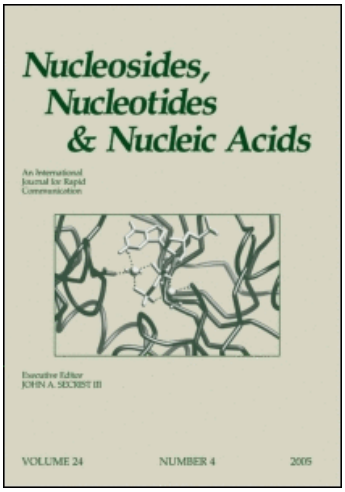

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Conformational Variability in Modified Nucleosides. Crystal and Molecular Structure of 2',3'-O-Isopropylidene Inosine

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CONFORMATIONAL VARIABILITY IN MODIFIED NUCLEOSIDES. CRYSTAL AND MOLECULAR STRUCTURE OF 2',3'-O-ISOPROPYLIDENE INOSINE

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ABSTRACT: The crystal structure of 2',3'-O-isopropylidene inosine shows a number of interesting features. The four independent molecules in the asymmetric unit exhibit significant conformational variations. Ribose puckers fall in the O(4')-**exo** region, unfavourable in unsubstituted nucleosides. Hypoxanthine bases show base-pairing (I.I) in a manner analogous to the guanine self pairs (G.G) in 2',3'-O-isopropylidene guanosine but with a C(2)-H...O(6) hydrogen bond instead of N(2)-H...O(6).

INTRODUCTION

Inosine is a minor constituent of tRNA and is known to occupy the 5' terminal (wobble position) of the anticodon triplet in some tRNAs. It differs from guanosine in the absence of an NH_2 group at position 2 of the base. It would therefore be of interest to study the effect of this change on molecular conformation and interactions. Crystal structures of inosine derivatives have often been found to be isomorphous with guanosine analogs e.g. guanosine and inosine[1], guanosine dihydrate and inosine dihydrate[2], S-(p-nitrobenzyl)-6-thioguanosine[3] and 6-4-(nitrobenzyl) thioinosine[4]. We describe here the structure of 2',3'-O-isopropylidene inosine (abbreviated Iso-I) which differs significantly from 2',3'-O-isopropylidene guanosine (Iso-G)[5,6].

EXPERIMENTAL

Thin needle shaped crystals were grown by slow evaporation of an aqueous solution of the compound (SIGMA Chemicals Co.) at

room temperature. Density measured by floatation in a mixture of carbon tetrachloride and benzene showed the presence of four molecules in the asymmetric unit.

Crystal Data: $C_{13}H_{16}N_4O_5 \cdot 0.5\ H_2O$; molecular weight: 317.30; $P2_12_12_1$; a= 10.895(1); b= 13.307(1); c= 40.577(3)Å; V=5882.84 Å^3; Z= 16; D_x= 1.43 Mg/m^3; D_m= 1.44 Mg/m^3; μ(CuKα)= 0.97 mm^{-1}; $\sin\theta_{max}/\lambda$ = 0.56 Å^{-1}.

Three dimensional CuKα intensity data were collected using a crystal 0.8 X 0.2 X 0.1 mm. with ω-2θ scan up to θ=60° on a CAD-4 diffractometer. A total of 5015 reflections were measured for h,k and l values between 0 and 12, 0 and 14, and 0 and 45, respectively. 3142 reflections with Io $\geq$ 2.5 σ (Io) were considered observed. The intensities were corrected for Lorentz and polarization effects. Absorption correction[7] was also applied.

The structure was solved by a combination of RANTAN[8], DIRDIF[9,10] and difference Fouriers. Full matrix refinement using SHELX400 (enhanced version of SHELX76[11]) with anisotropic temperature factors for non hydrogens and isotropic for hydrogens converged at R=0.072 (R_w=0.071). Hydrogen atoms not located from difference Fourier maps were fixed from geometrical criteria. The function minimized was $\Sigma\, w(|Fo|-|Fc|)^2$ where $w = 1/(\sigma^2(F)+0.000057|F|^2)$.

