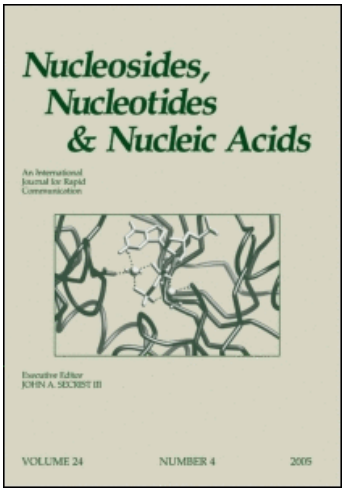

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STRUCTURE AND CONFORMATION OF 1-β-D-RIBO FURANOSYL PYRIDIN-2-ONE-5-CARBOXAMIDE: AN ANTI-INFLAMMATORY AGENT

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□ *The pyrimidine nucleoside, 1-β-D-ribofuranosyl pyridine-2-one-5-carboxamide, is an anti inflammatory agent used in the treatment of adjuvant-induced arthritis. It is the 2-one isomer of 1-β-D-ribofuranosyl pyridine-4-one 5-carboxamide, an unusual nucleoside isolated from the urine of patients with chronic myelogenic leukemia and an important cancer marker. Crystals of 1-β-D-ribofuranosyl pyridine-2-one-5-carboxamide are monoclinic, space group C2, with the cell dimensions* a = *31.7920(13),* b = *4.6872 (3),* c = *16.1838(11),* β = *93.071(3)°,* V = *2408.2(2)A^3,* D_{calc} = *1.496 mg/m^3 and* Z = *8 (two molecules in the asymmetric unit). The structure was obtained by the application of direct methods to diffractometric data and refined to a final R value of 0.050 for 1669 reflections with* $I \geq 3\sigma$. *The nucleoside exhibits an* anti *conformation across the glycosidic bond* ($\chi_{CN} = -15.5°, -18.9°$), *a C3′-*endo *C2′-*exo *[^3_2T] ribose pucker and* g^+ *across the C(4′)-C(5′) exocyclic bond. The amino group of the carboxamide group is distal from the 2-one and lacks the intramolecular hydrogen bonding found in the related 2-one molecule. Nuclear magnetic resonance studies shows also an* anti *conformation across the glycosidic bond but the solution conformation of the furanose ring is not the same as that found in the solid state.*

Keywords Anti-inflammatory agents; pyridine nucleosides; structure and conformation

INTRODUCTION

The presence of modified nucleosides and nucleotides in the urines of cancer patients have paved the way for the study of the anabolic and catabolic processes in carcinogenesis. As part of an ongoing program of the isolation, purification and characterization of these molecules, one of our senior colleagues, Chheda and coworkers have reported the presence of several urinary nucleosides[1–3] and published their structural characteristics

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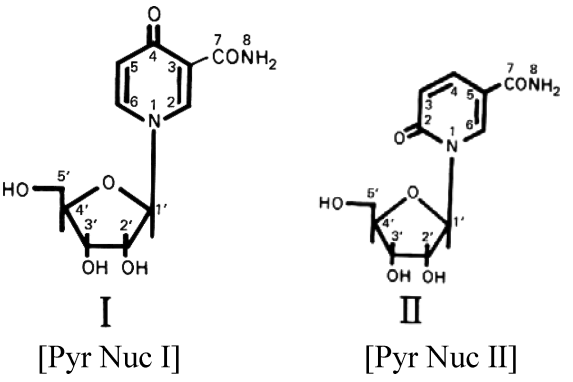
by x-ray diffraction and nuclear magnetic resonance techniques.[4,5] One of the unusual nucleoside isolated from the urine of patients with myelogenous leukemia was assigned the structure 1-β-D-ribofuranosyl pyridin-4-one-3-carboxamide[6] (I). The occurrence of this nucleoside has not been reported from any other biological source. This nucleoside and the previously reported methylated base, 1-methyl pyridine-4-one-3-carboxamide[6] has been structurally characterized and it has been suggested that these compounds are derived from the ubiquitous cofactor, nicotinamide adenine dinucleotide (NAD^+) or from the base nicotinamide,[7] which play a very important role in most biological oxidation and reduction processes. This nucleoside (I) is derived from NAD^+ after oxidation at the 4-position of its pyridine moiety followed by hydrolysis and dephosphorylation. This nucleoside is also the product of the reaction between nicotinamide and ribose-1-phosphate followed by oxidation at the 4-position. In addition to being a potential tumor marker in malignancy, this nucleoside is a novel metabolite in human biochemical pathways. This molecule has a quinonoid structure which can act as an electrophile and interact with DNA in a manner analogous to the quinines[8,9] and can take part in the production of superoxide radicals.[10] The chemical structure of this nucleoside suggested a potential interaction between the 4-keto oxygen and the 3-carboxamide group. The crystal structure of this nucleoside[11] characterized the β-configuration of the anomeric carbon and details of the intramolecular hydrogen bonding between the carboxamide group and the keto oxygen which was also found to exist in the solution state as confirmed from nmr study of the molecule.

