Purines. XXXI.¹⁾ An X-Ray Crystallographic Structure Analysis of 3-Methyladenosine *p*-Toluenesulfonate

Tozo FUJII,^{*,a} Tohru SAITO,^a and Tadamasa DATE^b

Faculty of Pharmaceutical Sciences, Kanazawa University,^a Takara-machi, Kanazawa 920, Japan and Research Laboratories, Tanabe Seiyaku, Co., Ltd.,^b Kawagishi-2-chome, Toda 335, Japan. Received October 3, 1988

The X-ray crystal structure of the title compound (3·TsOH) has been determined. In the crystal, this salt has the exocyclic iminium structure (type 5). The adenine moiety is almost planar, and the N(9)–C(1') bond is almost coplanar with the adenine ring. However, the N(3)-methyl group is displaced rather significantly from planarity, and the endocyclic and exocyclic angles at N(3), C(4), and N(9) notably depart from those of the usual adenosine systems. The ribose moiety is in the C(2')-*endo* puckering conformation and in the *high-anti* conformation [with the torsion angle O(1')–C(1')–N(9)–C(4), $\chi = -72.3^\circ$] with respect to the adenine moiety.

Keywords 3-methyladenosine *p*-toluenesulfonate; X-ray crystal structure; exocyclic iminium structure; *high-anti* conformation; hydrogen bonding; 3,9-dimethyladenine; molecular orbital calculation; protonation site

Since methylation of deoxyribonucleic acid (DNA) causes release of 3-methyladenine (1), 3-methyl-2'-deoxyadenosine (2) has been assumed to occur in methylated DNA molecules as a part structure.²⁾ Because of the extraordinary instability of its glycosidic bond to hydrolysis at the polynucleotide level,^{2b,c,j)} it is of prime importance to learn the nature of this part structure at the nucleoside level. We have synthesized 3-methyladenosine (3)³⁾ and 3-methyl-2'-deoxyadenosine (2),⁴⁾ both in the form of the *p*-toluenesulfonate salt, and found that they are readily susceptible to hydrolysis leading to 3-methyladenine (1) in aqueous acidic solution and to ring opening of the adenine moiety at the 2-position under basic conditions.^{3,4)} We have also synthesized a series of 3,9-dialkyladeninium salts (4)⁵⁾

No absorption corrections were applied.

Structure Determination and Refinement The structure was solved by direct methods using the program MULTAN⁶⁾ and the difference Fourier method. Refinement of atomic parameters was carried out using the block-diagonal least-squares method with anisotropic temperature factors. The hydrogen atoms were located on a difference Fourier map and refined with isotropic temperature factors. Throughout the refinement, the function $\sum w(|F_o| - |F_c|)^2$ was minimized. The weight $1/\sigma^2(F)$ was used during the final refinement stage and 7 reflections were deleted; the final *R* value was 0.048 ($R_w = 0.051$). The atomic scattering factors were taken from the literature.⁷⁾

Results

For convenience of designating the non-hydrogen atoms in the nucleoside salt 3·TsOH, they are numbered as shown

Fig. 1. Schematic Drawing of 3·TsOH Showing the Numbering of Non-hydrogen Atoms

and observed their easy ring opening¹⁾ at the 2-position under basic conditions.^{5b,c)} For a better understanding of such instability, it is essential to know the exact molecular structures of these 3,9-disubstituted adenine salts. This paper reports the crystal structure of 3-methyladenosine *p*-toluenesulfonate (3·TsOH), which was the only one that yielded an adequate single crystal for X-ray diffraction among the 3,9-disubstituted adenine salts (type 4) in hand.

