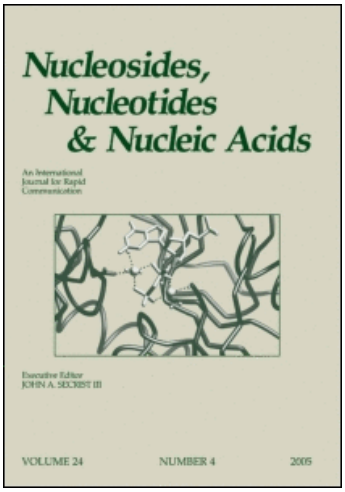

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Structure and Conformation of 1-β-D-Ribofuranosylpyridin-4-One-3-Carboxamide, A Novel Nucleoside from Human Urine with a Rare Ribose Pucker

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STRUCTURE AND CONFORMATION OF 1-β-D-RIBOFURANOSYLPYRIDIN-4-ONE-3-CARBOXAMIDE, A NOVEL NUCLEOSIDE FROM HUMAN URINE WITH A RARE RIBOSE PUCKER

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Abstract

The pyridine nucleoside, 1-β-D-ribofuranosylpyridin-4-one-3-carboxamide (PCR) is one of several novel nucleosides isolated in our laboratory from the urine of chronic myelogenous leukemia patients. Its crystal structure and conformation were studied to complete its characterization. This nucleoside exhibits an anti ($^{X}\chi_{CN}$ = 66.9°) conformation across the glycosidic bond, a rare pucker of the ribose ring, C(4')exo-C(3')endo ($_4T^3$), and g^+ across the C(4')-C(5') bond. The amino group of the carboxamide is proximal to and hydrogen bonded intramolecularly to O(4). Nuclear magnetic resonance studies show that the intramolecular hydrogen bond is present in the solution state also, but the solution conformation of the furanose ring is not the same as that observed in the solid.

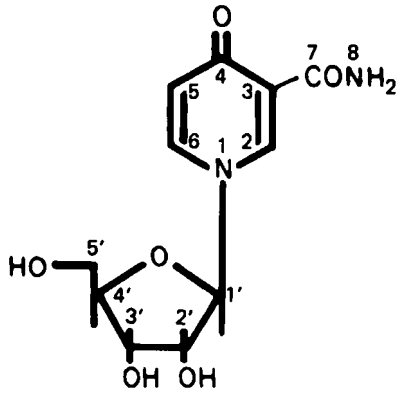
As a part of the program to investigate urines of cancer patients for the presence of novel substances derived from nucleic acid anabolic and catabolic processes, we have reported a number of urinary nucleosides and bases[1-3]. One of these unusual nucleosides isolated from the urine of patients with chronic myelogenous leukemia was assigned the structure 1-β-D-ribofuranosylpyridin-4-one-3-carboxamide (I) (PCR)[4]. The occurrence of this compound has not been reported previously from any biological source. Even though the origin of this nucleoside and the previously reported methylated base 1-methylpyri-

Abbreviations used are:

t^6A: 9-β-D-ribofuranosyl-N[purin-6-yl)-carbamoyl]-l-threonine.

g^6A: 9-β-D-ribofuranosyl-N[purin-6-yl)-carbamoyl]- glycine.

ac^4C: N^4-Acetylcytidine.



I

din-4-one-3-carboxamide[5] has not been fully established, it has been suggested that these compounds are derived either from the ubiquitous cofactor nicotinamide adenine dinucleotide (NAD^+) or from the base nicotinamide[6]. NAD^+ plays a key role in most biological oxidation and reduction reactions. It appears that the nucleoside (I) may arise from NAD^+ after oxidation at the 4-position of its pyridine moiety followed by hydrolysis and dephosphorylation. This nucleoside (I) may also result from the reaction between nicotinamide and ribose-1-phosphate followed by oxidation at the 4-position.

Aside from the potential of this nucleoside as a possible indicator of tumor burden in malignancy, the significance of this compound lies in the fact that it is a novel metabolic product in human biochemical pathways. Chemically this nucleoside has a quinonoid structure which can act as an electrophile and interact with DNA in a manner similar to the quinones[7,8]. It can also participate in the formation of the superoxide radical[9].

The chemical structure of this nucleoside suggested a potential interaction between 4-keto oxygen and 3-carboxamido group. The conformation of the sugar moiety and the proposed β-configuration of the anomeric carbon[4] needed to be confirmed. A preliminary nmr study indicated some unusual long range couplings: the proton at the C-6-

position of the pyridine moiety of (I) is coupled to both the C_5 and C_2 protons (J6,2 = 3 Hz; J6,5 = 8 Hz). Because of these interesting chemical features and because of its biochemical and mutagenic significance, X-ray crystallographic and nuclear magnetic resonance studies were undertaken on this compound.