RESULTS AND DISCUSSION

Figure 1 shows the nucleoside molecule schematically with atomic numbering. Tables 1 and 2 give the final positional parameters and selected torsion angles respectively. Fig. 2 shows views of the molecules perpendicular to their C(2'), C(3'), C(10) atom planes.

The glycosyl torsion angles χ_{CN}[O(4')-C(1')-N(9)-C(4))] for the four molecules are generally in the **syn** range with B and D however in the **high anti** region. NOE studies show that the solution structure of the compound is also predominantly **syn** (80%)[12, 13].

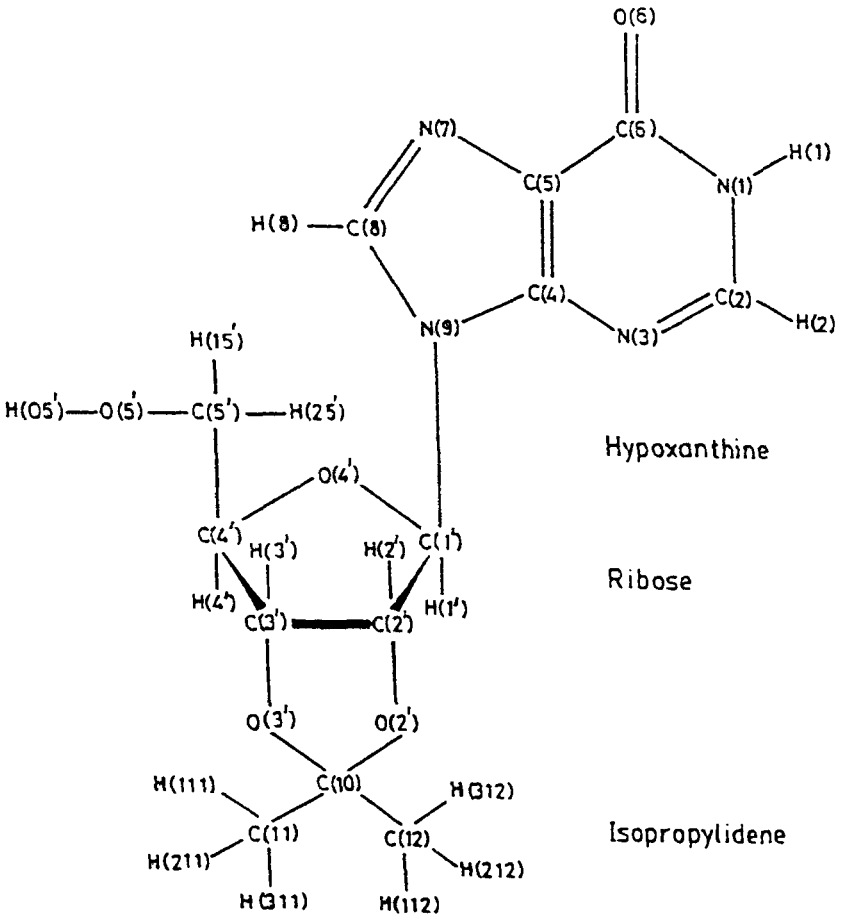

FIG. 1. Chemical structure and numbering.

The phase angle of pseudorotation and the amplitude of pucker for ribose[14] are

Molecule	P (°)	τ m (°)	Nature of pucker
A	278.6	38.0	O(4')-**exo**
B	262.9	17.8	marginally O(4')-**exo**
C	265.0	34.8	O(4')-**exo**
D	247.7	27.9	C(4')**endo**-O(4')**exo**

TABLE 1

Final positional parameters of non hydrogen atoms ($X10^4$) and equivalent temperature factors with e.s.d·s in parentheses.