Experimental

X-Ray Analysis 3-Methyladenosine *p*-toluenesulfonate (3·TsOH) was prepared as described previously,³⁾ and colorless transparent plates [mp ca. 150 °C (dec.)] of 3·TsOH were grown from MeOH. A crystal measuring 0.5 × 0.3 × 0.4 mm was selected and used for all data collection. Unit cell constants and intensity data were obtained with a Rigaku AFC/2 automatic diffractometer using graphite-monochromated CuK α radiation ($\lambda = 1.5418 \text{ \AA}$). The unit cell dimensions were determined from angular settings of 20 2θ -values in the range of 40–60°, giving the following crystal data: $a = 16.604(1) \text{ \AA}$; $b = 8.541(1) \text{ \AA}$; $c = 6.791(1) \text{ \AA}$; $\beta = 96.22(1)^\circ$; $U = 957.3 \text{ \AA}^3$; space group $P2_1$; $Z = 2$; $D_x = 1.576 \text{ g/cm}^3$; $F(000) = 478$; $\mu(\text{Cu K}\alpha) = 19.691 \text{ cm}^{-1}$. Out of 1964 unique reflections ($2\theta \leq 130^\circ$) measured by using the $\omega/2\theta$ scan technique, 1724 with $|F_{obs}| \geq 3\sigma(F)$ were considered reliable.

in Fig. 1. The final atomic positions and equivalent isotropic thermal parameters of the non-hydrogen atoms are listed in Table I. The bond lengths and angles are given in Tables II and III, respectively, and a stereoscopic view of the structure and conformation of the protonated nucleoside residue is presented in Fig. 2.

Discussion

Geometry of the Adenine Moiety It may be seen from

TABLE I. Positional Parameters ($\times 10^4$)^{a)} and Equivalent Isotropic Temperature Factors for Non-hydrogen Atoms of 3·TsOH

Atom	x	y	z	B_{eq} ($\text{\AA}^2$)	Atom	x	y	z	B_{eq} ($\text{\AA}^2$)
N(1)	898 (2)	6076 (5)	9462 (6)	1.97	O(2')	3731 (3)	11943 (5)	7892 (5)	3.30
C(2)	1650 (3)	5732 (6)	9403 (7)	1.98	O(3')	5105 (3)	10454 (5)	9260 (5)	3.30
N(3)	2291 (2)	6706 (5)	9564 (5)	1.72	C(5')	4484 (4)	10693 (7)	14279 (7)	3.08
C(4)	2098 (3)	8259 (6)	9773 (6)	1.69	O(5')	5227 (3)	11379 (5)	15110 (6)	4.04
C(5)	1321 (3)	8725 (6)	9840 (6)	1.77	C(1'')	7882 (3)	1215 (6)	5437 (7)	2.20
C(6)	687 (3)	7619 (5)	9706 (7)	1.87	C(2'')	7411 (3)	2573 (7)	5309 (7)	2.53
N(7)	1285 (2)	10338 (6)	10078 (7)	2.10	C(3'')	7789 (3)	4020 (6)	5181 (7)	2.40
C(8)	2039 (3)	10773 (5)	10157 (7)	2.20	C(4'')	8607 (3)	4132 (6)	5175 (7)	2.40
N(9)	2579 (2)	9557 (5)	9962 (5)	1.75	C(5'')	9065 (4)	2769 (7)	5191 (8)	2.86
N(10)	-77 (3)	7951 (5)	9765 (7)	2.67	C(6'')	8705 (3)	1318 (6)	5428 (7)	2.55
C(11)	3128 (3)	6107 (7)	9608 (7)	2.43	C(7'')	9032 (4)	5732 (7)	5064 (9)	3.78
C(1')	3457 (3)	9708 (6)	9960 (7)	1.84	S(8'')	7431 (1)	-661 (2)	5548 (2)	2.78
C(2')	3768 (3)	11376 (6)	9825 (7)	2.02	O(9'')	6592 (3)	-460 (6)	5696 (7)	4.96
C(3')	4647 (3)	11159 (6)	10677 (7)	2.17	O(10'')	7598 (3)	-1456 (5)	3741 (5)	3.12
C(4')	4583 (3)	9967 (6)	12279 (7)	2.09	O(11'')	7855 (4)	-1418 (5)	7253 (5)	5.02
O(1')	3834 (2)	9110 (4)	11732 (5)	2.50					

a) Estimated standard deviations are given in parentheses and denote the least significant digits.