The title compound, 1-β-D-ribofuranosyl pyridin-2-one-5-carboxamide (II) is the 2-one isomer of compound I. The chemical structure of this compound suggests that this molecule, unlike compound (I) cannot have the interaction between the 2-one and the carboxamide. This compound



SCHEME 1 Chemical structures of PyrNucI and PyrNucII.

has anti-inflammatory properties similar to several other nicotinamide related derivatives.[12] Structural studies of compound (II) have been undertaken in order to obtain the conformational and structural details of this compound so as to compare it with the structure of the 4-one molecule. A knowledge of the structures of the two isomers could lead to an understanding as to why the 4-one isomer is excreted in the human urine and not the 2-one thereby hopefully lead to a possible understanding of the catabolic processes involved in the pathways leading to the presence of pyridine nucleosides in human urine of patients with chronic myelogenous leukemia.

X-RAY CRYSTALLOGRAPHY

Compound (II) was obtained as a gift from R. K. Robins of the Nucleic Acid Research Institute, Irvine, California, USA. Excellent needle-like crystals of the compound were obtained by a slow evaporation of an aqueous solution of the compound. Crystals of II (C_{11} H_{15} N_2O_6) are monoclinic, space group C2, with cell dimensions $a = 31.7920$ (13), $b = 4.6872$ (3), $c = 16.1838$ (11) A, $\beta = 93.071$ (3)°, $V = 2408.2$ (2) A^3, $Z = 8$ (two molecules in the asymmetric unit). A crystal of approximate dimensions $0.55 \times 0.12 \times 0.07$ mm^3 was chosen for data collection. Diffraction data were collected at 298 K using a Nonius Kappa-CCD diffractometer with graphite-monochromated Mo Kα radiation ($\lambda = 0.71073$A). A total of 191 frames were collected using phi plus omega scans to fill the asymmetric unit with a scan range of 2° and a counting time of 400 seconds per degree. The first ten frames were used for indexing reflections using the DENZO-SMAN software program of Nonius Corp., Delft, the Netherlands,[13] and refined to obtain final cell parameters. Table 1 gives the crystal data and the parameters used in the refinement of the structure of Pyr Nuc II. Data reductions were performed using DENZO-SMN.[13] The structure of PyrNucII was solved by using the "dual space refinement method" in the XM program of the Bruker SHELXTL[14] package and refined by ful-matrix least squares method on F. Atomic scattering factors were taken from the "International Tables for X-ray Crystallography"[15] and SHEXTL package of Bruker.[16] Hydrogen atoms were included in idealized positions. The structure refined to a goodness of fit (GOF)* of 1.119 and final residuals** of $R_1 = 0.050\%$ ($I > 2\sigma(I)$). A total of 1,667 reflections were employed for 218 parameters determination, resulting in a data-to-parameter ratio of ~7.6. The crystallographic data are deposited

* $GOF = [\sum [w(F_o^2 - F_c^2)^2]/(n - p)]^{1/2}$, where n and p denote the number of data and parameters.

** $R_1 = (\sum ||F_o| - |F_c||)/\sum |F_o|$;

$wR_2 = [\sum [w(F_o^2 - F_c^2)^2]/\sum [w(F_o^2)^2]]^{1/2}$,

where

$w = 1/[\sigma^2(F_o^2) + (a \cdot P)^2 + b \cdot P]$ and $P = [(Max; 0, F_o^2) + 2 \cdot F_c^2]/3$.

TABLE 1 Crystal data and structure refinement for 1-β-D-Ribofuranosyl pyridine-2-one-5-carboxamide [Pyr NucII]

Compound name	1-β-D-Ribofuranosyl pyridine-2-one-5-carboxamide
CCDC deposit no.	CCDC- 673939
Color/shape	colorless/ needle
Chemical Formula	C_{11} H_{15} N_2O_6
Formula weight	542.50
Temperature	293(2) K
Crystal system	Monoclinic
Space group	C2
Unit cell dimensions	$a = 31.7920(13)$ Å $b = 4.6872(3)$ Å $c = 16.1838(11)$ Å $\alpha = 90.0^\circ$ $\beta = 93.071(3)^\circ$ $\gamma = 90.0^\circ$
Volume, A^3	2408.2(2)
Z	8 (two per asymmetric unit)
Density observed, mg/m^3	1.50
Density calculated, mg/m^3	1.496
Absorption coefficient, mm^{-1}	0.123
Diffractometer	Kappa CCD
Radiation/wavelength	MoKα / 0.71073 Å
Crystal size	0.55 × 0.12 × 0.07 mm^3
θ range for data collection	2.77 to 18.19°
Index ranges	−27<=h<=27,-4<=k<=4,-14<=l<=14
Independent / observed reflections	6142 (1669, $I \leq 3\sigma$)
Refinement method	Full-matrix least squares on F^2
Computing	SHELXTL (Bruker 2000)
Data / restraints/ parameters	1669 / 1 / 221
Goodness of fit on F^2	1.119
Function minimized	Σ [$\lvert F_o^2 \rvert$ - (1/k)$\lvert F_c^2 \rvert$]
Final R indices [$I > 2\sigma$ (I)]	R1 = 0.050, wR2 = 0.1188
R indices (all data)	R1 = 0.0577, wR2 = 0.1250
Large diff. peaks and hole	0.181 and −0.211

