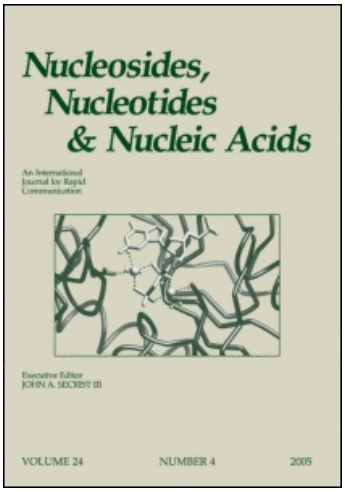

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Structure and Conformation of 5'-Chlorocyclocytidine, a Potent Inhibitor of Nucleic Acid Synthesis: X-Ray, ^{1}H and ^{13}C NMR Analyses

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STRUCTURE AND CONFORMATION OF 5'-CHLOROCYCLOCYTIDINE, A POTENT INHIBITOR OF NUCLEIC ACID SYNTHESIS: X-RAY, ^{1}H AND ^{13}C NMR ANALYSES[1]

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ABSTRACT: The structure of the hydrochloride of 5'-chlorocyclocytidine, a potent inhibitor of DNA synthesis, was determined by X-ray crystallography. The nucleoside crystallizes in the orthorhombic space group $P2_12_12_1$ with cell dimensions a = 10.413(4), b = 13.236(5), c = 17.064(6) Å and with two independent molecules in the asymmetric unit (Z = 8). Atomic parameters were refined by full-matrix least squares to a final value of R = 0.053 for 2490 observed reflections. In both molecules the furanose ring has a C4' *endo*/O4' *exo* ($^{4}T_{0}$) pucker. In molecule A the orientation of the -CH_2Cl side chain is *gauche*$^{-}$. In molecule B the side chain is disordered: in 70% of these molecules the orientation is *trans* and in 30% it is *gauche*$^{+}$. ^{1}H NMR spectra indicate a conformational equilibrium between C4' *exo*/O4' *endo* ($_{4}T^{0}$) and C4' *endo*/C3' *exo* ($^{4}_{3}T$) with a population ratio of 38:62. All three side chain rotamers occur in solution, the *trans* orientation contributing most. $^{1}J(C,H)$ values for C1' and C2' are significantly higher than normal and can therefore be used as a diagnostic tool for the assignment of bridgehead carbon atoms in cyclonucleosides.

INTRODUCTION

In order to study structure-activity relationships,[2-4] various derivatives of deoxynucleosides were synthesized. Of particular interest are those incorporating the structure of the cytostatic (cancerostatic) agent arabinosylcytosine.[5,6] From the 5'-deoxy-5'-chloronucleoside series X-ray, ^{1}H and ^{13}C NMR analyses were performed on 5'-chloroarabinosylcytosine (5'-Cl-ara-C)[7] and on the product of its conversion 2',5'-anhydroarabinosylcytosine.[8] Another interesting compound belonging to this nucleoside series is 5'-chlorocyclocytidine (5'-Cl-cC).[5] It is a prodrug of 5'-Cl-ara-C since the 2,2'-anhydro bond

of 5'-Cl-cC is cleaved even in *in vitro* conditions.[4] The product of this cleavage, 5'-Cl-ara-C[6] is a strong inhibitor of DNA synthesis,[2] and it is converted in biological conditions to 2',5'-anhydroarabinosylcytosine.[9] This route of transformation *in vivo* is seen as a reason for the very similar biological effects of 5'-Cl-cC when compared to 5'-arabinosylcytosine. The rate of penetration of 5'-Cl-cC through the intestinal wall is higher than that for cyclocytidine or arabinosylcytosine but it is comparable to measured rates for 5'-Cl-ara-C.[4] During the penetration 100% of 5'-Cl-cC is converted to 5'-Cl-ara-C.[4] Using thymidine and uridine incorporation to nucleic acids, it was found that 5'-Cl-ara-C is a good inhibitor of both DNA synthesis (ID_{50} = 5 μmol/l) and RNA synthesis (ID_{50} = 117 μmol/l). These properties are similar to those of cyclocytidine[10] which also shows inhibition of both DNA and RNA synthesis. In view of different physico-chemical properties, 5'-cyclocytidine may possess some advantages compared with arabinosylcytosine when used for therapy. Arabinosylnucleosides themselves do not inhibit RNA synthesis.[4,10,11] Immediate conversion of 5'-Cl-cC to 5'-Cl-ara-C is probably the reason for the similar activity of both compounds in the inhibition of L1210 cell growth.[11]