TABLE II. Bond Lengths in 3·TsOH

Bond	Length ^{a)} ($\text{\AA}$)	Bond	Length ^{a)} ($\text{\AA}$)	Bond	Length ^{a)} ($\text{\AA}$)
N(1)-C(2)	1.287 (6)	C(8)-N(9)	1.388 (6)	C(1'')-C(2'')	1.397 (7)
N(1)-C(6)	1.378 (7)	N(9)-C(1')	1.463 (5)	C(1'')-C(6'')	1.370 (7)
C(2)-N(3)	1.346 (6)	C(1')-C(2')	1.521 (7)	C(1'')-S(8'')	1.774 (5)
N(3)-C(4)	1.376 (6)	C(1')-O(1')	1.392 (5)	C(2'')-C(3'')	1.392 (7)
N(3)-C(11)	1.479 (6)	C(2')-C(3')	1.522 (6)	C(3'')-C(4'')	1.363 (7)
C(4)-C(5)	1.356 (6)	C(2')-O(2')	1.395 (6)	C(4'')-C(5'')	1.388 (8)
C(4)-N(9)	1.364 (6)	C(3')-C(4')	1.502 (7)	C(4'')-C(7'')	1.503 (8)
C(5)-C(6)	1.410 (6)	C(3')-O(3')	1.423 (6)	C(5'')-C(6'')	1.383 (8)
C(5)-N(7)	1.388 (6)	C(4')-O(1')	1.456 (5)	S(8'')-O(9'')	1.419 (4)
C(6)-N(10)	1.305 (6)	C(4')-C(5')	1.518 (7)	S(8'')-O(10'')	1.455 (4)
N(7)-C(8)	1.302 (6)	C(5')-O(5')	1.426 (6)	S(8'')-O(11'')	1.441 (4)

a) Estimated standard deviations are given in parentheses for the last digits.

TABLE III. Bond Angles in 3·TsOH

Bond	Angle ^{a)} ($^\circ$)	Bond	Angle ^{a)} ($^\circ$)
C(2)-N(1)-C(6)	118.8 (4)	C(2')-C(3')-C(4')	102.6 (4)
N(1)-C(2)-N(3)	128.1 (5)	C(2')-C(3')-O(3')	110.8 (4)
C(2)-N(3)-C(4)	114.4 (4)	C(4')-C(3')-O(3')	106.7 (4)
C(2)-N(3)-C(11)	121.4 (4)	C(3')-C(4')-O(1')	106.4 (3)
C(4)-N(3)-C(11)	124.1 (4)	C(3')-C(4')-C(5')	113.2 (4)
N(3)-C(4)-C(5)	121.3 (4)	O(1')-C(4')-C(5')	105.2 (4)
N(3)-C(4)-N(9)	130.7 (4)	C(1')-O(1')-C(4')	109.1 (4)
C(5)-C(4)-N(9)	108.0 (4)	C(4')-C(5')-O(5')	110.5 (4)
C(4)-C(5)-C(6)	120.5 (4)	C(2'')-C(1'')-C(6'')	119.9 (5)
C(4)-C(5)-N(7)	110.4 (4)	C(2'')-C(1'')-S(8'')	121.1 (4)
C(6)-C(5)-N(7)	129.2 (4)	C(6'')-C(1'')-S(8'')	119.0 (4)
N(1)-C(6)-C(5)	116.8 (4)	C(1'')-C(2'')-C(3'')	119.1 (4)
N(1)-C(6)-N(10)	118.2 (4)	C(2'')-C(3'')-C(4'')	121.3 (4)
C(5)-C(6)-N(10)	125.0 (5)	C(3'')-C(4'')-C(5'')	118.7 (5)
C(5)-N(7)-C(8)	103.6 (4)	C(3'')-C(4'')-C(7'')	121.9 (5)
N(7)-C(8)-N(9)	114.2 (4)	C(5'')-C(4'')-C(7'')	119.4 (4)
C(4)-N(9)-C(8)	103.8 (3)	C(4'')-C(5'')-C(6'')	121.1 (5)
C(4)-N(9)-C(1')	130.2 (4)	C(1'')-C(6'')-C(5'')	119.8 (5)
C(8)-N(9)-C(1')	126.0 (4)	C(1'')-S(8'')-O(9'')	108.4 (3)
N(9)-C(1')-C(2')	115.3 (4)	C(1'')-S(8'')-O(10'')	105.4 (2)
N(9)-C(1')-O(1')	108.8 (3)	C(1'')-S(8'')-O(11'')	105.5 (2)
C(2')-C(1')-O(1')	105.8 (3)	O(9'')-S(8'')-O(10'')	113.2 (2)
C(1')-C(2')-C(3')	100.4 (4)	O(9'')-S(8'')-O(11'')	113.3 (3)
C(1')-C(2')-O(2')	113.7 (4)	O(10'')-S(8'')-O(11'')	110.3 (2)
C(3')-C(2')-O(2')	110.0 (4)		