with the Cambridge Crystallography Data Center (CCDC), Cambridge UK as supplementary material no. CCDC 673939.*

The structure of the title compound is shown in Figure 1. The final fractional atomic coordinates are given in Table 2 and the bond distances and angles together with their standard deviations are given in Table 3. Table 4 gives the torsion angles in both the molecules and Table 5 gives the hydrogen bond distances and angles in both molecules A and B. Unlike

*Copies of the data may be obtained free of charge upon request from The Director, Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK. (Fax: +44–1223-336033; e-mail: deposit@ccdc.cam.ac.uk; WEB: http://www.csdc.cam.ac.uk.

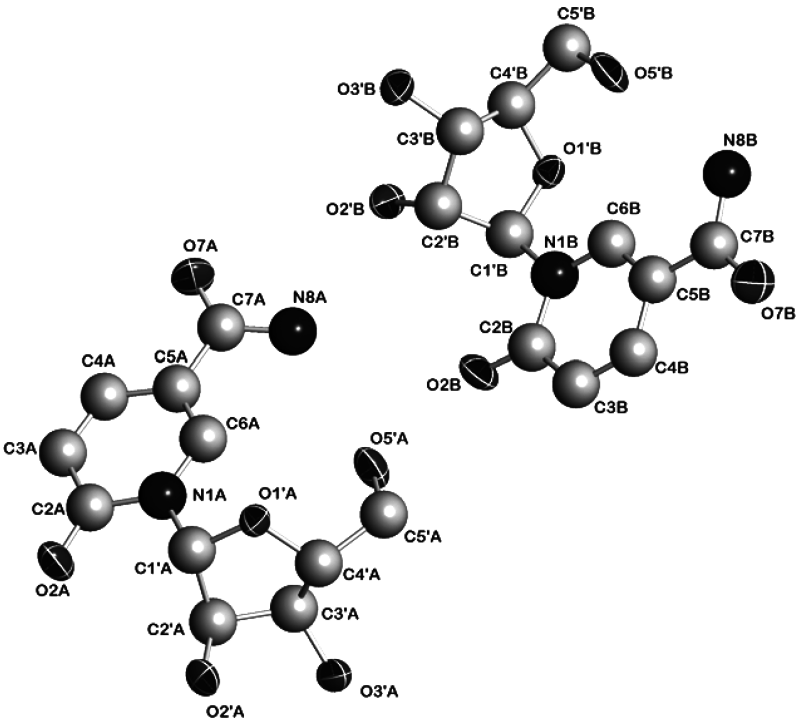


FIGURE 1 A drawing of the 1-β-D-Ribofuranosyl pyridine-2-one-5-carboxamide showing the conformational details and the atom numbering scheme used. The molecule has the anti conformation ($\chi_{CN} = -15.5$ and $-18.4°$) and has the ribose conformation $^{3}_{2}T$ in both the molecules.

the 4-one isomer, there is no internal hydrogen bonding between the keto oxygen and the amino group of the carboxamide group in this compound. Using the nucleic acid nomenclature of Sundaralingam,[17] PyrNucII has the preferred anti-conformation in both the molecules A and B and the preferred g^+ across the C(4′)-C(5′) bond. The ribose ring has a $^{3}_{2}T$ ribose pucker in both the molecules, as against the Pyr Nuc I which has the $_{4}T^{3}$ pucker.

Figure 2 illustrates a superposition of both the independent molecules in the asymmetric unit. The molecules show differences in the orientation of the carboxamide group and the conformation of the ribose ring including the exocyclic hydroxyl group. The molecules are connected by a network of hydrogen bonds involving the carboxamide group and the exocyclic hydroxyl groups of the ribose rings (Table 5 and Figure 3).

NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY

The nuclear magnetic resonance spectroscopy (NMR) 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 6

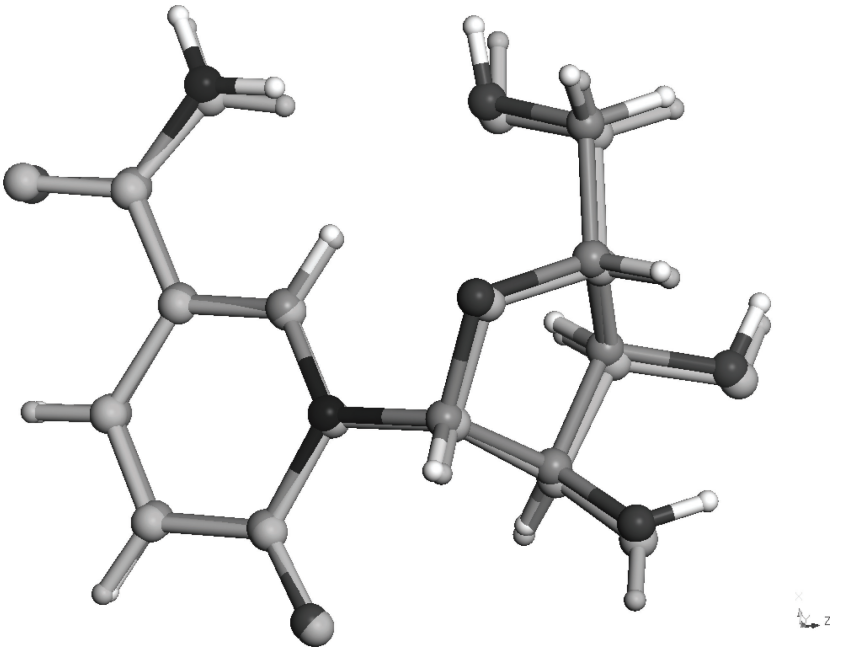


FIGURE 2 Superposition of the independent molecules A and B in the asymmetric unit. The differences are in the ribose ring and the exocyclic carboxamide group.

obtained in D_2O were refined using the interactive spin-simulation program NMR-LAOCN-4A. Selected chemical shifts and coupling constants are listed in Table 6. The data obtained in D_2O is generally typical of nucleosides, except for the amino resonance pattern. It is clear from the chemical shifts and $\Delta\delta$ values of Pyr Nuc I 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 bonded 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 PyrNuc II[12] [7.305 and 7.480 ppm at 30°C]. In Pyr NucII this type of intramolecular hydrogen bond is not possible and both NH resonances are similar to the NH_a chemical shifts of PyrNuc I. Intramolecular hydrogens of this general type are not as exposed to solvent and can be 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-acetylacetamide in dimethylsulfoxide, the coefficient is 0.0061 ppm/degree.[19] Table 7 lists the coefficients for PyrNucI, PyrNucII, and cytidine. The data indicate that solvent exposed

TABLE 2 Final fractional positional parameters and their estimated standard deviations

Atom	x/a	y/b	z/c	B(Å^2)*
Molecule A				
O(2A)	10230(2)	7562(18)	6983(4)	66(2)
O(7A)	8703(2)	483(17)	5467(4)	68(2)
O(1′A)	9154(2)	9679(14)	8025(3)	40(2)
O(2′A)	9987(2)	9949(15)	9041(3)	49(2)
O(3′A)	9493(2)	6503(16)	9961(3)	55(2)
O(5′A)	8549(2)	5350(15)	8397(4)	57(2)
N(1A)	9533(2)	6667(16)	7160(4)	37(2)
N(8A)	8447(2)	1910(20)	6651(5)	64(3)
C(2A)	9904(3)	6210(20)	6756(6)	51(3)
C(3A)	9861(3)	4130(20)	6087(5)	62(3)
C(4A)	9493(3)	2850(20)	5890(5)	49(3)
C(5A)	9135(3)	3356(19)	6328(5)	34(2)
C(6A)	9167(3)	5320(20)	6952(4)	38(2)
C(7A)	8745(3)	1800(20)	6112(6)	41(3)
C(1′A)	9562(3)	8850(20)	7843(5)	41(3)
C(2′A)	9780(3)	7660(20)	8631(5)	37(2)
C(3′A)	9403(3)	6630(20)	9089(5)	38(2)
C(4′A)	9063(3)	8840(20)	8856(5)	38(3)
C(5′a)	8615(3)	7850(20)	8888(6)	49(3)
Molecule B				
O(2B)	7776(2)	2750(16)	8323(4)	56(2)
O(7B)	6102(2)	8102(18)	9448(4)	68(2)
O(1′B)	6779(2)	−723(13)	7062(3)	38(2)
O(2′B)	7577(2)	61(15)	6179(3)	51(2)
O(3′B)	7011(2)	3167(16)	5230(3)	52(2)
O(5′B)	6044(2)	2662(17)	6602(4)	60(2)
N(1B)	7072(2)	2649(17)	8004(4)	35(2)
N(8B)	5852(3)	5782(19)	8320(5)	67(3)
C(2B)	7426(3)	3670(20)	8474(5)	42(3)
C(3B)	7330(3)	5590(20)	9104(5)	49(3)
C(4B)	6934(3)	6490(2)	9229(6)	53(3)
C(5B)	6593(3)	5490(20)	8725(5)	39(3)
C(6B)	6678(3)	3614(19)	8126(5)	38(2)
C(7B)	6166(3)	6550(20)	8858(6)	46(3)
C(1′B)	7157(3)	630(20)	7330(5)	36(2)
C(2′B)	7321(3)	2090(20)	6572(5)	42(3)
C(3′B)	6919(2)	2720(20)	6065(5)	36(2)
C(4′B)	6659(3)	80(20)	6206(5)	40(3)
C(5′B)	6189(3)	360(20)	6090(6)	50(3)