Because of the high activity of this compound in inhibition of nucleic acid synthesis and its good transport through intestinal membrane, 5'-Cl-cC belongs to a group of drugs which are candidates for formulation as a peroral drug form with cytotoxic agent. Preliminary results from pharmacokinetic analysis have agreed with this suggestion. Consequently, we considered it important to determine the conformation of 5'-Cl-cC in the solid state and in solution in order to gain more information about its structure.

EXPERIMENTAL

The hydrochloride of 5'-chlorocyclocytidine, $C_9H_{10}N_3O_3 \cdot HCl$, was synthesized as previously described.[12] Colorless crystals were obtained from a methanol-water (9:1) mixture. They belong to the orthorhombic space group $P2_12_12_1$ and have the following cell dimensions: a = 10.413(4), b = 13.236(5), c = 17.064(6) Å. Three-dimensional X-ray intensity data were measured on a CAD4 diffractometer with Mo Kα radiation. Of the 3025 unique reflections ($2\theta \leq 55^{\circ}$), 2494 (82.4%) had intensities $\geq 2.5\sigma(I)$ and were considered observed. The intensities

were corrected for Lorentz and polarization factors; absorption corrections were unnecessary (μ = 5.5 cm^{-1}).

There are two independent molecules in the asymmetric unit (Z = 8). The crystal structure was determined by direct methods.[13] Atomic parameters were refined by full-matrix least squares with anisotropic temperature factors for non-hydrogen atoms. A positional disorder was discovered in the -CH_2Cl side chain in molecule B. Therefore, coordinates and occupancy factors were refined for major and minor positions of C5'B and Cl5'B, respectively. Hydrogen atoms, except those attached to C5'B, were located on difference Fourier maps and refined with isotropic temperature factors. The refinement converged at R = 0.040 and R_w = 0.053 for 2490 observed reflections (four low-order reflections suffered from secondary extinction and were given zero weights). The coordinates and temperature parameters are listed in Table 1. A list of structure factors is available from GIB.

^1H and ^{13}C NMR spectra were measured on a Varian XL-200 FT-NMR spectrometer at 200 and 50.3 MHz, respectively. Solutions of ca. 5 mg of 5'-Cl-cC in 0.4 ml of D_2O and/or DMSO-d_6 were used for both ^1H and ^{13}C NMR spectra. DSS was used as an internal reference in D_2O solution. For referencing in DMSO-d_6 solution, TMS was used in ^1H NMR spectra and the solvent signal in ^{13}C NMR spectra (chemical shifts recalculated to a δ-scale using δ_C(DMSO-d_6) = 39.7 ppm). The proton chemical shifts and coupling constants were checked using a spectrum-simulation procedure (standard program SPIN on the Varian XL-200 instrument). Carbon-13 chemical shifts and the numbers of directly bonded protons were determined from the "attached proton test" ^{13}C NMR spectra.[14] Selective proton decoupled ^{13}C NMR spectra were used for the assignment of signals. Coupling constants J(C,H) were determined from a proton-coupled ^{13}C NMR spectrum. Carbon-13 relaxation times T_1 were measured, using the inversion recovery technique in D_2O. NMR parameters of 5'-Cl-cC are given in Table 2.

RESULTS AND DISCUSSION

X-Ray Analysis

Stereoscopic views of molecule A and of the two conformers of molecule B are shown in Fig. 1. In this crystal structure we observe the highly unusual occurrence of all three rotamers of the side chain

TABLE 1. Final atomic parameters and their standard deviations[a]

