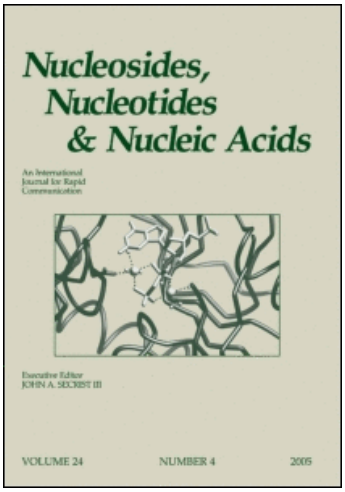

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Trifurcated (Four-Center) Hydrogen Bond in Solid State Crystal Structure of 5'-Amino-5'-deoxyadenosine p-Toluenesulfonate

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TRIFURCATED (FOUR-CENTER) HYDROGEN BOND IN SOLID STATE CRYSTAL STRUCTURE OF 5'-AMINO-5'-DEOXYADENOSINE p-TOLUENESULFONATE

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Abstract: The crystal structure of 5'-amino-5'-deoxyadenosine (5'-Am.dA) p-toluenesulfonate has been determined by X-ray crystallographic methods. It belongs to the orthorhombic space group $P2_12_12_1$ with a=7.754(3)Å, b=8.065(1)Å and c=32.481(2)Å. This nucleoside shows a *syn* conformation about the glycosyl bond and C2'-endo-C3'-exo puckering for the ribose sugar. The orientation of N5' atom is gauche-trans about the exocyclic C4'-C5' bond. The amino nitrogen N5' forms a trifurcated hydrogen bond with N3, O9T and O4' atoms. Adenine bases form A.A.A triplets through hydrogen bonding between N6, N7 and N1 atoms of symmetry related nucleoside molecules.

Introduction

Molecular biologists interest on aminopurines stems from its potentiality to introduce transition in DNA by altering the base pairing pattern. As part of our systematic X-ray crystallographic studies[1,2,3,4] on ribose/deoxyribose nucleosides and nucleotides we have now obtained the three dimensional structure of 5'-Am.dA p-toluenesulfonate. This investigation was taken up with a view to delineate the conformational differences between 5', 3' and 2' amino deoxyadenosines and unravelling the effect of amino substitution on the conformation of this nucleoside.

EXPERIMENTAL

Crystals of 5'-Am.dA p-toluenesulfonate were grown by direct evaporation from a 500mM water/ethanol solution of the compound obtained from Sigma Chemical Company(USA). Unit cell parameters were obtained by indexing 25 reflections in the