$$Beq = (4/3)\Sigma\Sigma\beta ij\ a_i.a_j$$
$$ij$$

ATOM	X	Y	Z	EQUIVALENT B ($Å^2$)	X	Y	Z	EQUIVALENT B ($Å^2$)
Molecule A					**Molecule C**			
N(1)	10802(7)	12553(7)	10099(2)	3.4(3)	10648(7)	10017(7)	10018(2)	3.3(2)
C(2)	11320(9)	12678(8)	10395(3)	3.5(3)	11321(9)	9946(9)	9716(2)	3.3(3)
N(3)	10738(6)	12829(7)	10680(2)	3.5(3)	10696(7)	9874(7)	9435(2)	3.6(3)
C(4)	9522(8)	12816(8)	10628(2)	2.5(3)	9447(9)	9887(8)	9498(2)	3.0(3)
C(5)	8920(8)	12715(8)	10343(2)	2.6(3)	8911(9)	9955(8)	9797(2)	3.0(3)
C(6)	9553(9)	12588(8)	10036(2)	2.6(3)	9585(9)	10036(8)	10087(2)	3.0(3)
O(6)	9152(6)	12475(6)	9756(2)	4.1(2)	9241(6)	10096(6)	10374(1)	4.4(2)
N(7)	7629(6)	12770(7)	10399(2)	3.3(3)	7613(7)	9919(7)	9761(2)	3.5(2)
C(8)	7523(8)	12908(8)	10708(2)	3.1(3)	7480(9)	9845(9)	9442(2)	3.7(3)
N(9)	8658(6)	12949(6)	10873(2)	2.7(2)	8518(7)	9841(7)	9262(2)	3.2(2)
C(1')	8966(9)	13038(9)	11216(2)	3.7(3)	8743(10)	9749(8)	8911(2)	3.2(3)
C(2')	7793(9)	13064(10)	11445(2)	4.0(3)	7526(9)	9853(9)	8713(2)	3.7(3)
O(2')	8094(7)	13619(6)	11731(2)	4.9(2)	7630(8)	9202(6)	8442(2)	5.3(3)
C(3')	7647(10)	11994(9)	11555(3)	4.5(4)	7574(9)	10877(9)	8560(3)	4.1(3)
O(3')	7720(9)	12051(7)	11905(2)	7.0(3)	7656(8)	10710(6)	8215(2)	5.0(3)
C(4')	8747(10)	11411(9)	11424(2)	3.9(3)	8769(9)	11365(8)	8690(2)	3.5(3)
O(4')	9024(6)	12210(6)	11334(2)	4.0(2)	9501(6)	10499(5)	8788(2)	3.5(2)
C(5')	8479(9)	10745(9)	11138(2)	3.9(3)	8535(9)	12098(9)	8962(2)	3.7(3)
O(5')	9583(7)	10202(6)	11040(2)	5.5(3)	9718(6)	12501(6)	9083(2)	4.4(2)
C(10)	7664(13)	13087(12)	11999(3)	5.5(4)	7324(12)	9720(9)	8157(2)	4.2(4)
C(11)	6319(11)	13404(14)	12072(3)	9.2(6)	7977(14)	9275(13)	7874(3)	8.8(6)
C(12)	8473(12)	13244(13)	12296(3)	7.4(5)	5921(13)	9650(13)	8096(4)	9.1(6)
