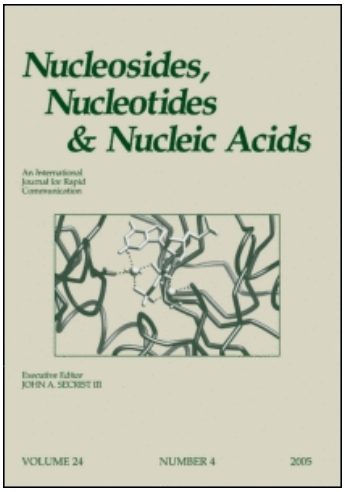

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Conformational Analysis of 6-Substituted Uridine Analogues: Crystal Structures of Uridine-6-Thiocarboxamide and 6-Cyanouridine

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CONFORMATIONAL ANALYSIS OF 6-SUBSTITUTED URIDINE ANALOGUES: CRYSTAL STRUCTURES OF URIDINE-6-THIOCARBOXAMIDE AND 6-CYANOURIDINE

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ABSTRACT. Crystal structure analyses of uridine-6-thiocarboxamide (I) and 6-cyanouridine (II) show that both structures adopt a syn conformation about the glycosyl bond. The conformation of I is similar to that of orotidine (III). The furanose ring conformation of I is C4'-exo, unusual for syn conformers, and is C3'-endo in II. These results have a bearing on the inhibition of orotidylate decarboxylase by the 5'-phosphate of I.

Most pyrimidine nucleosides and their 5-substituted derivatives exist in an anti conformation [1] due to the repulsive interaction between the furanose ring and the 2-oxo substituent of the pyrimidine base. In contrast, 6-substituted pyrimidine nucleosides, with the exception of 6-azauridine [2], must assume a syn conformation to avoid steric interference between the sugar and the 6-substituent. Since the molecule is forced into the less favorable syn conformation, an accommodating change in the furanose ring conformation must also take place.

We have been interested in 6-substituted pyrimidine nucleoside and nucleotide analogues as potential antimetabolites of pyrimidine biosynthesis and nucleotide metabolism [3,4]. Orotidine 5'-monophosphate (OMP) decarboxylase, a key enzyme of de novo pyrimidine biosynthesis [5] responsible for the formation of uridine 5'-monophosphate (UMP), is subject to inhibition by many 6-substituted pyrimidine analogues. The 5'-phosphate of 6-azauridine has been the prototype of OMP decarboxylase inhibitors [6] and was found to be useful in the chemotherapy of cancer and psoriasis. More recently, 6-OH-UMP [7,8] and UMP-6-thiocarboxamide [4] were shown to be potent, tight binding inhibitors of OMP decarboxy-

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lase. The latter has been successfully employed in the study of the molecular mechanism of this enzyme [9].

As a further investigation of the mechanism of OMP decarboxylase and its inhibition, the crystal and molecular structures of the orotidine analogue, uridine-6-thiocarboxamide (I), and its synthetic precursor [10], 6-cyanouridine (II) (FIG.1), were undertaken and are reported here as part of this study [11].

MATERIALS AND METHODS

Samples of I and II were synthesized as described [10] and recrystallized from aqueous ethanol. Crystal data for these compounds are listed in TABLE 1. Accurate cell dimensions were calculated from the least-squares analysis of 25 reflections for each crystal. Data for I were collected on a Nicolet P3 diffractometer using MoKα radiation with a Nb-filter and for II a Nonius CAD-4 diffractometer using CuKα radiation with a Ni-filter. The crystals were stable and showed no deterioration upon radiation. The data were corrected for Lorentz and polarization effects, but not for extinction or absorption effects. Both structures were solved by use of direct methods procedures using the programs MULTAN [12] and NQEST [13]. Parameters were refined by full matrix least-squares techniques using anisotropic thermal parameters for the non-hydrogen atoms. Hydrogen atom positions were located in difference Fourier syntheses and refined isotropically. Atomic scattering factors

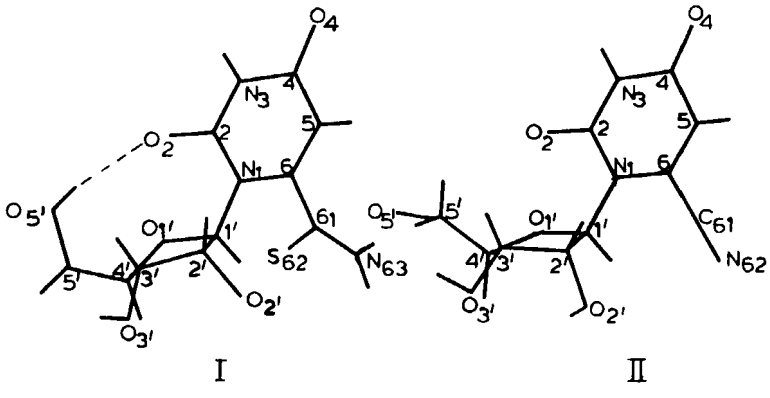

FIGURE 1. Molecular conformation and numbering scheme for I and II.