Anisotropically refined atoms are given in the form of the isotropic equivalent displacement parameter defined as (4/3) * [a2*B(1,1) + b2*B(2,2) + c2*B(3,3) + ab (cos gamma) * B(1,2) + ac (cos beta) * B(1,3) + bc (cos alpha) * B(2,3)].

exchangeable hydrogens have values ranging from about 0.004 to 0.009. However, the NHb resonance of PyrNuc I has a value of 0.0012 consistent with an intramolecular hydrogen bond where as that of PyrNuc II has a higher value of 0.0068.

The NMR results from an analysis in aqueous solution are also included in Table 6. All assignments are confirmed by spectral simulations. The base

TABLE 3 Final bond distances (inA) and bond angles (°) in 1-β-D-ribofuranosyl pyridine-2-one-5-carboxamide

Bond distance	Molecule A	Bond distance	Molecule B
O(2A)-C(2A)	1.251(11)	O(2B)-C(2B)	1.229(10)
O(7A)-C(7A)	1.213(10)	O(7B)-C(7B)	1.228(11)
O(1′A)-C(1′A)	1.401(10)	O(1′B)-C(1′B)	1.406(10)
O(1′A)-C(4′A)	1.445(10)	O(1′B)-C(4′B)	1.467(10)
O(2′A)-C(2′A)	1.405(11)	O(2′B)-C(2′B)	1.423(11)
O(3′A)-C(3′A)	1.427(9)	O(3′B)-C(3′B)	1.413(9)
O(5′A)-C(5′A)	1.424(12)	O(5′B)-C(5′B)	1.449(12)
N(1A)-C(6A)	1.352(10)	N(1B)-C(6B)	1.354(10)
N(1A)-C(2A)	1.395(11)	N(1B)-C(2B)	1.410(11)
N(1A)-C(1′A)	1.503(11)	N(1B)-C(1′B)	1.481(11)
N(8A)-C(7A)	1.325(10)	N(8B)-C(7B)	1.338(11)
C(2A)-C(3A)	1.458(14)	C(2B)-C(3B)	1.407(12)
C(3A)-C(4A)	1.338(13)	C(3B)-C(4B)	1.351(12)
C(4A)-C(5A)	1.392(11)	C(4B)-C(5B)	1.401(12)
C(5A)-C(6A)	1.366(11)	C(5B)-C(6B)	1.346(11)
C(5A)-C(7A)	1.465(12)	C(5B)-C(7B)	1.472(12)
C(1′A)-C(2′A)	1.524(12)	C(1′B)-C(2′B)	1.523(12)
C(2′A)-C(3′A)	1.524(11)	C(2′B)-C(3′B)	1.511(11)
C(3′A)-C(4′A)	1.528(13)	C(3′B)-C(4′B)	1.511(14)
C(4vA)-C(5′A)	1.502(12)	C(4′B)-C(5′B)	1.502(11)
Bond angle	Molecule A	Bond angle	Molecule B
C(1′A)-O(1′A)-C(4′A)	110.7(6)	C(1′B)-O(1′B)-C(4′B)	110.6(6)
C(6A)-N(1A)-C(2A)	123.3(8)	C(6B)-N(1B)-C(1′B)	122.0(7)
C(6A)-N(1A)-C(1′A)	121.2(7)	C(6B)-N(1B)-C(2B)	121.8(8)
C(2A)-N(1A)-C(1′A)	115.5(7)	C(2B)-N(1B)-C(1′B)	116.1(7)
O(2A)-C(2A)-N(1A)	119.4(8)	O(2B)-C(2B)-N(1B)	118.7(8)
O(2A)-C(2A)-C(3A)	126.9(9)	O(2B)-C(2B)-C(3B)	127.1(9)
N(1A)-C(2A)-C(3A)	113.7(9)	N(1B)-C(2B)-C(3B)	114.1(9)
C(4A)-C(3A)-C(2A)	121.6(10)	C(4B)-C(3B)-C(2B)	123.3(9)
C(3A)-C(4A)-C(5A)	122.0(9)	C(3B)-C(4B)-C(5B)	120.3(9)
C(6A)-C(5A)-C(4A)	117.4(8)	C(6B)-C(5B)-C(4B)	117.3(8)
C(6A)-C(5A)-C(7A)	122.8(8)	C(6B)-C(5B)-C(7B)	123.1(8)
C(4A)-C(5A)-C(7A)	119.8(8)	C(4B)-C(5B)-C(7B)	119.5(9)
N(1A)-C(6A)-C(5A)	121.9(8)	N(1B)-C(6B)-C(5B)	123.1(8)
O(7A)-C(7A)-N(8A)	122.6(9)	O(7B)-C(7B)-N(8B)	121.0(9)
O(7A)-C(7A)-C(5A)	120.7(8)	O(7B)-C(7B)-C(5B)	120.5(8)
N(8A)-C(7A)-C(5A)	116.7(9)	N(8B)-C(7B)-C(5B)	118.5(10)
O(1′A)-C(1′A)-N(1A)	109.0(7)	O(1′B)-C(1′B)-N(1B)	109.1(6)
O(1′A)-C(1′A)-C(2′A)	108.1(6)	O(1′B)-C(1′B)-C(2′B)	106.1(6)
N(1A)-C(1′A)-C(2vA)	112.0(7)	N(1B)-C(1′B)-C(2′B)	112.9(8)
O(2′A)-C(2′A)-C(1′A)	107.4(7)	O(2′B)-C(2′B)-C(3′B)	112.0(7)
O(2′A)-C(2′A)-C(3′A)	112.0(7)	O(2′B)-C(2′B)-C(1′B)	106.6(7)
C(1′A)-C(2′A)-C(3′A)	100.8(7)	C(3′B)-C(2′B)-C(1′B)	102.1(7)
O(3′A)-C(3′A)-C(4′A)	112.0(7)	O(3′B)-C(3′B)-C(4′B)	114.1(7)
O(3′A)-C(3′A)-C(2′A)	111.8(7)	O(3′B)-C(3′B)-C(2′B)	109.5(6)
C(4′A)-C(3′A)-C(2′A)	103.2(7)	C(4′B)-C(3′B)-C(2′B)	102.2(7)
O(1′A)-C(4′A)-C(5′A)	110.7(7)	O(1′B)-C(4′B)-C(5′B)	110.3(7)
O(1′A)-C(4′A)-C(3′A)	104.0(6)	O(1′B)-C(4′B)-C(3′B)	103.7(7)
C(5′A)-C(4′A)-C(3′A)	116.2(8)	C(5′B)-C(4′B)-C(3′B)	117.2(8)
O(5′A)-C(5′A)-C(4′A)	110.2(8)	O(5′B)-C(5′B)-C(4′B)	109.9(8)