Molecule B					**Molecule D**			
N(1)	10803(7)	15009(7)	9750(2)	3.4(2)	10622(7)	17500(7)	10133(2)	3.4(3)
C(2)	11264(8)	14973(9)	9447(2)	3.3(3)	11089(9)	17615(9)	10433(2)	3.2(3)
N(3)	10663(7)	14912(7)	9169(2)	3.7(3)	10461(7)	17717(7)	10709(2)	3.7(3)
C(4)	9444(8)	14890(8)	9233(2)	2.8(3)	9228(10)	17656(8)	10644(2)	3.0(3)
C(5)	8865(9)	14922(9)	9532(2)	3.2(3)	8640(8)	17553(8)	10343(2)	2.4(3)
C(6)	9558(9)	15005(9)	9825(2)	3.3(3)	9339(9)	17450(8)	10052(3)	3.0(3)
O(6)	9217(6)	15043(6)	10114(2)	4.0(2)	9007(6)	17354(6)	9767(2)	3.9(2)
N(7)	7565(6)	14845(7)	9487(2)	3.2(2)	7381(6)	17565(6)	10359(2)	3.2(2)
C(8)	7444(8)	14807(9)	9172(2)	3.7(3)	7215(9)	17681(8)	10699(2)	3.1(3)
N(9)	8531(7)	14816(6)	8993(2)	3.0(2)	8283(6)	17761(6)	10877(2)	2.7(2)
C(1')	8757(9)	14815(9)	8640(2)	3.4(3)	8523(10)	17806(8)	11227(2)	3.2(3)
C(2')	7702(11)	14297(8)	8442(2)	3.7(3)	7510(10)	18220(9)	11435(2)	3.6(3)
O(2')	8223(8)	13623(7)	8222(2)	6.2(3)	7930(8)	18983(6)	11643(2)	5.2(3)
C(3')	7202(10)	15119(9)	8235(3)	4.5(4)	7114(9)	17402(9)	11658(2)	3.4(3)
O(3')	7479(12)	14826(7)	7908(2)	9.0(4)	7577(8)	17682(6)	11967(2)	4.7(2)
C(4')	7574(11)	16095(9)	8314(3)	4.5(4)	7719(11)	16465(8)	11536(2)	3.6(3)
O(4')	8937(6)	15777(6)	8517(2)	4.5(2)	8763(6)	16844(6)	11355(2)	3.9(2)
C(5')	7143(14)	16873(10)	8498(3)	6.5(5)	6866(13)	15923(10)	11307(3)	8.1(5)
O(5')	6815(11)	16520(10)	8782(3)	11.8(5)	7322(8)	15297(8)	11099(2)	8.2(4)
C(10)	7731(14)	13814(11)	7914(3)	5.9(5)	7645(12)	18721(10)	11971(3)	4.5(4)
C(11)	8641(16)	13579(16)	7646(4)	13.2(9)	6457(14)	19241(12)	12070(3)	7.6(5)
C(12)	6594(13)	13206(12)	7562(3)	8.0(6)	5666(14)	19034(11)	12198(3)	7.8(5)
OW(1)	9752(6)	5247(6)	8501(2)	4.6(2)				
OW(2)	9375(7)	7396(6)	9077(2)	5.2(3)				