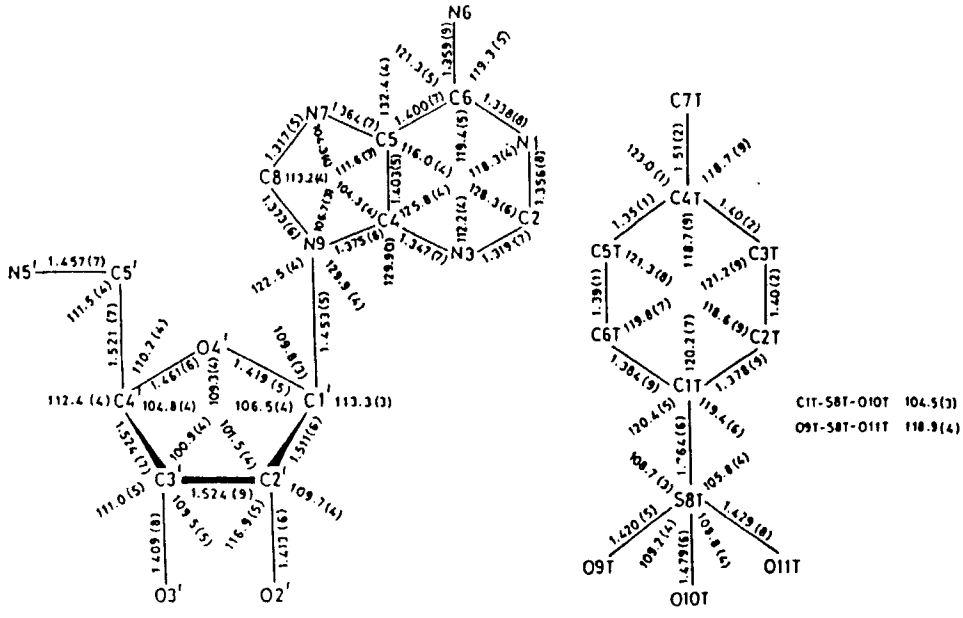

Fig. 1. Bond lengths and bond angles in 5'-Am.dA p-toluenesulfonate

range $12° \leq \theta \leq 25°$ obtained from an automatic search on a CAD-4 diffractometer. They were later refined by least square calculations. CuKα three dimensional intensity data upto $\sin\theta/\lambda$=0.62Å^{-1} were collected on a diffractometer and was corrected for Lorentz, polarisation and absorption errors (μ=17.9cm^{-1}). 2083 out of 2248 reflections having $F_o \geq 3\sigma(F_o)$ were considered observed. Two monitor reflections 1 4 0 and 2 0 -8 showed negligible variation during data collection indicating crystal and instrument stability.

Crystal data are:

C_{17} N_6 O_6 H_{22} S, Mw 438.50, orthorhombic, $P2_12_12_1$,

a = 7.754(3) Å, b = 8.065(1) Å, c = 32.481(2) Å, V=2031.4 Å^3, Z=4,

Dc = 1.42 g/cm^3, Dm = 1.42 g/cm^3 (acetone/bromoform mixtures).

The structure was solved using SHELX86[5]. All the molecular atoms were located from a Fourier map, except the methyl carbon of the p-toluenesulfonate ion, which was identified from a difference Fourier computed subsequently. The structure was refined using SHELX76[6] by full matrix least square refinement with isotropic

TABLE 1.

Final atomic coordinates with e. s. d's in parentheses and equivalent isotropic values of the anisotropic thermal parameters for non hydrogen atoms $B_{eq}=\frac{4}{3}\Sigma_i\Sigma_j\beta_{ij}a_ia_j$.

ATOM	x	y	z	B_{eq}
N1	-0.6137(6)	0.3818(9)	0.9526(1)	4.57(1)
C2	-0.5837(7)	0.4291(10)	0.9132(2)	4.20(1)
N3	-0.6899(6)	0.4231(7)	0.8815(1)	3.83(1)
C4	-0.8441(6)	0.3580(6)	0.8916(1)	2.77(1)
C5	-0.8931(6)	0.3032(7)	0.9309(1)	3.37(1)
C6	-0.7681(7)	0.3176(10)	0.9618(2)	3.98(1)
N6	-0.8011(7)	0.2694(12)	1.0011(2)	5.89(2)
N7	-1.0574(6)	0.2427(8)	0.9310(1)	3.97(1)
C8	-1.1091(6)	0.2590(8)	0.8926(1)	3.45(1)
N9	-0.9860(5)	0.3274(5)	0.8674(1)	2.71(1)
C1'	-1.0230(6)	0.3796(5)	0.8255(1)	2.21(1)
C2'	-1.0593(6)	0.5631(6)	0.8218(2)	3.20(1)
C3'	-1.0074(7)	0.5964(7)	0.7773(2)	3.90(1)
C4'	-0.8451(6)	0.4908(7)	0.7739(1)	3.04(1)
O4'	-0.8788(4)	0.3466(4)	0.7999(1)	2.63(1)
O3'	-1.1372(7)	0.5376(8)	0.7507(2)	6.74(2)
O2'	-1.2327(5)	0.5952(5)	0.8325(2)	4.48(1)
C5'	-0.6850(6)	0.5817(6)	0.7888(2)	3.18(1)
N5'	-0.5395(6)	0.4692(7)	0.7937(2)	4.12(1)
C1T	0.4242(8)	0.9491(7)	0.8666(2)	3.81(1)
C2T	0.4874(12)	0.9229(15)	0.9057(2)	6.82(2)
C3T	0.3723(20)	0.8761(18)	0.9367(3)	9.78(4)
C4T	0.1956(15)	0.8648(12)	0.9291(3)	6.71(2)
C5T	0.1368(10)	0.8986(10)	0.8908(3)	5.42(2)
C6T	0.2486(8)	0.9418(8)	0.8592(2)	4.12(1)
C7T	0.0784(21)	0.8112(19)	0.9636(4)	10.46(5)
S8T	0.5689(2)	0.9952(2)	0.8263(0)	4.08(0)
O9T	0.5843(8)	1.1701(6)	0.8229(2)	6.15(2)
O10T	0.7354(7)	0.9252(7)	0.8401(2)	6.35(2)
O11T	0.5095(11)	0.9030(11)	0.7915(2)	8.45(2)

thermal parameters in the initial stages and anisotropic temperature factors in the final cycles for all non-hydrogen atoms to a R of 0.068. 11 out of 22 hydrogens, which includes the 5'-aminohydrogen (N5'H) participating in the trifurcated hydrogen bond, were located from difference Fourier maps and remaining hydrogens were fixed from stereochemical considerations. The other two 5'-aminohydrogens (N5'H1 &

