR5) are 27.9 (CH), 23.2 and 23.2ppm (2xCH_3).

The **NOESY** spectrum was performed in phase-sensitive mode using time proportional phase incrementation (TPPI). No special sample preparation was carried out and no attempt to quantify accurately the nOe size was made. A spectral width of 10.1 ppm was observed and 1K increments of 2K data points were acquired with 16 transients per increment. Solvent presaturation was carried out and a mixing time of 400 ms was used. The data was processed with a QSINE window function shifted by $\pi/2$.
The **ROESY** spectrum was performed and processed exactly as described above using a CW spin-lock field of 2 kHz and a spin-lock mixing time of 500 ms. To reduce the possibility of Homonuclear Hartman-Hahn (HOHAHA) crosspeaks, the transmitter was offset to the high frequency end of the spectrum.

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