X-ray Crystallography: The material (I) was prepared as described earlier[4], and crystallized from 95% ethanol to give transparent needles, M.P. 212-213° . Crystals of this nucleoside ($C_{11}H_{14}N_2O_6$) are orthorhombic, space group $P2_12_12_1$ with unit cell dimensions (at 22± 3°C) a = 8.689(1), b = 18.839(3), c = 6.971(1)Å, Z = 4, V = 1141Å^3, μ = 11.2 cm^{-1}, D_m = 1.57 g.cm^{-3} (flotation in a mixture of bromoform and benzene), D_c = 1.567 g.cm-3, F(000) = 564.

Complete three-dimensional intensity data were collected using a crystal of dimensions 0.35 x 0.35 x 0.1 mm on a CAD-4 diffractometer by the $\omega/2\theta$ technique. The intensities of 2118 reflections (1908 with their $I>3\sigma$) to the limit $2\theta<150°$ for CuKα (λ = 1.5418A) were measured. Scan widths were calculated according to the relation (A+B tanθ) with values of 0.5 and 0.15° for A and B respectively. Aperture widths were calculated using the relation (3.0 + 1.2 tan θ) mm where θ is the Bragg angle. The maximum time spent on any reflection measured was 100 seconds and the background count time was half the scan time. A faster scan was used for strong reflections. The intensities of three reflections were monitored after every hour of X-ray exposure and the variation in intensities was less than 3% during the time of complete data collection. The orientation matrix was checked every 100 reflections. Lorentz and polarization corrections were applied to all reflections. The intensities of three reflections close to χ of 90° were measured for all values of ϕ from 0 to 360° and the resultant curve of transmission was used to correct for absorption effects. The minimum and maximum transmission factors were 0.79 and 0.99 respectively with an average value of 0.92.

The structure was solved by application of the multisolution technique[10] and was refined by a full-maxtrix-least-squares procedure initially with isotropic thermal parameters and later with anisotropic thermal parameters for the non-hydrogen atoms. A difference electron density map was used to locate the positions of all hydrogen

atoms in the molecule. The structure was further refined with isotropic temperature factors for the hydrogen atoms and anisotropic factors for the other atoms. The function minimized was $w(|Fo|-(1/k)|Fc|)^2$ in which the weight $w = 4|Fo|^2/\sigma(|Fo|^2)^2$ and $\sigma(|Fo|)^2 = [\sigma^2(I) + p^2I^2]^{1/2}/LP$, where $\sigma(I)$ is the standard deviation of intensity I based upon counting statistics; k is the scale factor and p is an ignorance factor used to down weight intense inflections (p = 0.05). The final R factor was 0.038 for the significant 1908 reflections ($I \geq 3\sigma$). The calculations were done on PDP 11/34 computer with the aid of the Enraf Nonius structure determination package[11]. Atomic scattering factors were taken from the "International Tables for X-ray Crystallography"[12], Fourier and torsion-angle programs by Dr. S.T. Rao and ORTEP by Johnson (1965)[13].

The structure of the title compound is shown in Fig. 1. The final fractional atomic coordinates are given in Table 1 and the bond distances and angles together with their standard deviations are given in Table 2.

The amino group of the carboxamide is proximal to and hydrogen bonded to O(4) internally (H...O, 1.90(3)Å, N-H...O, 149°). O(4) is also hydrogen bonded intermolecularly (Fig. 2) to O(2') (H...O, 1.86(3)Å, O(2')-H...O(4), 168°). Using the nucleic acid nomenclature of Sundarlingam[14], this nucleoside has the anti conformation with χ_{CN} (C(6)-N(1)-C(1')-O(1') equal to 66.9°. The conformation across C(4')-C(5') is g^+. When C(4') is out of the mean plane of the other atoms, the usual C(4') endo/exo notation leads to an inconsistent description of the pucker and the pseudorotation path due to the large movement of reference atom C(5') to near equatorial disposition and the pucker description turns out to be C(4')endo-C(3')endo. On the other hand, phase value of 41.2° corresponds to C(4')-exo-C(3')endo from the pseudo rotation circle. Consistency in nomenclature can be achieved by using N(1) rather than C(5') as a reference atom for C(4') endo/exo puckers only[15]. Referring to the twist conformation with respect to the three atom plane through C(1'), C(2') and O(1') the ribose has a rare pucker, C(4')exo-C(3')-endo ($_4T^3$), with C(4') and C(3') deviating, respectively, from the plane of the other three atoms by 0.36Å in the direction opposite to and 0.26Å in the same direction of