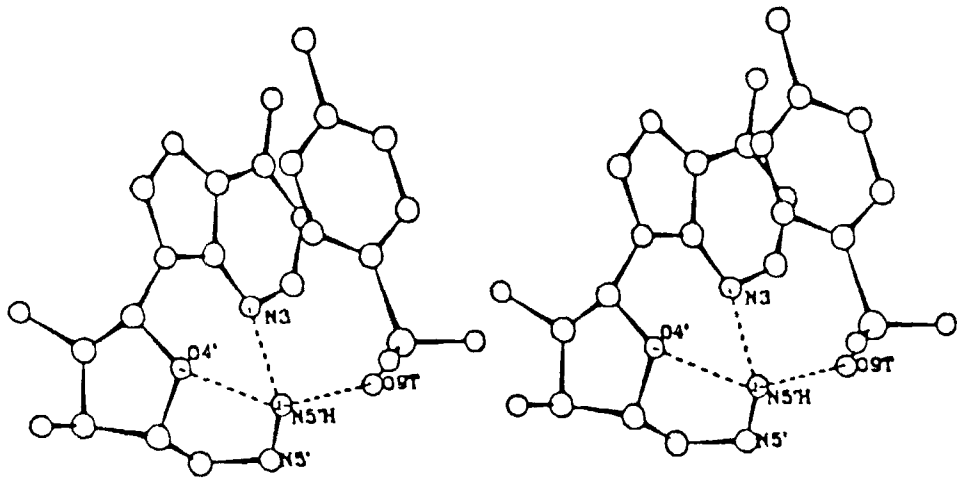

Fig. 2. A stereoview of 5′-Am.dA p-toluenesulfonate showing its *syn* conformation and the trifurcated hydrogen bond

N5′H2) were fixed tetrahedrally to N5′. All hydrogens were refined isotropically along with non-hydrogen atoms to a final R of 0.055. Atomic numbering in 5′-Am.dA p-toluenesulfonate is shown in Fig.1.

RESULTS AND DISCUSSION

Atomic coordinates are given in Table 1. The molecular conformation of 5′-Am.dA p-toluenesulfonate is shown in Fig.2.

Adenine base

The adenine base is planar including the N9 atom. The bond lengths and bond angles depicted in Fig.1 agree with the average values reported for purine structures.[7] The orientation of the adenine base with respect to the deoxyribose moiety is *syn* as shown in Fig.2 with glycosyl torsion angle χ(O4′-C1′-C9-C4) = 48.4(6)°. It is further stabilized by an intramolecular hydrogen bond between N5′ and N3 atom of the base as in 5′-methylammonium-5′-deoxyadenosine[8] (5′-Me.Am.dA) and similar to that between O5′ and N3 atoms in A2′p5′A[1], G2′p5′C[2] and other purine structures reported in literature[9]. In contrast the conformation about the glycosyl bond in α-D-

TABLE 2.

Selected torsion angles(°) in 5'Am.dA

N5' -C5' -C4' -O4'	51.2(6)	N5' -C5' -C4' -C3'	167.7(5)
O4' -C1' -C2' -C3'	33.1(5)	C1' -C2' -C3' -C4'	-40.4(5)
C2' -C3' -C4' -O4'	34.4(5)	C3' -C4' -O4' -C1'	-14.7(5)
C2' -C1' -O4' -C4'	-11.7(5)	C4 -N9 -C1' -O4'	48.4(6)

2'-amino-2'-deoxyadenosine[10] (2'-Am.dA), 3'-amino-3'-deoxyadenosine[11] (3'-Am.dA) and 3'-amino-3'-deoxy-N,N-dimethyladenosine[12] (3'-Am.dime.dA) is anti (Table 4).

