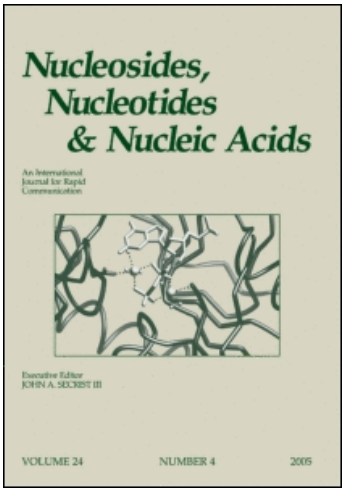


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# CONFORMATION OF 3-SUBSTITUTED PURINE NUCLEOSIDE STUDIED BY X-RAY CRYSTALLOGRAPHY AND THEORETICAL CALCULATIONS†

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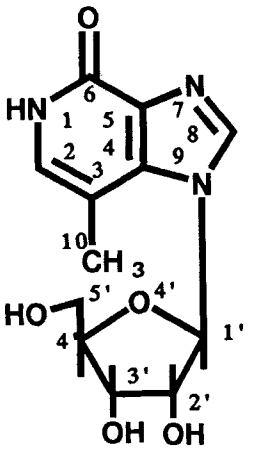
**ABSTRACT**: The molecular conformation of 3-methyl-3-deazainosine has been investigated by X-ray crystallographic and theoretical studies. In the crystal state the molecule has the ***high anti*** conformation about the glycosidic bond with the torsion angle of -79°. The sugar ring is puckered with C(1')-*exo*, C(2')-*endo*, and the conformation about the C(4')-C(5') bond is *gauche-trans*. The quantum chemical calculations show that the lowest energy conformation about the glycosidic bond of 3-methyl-3-deazainosine is *anti* shifted to ***high anti***, whereas in inosine the *syn* conformation is stable as well as the *anti* conformation.

## INTRODUCTION

The purine nucleosides having a substituent at the 3-position have been used as model compounds for investigations of physicochemical properties in the nucleosides with the glycosidic conformation fixed *anti*[1]. The 3-substituted purine nucleosides and nucleotides will be also useful for studies on glycosidic conformational aspects of nucleosides-enzyme interactions. In cellular nucleic acids, 3-substituted purines, for example 3-methyladenine and 3-methylguanine are produced by endogenous genotoxic

†This paper is dedicated with appreciation to Professor Morio Ikehara on the occasion of his 70th birthday.

agents such as S-adenosyl-methionine and by chemical alkylating agents. In DNA 3-methyladenine is a cytotoxic DNA lesion that blocks replication[2]. Therefore, the conformation of 3-substituted purine nucleosides is of interest. However, their X-ray crystal structures have rarely been reported,[3] and the details of their conformational properties have not been known well. In this paper, we describe the crystal structure of 3-methyl-3-deazainosine (3MDI) and the conformational analysis by the quantum chemical method.




3MDI

## EXPERIMENTAL

3MDI was synthesized as previously described[4] and recrystallized from water solution. The crystal data are as follows: $C_{12}H_{15}N_3O_5 \cdot 2H_2O$, space group $P2_12_12_1$, $a$ = 14.581(3), $b$ = 13.219(3), $c$ = 7.566(1) Å, $V$ = 1458.3(5) Å$^{-3}$, $Z$ = 4, $D_x$ = 1.444 Mg m$^{-3}$ , $F(000)$ = 672, $\mu$(Cu K$\alpha$) = 0.92 mm$^{-1}$. Intensity data were measured on a Rigaku AFC-5R with graphite-monochromated Cu $K\alpha$ radiation using the $\omega$-2$\theta$ scan method. Of the 1379 unique reflections within $\sin\theta/\lambda$ = 0.56, 1324 had $|Fo| > 1.0\sigma(Fo)$ and were considered as observed. The intensities were corrected for Lorentz and polarization but not for absorption because of a small crystal (crystal size, 0.3x0.3x0.1 mm$^3$). The structure was solved by direct methods using the program SHELXS-86[5] and refined by the full matrix least-squares method with anisotropic temperature factors for non-hydrogen atoms using the program SHELX-76[6]. All hydrogen atoms were located on difference Fourier maps and refined with fixed isotropic temperature factors. The final R value was 0.038 (unit weight). The final atomic parameters for non-hydrogen atoms are listed in Table 1. All numerical calculations were carried out on an ACOS 930 computer at the Research Center for Protein Engineering, Institute for Protein Research, Osaka University.