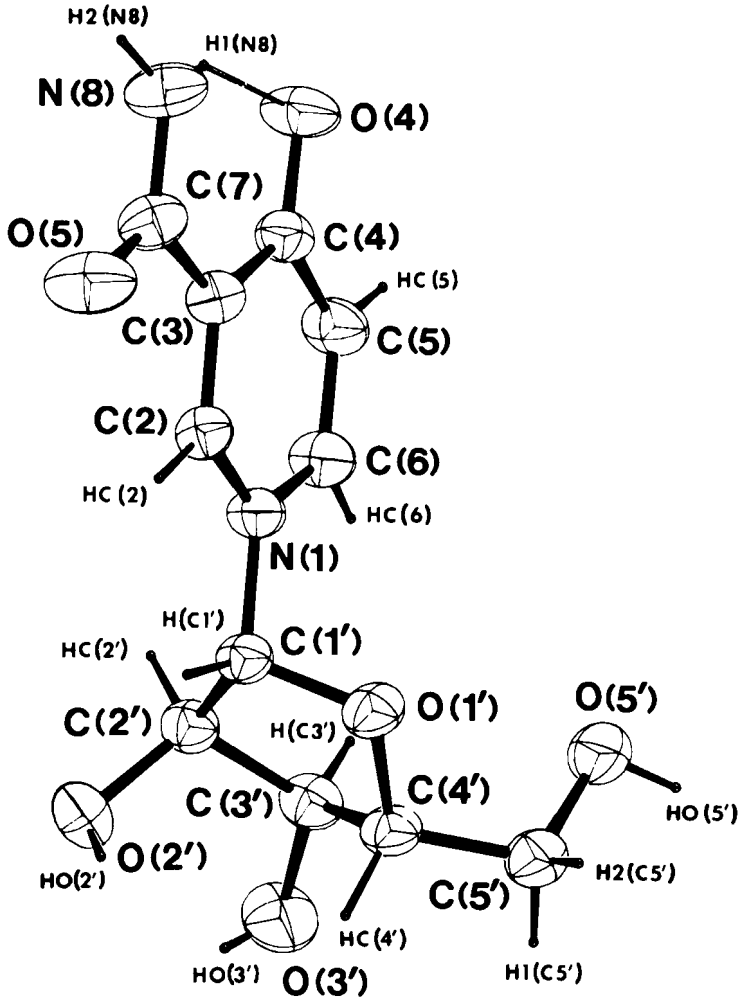


FIG. 1. An ORTEP drawing of the 1-β-D-ribofuranosylpyridin-4-one-3-carboxamide molecule showing the conformational details and the numbering scheme used. The molecule has the anti conformation (χ_{CN} = 66.9°) and has a novel ribose conformation with C(4') deviating from the plane of the other four atoms of the ribose ring. O(5') is g^+.

TABLE 1

Final Fractional Positional Parameters with Estimated Standard Deviations Given in Parentheses

Atom	X	Y	Z	B_{eq}
O(4)	-0.4704(2)	-0.0183(1)	-0.4533(3)	3.94(3)
O(1')	0.1880(2)	-0.0956(1)	-0.6765(2)	2.93(3)
O(2')	0.3462(2)	-0.0986(1)	-0.2428(2)	3.42(3)
O(3')	0.3720(2)	-0.2308(1)	-0.3950(3)	4.49(4)
O(5')	0.1384(2)	-0.2318(1)	-0.8488(3)	4.25(3)
O(5)	-0.1516(2)	0.1501(1)	0.4375(3)	4.34(4)
N(1)	-0.0086(2)	-0.0544(1)	-0.4774(2)	2.55(3)
N(8)	-0.4042(2)	0.1208(1)	-0.4422(4)	3.95(4)
C(6)	-0.1104(2)	-0.1094(1)	-0.4800(3)	3.06(4)
C(5)	-0.2633(2)	-0.0984(1)	-0.4679(4)	3.30(4)
C(4)	-0.3266(2)	-0.0286(1)	-0.4592(3)	2.72(4)
C(3)	-0.2147(2)	0.0281(1)	-0.4573(3)	2.55(3)
C(2)	-0.0618(2)	0.0127(1)	-0.4656(3)	2.46(3)
C(1')	0.1578(2)	-0.0684(1)	-0.4915(3)	2.46(4)
C(2')	0.2159(2)	-0.1232(1)	-0.3443(3)	2.67(4)
C(3')	0.2550(2)	-0.1874(1)	-0.4696(4)	2.86(4)
C(4')	0.2962(2)	-0.1537(1)	-0.6611(3)	2.71(4)
C(5')	0.2862(2)	-0.2006(1)	-0.8334(4)	3.37(4)
C(7)	-0.2556(2)	0.1049(1)	-0.4460(4)	3.08(4)
H(C6)	-0.066(3)	-0.153(2)	-0.479(4)	4.1(6)
H(C5)	-0.338(4)	-0.139(2)	-0.473(5)	6.3(8)
H(C2)	0.016(2)	0.046(1)	-0.462(3)	1.9(4)
H(C1')	0.205(3)	-0.026(1)	-0.471(3)	2.7(4)
H(C2')	0.134(3)	-0.131(1)	-0.239(4)	3.6(5)
H(C3')	0.153(3)	-0.213(1)	-0.476(3)	3.6(5)
H(C4')	0.409(3)	-0.134(1)	-0.646(4)	3.7(5)
H1(C5')	0.301(3)	-0.176(2)	-0.944(4)	4.1(6)
H2(C5')	0.367(4)	-0.236(2)	-0.807(5)	4.1(6)
H(O2')	0.404(4)	-0.080(1)	-0.316(4)	4.7(6)
H(O3')	0.427(4)	-0.209(2)	-0.357(5)	6.5(8)
H(O5')	0.147(5)	-0.268(2)	-0.929(6)	8.0(1)
H1(N8)	-0.438(5)	0.157(2)	-0.411(6)	9.0(1)
H2(N8)	-0.461(3)	0.081(2)	-0.432(4)	4.3(8)