TABLE 2

Selected torsion angles (°) with e.s.ds in parentheses.

		Mol. A	Mol. B	Mol. C	Mol. D
χ_{CN}	C(4) - N(9) -C(1')-O(4')	-57.2(1.2)	-80.3(1.1)	-55.4(1.2)	-77.6(1.1)
ϕ_{oc}	C(3')-C(4')-C(5')-O(5')	-178.1(0.8)	61.7(1.5)	177.6(0.8)	158.3(1.1)
ϕ_{oo}	O(4')- C(4')-C(5')-O(5')	65.0(1.0)	-55.8(1.4)	61.0(1.0)	46.0(1.4)
	Ribose				
θ_1	C(1')-C(2')-C(3')-C(4')	5.2(1.1)	-2.5(1.1)	-3.4(1.0)	-10.8(1.1)
θ_2	C(2')-C(3')-C(4')-O(4')	16.1(1.1)	11.7(1.2)	21.5(1.0)	22.9(1.0)
θ_3	C(3')-C(4')-O(4')-C(1')	-34.8(1.0)	-17.5(1.1)	-34.6(1.0)	-28.2(1.0)
θ_4	C(2')-C(1')-O(4')-C(4')	38.0(1.0)	16.5(1.1)	32.7(1.0)	21.8(1.0)
θ_5	O(4')-C(1')-C(2')-C(3')	-25.9(1.0)	-8.4(1.1)	-17.0(1.0)	-6.0(1.1)
	Dioxolane				
θ_1	O(2')-C(2')-C(3')-O(3')	7.4(1.1)	0.6(1.1)	2.0(1.1)	-11.9(1.0)
θ_2	C(3')-C(2')-O(2')-C(10)	-23.6(1.2)	-18.9(1.2)	-19.6(1.1)	-8.1(1.1)
θ_3	C(2')-O(2')-C(10)-O(3')	30.4(1.2)	30.8(1.3)	30.4(1.1)	25.5(1.2)
θ_4	C(3')-O(3')-C(10)-O(2')	-25.7(1.3)	-30.2(1.3)	-28.3(1.1)	-33.3(1.1)
θ_5	C(2')-C(3')-O(3')-C(10)	10.7(1.2)	18.0(1.2)	16.0(1.1)	28.1(1.1)