Furanose ring

The C2' and C3' atoms are displaced by 0.29Å and 0.32Å from the plane constituted by C1'-C4'-O4' atoms on the same and opposite sides of C5' atom respectively indicating C2'-endo-C3'-exo puckering of the ribose moiety. Pseudorotation parameters[13] for the furanose ring are τ_m = 40.4(3)° and P = 181.9(4)°. The conformation about the C4'-C5' exocyclic bond is gauche-trans(g^-) with ϕ_{NO}=51.2(6)° and ϕ_{NC}=167.8(1)° (Table 2). Similar puckering is observed in 5'-Me.Am.dA. On the other hand the furanose ring puckering in 3'-Am.dA is C3'-endo, C3'-exo in 2'-Am.dA and C3'-endo-C2'-exo in 3'-Am.dime.dA (Table 4). A comparison of the structural features of 5'-Am.dA with other structures suggests the earlier observation by Rao and Sundaralingam[9] that there is a definite correlation between the *syn* geometry and C2'-endo puckering of the ribose moiety still holds good since both C2'-endo-C3'-exo and C2'-endo belong to S-type puckering with hardly any energy difference between them.

Charge neutralisation

The negatively charged SO_3 group of the (toluenesulfonate) solvate molecule necessarily requires a positive charge in the asymmetric unit for neutralisation. However, the difference Fourier map did not reveal any hydrogen near N1 atom of the base. In addition, the neutrality of the base was evident from the C6-N1-C2 bond angle of 118.3(4)° being significantly less than the lower limit of 121.3° expected for base protonated structures.[7] This implies protonation of th N5' amino group.

TABLE 3.
Hydrogen bonding in 5'Am.dA p-toluenesulfonate
distances(Å) and angles(°)

X -H...Y	X...H	X -Y	H...Y	X -H...Y (°)	SYMM
N6 -N6H1...N7	0.8	2.91(1)	2.1	151(8)	1
N5' -N5'H..O9T	1.1	2.76(1)	2.1	120(1)	6
N5' -N5'H..N3	1.1	3.10(1)	2.4	125(0)	0
N5' -N5'H..O4'	1.1	2.82(1)	2.4	99(0)	0
N5' -N5'H1..O2'	1.1	2.88(1)	1.8	162(1)	5
N5' -N5'H2..O11T	1.1	2.83(1)	1.8	154(1)	4
N6 -N1	—	3.16(2)	—	—	2
O3' -O4'	—	2.99(1)	—	—	3
O2' -O10T	—	2.68(1)	—	—	7

symmetry codes: 0. x,y,z 1. x+0.5,-y+0.5,-z+2 2. x-0.5,-y+0.5,-z+2 3. -x-2,y+0.5,-z+1.5 4. -x,y-0.5,-z+1.5 5. x+1,y,z 6. x-1,y-1,z 7. x-2,y,z

Trifurcated hydrogen bond

A spectacular feature of 5'-Am.dA nucleoside is the trifurcated hydrogen bond formation observed in less than 1% crystal structures studied so far.[14,15] In this structure the aminonitrogen N5' forms a trifurcated hydrogen bond with the sulfonyl oxygen (O9T), the base nitrogen (N3) and the ribose oxygen (O4') at distances 2.76Å, 3.10Å and 2.82Å respectively as illustrated in Table 3 and Fig.2. Never before a trifurcated hydrogen bond has been reported with O-S as acceptor in nucleoside/nucleotide crystal structures. However, in the neutron diffraction studies[16] of sulfamic acid all three amino protons form bifurcated hydrogen bonds with sulfonyl group with O-S as acceptor. Perhaps, justification for classifying the linkage between N5' and N3, O9T and O4' as trifurcated hydrogen bond based on geometrical considerations will be worthwhile. In this case, two important hydrogen bond criteria namely donor-acceptor distance lower than sum of van der Waals radii, distance and angles involving hydrogen atom within prescribed limits are satisfied. The deviation of 0.58Å for the hydrogen atom from the plane constituted by N5', O9T and N3 is significantly larger than the putative value of 0.2Å for bifurcated hydrogen bonds. Moreover, the difference of 5° between the angles N5' -N5'H..O9T(120°) and N5' -N5'H..N3(125°) is