a) Estimated standard deviations are given in parentheses for the last digits.

Fig. 2. Stereoview of the Structure of the Nucleoside Cation Moiety in 3·TsOH

Tables II and III that the bond lengths and angles are not always in agreement with the average values found for the natural purine bases.⁸⁾ One of the most notable departures from the dimensions of the usual adenine systems, mainly occurring in the pyrimidine part, indicates the localization of double bonds at N(1)=C(2), C(4)=C(5), and C(6)=N(10). The C(6)=N(10) bond length of 1.305 Å is shorter than the corresponding bond length (1.332 Å)⁹⁾ in adenosine (6), but longer than that (1.287 Å)¹⁰⁾ in *N*⁶-methoxy-2',3',5'-tri-*O*-methyladenosine (7) (Fig. 3). The angles of C(2)-N(1)-C(6) (118.8°) and N(1)-C(6)-C(5) (116.8°) are not similar to those (123.9° and 111.1°, respectively)¹⁰⁾ in 7, but are rather similar to those (119.3° and 117.4°, respectively)⁹⁾ in 6 (Fig. 4). These results suggest protonation at the exocyclic imino nitrogen [N(10)] in 3·TsOH, and this was clearly recognized in the difference electron density maps. The positional parameters ($\times 10^3$, with estimated standard deviations in parentheses) and isotropic temperature factor for N(10)-H [*syn* with respect to N(1)] are $x = -31(4)$, $y = 883(9)$, $z = 1008(10)$, and $B_{iso} = 0.4$; those for N(10)-H (*anti*) are $x = -54(4)$, $y = 710(10)$, $z = 1029(11)$, and $B_{iso} = 0.2$.

Interestingly, the adenine moiety in 3·TsOH is almost planar, with the largest deviation (-0.015 Å) from the least-squares plane being that of the N(10) atom. The

Fig. 5. A Perspective View of 3-TsOH along the *c* Axis¹²⁾

is usual *trans-gauche* ($\psi_{OO} = 171.6^\circ$, $\psi_{OC} = -72.7^\circ$), and the 5'-hydroxy group O(5')-H forms an intermolecular hydrogen bond with the O(9'') atom of tosylate ion, as shown in Table VI and Fig. 5.

Hydrogen Bonding and Packing of the Molecules All protons attached to the O and N atoms are involved in hydrogen bonds (Table VI), forming an extensive intermolecular network (Fig. 5).¹²⁾ The adenine moiety lies nearly parallel to the *ab* plane, as shown in Fig. 5, to form a stacked column with the tosylate molecule. The interplanar separation is 3.6 Å, and the dihedral angle is 10.6° .