TABLE 1. Crystal Data for Compounds (I) and (II).

	I	II
Molecular Formula	$C_{10}H_{13}O_6N_3S$	$C_{10}H_{11}O_6N_3$
Molecular Weight	303.3	269.2
Crystal System	Orthorhombic	Orthorhombic
Space Group	$P2_12_12_1$	$P2_12_12_1$
Cell Dimensions		
a	9.201(3)Å	10.068(2)Å
b	14.522(5)	16.219(2)
c	9.033(3)	7.048(1)
z	4	4
Volume	1206.8Å^3	1150.9Å^3
Density (calc)	1.67 g/cc	1.55 g/cc
Crystal Size	0.12x0.24x0.58mm	0.5x0.6x1.0mm
Radiation (λ)	0.71069Å	1.5418Å
Absorption coeff (μ)	3.03	11.4
R	4.5%	4.0%

TABLE 2. Positional and Isotropic Thermal Parameters for I

ATOM	X/a(σ)	Y/b(σ)	Z/c(σ)	BISO(σ)
N(1)	0.9356(3)	0.3763(2)	0.8780(3)	0.85(5)
C(2)	1.0098(3)	0.4512(2)	0.9347(3)	0.99(6)
N(3)	0.9607(3)	0.4827(2)	1.0694(3)	1.07(5)
C(4)	0.8401(4)	0.4524(2)	1.1460(3)	1.13(6)
C(5)	0.7700(3)	0.3729(2)	1.0825(4)	1.08(6)
C(6)	0.8203(3)	0.3374(2)	0.9538(3)	0.94(5)
O(1')	1.1141(3)	0.3036(2)	0.7369(3)	1.12(4)
C(1')	0.9746(3)	0.3446(2)	0.7296(3)	0.89(6)
C(2')	0.9734(3)	0.4170(2)	0.6053(3)	0.86(5)
C(3')	1.1258(3)	0.4064(2)	0.5355(3)	0.91(6)
C(4')	1.1790(3)	0.3131(2)	0.5922(4)	0.98(6)
C(5')	1.3415(3)	0.3055(2)	0.6148(4)	1.19(6)
O(5')	1.1395(3)	0.3745(2)	0.7135(3)	1.17(5)
O(3')	1.1208(3)	0.4094(2)	0.3785(3)	1.28(5)
O(2')	0.8587(3)	0.3912(2)	0.5110(3)	1.20(5)
O(2)	1.1127(3)	0.4863(2)	0.8698(3)	1.28(5)
O(4)	0.8034(3)	0.4914(2)	1.2614(3)	1.30(5)
C(61)	0.7594(3)	0.2496(2)	0.8927(3)	0.92(6)
S(62)	0.8498(1)	0.1522(1)	0.9167(1)	1.33(2)
N(63)	0.6358(3)	0.2568(2)	0.8198(3)	1.46(6)

were taken from the International Tables for X-ray Crystallography [14]. All computations were performed on a VAX 11/780 computer using the Nonius least-squares package. Positional parameters and isotropic thermal parameters for both structures are listed in TABLES 2 and 3 [23].