Atom	x	y	z	B_{eq}/B
Cl^- 1	0.24583(10)	0.04715(7)	0.48040(5)	2.97(3)
Cl^- 2	0.68049(10)	0.00978(7)	0.55069(6)	3.40(3)
N 1A	0.9858(3)	0.6422(2)	0.5959(2)	2.55(11)
C 2A	1.0909(4)	0.7002(3)	0.5908(2)	2.59(12)
O 2A	1.1942(3)	0.6538(2)	0.6167(2)	3.01(10)
N 3A	1.0950(3)	0.7933(3)	0.5651(2)	2.86(11)
C 4A	0.9806(4)	0.8346(3)	0.5474(2)	2.74(13)
N 4A	0.9792(4)	0.9297(3)	0.5237(2)	3.57(14)
C 5A	0.8621(4)	0.7785(3)	0.5536(3)	3.07(15)
C 6A	0.8680(4)	0.6822(3)	0.5767(3)	3.00(14)
C 1'A	1.0145(4)	0.5435(3)	0.6318(2)	2.79(13)
C 2'A	1.1573(4)	0.5577(3)	0.6515(2)	2.90(13)
C 3'A	1.1614(4)	0.5628(4)	0.7415(3)	3.31(15)
O 3'A	1.2171(5)	0.4731(3)	0.7687(2)	5.51(18)
C 4'A	1.0205(4)	0.5741(3)	0.7657(2)	3.25(16)
O 4'A	0.9477(3)	0.5301(2)	0.7022(2)	3.31(10)
C 5'A	0.9758(5)	0.6812(4)	0.7795(3)	3.85(17)
Cl 5'A	1.0356(2)	0.7233(1)	0.8722(1)	5.37(6)
N 1B	0.4091(3)	-0.1663(2)	0.8424(2)	2.34(10)
C 2B	0.5000(4)	-0.0973(3)	0.8552(2)	2.41(12)
O 2B	0.4771(3)	-0.0110(2)	0.8190(2)	3.03(10)
N 3B	0.6035(3)	-0.1095(2)	0.8972(2)	2.69(11)
C 4B	0.6190(4)	-0.2037(3)	0.9274(2)	2.47(12)
N 4B	0.7249(4)	-0.2193(3)	0.9686(2)	3.27(13)
C 5B	0.5261(4)	-0.2808(3)	0.9169(2)	2.67(13)
C 6B	0.4204(4)	-0.2604(3)	0.8743(2)	2.62(12)
C 1'B	0.3043(4)	-0.1250(3)	0.7939(2)	2.59(13)
C 2'B	0.3558(4)	-0.0178(3)	0.7779(2)	2.88(14)
C 3'B	0.3727(4)	-0.0148(4)	0.6888(2)	3.30(16)
O 3'B	0.2664(4)	0.0416(3)	0.6607(2)	4.28(14)
C 4'B	0.3664(5)	-0.1242(4)	0.6640(2)	3.44(16)
O 4'B	0.2906(3)	-0.1754(2)	0.7227(1)	3.16(10)
C 5'BM	0.5095(6)	-0.1597(5)	0.6629(4)	3.16(24)
C 5'Bm	0.4630(17)	-0.1964(14)	0.6330(10)	3.8(3)
Cl 5'BM	0.5144(2)	-0.2933(1)	0.6549(1)	4.85(9)
Cl 5'Bm	0.5922(4)	-0.2174(4)	0.7002(2)	4.30(17)
H N41A	1.057(5)	0.968(4)	0.519(3)	3.9(11)
H N42A	0.923(6)	0.965(4)	0.520(3)	3.3(11)
H 5A	0.786(6)	0.803(4)	0.545(3)	4.4(12)
H 6A	0.787(6)	0.651(4)	0.588(3)	4.2(12)
H 1'A	1.005(5)	0.486(4)	0.596(3)	3.4(10)
H 2'A	1.206(5)	0.507(4)	0.633(3)	3.7(11)
H 3'A	1.210(7)	0.602(5)	0.747(4)	5.4(16)
H O3'A	1.223(11)	0.496(8)	0.805(6)	12.3(30)
H 4'A	1.002(5)	0.538(4)	0.802(3)	3.7(11)
H 5'A	0.996(5)	0.742(4)	0.756(3)	2.8(9)
H 5"A	0.878(8)	0.665(6)	0.776(4)	6.9(17)
H N41B	0.738(5)	-0.283(4)	0.986(3)	3.4(10)
H N42B	0.770(5)	-0.166(4)	0.977(3)	2.9(9)
H 5B	0.531(5)	-0.352(4)	0.944(3)	3.9(10)
H 6B	0.358(4)	-0.309(3)	0.860(3)	2.9(9)
H 1'B	0.237(5)	-0.125(3)	0.818(3)	2.6(9)
H 2'B	0.299(8)	0.035(6)	0.795(4)	7.4(18)
H 3'B	0.438(6)	0.018(5)	0.667(4)	4.7(13)
H O3'B	0.259(7)	0.034(5)	0.610(4)	6.4(16)
H 4'B	0.302(5)	-0.140(3)	0.619(3)	2.9(9)
H 5'BM	0.548(8)	-0.134(6)	0.607(6)	6.2(20)
H 5"BM	0.554(8)	-0.145(6)	0.706(5)	5.8(17)

[a] M and m refer to the major and minor conformers, respectively, of the side chain in molecule B.