An Approach through Molecular Orbital (MO) Calculation In order to predict the site of protonation in the adenine moiety of 3-methyladenosine *p*-toluenesulfonate (3-TsOH), the semiempirical MO calculation method MNDO (modified neglect of diatomic overlap)^{13,14)} was applied to 6-imino-3,9-dimethylpurine, which is structurally analogous to 3 and the simplest in the 3,9-disubstituted adenine series. For this base, the *N*⁶-H *syn* (8) and *anti* (9) configurations are possible with respect to N(1). The geometries were optimized by the DFP (Davidon-Fletcher-Powell) optimization procedure¹⁵⁾ using the re-

TABLE VII. Atomic Orbital Coefficients for the HOMO of the *Syn* (8) and *Anti* (9) Forms of 6-Imino-3,9-dimethylpurine

<i>Syn</i> form (8) ^{a)}				<i>Anti</i> form (9) ^{a)}			
$\epsilon = -8.47464$ kcal/mol				$\epsilon = -8.54276$ kcal/mol			
N(1)(<i>p</i> _z)	0.2336	C(6)(<i>p</i> _z)	-0.1251	N(1)(<i>p</i> _z)	0.2452	C(6)(<i>p</i> _z)	-0.1361
C(2)(<i>p</i> _z)	0.0331	N(7)(<i>p</i> _z)	-0.1166	C(2)(<i>p</i> _z)	0.0598	N(7)(<i>p</i> _z)	-0.1152
N(3)(<i>p</i> _z)	-0.3964	C(8)(<i>p</i> _z)	-0.4081	N(3)(<i>p</i> _z)	-0.4099	C(8)(<i>p</i> _z)	-0.3961
C(4)(<i>p</i> _z)	0.4134	N(9)(<i>p</i> _z)	-0.0467	C(4)(<i>p</i> _z)	0.3852	N(9)(<i>p</i> _z)	-0.0314
C(5)(<i>p</i> _z)	0.4933	N ⁶ (<i>p</i> _z)	-0.4025	C(5)(<i>p</i> _z)	0.4937	N ⁶ (<i>p</i> _z)	-0.4154

a) The *z* axis was taken in the direction of the molecular plane. Coefficients for the *s*, *p*_x, and *p*_y orbitals are substantially 0.

sults of the present X-ray analysis as initial geometry, and assuming *C*_s symmetry. The heats of formation thus estimated for 8 and 9 were 82.970 and 84.238 kcal/mol, respectively. It may be seen from Table VII that the HOMO density at N⁶ [N(10)] is greater than that at N(1) in both configurations. This supports preferential protonation of 3 at N(10) in the salt form (3-TsOH), as evidenced by the above X-ray crystallographic results.

Conclusion

In the crystal, the *p*-toluenesulfonate salt of 3-methyladenosine (3) has the oxocyclic iminium structure (type 5). The adenine moiety is almost planar, and the N(9)-C(1') bond is almost coplanar with the adenine ring. However, the displacement of the N(3)-methyl group from planarity and the endocyclic and exocyclic angles at N(3), C(4), and N(9) are in favor of keeping the N(3)-methyl and N(9)-ribose groups away from each other, representing a notable departure from those of the usual adenosine systems. The ribose moiety is in the C(2')-*endo* (²E) puckering conformation and in the *high-anti* (part of *syn*) conformation with respect to the adenine moiety. By analogy with this as well as from the results of the semiempirical calculation of the atomic orbital coefficients for the HOMO of 6-imino-3,9-dimethylpurine (8 or 9), similar exocyclic iminium structures (type 5) are inferred for the previously synthesized salts of 3-methyl-2'-deoxyadenosine (2)⁴⁾ and 3,9-dialkyladenines.⁵⁾ Thus, the present findings may shed light on factors responsible for the previously described, extraordinary instability^{3,4,5b,c)} of 3,9-disubstituted adenines at both the base and the nucleoside levels.

Acknowledgment We wish to thank Emeritus Professor Dr. Shigehiko Sugawara (University of Tokyo) and Drs. Seiichi Saito, Keishi Kotera, and Mikio Takeda (Tanabe Seiyaku, Co.) for their interest and encouragement. Financial support provided by the Japan Research Foundation for Optically Active Compounds is deeply appreciated.