TABLE 2

Bond Distances (Å) and Bond Angles (°) Between the Non-hydrogen Atoms With Estimated Standard Deviations Given in Parentheses

Bond Distances

N(1)-C(2)	1.362(3)	N(1)-C(1')	1.472(2)
C(2)-C(3)	1.361(3)	C(1')-O(1')	1.414(3)
C(3)-C(4)	1.444(3)	C(1')-C(2')	1.539(3)
C(4)-C(5)	1.429(3)	C(2')-C(3')	1.529(3)
C(5)-C(6)	1.346(3)	C(3')-C(4')	1.521(3)
C(4)-O(4)	1.264(2)	C(4')-O(1')	1.447(2)
C(3)-C(7)	1.495(3)	C(4')-C(5')	1.493(3)
C(7)-O(5)	1.241(3)	C(5')-O(5')	1.416(3)
C(7)-N(8)	1.325(3)	N(1)-C(6)	1.362(3)
C(2')-O(2')	1.414(3)	C(3')-O(3')	1.404(3)

Bond Angles

N(1)-C(2)-C(3)	122.4(2)	N(1)-C(1')-C(2')	113.4(2)
C(2)-C(3)-C(4)	120.1(2)	N(1)-C(1')-O(1')	107.9(2)
C(3)-C(4)-C(5)	114.9(2)	C(1')-C(2')-C(3')	103.0(2)
C(4)-C(5)-C(6)	121.7(3)	C(2')-C(3')-C(4')	102.9(2)
C(5)-C(6)-N(1)	121.6(2)	C(3')-C(4')-O(1')	103.2(2)
C(6)-N(1)-C(2)	119.4(2)	C(4')-O(1')-C(1')	109.1(2)
C(3)-C(4)-O(4)	123.6(2)	C(1')-C(2')-O(2')	112.2(2)
C(5)-C(4)-O(4)	121.5(3)	C(3')-C(2')-O(2')	111.5(2)
C(2)-C(3)-C(7)	116.1(2)	C(4')-C(3')-O(3')	113.4(2)
C(4)-C(3)-C(7)	123.8(2)	C(2')-C(3')-O(3')	114.3(2)
C(3)-C(7)-O(5)	119.6(2)	C(2')-C(1')-O(1')	107.6(2)
C(3)-C(7)-N(8)	116.9(2)	C(3')-C(4')-C(5')	116.4(2)
N(8)-C(7)-O(5)	123.6(2)	O(1')-C(4')-C(5')	110.5(2)
C(6)-N(1)-C(1')	120.1(2)	C(4')-C(5')-O(5')	111.2(2)
C(2)-N(1)-C(1')	120.5(2)		