TABLE 2. Proton and carbon-13 NMR data of 5'-Cl-cC

Chemical shifts (ppm)			Coupling constants (Hz)[a]		
Proton	DMSO-d_6	D_2O	H,H	DMSO-d_6	D_2O
H5	6.63	6.64	5,6	7.4	7.4
H6	8.36	8.17			
N(4)H_2	9.57;9.40	--			
H1'	6.56	6.67	1',2'	6.0	6.0
H2'	5.49	5.66	2',3'	1.5	1.4
H3'	4.44	4.79	3',4'	3.5	3.0
H4'	4.38	4.61	4',5'	4.6	5.1
H5'	3.71	3.71	5',5"	12.2	12.4
H5"	3.59	3.61			
OH	6.36	--	3',OH	4.5	--

Chemical shifts (ppm)				Coupling constants (Hz) in D_2O[a,b]			
Carbon	DMSO-d_6	D_2O	NT_1 (s)	^1J(C,H)		^2J and ^3J(C,H)	
C2	159.64	156.21	[c]		--		
C4	172.17	170.28	[c]		--	C4,H6	8.7
C5	102.55	105.96	1.02	C5,H5	178.6	C5,H6	3.6
C6	140.92	142.97	1.26	C6,H6	194.3	C6,H5	3.9
C1'	90.74	93.88	1.32	C1',H1'	189.7		
C2'	90.74	93.44	1.31	C2',H2'	169.7		
C3'	87.31	90.44	1.49	C3',H3'	153.3		
C4'	75.80	78.50	1.14	C4',H4'	156.2		
C5'	44.53	46.26	1.62	C5',H5'	152.7	C5',H3'	4.1
				C5',H5"	152.7		

[a]Absolute values of J are given.
[b]Only some of long-range J(C,H) could be determined.
[c]The high T_1 values of quarternary carbons were not determined.

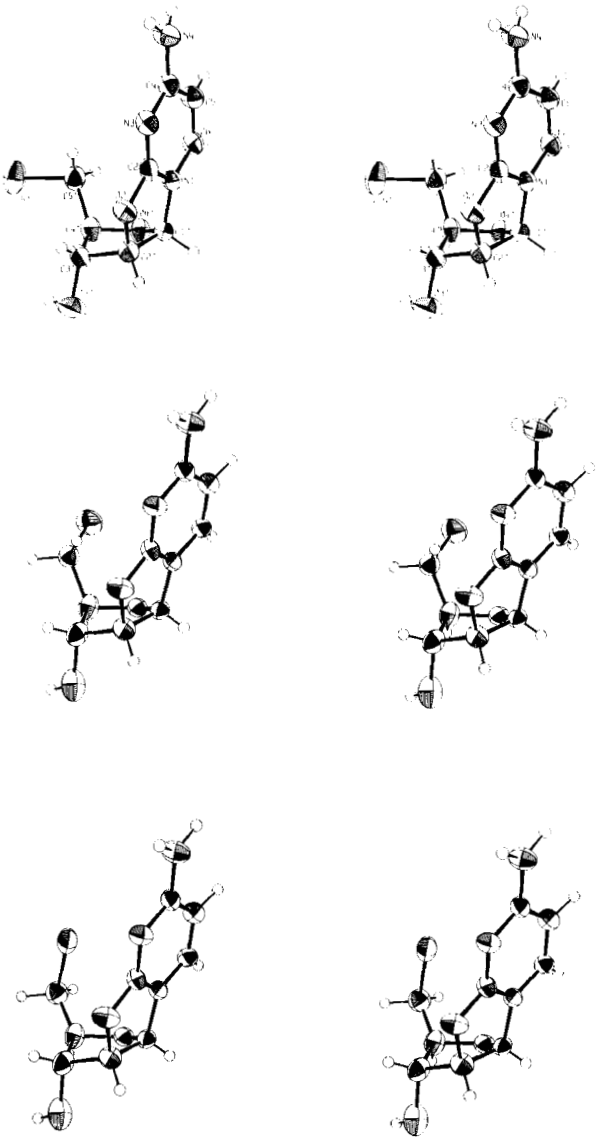


FIG. 1. Stereoscopic views of molecule A (top), molecule B, major conformer (center), and molecule B, minor conformer (bottom).