References and Notes

- 1) Paper XXX in this series, T. Fujii, T. Saito, I. Inoue, Y. Kumazawa, and K. Tamura, *Chem. Pharm. Bull.*, **36**, 107 (1988).
- 2) a) P. D. Lawley and P. Brookes, *Biochem. J.*, **89**, 127 (1963); b) G. P. Margison and P. J. O'Connor, *Biochim. Biophys. Acta*, **331**, 349 (1973); c) A. M. Maxam and W. Gilbert, *Proc. Natl. Acad. Sci. U.S.A.*, **74**, 560 (1977); d) S. Riazuddin and T. Lindahl, *Biochemistry*, **17**, 2110 (1978); e) B. Singer and T. P. Brent, *Proc. Natl. Acad. Sci. U.S.A.*, **78**, 856 (1981); f) M. D. Mamet-Bratley and B. Karska-Wysocki, *Biochim. Biophys. Acta*, **698**, 29 (1982); g) P. Karran, T. Hjelmgren, and T. Lindahl, *Nature* (London), **296**, 770 (1982); h) P. E. Gallagher and T. P. Brent, *Biochemistry*, **21**, 6404 (1982); i) M. Szyf, Y. Gruenbaum, S. Urieli-Shoval, and A. Razin, *Nucleic Acids Res.*, **10**, 7247 (1982); j) J. Hindley, "DNA Sequencing," Elsevier Biomedical Press, Amsterdam, 1983; k) P. E. Gallagher and T. P. Brent, *Biochim. Biophys. Acta*, **782**, 394 (1984).
- 3) T. Saito and T. Fujii, *J. Chem. Soc., Chem. Commun.*, **1979**, 135.
- 4) T. Fujii, T. Saito, and T. Nakasaka, *J. Chem. Soc., Chem. Commun.*, **1980**, 758.
- 5) a) T. Fujii, T. Itaya, K. Mohri, and T. Saito, *J. Chem. Soc., Chem. Commun.*, **1973**, 917; b) T. Fujii, T. Saito, and M. Kawanishi, *Tetrahedron Lett.*, **1978**, 5007; c) T. Fujii, T. Saito, and T. Nakasaka, *Heterocycles*, **15**, 195 (1981); d) T. Fujii, T. Saito, I. Inoue, Y. Kumazawa, and N. J. Leonard, *Chem. Pharm. Bull.*, **34**, 1821 (1986).
- 6) G. Germain, P. Main, and M. M. Woolfson, *Acta Crystallogr., Sect. A*, **27**, 368 (1971).
- 7) J. A. Ibers and W. C. Hamilton (eds.), "International Tables for X-Ray Crystallography," Vol. IV, Kynoch Press, Birmingham, 1974.
- 8) R. Taylor and O. Kennard, *J. Mol. Struct.*, **78**, 1 (1982).

- 9) T. F. Lai and R. E. Marsh, *Acta Crystallogr., Sect. B*, **28**, 1982 (1972).
- 10) G. I. Birnbaum, B. Kierdaszuk, and D. Shugar, *Nucleic Acids Res.*, **12**, 2447 (1984).
- 11) For the nomenclature and notations used, see a) C. Altona and M. Sundaralingam, *J. Am. Chem. Soc.*, **94**, 8205 (1972); b) M. Sundaralingam, "Conformation of Biological Molecules and Polymers," ed. by E. D. Bergmann and P. Pullman, The Israel Academy of Sciences and Humanities, 1973, p. 417; c) H. P. M. de Leeuw, C. A. G. Haasnoot, and C. Altona, *Israel J. Chem.*, **20**, 108 (1980); d) W. Saenger, "Principles of Nucleic Acid Structure," ed. by C. R. Cantor, Springer-Verlag, New York, 1984, pp. 9—24.
- 12) In Fig. 5, hydrogen atoms have been deleted for clarity, but hydrogen bonds are indicated by broken lines.
- 13) a) M. J. S. Dewar and W. Thiel, *J. Am. Chem. Soc.*, **99**, 4899 (1977); b) *Idem, ibid.*, **99**, 4907 (1977).
- 14) Program No. 464, Quantum Chemistry Program Exchange (MOPAC), Indiana University, Bloomington, IN.
- 15) a) R. Fletcher and M. J. D. Powell, *Comput. J.*, **6**, 163 (1963); b) W. C. Davidon, *ibid.*, **10**, 406 (1968).