TABLE 4 Selected torsion angles in 1-β-D-ribofuranosyl pyridine-2-one-5-carboxamide

Torsion angle		(°)
Molecule A		
C(6A)-N(1A)-C(1′A)-O(1′A)	χ	−15.3(8)
C(1′A)-O(1′A)-C(4′A)-C(3′A)	θ_0	−16.5(7)
C(2′A)-C(3′A)-C(4′A)-O(1′A)	θ_1	32.5(6)
C(1′A)-C(2′A)-C(3′A)-C(4′A)	θ_2	−35.3(7)
O(1′A)-C(1′A)-C(2′A)-C(3′A)	θ_3	26.7(7)
C(4′A)-O(1′A)-C(1′A)-C(2′A)	θ_4	−6.6(9)
O(1′A)-C(4′A)-C(5′A)-O(5′A)	φ_{oo}	64.0(7)
C(3′A)-C(4′A)-C(5′A)-O(5′A)	φ_{oc}	−54.3(8)
	P	**187.8**
	τ_m	**35.1**
Molecule B		
C(6B)-N(1B)-C(1′B)-O(1′B)	χ	−18.9(8)
C(1′B)-O(1′B)-C(4′B)-C(3′B)	θ_0	−17.3(6)
C(2′B)-C(3′B)-C(4′B)-O(1′B)	θ_1	34.4(6)
C(1′B)-C(2′B)-C(3′B)-C(4′B)	θ_2	−38.5(6)
O(1′B)-C(1′B)-C(2′B)-C(3′B)	θ_3	28.8(6)
C(4′B)-O(1′B)-C(1′B)-C(2′B)	θ_4	−7.5(7)
O(1′B)-C(4′B)-C(5′B)-O(5′B)	φ_{oo}	63.6(7)
C(3′B)-C(4′B)-C(5′B)-O(5′B)	φ_{oc}	−54.6(8)
	P	**187.5**
	τ_m	**38.9**

TABLE 5 Hydrogen bond distances (Å) and angles (°)