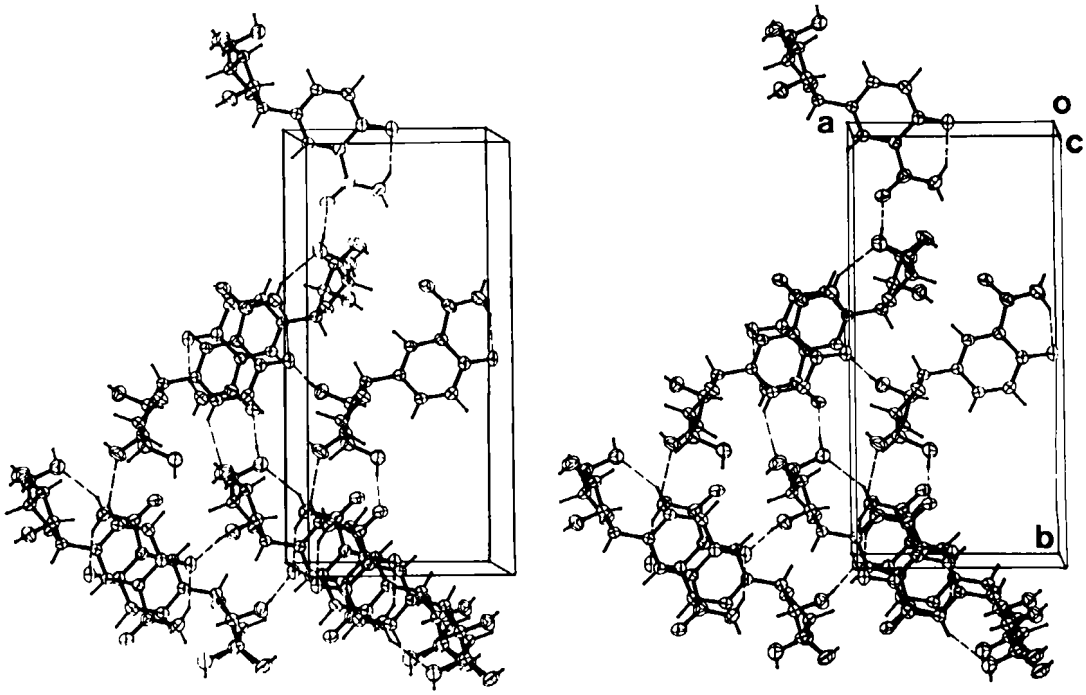


FIG. 2. A stereoscopic representation of the packing of the molecules in the unit cell showing details of (thin lines) of hydrogen bonding and partial stacking of the pyridine molecules.

C(5'), with a P of 41.2(2) and τ_m = 38.2(2)°. Although very common in nucleoside 3',5'-cyclic phosphates and anhydronucleosides (where the sugar and base are fused together), this pucker is rare for normal nucleotide and nucleoside structures. To the best of our knowledge a similar conformation has been found only in two out of 179 other nucleoside structures, 8-bromo 9-β-D-xylofuranosyladenine[16] and adenosine-5'-(methylphosphonate)[17].

Figure 2 shows the hydrogen bonding and partial stacking of the pyridine rings in the crystal structure. In addition to the internal hydrogen bond to O(4), the amino group is hydrogen bonded to O(5) of another molecule. Table 3 gives a list of hydrogen bond distances and angles. The crystal structure is stabilized by a number of other hydrogen bonds involving O(5') and O(2'), and a C-H...O hydrogen bond.

TABLE 3

Hydrogen Bond Distances (in Å) and Angles (°)

Donor D	H	Acceptor A	Distance in A D-H	H...A	D...A	Angle D-H...A	Data Set
N(8)	H1(N8)	O(4)	0.87	1.90	2.687	149°	(x y z)
	H2(N8)	O(5')	0.80	2.26	2.990	151°	($\bar{x}$-1/2 -y 1/2+z)
O(5')	H(O5')	O(5)	0.90	1.79	2.679	172°	($\bar{x}$ y-1/2-z-3/2)
O(2')	H(O2')	O(4)	0.80	1.86	2.644	168°	(1+x y z)
C-H...O Interactions							
C(6)	H(C6)	O(3')	0.91	2.41	3.137	138°	(x-1/2 -y-1/2 -z-1)

Nuclear Magnetic Resonance Spectroscopy: The NMR spectra were acquired at 200 MHz with a Bruker WP-200 operating in the pulsed FT/quadrature phase acquisition mode. Chemical and coupling constants listed in Table 4 obtained in D_2O were refined using the interactive spin-simulation program NMR-LAOCN-4A. The NOE-difference spectra were obtained by subtracting on-resonance and off-resonance time-domain spectra. The H-1 NMR spectrum of I in DMSO-d6 is illustrated in Figure 3. Selected chemical shifts and coupling constants are listed in Table 4. In addition, chemical shifts and coupling constants of the non-exchangeable protons obtained in D_2O are also included in this table. The data obtained in DMSO-d6 is generally typical of nucleosides, except for the amino resonance pattern. It is clear from the chemical shifts and $\Delta\delta$ values that the NH_2 resonances occur at 7.5 ppm and at 9.5 ppm. Since the two protons on NH_2 are magnetically nonequivalent and separated by only two bonds, a 4.6 Hz coupling constant is observed. The large downfield shift is indicative of a strong hydrogen-bond interaction formed by one of the amino hydrogens. Such an interaction can occur with the O(4) atom when the NH_2 of the carboxamide group is proximal to O(4). Data supporting this contention comes from the similarity of chemical shift values of the NH_2 resonances of 1- β-D-ribofuranosylpyridin-2-one-5- carboxamide[18](compound II)(7.305 and 7.480 ppm at 30°). In compound II this type of intramolecular hydrogen bond is not possible and both NH resonances are similar to the NHa chemical shift of I. Intramolecular hydrogens of this general type are not as exposed to solvent and can be

