Purines. LXVI.[1] Adenine 7-Oxide: Its Synthesis, Chemical Properties, and X-ray Molecular Structure

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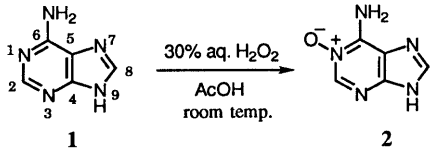
A detailed account is given of the first unequivocal synthesis of adenine 7-oxide (8). The synthesis started with peroxycarboxylic acid oxidation of 3-benzyladenine (6), readily obtainable from adenine (1) by benzylation, and proceeded through nonreductive debenzylation of the resulting 3-benzyladenine 7-oxide (7). The location of the oxygen function in 7 and 8 was confirmed by their chemical reactions including deamination and methylation and by X-ray crystallographic analysis. A UV spectroscopic approach suggested that the neutral species of 8 exists in H_2O as an equilibrated mixture of the N(7)-oxide (8) and N(7)-OH (21) tautomers. Treatment of 6 with 30% aqueous H_2O_2 in MeOH in the presence of MeCN and $KHCO_3$ at 30 °C produced the N(7)-oxide 7 and 7-acetamido-3-benzyladenine (15) in 12% and 1% yields, respectively.

Keywords adenine 7-oxide; synthesis; X-ray analysis; 3-benzyladenine *N*-oxidation; debenzylation nonreductive; reaction adenine 7-oxide

Adenine (**1**) is an important fundamental biomolecule, which has a bicyclic ring system consisting of a 4-aminopyrimidine and an imidazole ring in juxtaposition.[2] On treatment with 30% aqueous H_2O_2 in AcOH at room temperature, it undergoes *N*-oxidation preferentially at the 1-position to produce adenine 1-oxide (**2**) in good yield (Chart 1).[3,4] This regioselectivity appears to reflect the generalization[5] that on *N*-oxidation pyrimidine compounds form only mono-*N*-oxides, whereas imidazoles are resistant to *N*-oxidation.

In 1968, however, Rhaese[6] claimed that treatment of **1** with 0.1 M H_2O_2 in 0.01 M phosphate buffer (pH 7.0) at 37 °C for 5 d afforded adenine 7-oxide (**8**) (isolated as a monohydrate sensitive to UV light) in 5% yield without any detectable formation of the N(1)-oxide **2**. He further claimed that the N(7)-oxide **8** was among the products of X-ray irradiation of **1** in 0.05 M phosphate buffer (pH 7.0).[6] Later on, these results were reportedly reproduced by Yamamoto,[7] who further asserted that **8** bound noncovalently to urease, an SH protein, in an experiment using a sample of **8** prepared by the method of Rhaese. This unusual regioselectivity of *N*-oxidation of **1** was so striking as to appear questionable. Moreover, the chemical and spectroscopic evidence adduced by both authors appeared insufficient to allow definitive assignment of the N(7)-oxide structure to their samples, which they thought to be the new *N*-oxide **8**.



1 **2**

Chart 1

We therefore reexamined the H_2O_2/buffer oxidation procedure[6] of Rhaese for **1**, but completely failed to reproduce his results; we were unable to obtain any *N*-oxide from **1**. This led us to design a three-step route for the synthesis of adenine 7-oxide (**8**) from adenine (**1**) in the present work. In order to establish the structure of the target compound (**8**), its chemical behavior and X-ray molecular structure were also investigated. A brief account of a part of the results reported here has been published in a preliminary form.[8]

The basis for the new synthetic design was provided by the following consideration. The direct alkylation of **1** at the 3-position in the absence of added base presents the most convenient method of securing 3-alkyladenines (type **6**),[9] and the second stage of alkylation of the 3-substituted adenines (type **6**) provides a ready access to 3,7-disubstituted adenines (type **3**).[9,10] If there were a parallelism between *N*-alkylation and *N*-oxidation in regioselectivity at the second stage and if a similar two-step reaction sequence involving *N*-oxidation could be coupled with selective removal of the 3-substituent, it should conclude a three-step synthesis of adenine 7-oxide (**8**) from adenine (**1**). This was neatly realized by the use of a benzyl group at the 3-position (Chart 2).

Treatment of 3-benzyladenine (**6**), easily obtainable from **1** in 66% yield according to the literature procedure,[9] with 0.75 molar equivalent of magnesium monoperoxyphthalate hexahydrate (MMPP·$6H_2O$)[11,12] in MeOH at 30 °C for 20 h afforded 3-benzyladenine 7-oxide (**7**) in the form of a monohydrate (**7**·H_2O) in 40% yield, together with 51% recovery of **6**. Replacement of MMPP·$6H_2O$ by *m*-chloroperoxybenzoic acid (MCPBA) in this oxidation, but in MeOH–1 M acetate buffer (pH 5.0) (1 : 1, v/v) at 30 °C for 15 h,[13] also produced **7**·H_2O in 24% yield, along with 29% recovery of **6**. The use of plain MeOH as the solvent in this case retarded the reaction because of deposition of the salt formed from unaltered

This paper is dedicated to the memory of Emeritus Professor Dr. Yoshio Ban (Hokkaido University), whose most distinguished, constantly inspiring scholar's life suddenly ended at the age of 73 on July 16, 1994.