Serial No.	Donor D	Hydrogen H	Acceptor A	Distances in Å D-H	Distances in Å H. . .A	Angle (°) D. . .A	Symmetry D-H. . .A
1	N8A	H2A1	O2′B	0.860	2.126	2.959	162.9 [x, y, z]
2	N8A	H2A3	O1′A	0.860	2.400	3.258	175.2 [x, y, z]
3	O2′A	HO2′A	N8B	0.913	2.399	3.067	130.1 [x+1/2, y+1/2,z]
4	O3′A	H3′A	O7B	0.820	1.872	2.689	174.1[−x+3/2, y−1/2, −z+2]
5	O5′A	H5′A	O2B	0.820	1.936	2.738	165.5 [x, y, z]
6	N8B	H1B2	O2′A	0.860	2.361	3.067	139.5 [x−1/2, y−1/2,z]
7	N8B	H2B2	O5′B	0.860	2.385	3.228	167.0 [x, y, z]
8†	O2′B	H2′B	O3′B	0.820	2.331	2.832	120.0 [−x+3/2, y−1/2, −z+1]
		HO2′B	O3′B	0.820	2.257	2.720	116.2 [x, y, z]
9	O3′B	H3′B	O7A	0.820	1.953	2.712	153.5 [−x+3/2, y+1/2, −z+1]
10	O5′B	H5′B	O2A	0.820	1.894	692	162.0 [x−1/2, y−1/2, z]

†This is a bifurcated hydrogen bond.[18]

TABLE 6 Comparison of chemical shifts and coupling constants[a]

Compound (ppm)	H(1′)	H(2′)	H(3′)	H(4′)	H2(5′)	H1(5′)	HC(2)	HC(5)	HC(6)	
Pyr NucII	6.120	4.321	4.331	4.232	4.072	3.910	8.211	8.50	8.011	
Pyr Nuc I	5.672	4.361	4.310	4.278	3.939	3.862	8.794	6.726	8.066	
Uridine[b]	5.955	4.391	4.267	4.173	3.951	3.846	—	5.943	7.916	
Cytidine[b]	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		2.2	2.2	3.0	2.0	3.4	−12.9			
II		5.5	5.3	3.4	3.4	4.9	−12.6	2.5	7.7	
Uridine		4.5	5.4	5.4	3.0	4.4	−12.8	—	8.1	
Cytidine		4.0	5.2	6.0	2.8	4.3	−12.7	—	7.6	
(ppm)	H(1′)	H(2′)-H(4′)	H1(5′)-H(4′)	H(2)	H(5)	H(6)	HO(2′)-HO(3′)	HO5′	H1(N8)	H2(N8)
Pyr Nuc II	5.41	4.11	3.54	8.675	6.32	8.054	5.354	5.245	9.521	7.632
PyrNuc I	5.512	4.01	3.63	8.665	6.48	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
Pyr Nuc II	5.7			3.5;	6.1	4.7	4.5		2.3	7.8
Pyr Nuc I	5.8			3.6;	6.0	4.8	4.6		2.4	7.7

[a]In D_2O at 30åC unless otherwise indicated.
[b]Unpublished results.

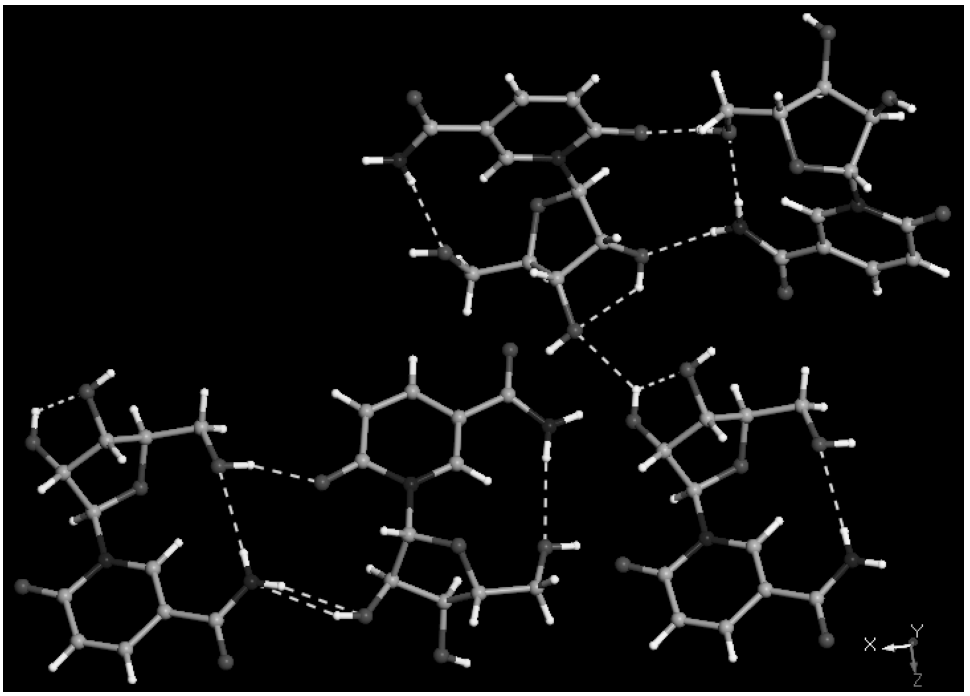
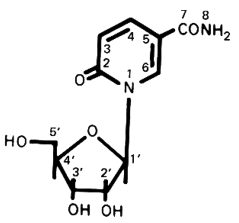