It is seen that the ribose pucker is predominantly O(4')-**exo**, an unfavourable conformation for unsubstituted furanose rings, due to steric clashes between C(5') and N(9)/N(1) atoms of the purine/pyrimidine base[15]. This is in contrast to C(3')-**endo** in solutions, observed from NOE studies[12]. It is also different from C(2')-**endo**/ C(3')-**endo**, commonly found in inosine crystals. Conformation about the C(4')-C(5') bond (Table 2) in molecule B is g^+, different from g^- in the remaining molecules. Inosine compounds normally have shown g^+ although there are exceptions like inosine[16] and 2-ethylthio-8-methyl inosine monohydrate[17].

The dioxolane ring in molecules A and D show a twist conformation unlike in molecules B and C (envelope). The pseudorotation parameters for the dioxolane ring[18] are

Molecule	P_λ(°)	λ_m (°)	Nature of pucker
A	75.3	30.4	C(10)**exo**-O(2')-**endo**
B	88.9	31.9	C(10)-**exo**
C	85.9	30.8	C(10)-**exo**
D	111.6	33.2	O(3')**endo**-C(10)**exo**

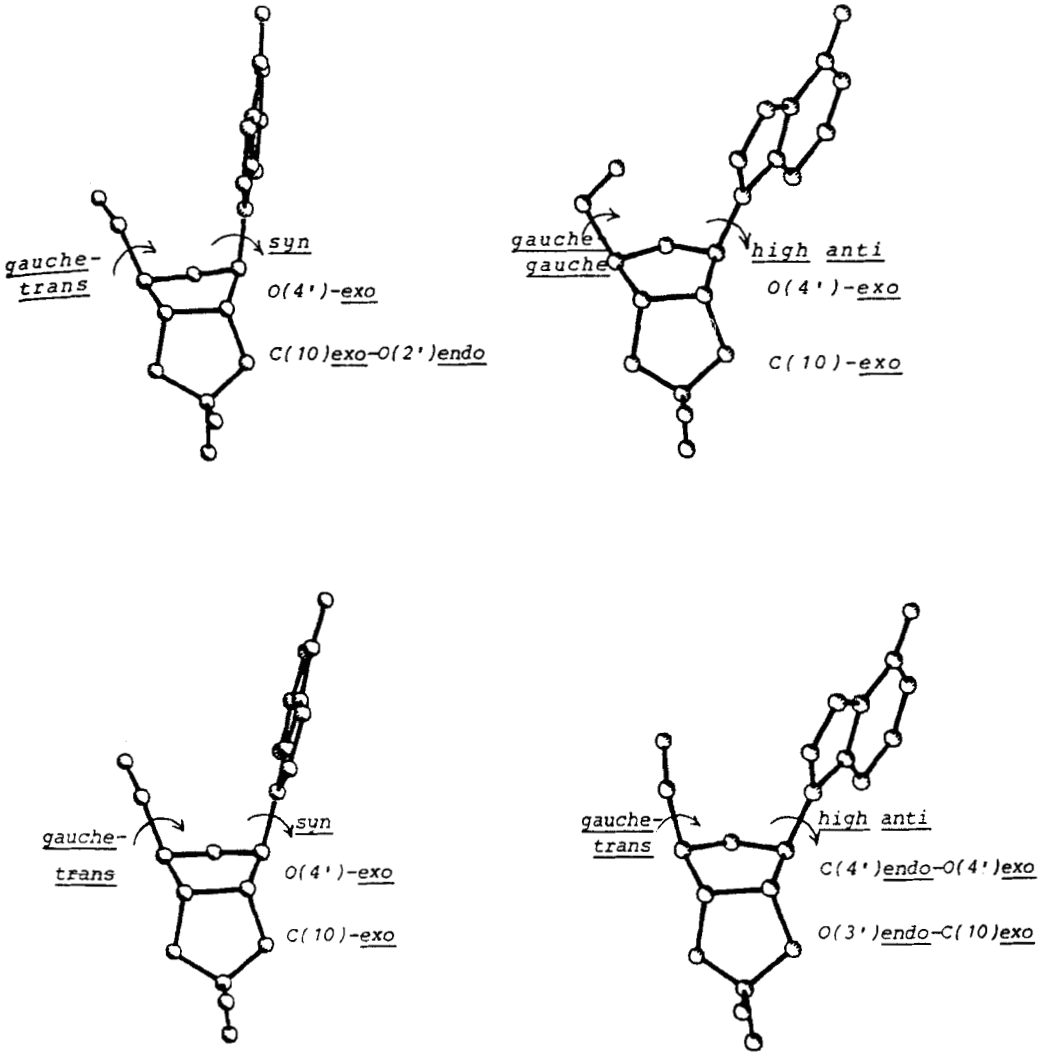

FIG. 2 Views of the four independent molecules showing conformational differences.

Thus the four independent molecules in the asymmetric unit of the crystal differ significantly in their conformational features. Application of Rossmann and Argos algorithm[19] shows that the independent molecules are related by pseudo translations (-0.04, 0.51. 0.00 for molecules A and D; 0.02, -0.47, 0.03 for B