Molecular orbital and mechanics calculations of nucleosides were performed on a personal IRIS4D/25 using the program SPARTAN[7].

TABLE 1. Fractional coordinates of non-hydrogen atoms and equivalent isotropic temperature factors with e.s.d.'s in parentheses

$$Beq=8\pi^2/3\ \Sigma_i\ \Sigma_j\ (\ U_{ij}\ a^*_i\ a^*_j\ \mathbf{a}_i\ \mathbf{a}_j\ )$$

| atom | x | y | z | Beq(Å$^2$) |
|---|---|---|---|---|
| N(1) | 0.3378(2) | 1.1364(2) | 0.2841(5) | 3.35(9) |
| C(2) | 0.2470(3) | 1.1064(3) | 0.2832(6) | 3.38(10) |
| C(3) | 0.2208(2) | 1.0087(3) | 0.2776(6) | 2.97(9) |
| C(4) | 0.2942(2) | 0.9390(3) | 0.2699(5) | 2.40(8) |
| C(5) | 0.3862(2) | 0.9692(3) | 0.2683(6) | 2.66(9) |
| C(6) | 0.4112(3) | 1.0726(3) | 0.2773(6) | 3.09(10) |
| N(7) | 0.4436(2) | 0.8863(2) | 0.2564(5) | 3.22(8) |
| C(8) | 0.3891(3) | 0.8077(3) | 0.2529(7) | 3.13(10) |
| N(9) | 0.2976(2) | 0.8343(2) | 0.2600(5) | 2.49(7) |
| O(6) | 0.4916(2) | 1.1070(2) | 0.2784(5) | 4.10(8) |
| C(10) | 0.1207(3) | 0.9807(3) | 0.2785(9) | 4.06(13) |
| C(1') | 0.2205(2) | 0.7644(3) | 0.2541(6) | 2.53(8) |
| C(2') | 0.2443(3) | 0.6567(3) | 0.1962(5) | 2.62(9) |
| C(3') | 0.1592(3) | 0.6001(3) | 0.2624(5) | 2.98(9) |
| C(4') | 0.1345(3) | 0.6574(3) | 0.4312(5) | 2.54(9) |
| C(5') | 0.1590(3) | 0.6005(3) | 0.5968(5) | 2.84(9) |
| O(4') | 0.1854(2) | 0.7526(2) | 0.4269(3) | 3.38(7) |
| O(2') | 0.2641(2) | 0.6470(3) | 0.0157(4) | 3.25(7) |
| O(3') | 0.0866(2) | 0.6095(3) | 0.1376(4) | 4.21(8) |
| O(5') | 0.1299(2) | 0.6512(2) | 0.7527(4) | 2.66(6) |
| O(W1) | 0.0899(2) | 0.4154(4) | 0.0269(6) | 6.37(13) |
| O(W2) | 0.9524(5) | 0.7834(4) | 0.1749(8) | 10.8(2) |



## RESULTS AND DISCUSSION

Bond lengths and angles are given in Table 2. An *ORTEP*[8] drawing of 3MDI molecule is shown in Fig. 1. The selected torsion angles are given in Table 3.

***3-Methyl,3-deazaxanthine moiety*** --- The 3-deazaxanthine moiety is essentially planar with the maximum deviation of 0.015(5) Å at C(2) from the least-squares plane. The C(10) atom of the methyl group is just on the plane and is only displaced by 0.007(8) Å.

The bond lengths in the 3-deazaxanthine moiety are in good agreement with ones observed in 3-deazaguanosine[9]. As expected, the C(2)-C(3) and C(3)-C(4) bonds are

TABLE 2. Bond lengths (Å) and angles (°)