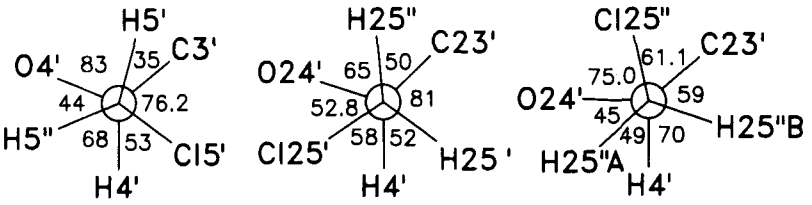


FIG. 2. Newman projections along C4'-C5': molecule A (left), molecule B, major conformer (center), and molecule B, minor conformer (right).

(Fig. 2). In molecule A the $-CH_2Cl$ side chain is in the relatively rare *gauche*$^-$ conformation. As mentioned above, the side chain in molecule B is disordered. In 70% of these molecules $-CH_2Cl$ is in the *trans* conformation while in 30% it is *gauche*$^+$. Normally, both conformers are favored by the *gauche* effect,[15] but in this structure the latter may be further stabilized as a result of an attraction between Cl and the positively charged pyrimidine ring. The intramolecular distances N1B···Cl5'Bm (3.159 Å) and C2B···Cl5'Bm (3.233 Å) are ~0.3 Å shorter than the sum of van der Waals radii. The rotamer population in the crystal structure is in agreement with that found in solution (see below).

The puckers of the furanose rings are very similar in both molecules (Fig. 3). In both molecules the ring has a C4' *endo*/O4' *exo* ($^4T_0$) pucker with a pseudorotation angle[16] of 247.1° in molecule A and 238.0° in molecule B. In the minor conformer of molecule B, the ring is somewhat more puckered at C4', giving rise to a major (70%) and a minor (30%) site for C5' (C5'BM and C5'Bm, respectively), but it was not possible to resolve two sites for C4'. In both molecules the furanose rings are flatter than normal, the maximum degree of pucker being 30.3° in molecule A and 28.5° in molecule B rather than the usual 37°. The type of pucker along the pseudorotation pathway is correlated with the shape of the 2,2'-anhydro (oxazolidine) ring. In molecule A this ring deviates significantly from planarity (χ^2 = 278.8°), the maximum deviation, for C2', being 0.057 Å. In molecule B the ring is much flatter (χ^2 = 24.5°) and the maximum deviation from the plane is 0.014 Å for C2. The flatter the anhydro ring, the more likely that the

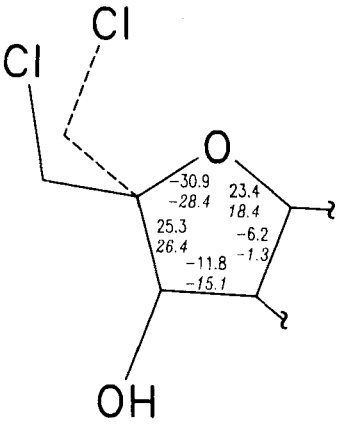


FIG. 3. Endocyclic torsion angles in the sugar ring; the lower values in italics refer to molecule B.