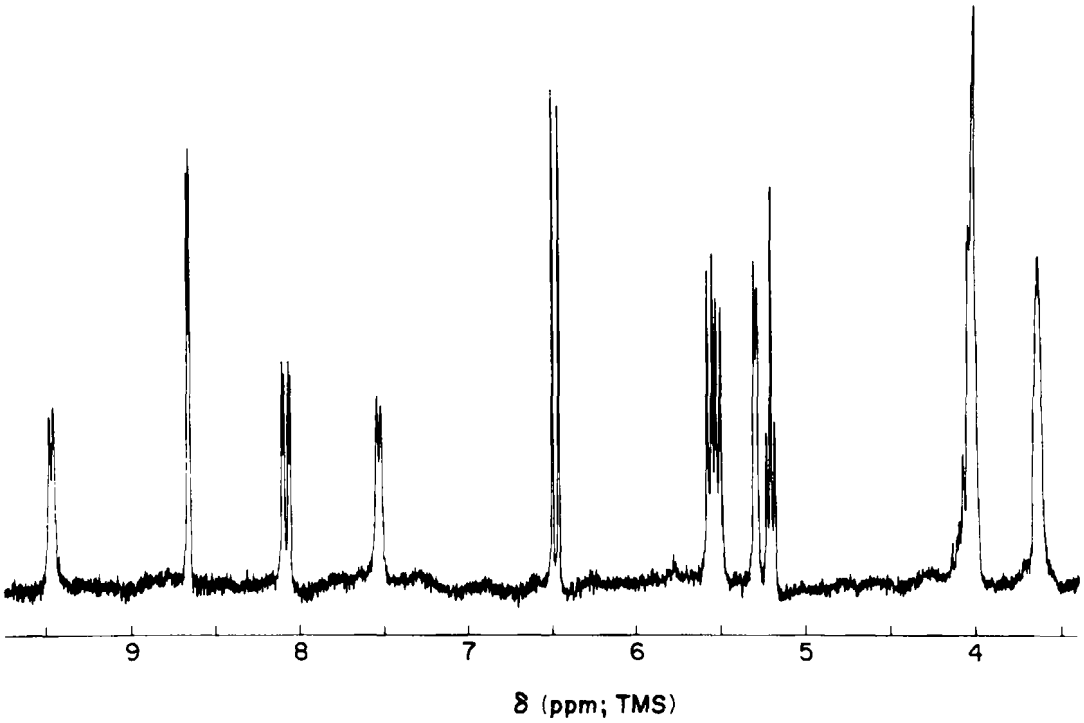


FIG. 3. 200 MHz H-1 NMR spectrum of I in DMSO-d_6 at 30°C.

characterized by their reduced sensitivity to temperature perturbation. The change in chemical shift with temperature change ($d\delta/dT$) is referred to as the chemical shift temperature coefficient. For a representative solvated amide, N-methylacetamide in dimethylsulfoxide, the coefficient is 0.0061 ppm/degree[19]. In Table 5 are listed these coefficients for compounds(I),(II) and cytidine. The data indicate that solvent exposed exchangeable hydrogens have values ranging from about 0.004 to 0.009. However the NHb resonance of (I) has a value of 0.0012, consistent with an intramolecular hydrogen bond. Thus NHb is assigned as the H1(N8) atom in Figure 1.

The NMR results from analysis in aqueous solution are also included in Table 4. All assignments are confirmed by spectral simulation. The base hydrogens are readily assigned by their coupling patterns. The HC(2) resonance is a doublet (J = 2.5 Hz) due to splitting by the HC(6). The HC(5) resonance is also a doublet (J = 7.7 Hz)

TABLE 4

Comparison of Chemical Shifts and Coupling Constants[a]

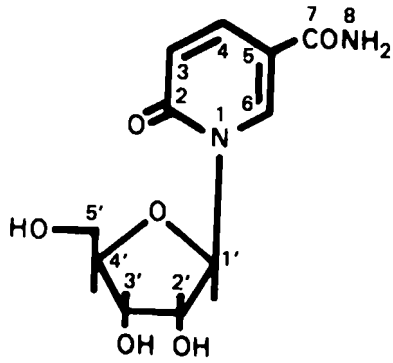
Compound	(ppm):	HC(1')	HC(2')	HC(3')	HC(4')	H2(C5')	H1'(C5')	HC(2)	HC(5)	HC(6)
I		5.672	4.361	4.310	4.278	3.939	3.862	8.794	6.726	8.066
II[b]		6.120				4.072	3.910			
Uridine[c]		5.955	4.391	4.267	4.173	3.951	3.846	---	5.943	7.916
Cytidine[c]		5.920	4.323	4.223	4.150	3.950	3.836	---	6.062	7.865