| | | | |
|---|---|---|---|
| N(1)–C(2) | 1.382(6) | C(3)–C(10) | 1.506(8) |
| C(2)–C(3) | 1.347(6) | N(9)–C(1') | 1.456(6) |
| C(3)–C(4) | 1.413(6) | C(1')–C(3') | 1.529(6) |
| C(4)–C(5) | 1.400(6) | C(2')–C(3') | 1.533(6) |
| C(5)–C(6) | 1.416(6) | C(3')–C(4') | 1.528(6) |
| N(1)–C(6) | 1.364(6) | O(4')–C(1') | 1.413(5) |
| C(5)–N(7) | 1.382(6) | C(4')–O(4') | 1.461(5) |
| N(7)–C(8) | 1.308(7) | C(4')–C(5') | 1.504(6) |
| C(8)–N(9) | 1.381(6) | C(2')–O(2') | 1.402(5) |
| C(4)–N(9) | 1.387(5) | C(3')–O(3') | 1.424(5) |
| C(6)–O(6) | 1.257(6) | C(5')–O(5') | 1.421(5) |
| | | | |
| C(2)–N(1)–C(6) | 125.0(4) | C(3)–C(4)–N(9) | 132.8(4) |
| N(1)–C(2)–C(3) | 123.1(4) | C(6)–C(5)–N(7) | 127.8(4) |
| C(2)–C(3)–C(4) | 114.3(4) | O(4')–C(1')–C(2') | 104.2(3) |
| C(3)–C(4)–C(5) | 122.7(4) | C(1')–C(2')–C(3') | 100.2(3) |
| C(4)–C(5)–C(6) | 121.4(4) | C(2')–C(3')–C(4') | 102.8(3) |
| N(1)–C(6)–C(5) | 113.4(4) | C(3')–C(4')–O(4') | 106.8(3) |
| C(5)–C(4)–N(9) | 104.5(3) | C(4')–O(4')–C(1') | 107.4(3) |
| C(4)–N(9)–C(8) | 106.9(4) | N(9)–C(1')–C(2') | 115.1(3) |
| N(7)–C(8)–N(9) | 112.6(4) | N(9)–C(1')–O(4') | 108.8(3) |
| C(5)–N(7)–C(8) | 105.3(4) | C(1')–C(2')–O(2') | 114.3(3) |
| C(4)–C(5)–N(7) | 110.8(4) | C(3')–C(2')–O(2') | 116.1(3) |
| N(1)–C(6)–O(6) | 120.5(4) | C(2')–C(3')–O(3') | 110.0(3) |
| C(5)–C(6)–O(6) | 126.1(4) | C(4')–C(3')–O(3') | 109.6(3) |
| C(2)–C(3)–C(10) | 120.7(4) | C(5')–C(4')–C(3') | 113.1(3) |
| C(4)–C(3)–C(10) | 125.0(4) | C(5')–C(4')–O(4') | 109.2(3) |
| C(4)–N(9)–C(1') | 127.4(3) | C(4')–C(5')–O(5') | 112.6(3) |
| C(8)–N(9)–C(1') | 125.7(4) | | |



longer by 0.04–0.05 Å than corresponding ones (C(2)–N(3); 1.308 Å, N(3)–C(4); 1.363 Å) in the usual xanthine moiety[10]. The 0.03 Å lengthening of the C(4)–C(5) and C(6)–O(6) bonds is also observed. These bond lengths provide the evidence of increasing delocalization of electrons in a O(6)–C(6)–C(5)–C(4)–C(3)–C(2) fragment as compared to the xanthine moiety. The C(2)–C(3)–C(4) angle is 3° larger than in the xanthine moiety, whereas the C(3)–C(4)–C(5) angle is 6° smaller. A similar difference is observed in the angles around N(3) and C(4) between the 3-alkyladenine and adenine moieties[11]. However, it is unknown whether the difference between 3MDI and the xanthine moieties is due to

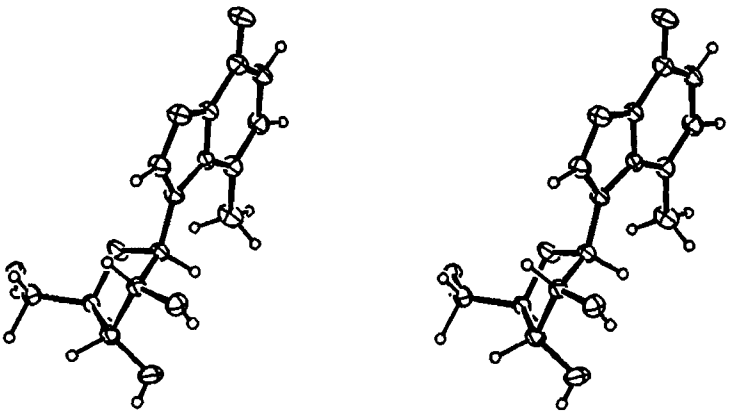


FIG. 1. An ORTEP drawing of 3MDI molecule



TABLE 3. Selected torsion angles (°)

| | | |
|---|---|---|
| $\chi$ | C(4)–N(9)–C(1')–O(4') | –78.7(5) |
| | C(8)–N(9)–C(1')–O(4') | 102.8(5) |
| $\tau_0$ | C(4')–O(4')–C(1')–C(2') | –35.5(4) |
| $\tau_1$ | O(4')–C(1')–C(2')–C(3') | 43.5(4) |
| $\tau_2$ | C(1')–C(2')–C(3')–C(4') | –34.2(4) |
| $\tau_3$ | C(2')–C(3')–C(4')–O(4') | 14.8(4) |
| $\tau_4$ | C(3')–C(4')–O(4')–C(1') | 12.8(4) |
| $\psi_{oc}$ | O(5')–C(5')–C(4')–C(3') | –175.8(3) |
| $\psi_{oo}$ | O(5')–C(5')–C(4')–O(4') | 65.5(4) |

the effect of methylation or/and deazation, because of the lack of the bond angles and coordinates of 3–deazaguanosine in ref. 9.