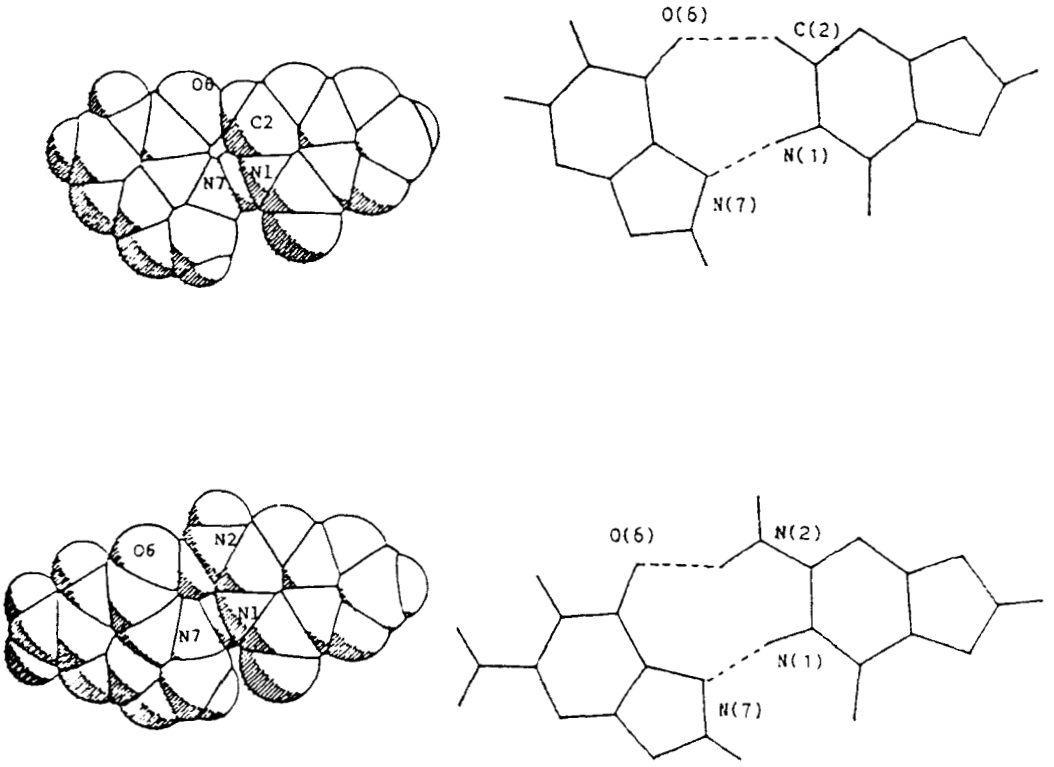

FIG. 3 I.I base pair observed in the present structure (top) is analogous to the G.G base pair observed in Iso-I (bottom) with a C(2)-H...O(6) interaction instead of N(2)-H...O(6) bond contributing to the stability of I.I base pairs.

Molecular packing:

I.I self base pairing:

Hypoxanthine bears a close similarity to guanine except for the presence of NH_2 attached to the C(2) atom in guanine. It is of interest to know how this affects the base pairing interactions in the two cases. The present study shows hypoxanthines form self base pairs (I.I) analogous to the guanine base pairs (G.G) found in Iso-G[5,6], with C(2)-H...O(6) however replacing the N(2)-H...O(6) hydrogen bond (Fig. 3). This unusual

TABLE 3

Hydrogen bond distances (Å) and angles (°).

Donor (A)	Acceptor (B)	A...B (Å)	H...B (Å)	A-H...B (°)	Symmetry of B *	Translation of B	
N(1)A	N(7)A	2.87(1)	1.81(8)	172(7)	2	0 2 2	
O(5')A	O(6)C	2.73(1)	1.65(1.2)	156(9)	1	0 0 0	
N(1)B	N(7)C	2.80(1)	1.90(8)	150(6)	2	0 2 2	
O(5')B	OW(2)	3.25(1)	-	-	1	0 1 0	#
N(1)C	N(7)B	2.77(1)	1.71(7)	168(6)	2	0 2 2	
O(5')C	O(6)A	2.80(1)			1	0 0 0	
N(1)D	N(7)D	2.86(2)	1.81(8)	174(7)	2	0 3 2	
O(5')D	N(3)C	2.81(1)	-	-	2	-1 2 2	#
O(5')D	OW(1)	2.91(1)	-	-	1	0 1 1	#
OW(1)	O(6)B	2.86(1)	-	-	1	0 -1 -1	#
OW(2)	O(6)D	2.83(1)	-	-	1	0 -1 0	#
OW(2)	O(4')B	3.17(1)	-	-	1	0 -1 0	#
C-H...O hydrogen bonds:							
C(8)A	O(5')C	3.22(1)	2.5(2)	138(1.2)	2	-1 2 2	
C(2)A	O(6)A	3.15(1)	2.2(1)	145(7)	2	0 2 2	
C(2)B	O(6)C	3.33(1)	2.44(9)	148(7)	2	0 2 2	
C(8)B	O(5')A	3.23(1)	2.4(1)	147(1.0)	2	-1 2 2	
C(5')B	O(2')C	3.15(1)	2.4(1)	138(1.0)	1	0 1 0	
C(2)C	O(6)B	3.23(1)	2.3(1)	141(8)	2	0 2 2	
C(8)C	OW(1)	3.13(1)	2.28(9)	148(7)	2	-1 1 1	
C(1')C	OW(2)	3.27(1)	2.52(9)	158(8)	1	0 0 0	
C(2)D	O(6)D	3.28(1)	2.42(9)	158(7)	2	0 3 2	
C(8)D	OW(2)	3.23(1)	2.3(1)	145(1.0)	2	-1 2 2	

* Symmetry codes:

1. x, y, z; 2. x+0.5, -y+0.5, -z

\# Hydrogens connected to O(5')B, O(5')D, OW(1) and OW(2) could not be located

base pairing has been seen in only one other inosine structure solved so far[20]. The crystal packing has several other short C-H...O contacts. Considering their geometrical characteristics and the fact that the C-H groups are adjacent to electronegative atoms, they are described as C-H...O hydrogen bonds[21,22]. Hydrogens involved in these have also been unambiguously located