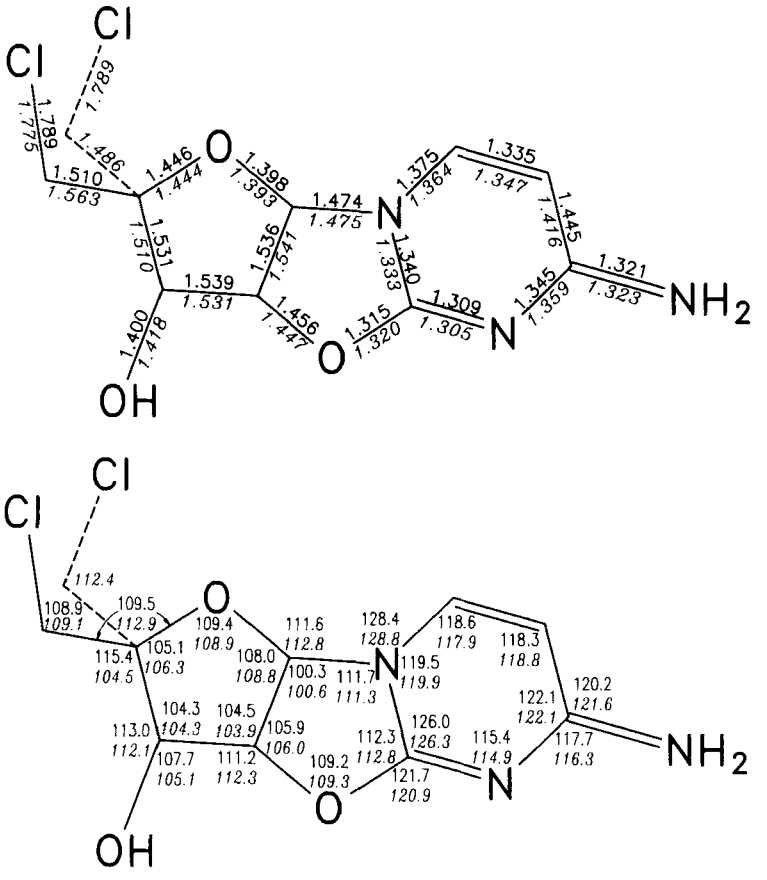
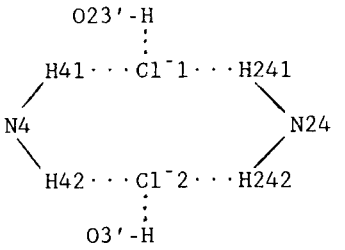


FIG. 4. Bond lengths (top) and bond angles (bottom); the lower values in italics refer to molecule B and the values along dashed lines to the minor conformer of molecule B.

furanose ring atom opposite the common C1'-C2' bond, i.e. C4', will be puckered. This observation is borne out by results obtained from X-ray analyses of other 2,2'-anhydro nucleosides.[17-24] As we pointed out previously,[20] the preferred conformation is C4' *endo* in β-anhydro-nucleosides and C4' *exo* in α-anhydronucleosides.

The glycosidic torsion angles (C6-N1-C1'-O4') in molecules A and B are 305.1 and 296.7°, respectively. Values of 300 ± 10° have been observed in analogous molecules.[17,18,21-24] The formation of the oxazolidine ring changes the geometry of the pyrimidine moiety (Fig. 4). In particular, N1-C2 and C2-N3 are shorter (by 0.05 and 0.08 Å, respectively) than the corresponding bonds in protonated cytosines,[25] while C2-O2 is 0.11 Å longer. Furthermore, the bond angle N1-C2-N3 is 11° larger, while N1-C2-O2 and C2-N3-C4 are each 10° smaller. These changes reflect the fact that the cytosine moiety is protonated at N4 rather than at N3 and that the double bond system is cross-conjugated. The pyrimidine ring in molecule A is somewhat less planar (χ^2 = 55.5) than in molecule B (χ^2 = 33.2).

All available protons participate in a hydrogen bonding scheme which can be represented schematically as follows:



The distances and angles are given in Table 3; the corrected H···*A* distances were obtained after normalizing the O-H and N-H bond lengths to their nominal values of 0.97 and 1.04 Å, respectively. As can be seen, the N-H···Cl⁻ angles deviate more from linearity in the case of Cl⁻2. Consequently, the hydrogen bonds to that ion are weaker and the thermal motion of Cl⁻2 is higher than that of Cl⁻1. However, all four N-H···Cl⁻ bonds are stronger than the N-H···Cl5' bond in 5'-Cl-ara-C,[7] reflecting the fact that Cl⁻ is a better proton acceptor than covalently