	J(Hz):	1',2'	2',3'	3',4'	4'5'2	4'5'1	5'2,5'1	2,6	5,6
I		5.5	5.3	3.4	3.4	4.9	-12.6	2.5	7.7
II		2.2			2.0	3.4	-12.9		
Uridine[c]		4.5	5.4	5.4	3.0	4.4	-12.8	---	8.1
Cytidine[c]		4.0	5.2	6.0	2.8	4.3	-12.7	---	7.6

	(ppm):	HC(1')	HC(2')-HC(4')	H1(C5')-H2(C5')	HC(2)	HC(5)	HC(6)	HO(2')-HO(3')	HO5'	H1(N8)	H2(N8)
I[d]		5.512	4.01	3.63	8.665	6.480	8.085	5.292; 5.567	5.201	9.474	7.532

	J(Hz):	1',2'	2',OH ; 3',OH	5',OH	H1',H2(N8)	2,6	5,6
I[d]		5.8	3.6 ; 6.0	4.8	4.6	2.4	7.7

a. In D_2O at 30°C unless otherwise indicated.
b. 1-β-D-ribofuranosylpyridine-2-one-5-carboxamide (2-one isomer of I).
c. Unpublished results.
d. In DMSO-d_6.



II

Table 5

Chemical Shift Temperature Coefficient

Proton	d δ/dT (ppm/degree)[a]		
	Compound I	Compound II[b]	Cytidine
HO(2')	0.0049[c]	0.0078[d]	0.0088
HO(3')	0.0043[c]	0.0084[d]	0.0089
HO(5')	0.0043	0.0070	0.0085
NHa	0.0056	0.0068	0.0076
NHb	0.0012		

a. Resonances move upfield with increasing temperature.
b. Compound II is the 2-one isomer of I.
c,d. Due to tentative resonance assignment of HO(2') and HO(3'), these values may be interchanged.

from interaction with HC(6), while HC(6) is a doublet of doublets from interactions with both HC(2) and HC(5). With regard to the furanose chemical shifts, the only unusual δ value is HC(1'). The resonance position of this hydrogen is at unusually high field. A contributing factor is the absence of a carbonyl function usually located at the C2 position of pyrimidine bases. The exocyclic conformation of C(4')-C(5') can be analyzed from J4',5'1 and J4,5'2 values[20]. This calculation yields a g+ population of 67% for (I) compared to 66% and 68% for uridine and cytidine, respectively. The furanose conformation (a C2'-endo and C3'-endo equilibrium) is usually determined by analysis of the J1',2' and J3',4' coupling constants[20]. For normal 5'-nucleotides, the sum (Σ) of these two coupling constants is 9.3 Hz (±0.3)[20]. In the cases of uridine and cytidine (Table 4), Σ is 9.9 and 10.0 Hz, respectively. The unusual values for these parameters in I suggests that the furanose conformation is not confined to the normal C2'- and C3'-endo forms. Furthermore the solid state conformation (C4'-exo-C3'-endo) is not a major solution conformation since the $J_{1',2'}$ and $J_{3',4'}$ arising from the solid would be about 1.0 and 11.3 Hz, respectively.

The possibility that other conformations may contribute to the solution structure of (I) has been considered by evaluating 15 different furanose conformations. This includes all paired combinations of C1'-exo, C2'-endo, C2'-exo, C3'-endo, C3'-exo and C4'-exo. Two best fit classes (C3'-endo/C3'-exo and C2'-endo/C2'-exo) are obtained with a 32% C3'-endo-68% C3'-exo conformational blend deviating from the sum of the experimental coupling constants by only 0.21 Hz. Although the nmr data does not indicate the same conformation (C4'-exo/C3'-endo twist) as the x-ray data, it is apparent that an unusual conformation is also present in solution.

To obtain conformational information regarding the sugar-base torsion angle, one-dimensional NOE difference spectroscopy was employed. Figure 4 shows the NOE effects on H1'[H2], H1'[H6] and H5-[H6], where the bracket indicates the irradiated atom. The slope of these curves indicates their relative cross-relaxation rates (σ_{ij})[21]. The σ_{ij} values are: H1'[H2], 7.98; H1'[H6], 4.43; and H5 [H6], 6.25. If the internuclear-vector correlation times are assumed to be similar, then ratios of these values can provide

internuclear distances using the equation:

$$\left(\frac{\sigma_{ij}}{\sigma_{mn}}\right)^{1/6} = \frac{r_{mn}}{r_{ij}}$$

These data indicate a ratio of slopes σ(H1',H6)/ σ(H1',H2) to be 1.80. Using the above equation and the distances (H1'-H6: 3.37Å; H1'-H2: 2.15Å) obtained from the crystal data with χ of 66.9°, the ratio of slopes is predicted to be 10.91. The immediate conclusion is that the solution conformation can not be the same as the solid-state. Using the same equation, r(H5-H6) from the crystal (2.37Å) and σ (H5-H6) from the nmr study, values obtained for r(H1'-H6) and r(H1'-H2) are 2.51 and 2.27Å, respectively. The ratio, r(H1'-H6)/r(H1'-H2), is 1.103. There are two general interpretations for this data. The first is that the solution structure is qualitatively similar to the crystal except for rotation of the base. Such a rotation of the base around the sugar produces a r(H1'-H6)/r(H1'-H2) value of 1.108 when χ is 135°. Alternatively, the base could be oscillating rapidly over a range of χ values to produce the observed ratio. If it is assumed that all χ values are equi-energetic, then a range of 135±20 would give a ratio of 1.106, or a range of 135±50 gives a ratio of 1.095. The general conclusion from these NOE results is that in aqueous solution the base is not fixed at a χ angle of 66.9°, but is more consistent with an angle (probably time averaged) around 135°.

Comparison of the X-ray and nmr results: The X-ray and NMR results indicate that the N(8)-H1(N)...O(4) intramolecular bond is present both in the solid and solution state. In the solid state, the nucleoside is in the anti conformation, but the NMR results suggest high anti conformation across the glycosidic bond. The conformation across C(4')-C(5') is g^+, both in the solid and solution state. While the solid state shows the furanose ring has the C(4')-exo-C(3')-endo pucker, the solution studies suggest that the furanose conformation is 32% C3'-endo/68% C3'-exo.

In many modified nucleosides[22] such as t^6A, g^6A, ac^4C, intramolecular hydrogen bonds are present. It has been suggested[22] that the distal conformation of ureido purines that occur adjacent to the anticodons in tRNA is stabilized by intramolecular hydrogen bonds, giving rise to difficulty in Watson-Crick base pairing by bases adja-

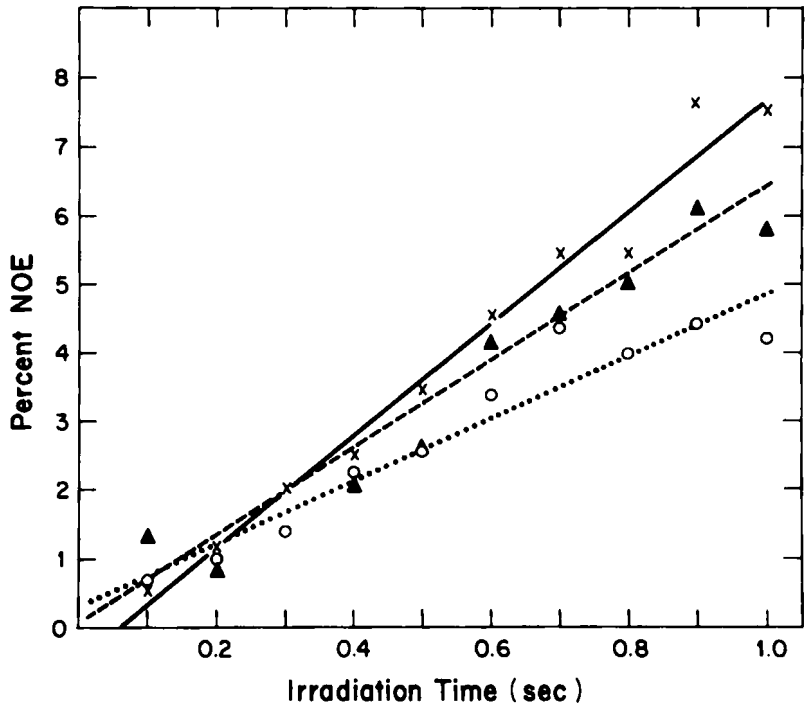


Fig. 4. NOE enhancement of H5[H6 irradiated] (Δ---Δ), H1'[H2 irradiated] (X——X) and H1'[H6 irradiated] (O•••O) of compound (I) as a function of irradiation time; in D_2O at 30°C.

cent to anticodons. For ac^4C, the modified nucleoside that occurs at the first position of the anticodon in some tRNA's there is an intramolecular hydrogen bond that stabilizes the proximal conformation of the N-acetyl group and enables this base to take part in codon-anticodon reading[23]. Our X-ray and nmr results in PCR show that such intramolecular hydrogen bonds persist in the solution state also and is likely to be present in the macromolecules as well, lending support to our earlier hypotheses on the biological roles of modified nucleosides[24].

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