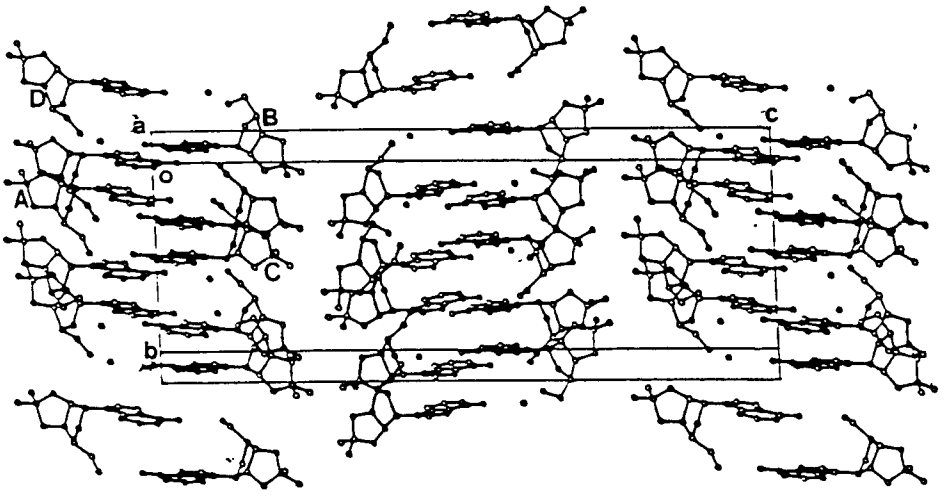

FIG. 4 Crystal packing showing bases stacked along the b-direction.

from difference Fourier- maps. Table 3 gives the details of hydrogen bonds in the structure.

Base stacking interaction:

We had noticed during data collection that reflection (040) with a Bragg spacing of 3.33 Å was the most intense of all the reflections. This suggested that the bases in the crystal structure essentially stacked perpendicular to the b-axis of the crystal. The X-ray analysis clearly demonstrates this feature (Fig. 4). It is seen that the base atoms are confined to the (040) planes which are separated at 3.3 Å intervals. Although the hypoxanthine rings do not show any direct ring-ring overlap, some of the exocyclic atoms show close contacts (3.2-3.5Å) with neighbouring ring atoms. The base stacking in Iso-I is different from that observed in Iso-G[5,6] and has also little in common with those found earlier in crystals containing hypoxanthine base.

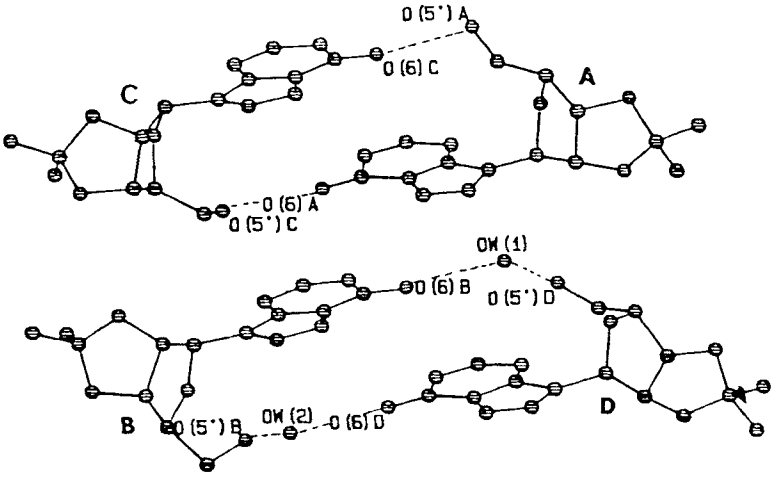

FIG. 5 View of the four independent molecules showing dimer formation.

An interesting feature of the structure is that the independent molecules in the asymmetric unit form dimers through hydrogen bonds, either directly or through water bridges as shown in Fig. 5. Base stacking between molecules B and D is less pronounced compared to that between A and C and can be attributed to the presence of water molecules between them.

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