TABLE 3. Distances and angles for hydrogen bonds

			Distances (Å)		Angles (deg)
D	*A*	at	*D*···*A*	*H*···*A* corr	*D*-H···*A*
N4A-H41···Cl⁻1		1+*x*,1+*y*,*z*	3.267(4)	2.25	167(4)
N4A-H42···Cl⁻2		*x*,1+*y*,*z*	3.318(4)	2.40	150(4)
N4B-H41···Cl⁻1		1-*x*,-1/2+*y*,3/2-*z*	3.226(4)	2.19	173(4)
N4B-H42···Cl⁻2		3/2-*x*,$\bar{y}$,1/2+*z*	3.260(4)	2.31	153(4)
O3'A-H···Cl⁻2		2-*x*,1/2+*y*,3/2-*z*	3.298(5)	2.42	153(9)
O3'B-H···Cl⁻1		*x*,*y*,*z*	3.085(3)	2.13	169(6)

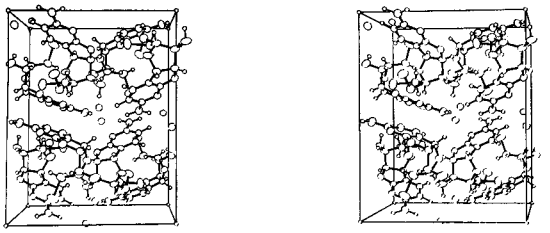


FIG. 5. Stereoscopic view of the molecular packing in the unit cell. The directions of the axes are *x* up, *y* →, *z* ↑.

bonded Cl. These hydrogen bonds hold the molecules together in the crystal, as shown in the packing diagram (Fig. 5). There are no stacking interactions between the pyrimidine bases.

Solution Conformation

^{1}H NMR spectra

^{1}H NMR spectra were studied in order to compare the conformation in solution with that determined by the X-ray analysis. From the inspection of molecular models it is possible to expect some limitation of the flexibility of the pentose ring due to the 2,2'-anhydro bond in 5'-Cl-cC. Information about the geometry of the pentose ring in

solution can be obtained from interproton coupling constants $J(1',2')$, $J(2',3')$ and $J(3',4')$. The use of the commonly accepted two-state model[26] (based on the generalized Karplus relation derived by Haasnoot *et al.*[27]) with a maximum pucker value $\tau_{max} = 30^{\circ}$ (found in the crystal) gave an excellent agreement (rms = 0.00 Hz !) between calculated and observed J-values (Table 4) for similar conformation equilibria in two solvents: in DMSO-d_6 ($P(N) = 67^{\circ}$, $P(S) = 219^{\circ}$, x(N):x(S) = 38:62) and in D_2O ($P(N) = 63^{\circ}$, $P(S) = 217^{\circ}$, x(N):x(S) = 31:69). The major conformer with $P \approx 218^{\circ}$ is quite similar to each of the two forms (molecules A: $P = 247^{\circ}$, B: $P = 238^{\circ}$) found in the crystal. Both conformers present in solution (C4' *exo*/O4' *endo*, ${}_4T^0$, and C4' *endo*/C3' *exo*, ${}^4_3T$) are, of course, rather different from the conformation equilibrium C3' *endo* ↔ C2' *endo* in many arabinose nucleosides described in the literature.[26] This is not surprising when we take into account the effect of the 2,2'-anhydro bond in 5'-Cl-cC.

The conformation about the C4'-C5' bond in nucleosides can be interpreted in terms of rapid rotational averaging among three staggered rotamers - *gauche*$^+$ (g^+), *trans* (t), and *gauche*$^-$ (g^-).[28] The rotamer populations can be calculated[29] from the observed $J(4',5')$ and $J(4',5'')$ and the values for $J(trans)$ and $J(gauche)$, respectively. In the case of 5'-Cl-cC, the $J(trans)$ and $J(gauche)$ values 11.5 and 1.5 Hz, generally used for 5'-OH nucleosides, were corrected for different substituent electronegativity (as described in our previous paper),[7] giving $J(trans) = 11.9$ and $J(cis) = 1.55$ Hz. The calculated rotamer populations are given in Table 5. The values for the *gauche*$^-$ and *trans* rotamers were obtained by assigning the H5' and H5" protons according to the published convention.[30] Rotamer populations found in DMSO-d_6 and D_2O are very similar, with a small increase of g^- at the expense of t when going from DMSO-d_6 to D_2O solution. A comparison with ara-C and 5'-Cl-ara-C shows a continuing increase of the g^- rotamer and decrease of the g^+ form in 5'-Cl-cC (see Table 5).