TABLE 3. Positional and Isotropic Thermal Parameters for II

ATOM	X/a(σ)	Y/b(σ)	Z/c(σ)	BISO(σ)
N(1)	0.9765(2)	0.0544(1)	1.0373(3)	2.62(5)
C(2)	1.0692(3)	0.0877(2)	1.1618(4)	2.85(7)
N(3)	1.1052(3)	0.0393(1)	1.3124(4)	3.04(6)
C(4)	1.0573(3)	-.0375(2)	1.3558(5)	3.46(8)
C(5)	0.9623(3)	-.0690(2)	1.2221(5)	3.45(8)
C(6)	0.9254(3)	-.0234(2)	1.0733(5)	2.96(7)
O(1')	1.0428(2)	0.1049(1)	0.7416(3)	3.32(5)
C(1')	0.9363(3)	0.1032(2)	0.8703(4)	2.60(6)
C(2')	0.8919(3)	0.1924(2)	0.9071(4)	2.71(6)
C(3')	1.0026(3)	0.2449(2)	0.8191(4)	2.46(6)
C(4')	1.0685(3)	0.1868(2)	0.6747(4)	2.59(6)
C(5')	1.2161(3)	0.1969(2)	0.6629(5)	3.26(8)
O(5')	1.2724(2)	0.1371(1)	0.5392(3)	3.33(5)
O(3')	0.9545(2)	0.3171(1)	0.7270(3)	3.19(5)
O(2')	0.7701(2)	0.2013(2)	0.8085(5)	3.97(6)
O(2)	1.1149(2)	0.1562(1)	1.1392(3)	3.67(6)
O(4)	1.0976(3)	-.0741(1)	1.4968(4)	4.92(7)
C(61)	0.8255(3)	-.0544(2)	0.9464(6)	4.15(9)
N(62)	0.7439(4)	-.0817(2)	0.8543(7)	6.87(13)

RESULTS AND DISCUSSION

The molecular conformation of uridine-6-thiocarboxamide (I) and 6-cyanouridine (II) are illustrated in FIG. 1 and their conformational parameters defined in TABLE 4, along with those for other 6-substituted uridine analogues. These data show that both I and II adopt a syn conformation, as observed in the other 6-substituted uridine structures. The pucker of the furanose ring is defined by the pseudorotation parameter P and the degree of ring flattening, ν_m [18]. Although the values of ν_m normally cluster near 40° [18], that of II is unusually small. In I the furanose ring is C4'-exo, an unusual observation for syn conformations, while in II it is C3'-endo. The pseudorotational parameter P in I differs considerably from those listed in TABLE 4 and is outside the usual range (P = 0-34° and 144-190°) observed in similar structures [18,19]. Variations in the endocyclic angles (FIGS. 2,3) of the furanose ring have been correlated with the phase angle P [18,20]. As shown (TABLE 5), the largest deviations from the theoretically expected values [20] involve O1' in I and O1' and C2' in II. These variations are probably a result of the steric strain imposed by the unusual C4'-exo conformation in I and the flattening of II. The conformation 5'-hydroxyl

TABLE 4. Conformational Comparison of 6-Substituted Uridines

6-X	χ_{cn}	Pucker	P	ν_m	γ	HB	Ref
$CSNH_2$ (I)	71°	C4'-exo	62°	35°	g,g	yes	this work
CN (II)	72	C3'-endo	29	25	g,t	no	this work
COO^- (III)	69	C4'-exo	42	38	g,t	no	[15]
CH_3 (1)	70	C2'-endo	159	35	g,g	yes	[16]
(2)	69	C2'-endo	169	37	g,t	no	[16]
CH_3 (2'-deoxy)	60	C1'-exo-C2'-endo	139	31	g,g	yes	[17]

χ = O1'-C1'-N1'-C2; P = pseudorotation angle; ν = maximum sugar torsion angle; γ = rotation about C4'-C5'; HB = O5'...O2 hydrogen bond

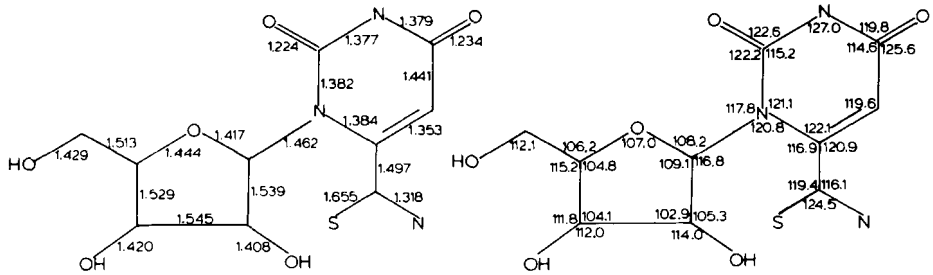

FIGURE 2. Bond distances and angles for I.

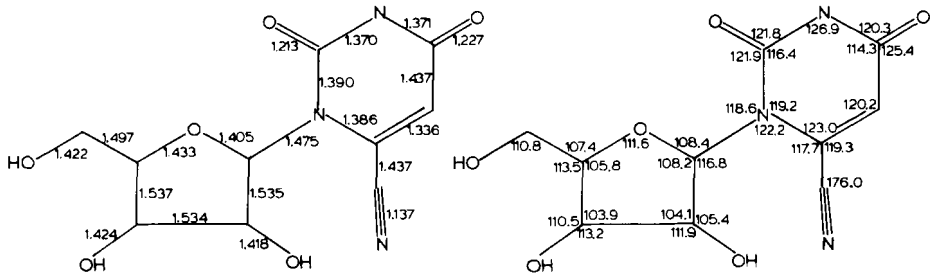

FIGURE 3. Bond distances and angles for II.