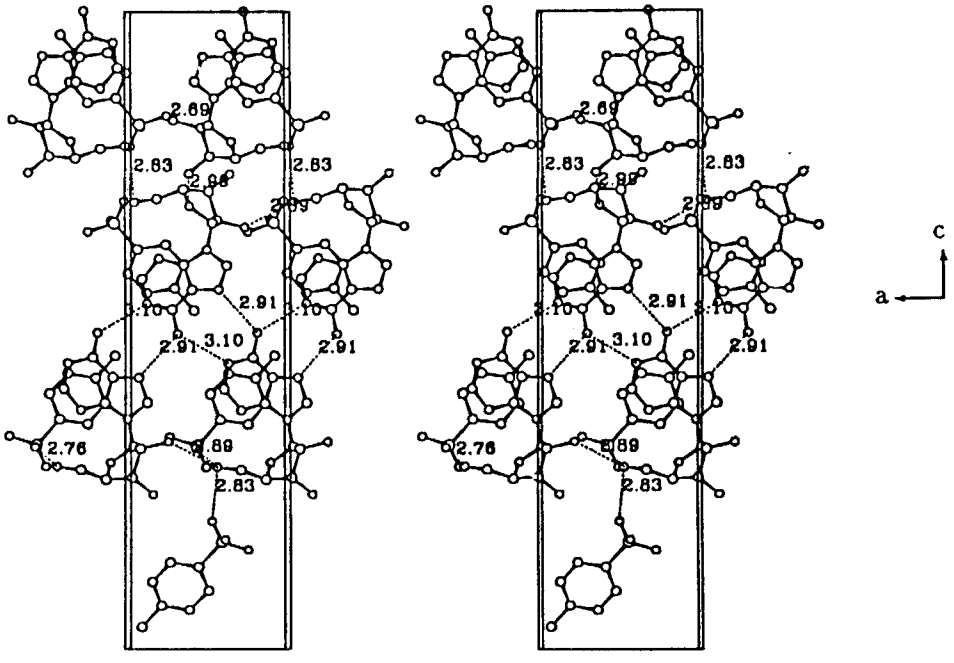

Fig. 3. A stereoview of packing of 5'-Am.dA p-toluenesulfonate in the orthorhombic lattice viewed down b axis. Hydrogen bonds are shown by dashed lines

significantly less than $\simeq 70°$ expected for asymmetric bifurcated hydrogen bonds.[15] Finally, participation of O4' in intramolecular bifurcated hydrogen bonding in nucleosides and nucleotides crystal structures has been well established.[15]

Hydrogen bonding and packing

The adenine base of 5'-Am.dA and the tosylate molecule are oriented nearly parallel to the ac plane (Fig.3) and stack along the b-axis alternately with an interplanar separation of 3.57Å and 14.5(2)° dihedral angle between them. Similar stacking has been observed in 3-methyladenosine p-toluenesulfonate[17]with an interplanar distance of 3.60Å and 10.6° dihedral angle. The N6 atom of the base forms hydrogen bonds with the N1 and N7 atoms of the symmetry related nucleoside molecules generating a A.A.A triplet by utilising both Watson-Crick and Hoogsteen sites of adenine as shown Fig.3. It may be mentioned that the hydrogen bonding pattern in 5'-Am.dA forming A.A.A resembles those observed in other adenosine structures[17,18,19,20] and

TABLE 4.

Comparison of conformational features of aminoadenosines

Name	Base conformation about glycosidic bond	Sugar pucker	Orientation about C5′-C4′
3′-Am.dA	Anti	C3′-endo	gt
3′-Am.dime.dA	Anti	C3′-endo-C2′-exo	gg and gt
2′-Am.dA	Anti	C3′-exo	gt
5′-Me.Am.dA	Syn	C2′-endo-C3′-exo	gt
5′-Am.dA	Syn	C2′-endo-C3′-exo	gt

is similar to the schemes proposed for linking A with its adjacent models for U.AU/ T.AT /A.AT/ triple helices.[21,22,23] The dihedral angle between the bases is 40° indicating a significantly higher propeller like twist between the hydrogen bonded bases. No base paring is observed in 2′-Am.dA, 3′-Am.dA and 5′-Me.Am.dA. And the amino protons in both 3′-Am.dA and 3′-Am.dime.dA are not involved in hydrogen bonding (but in both cases the N3′ atom accepts a hydrogen bond from the symmetry related O2′ atom of the sugar) unlike in 2′-Am.dA in which one of the amino protons makes an intramolecular hydrogen bond with the adjacent O3′ atom of sugar but does not interact with the adenine base as in 5′-Am.dA.

Summary and Conclusion

Table 4 presents a comparison of conformational features of 5′-Am.dA with its 2′ and 3′ analogous. It is obvious from Table.4 that the effect of amino substitution at 5′ on the conformation of the nucleoside is certainely not the same as at 2′ and 3′ sites of the ribose moiety. However it may be necessary to exercise some caution because the contribution of the sulfonate ion which is non-covalently bound to the nucleoside to the molecular conformation of the nucleoside could not be ignored. Perhaps NMR solution studies on these aminonucleosides may provide more information cn this aspect.

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