The vicinal coupling of the C3'-OH proton ($J(H3',OH) = 4.5$ Hz) indicates free rotation about the C3'-O3' bond without a distinct preferred orientation of the OH group. This is in agreement with the fact that this group cannot participate in intramolecular H-bonding.

TABLE 4. Comparison of experimental values of interproton coupling constants J (in Hz) with those calculated from X-ray data in the crystal and from a "two-state" conformation model in solution. Conformations of the furanose ring are described by phase angles P (deg), maximum pucker τ_{max} (deg) and populations x (%).

	Experimental		Calculated					
			Crystal[a]		Solution			
	DMSO	D_2O	A	B	DMSO		D_2O	
$J(1',2')$	6.0	6.0	7.15	7.02	6.00		6.00	
$J(2',3')$	1.5	1.4	0.44	0.54	1.50		1.40	
$J(3',4')$	3.5	3.0	1.94	1.02	3.50		3.00	
Conformation parameters:								
P			247	238	219	67	217	63
τ_{max}			30.3	28.5	30	30	30	30
x			100	100	62	38	69	31

[a]Calculated values for molecules A and B.

^{13}C NMR spectra

The proton decoupled "attached proton test" ^{13}C NMR spectrum in D_2O afforded nine signals corresponding to nine carbon atoms present, while in DMSO-d_6 the signals of two carbon atoms coincide at δ 90.74. The signal assignment of cytosine and C5' carbon atoms can be easily made on the basis of directly bonded protons, chemical shift arguments and comparison with ^{13}C NMR data of cytosine nucleosides. For pentose ring carbon atoms the assignment is not straightforward and literature data on cyclonucleosides are very scarce. Therefore, we used proton-coupled ^{13}C NMR spectra and selective proton decoupling experiments for

TABLE 5. Rotamer populations about the C4'-C5' bond in 5'-Cl-cC, 5'-Cl-ara-C and ara-C

Compound	Solvent	Exocyclic 5'-CH_2-X population		
		g^+	g^-	t
5'-Cl-cC	DMSO-d_6	0.28	0.29	0.43
	D_2O	0.27	0.34	0.39
5'-Cl-ara-C[a]	D_2O	0.31	0.27	0.42
ara-C[b]	D_2O	0.37	0.19	0.44

[a]Data taken from ref. 7.

[b]Data taken from refs. 28 and 31.

correct assignment of these carbon atoms and for determination of J(C,H) values (see Table 2).

Pentose carbon atoms C1' and C2' belonging to two fused five-membered rings in 5'-Cl-cC showed significantly increased values of ^1J(C,H) (189.7 and 169.7 Hz) in comparison with "normal" 5'-Cl-ara-C (167 and 154 Hz; ref. 7). We described [7,8] a similar increase of ^1J(C,H) for analogous bridgehead carbon atoms C2' and C4' in 2',5'-anhydroarabinosylcytosine (168.5 and 167.2 Hz in comparison with 151 and 154 Hz in 5'-Cl-ara-C). It seems that these higher values of ^1J(C,H) can be used as a diagnostic tool for the assignment of bridgehead carbon atoms of cyclonucleosides. The only well-resolved long-range J(C,H) in the pentose ring was the vicinal coupling J(C5',H3') = 4.1 Hz, in agreement with a *synperiplanar* arrangement of C5' and H3' atoms (the torsion angle is close to 0^o in a nearly planar form of the pentose ring).

Relaxation times T_1 of proton-bearing carbon atoms are given in Table 2. It is well known that the different NT_1 values of such atoms can reflect the internal or segmental motion in certain parts of a molecule (see e.g. ref. 32). In the case of 5'-Cl-cC, the NT_1 values are not significantly different. Nevertheless, the highest NT_1 value

(1.62 s) was observed for C5' which has undoubtedly the highest mobility due to rotation about the C4'-C5' bond. Other pentose ring atoms have NT_1 values (1.14 to 1.49 s) generally slightly higher than those of the carbon atoms in the cytosine ring (1.02 and 1.26 s). This agrees with partial mobility of the pentose ring as opposed to the cytosine moiety which cannot have any segmental motion.

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