TABLE 5. Comparison of Endocylic Furanose Angle With P.

Angle	I(62°) Obs.	I(62°) Theo.	II(29°) Obs.	II(29°) Theo.	III(42°) Obs.	III(42°) Theo.
C4'-O1'-C1'	107.0	105.5	111.6	108.5	108.0	109.0
O1'-C1'-C2'	109.1	108.5	108.2	108.8	108.0	109.0
C1'-C2'-C3'	102.9	103.5	104.1	101.5	103.0	102.5
C2'-C3'-C4'	104.1	103.0	103.9	102.5	103.0	102.5
C3'-C4'-O1'	104.8	104.0	105.8	103.5	104.0	103.5

oxygen is gauche-gauche with respect to the furanose ring in I and gauche-trans in II and III. There is also an intramolecular hydrogen bond in I between O5'...O2 (2.84Å).

The plane of the thiocarboxamide in I is nearly perpendicular to that of the pyrimidine ring. The C-S bond (1.655Å) and the C-N bond (1.318Å) lengths are typical for such functional groups [21]. The shortest intramolecular contacts that the thiocarboxamide makes are between S...O1' = 3.66Å and N...O2' = 3.98Å. There are no close intramolecular contacts between the cyano group and the sugar in II. In both structures the furanose hydroxyl oxygen atoms form a network of intermolecular hydrogen bonds with adjacent molecules in the crystal (TABLE 6).

As illustrated (FIG. 4, TABLE 4), the conformation of uridine-6-thiocarboxamide is similar to that of the natural substrate, orotidylic acid (III). These data show that the 6-carboxylate ion results in a syn conformation and a gauche-trans orientation of the glycoside O5'. The intramolecular contacts between O5' and the 6-substituents of I (O5'...S = 6.24Å, ...N6 = 7.26Å) and II (O5'...N6 = 6.77Å) are similar to those of orotidine (O5'...OA = 7.41Å, ...OB = 6.12Å). These data are consistent with the proposal [22] that the OMP decarboxylase contains a pocket which is able to bind the O5' and the 6-substitutent oxygen when they are about 6Å apart.

The observed similarity between the conformations of orotidine and I may account for the ability of the 5'-phosphate of the latter to bind to the active site of OMP decarboxylase. However, the exceptional inhibi-

TABLE 6. Hydrogen Bonding in (I) and (II).

Structure	D-H...A	D-H	H...A	‹D-H...A
(I)				
N3-H ...O5'1	2.785(4)	0.84(4)	1.95(4)	173(3)°
N62-HA...O3'1	3.007	0.86	2.21	154
HB...O5'1	2.957	0.85	2.22	146
O2'1-H...O4	2.731	0.85	1.90	164
O3'1-H...O2	2.884	0.81	2.12	156
O5'1-H...O2	3.110	0.79	2.84	134
(II)				
N3-H...O5'1	2.811(3)	0.89(3)	1.94(3)	164(2)
O2'1-H...O2	2.814	0.70	2.19	148
O3'1-H...O4	2.679			
O5'1-H...O3'1	2.726	1.01	1.80	152
N62...N1	3.181			

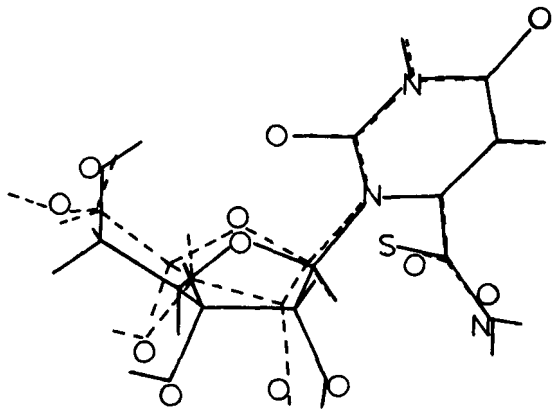

FIGURE 4. Comparison of uridine-6-thiocarboxamide with orotidine (--).

tory potency of this nucleotide analogue [4,9,10] cannot be adequately explained in terms of its conformational properties alone. Therefore, the results of this work support our previous studies [4,9] implicating the sulfur of the thioamide side chain in a specific interaction with OMP decarboxylase.

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