***Glycosidic bond*** --- The glycosidic torsion angle of –78.7(5)° indicates that the glycosidic conformation is in *anti* ($\chi$: –180° ~ –60°), especially ***high anti*** ($\chi$: –90° ~ –60°) region. A similar ***high anti*** conformation is found in 3–methyladenosine *p*–toluenesulfonate[3]. Many crystallographic, spectroscopic and theoretical studies on β–purine nucleosides have shown that the rotational barrier around the glycosidic bond is small and three kinds of

conformations such as *anti, high-anti* and *syn* are preferred[12]. On the other hand, in the cases of 3-substituted purine nucleosides, in both of the nucleosides reported the crystal structures take the *high-anti* conformation. These might mean a preference for the *high anti* conformation for 3-substituted purine nucleosides.

In order to investigate the conformational properties of the glycosidic bond for 3-substituted purine nucleosides, the conformational analysis around the glycosidic bond for 3MDI and inosine was performed using the program SPARTAN[7] with the molecular mechanics and orbital methods. First, the stable conformation was searched by molecular mechanics calculations using the TRIPOS5.2 force field when the glycosidic torsion angles were rotated by every 30°. Then they were optimized by molecular orbital calculations with the PM3 method. The result is shown in Table 4. In the case of 3MDI, the conformational search revealed that the initial twelve conformers degenerated into seven kinds of conformers. After optimization the seven conformers converged into only two conformers. One of them is *anti* with a torsion angle of -111°, and the other is *syn* with a torsion angle of 70°. The difference of total energy between *anti* and *syn* conformers is 8.0 kcal/mol. In inosine, three conformers ($\chi$ : -160°, -136° and 58°) appeared as optimized conformers, and the total energies of each of them are within 0.7 kcal/mol of one another. As compared to inosine, the *anti* conformer of 3MDI is considerably more stable than the *syn*, which is consistent with the observation by NMR spectroscopy[4], and the optimized *anti* conformer of 3MDI trends to approximate to the *high anti* conformer as found in the crystal. The results support the view that 3MDI has limited flexibility about the glycosidic conformation because of steric hindrance between the methyl group at the 3 position of purine and the sugar moiety.

There are interesting features in the bond angles around N(9). It is known that the bond angles around N(9) depend on the glycosidic conformation[12]. Generally, the C(8)-N(9)-C(1') angle is larger than the C(4)-N(9)-C(1') angle in nucleosides in the *anti* conformation, whereas nucleosides with the *syn* conformation have the larger C(4)-N(9)-C(1') angle than the C(8)-N(9)-C(1') angle[13]. In 3MDI, the C(4)-N(9)-C(1') angle is larger than the C(8)-N(9)-C(1') in spite of being the *anti* conformation. A similar observation is found in 3-methyladenosine *p*-toluenesulfonate[3]. This results from avoiding the short contact between the methyl group at the 3-position of base and the sugar moiety.

TABLE 4. Conformational analysis for the glycosidic bond by the program SPARTAN

| | 3MDI | | | | inosine | | | |
|---|---|---|---|---|---|---|---|---|
| Start model | Conformer search | | Optimization | | Conformer search | | Optimization | |
| χ (°) | χ (°) | type | χ (°) | ΔE(kcal/mol) | χ (°) | type | χ (°) | ΔE(kcal/mol) |
| -180 | -166 | A | -111 | 0 | -171 | A | -160 | 0.7 |
| -150 | -142 | B | -111 | 0 | -167 | A | -160 | 0.7 |
| -120 | -142 | B | -111 | 0 | -171 | A | -160 | 0.7 |
| -90 | -91 | C | -111 | 0 | -145 | B | -137 | 0.7 |
| -60 | -48 | D | -111 | 0 | -63 | D | -135 | 0.7 |
| -30 | -29 | E | -111 | 0 | -7 | F | 58 | 0 |
| 0 | 2 | F | -110 | 0 | -9 | F | 58 | 0 |
| 30 | 31 | H | 70 | 8.0 | 10 | G | 58 | 0 |
| 60 | 34 | H | 70 | 8.0 | 27 | H | 58 | 0 |
| 90 | 35 | H | 70 | 8.0 | 9 | G | 58 | 0 |
| 120 | -173 | A | -111 | 0 | 116 | I | 58 | 0 |
| 150 | -173 | A | -111 | 0 | 142 | J | -160 | 0.7 |