FIGURE 3 Representation of the packing of the molecules in the unit cell of 1-β-D-ribofuranosyl pyridine-2-one-5-carboxamide showing the network of hydrogen bonds involving the exocyclic hydroxyl groups of the ribose and the amino group of the carboxamide group.

hydrogens are readily assigned by their coupling patterns. With regard to the furanose chemical shifts, the only unusual δ value is HC(1′). The resonance position of this hydrogen is unusually high filed (6.07 ppm) due to the presence of the carbonyl function located at O(2). The exocyclic conformation of C(4′)- C(5′) can be analyzed from $J_{4',5'1}$ and $J_{4,5,2}$ values.[19] This calculation yields a g+ population of 58% for PyrNucII, 67% for PyrNucI as compared to 66% and 68% for uridine and cytidine respectively. The furanose conformation (a C2′-*endo* and C3′-*endo* equilibrium) is usually determined by an analysis of $J_{1',2'}$ and $J_{3',4'}$ coupling constants.[20] For an ideal standard North furanose puckering, $J_{1',2'}$ should be very small or nonexistent. For normal 5′-nucleosides, the sum (Σ) of these two coupling constants is 9.3 ± 0.3 Hz.[20] In the case of uridine and cytidine (Table 6), Σ is 9.9 and 10.0 Hz, respectively. The values for PyrNucI and Pyr NucII are 8.9 and 4.4, respectively. These values are unusual compared to uridine and cytidine and they suggest that the furanose ring is not confined to the normal C2′ and C3′-*endo* forms. Two best fit classes (C3′-endo/C3′-exo and C2′-endo/C2′exo) are obtained with a 32%-endo-68% C3′-exo conformational blend deviating from the sum of the experimental coupling constants by 0.23 Hz.

TABLE 7 Chemical shift temperature coefficient



II

	dδ/dT (ppm/degree)[a]		
Proton	Pyr Nuc II	Pyr Nuc I	Cytidine
HO(2′)	0.0078[b]	0.0049[c]	0.0088
HO(3′)	0.0084[b]	0.0043[c]	0.0089
HO(5′)	0.0070	0.0043	0.0085
NH_a		0.0056	
	0.0068		0.0076
NH_b		0.0012	

[a]Resonances move up field with increasing temperature.

[b,c]Due to the tentative resonance assignment of HO(2') and HO(3'), these values may be interchanged.

COMPARISON OF THE X-RAY AND NMR RESULTS

The x-ray and NMR results indicate that nucleoside is in the *anti* conformation. The conformation across C(4′)-C(5′) is g+ both in the solid and solution states. While the solid state shows that the furanose ring has the C3′-endo C2′-exo [$^3{}_2$T] pucker, the conformation from NMR seems to indicate that the conformation is not confined to the normal C2′- and C3′-endo forms. We are currently working on the structure-function relationship of many other biomarkers which we hope to publish soon.

CANCER BIOMARKERS

The discovery of new and more efficient biomarkers is central to the research development of our cancer institute. In our department of molecular and cellular biophysics, the discovery of several metabolites from human urine as biomarkers has been the key contribution of one of our colleagues, late Dr. Girish Chheda. With his collaboration, we have carried the crystal structure and nmr investions of several of these nucleic acid metabolites, most of which have been published in earlier issues of this journal. In addition to the work cited earlier in the introduction, two other nuclesides, 5-carbaoyluridine [ncm(5)U] and 5-carmoylmethyl-

2-thiouridine[ncm(5)s(2)U][21] isolated from the urine of chronic myelogenic leukemia and lung cancer patients, cis-dichloro-bis-isoprppylamine Platinum (II),[22] 1-α-D-ribofuranosyl-4-pyridone-3-carboxamide[23] from the urine of leukemic patients, 3-(3-amino-3-carboxylpropyl)uridine,[24] 6-amino-3- methyl- 5- (N-formylamino) uracil.[25] Creatinine and its 5-alkoxy analogs[26] and 5-trifluorothymine[27] have been reported from our department. An excellent review of the human urinary carcinogen biomarkers has been published recently.[28] Currently, our research is focused on the development of gelsolin as a biomarker for breast cancer.[29]

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