***Sugar moiety*** --- The sugar pucker is C(1')-*exo*, C(2')-*endo* with the pseudorotation phase angle $P$ = 143.8° and the degree of pucker $\tau_m$ = 44.0°. The C(1')-*exo*, C(2')-*endo* conformation belongs to the S-type pucker but slightly deviates from a typical S-type. This fact is not surprising, because the tendency of sugar conformations shifting from a typical S-type, C(2')-endo, to C(1')-exo conformations has been observed in nucleosides with the ***high anti*** glycosidic conformer[14]. The orientation of the exocyclic C(5')-O(5') bond described by the torsion angles $\psi_{oo}$ and $\psi_{oc}$, is *gauche-trans*.

The bond lengths and angles in the sugar moiety are similar to those in the ribose with C(2)-*endo*[12].

***Hydrogen bonds and molecular arrangement*** --- The molecular packing is shown in Fig. 2. The hydrogen bond parameters are listed in Table 5. All hydrogen atoms attached to the oxygen and nitrogen atoms except one hydrogen atom attached to O(W2) participate in hydrogen bonds. The hydrogen atom at C(8) also participates in the C(8)-H---O(6) hydrogen bond. Their hydrogen bond network elongates parallel to *b*. Among the hydrogen

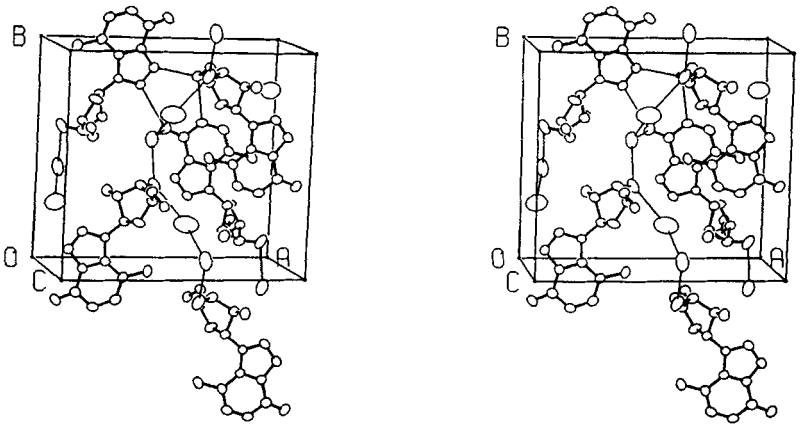



FIG. 2. Stereoview of the molecular arrangement in a unit-cell.
Thin lines indicate hydrogen bonds.



TABLE 5. Hydrogen bond parameters

| | | A-H--D (Å) | H--D (Å) | A-H--D (°) |
|---|---|---|---|---|
| N(1)-H---O(5') | -x+1/2,-y+2,z-1 | 2.857(5) | 1.94(4) | 170(4) |
| O(2')-H---O(5') | x,y,z-1 | 2.791(5) | 2.21(6) | 176(6) |
| O(3')-H---O(W1) | x,y,z | 2.699(6) | 1.85(5) | 173(5) |
| O(5')-H---N(7) | x-1/2,-y+3/2,-z+1 | 2.762(5) | 1.85(5) | 167(5) |
| O(W1)-H---O(6) | z-1/2,-y+3/2,-z | 2.735(6) | 1.90(5) | 171(5) |
| O(W1)-H---O(W2) | -x+1,y-1/2,-z+1/2 | 2.918(8) | 1.95(5) | 175(4) |
| O(W2)-H---O(3') | x-1,-y+1/2,-z | 3.032(8) | 2.32(5) | 119(3) |
| C(8)-H---O(6) | -x,+1,y-1/2,-z+1/2 | 3.181(7) | 2.28(5) | 160(4) |

bonded layers, 3-methyl-3-deazaxanthine rings form the hydrophobic columns parallel to *c*. The separation between successive aromatic rings is 3.7 Å, which is longer than the normal van der Waals separation distance (3.4 Å). Totally, the molecular arrangement is stabilized by the hydrogen bonds and the hydrophobic interactions.

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