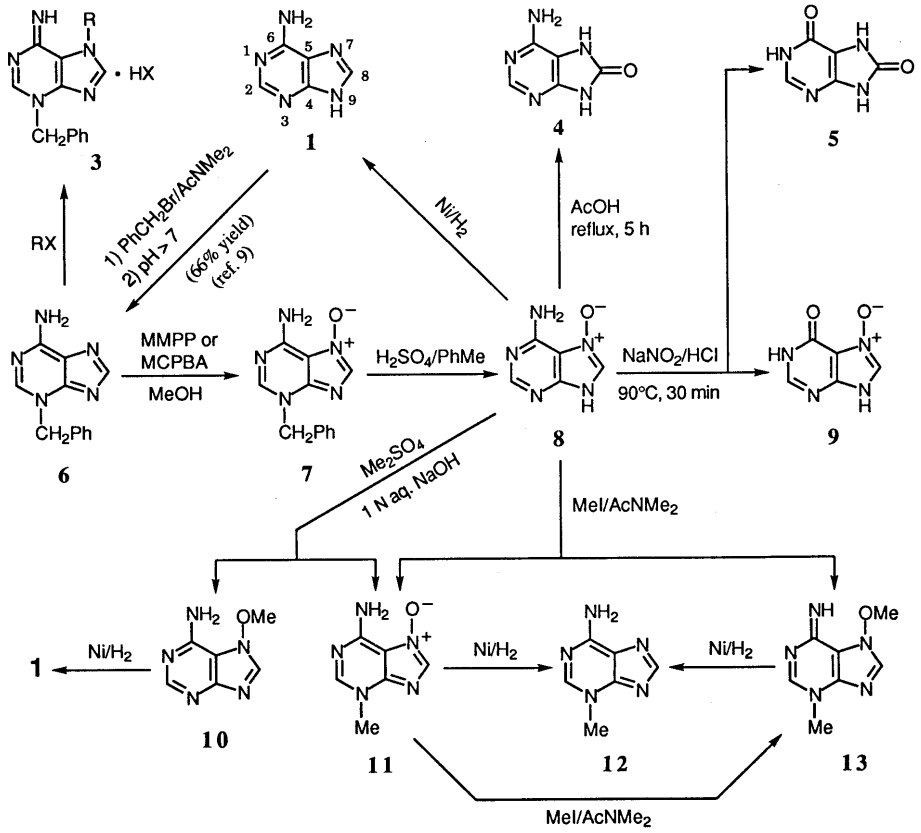


Chart 2

TABLE I. *N*-Oxidation of 3-Benzyladenine (**6**) to Form 3-Benzyladenine 7-Oxide (**7**)

Entry	Oxidizing agent		Solvent	Temp. (°C)	Time (h)	Yield (%)	Recovery (%)
	Reagent[a]	Amt. (molar eq)				7	6
1	MMPP·6H$_2$O	0.6[b]	MeOH	30	20	31	58
2	MMPP·6H$_2$O	0.75	MeOH	30	20	40	51
3	MMPP·6H$_2$O	1.0	MeOH	30	20	40	38
4	MMPP·6H$_2$O	1.3	MeOH	30	20	35	32
5	MMPP·6H$_2$O	1.5	MeOH–A[c]	30	60[d]	11	29
6	MCPBA	1.5	MeOH–B[e]	30	15[d]	24	29
7	MCPBA	1.5	MeOH–B[e]	30	19	12	—[f]
8	MCPBA	1.5	MeOH–C[g]	30	17[d]	18	27
9	MCPBA	1.5	MeOH–D[h]	30	18	16	63
10	MCPBA	1.5	AcOH	30	20	—	75
11	MCPBA	2.0	MeOH–B[e]	30	13[d]	20	54
12	H_2O_2[i]/MeCN/KHCO$_3$	30	MeOH	25	22	12[j]	28

a) MMPP = magnesium monoperoxyphthalate; MCPBA = *m*-chloroperoxybenzoic acid. *b*) See ref. 12 for the theoretically required amount. *c*) MeOH–1 M acetate buffer (pH 5.6) (1 : 1, v/v) was used. *d*) At the beginning of the reaction, a solution of the reagent in MeOH was added dropwise to a solution of **6** over a period of 5 h. *e*) MeOH–1 M acetate buffer (pH 5.0) (3 : 2, v/v) was used. *f*) No attempt was made to recover **6**. *g*) MeOH–0.2 M acetate buffer (pH 5.0) (3 : 2, v/v) was used. *h*) MeOH–0.5 M phosphate buffer (pH 6.2) (1 : 2, v/v) was used. *i*) A 30% aqueous solution was used. *j*) The 7-acetamido derivative **15** was also isolated in 1% yield.

6 and *m*-chlorobenzoic acid in the course of the reaction. It may be seen from Table I that modification of the reaction conditions for each peroxycarboxylic acid oxidation did not improve the yield of **7**. Application of a fairly large excess of the oxidizing agent tended to decrease both the yield of **7** and the recovery of **6**. This may be attributable to deep-seated oxidative degradation of **7** as well as **6**.[3a,14] The use of 30% aqueous H_2O_2 in AcOH at room temperature or MCPBA in AcOH at 30 °C as the oxidizing agent was found to be ineffective.

For removal of the benzyl group from **7**, we applied the previously reported, nonreductive debenzylation procedure.[10b,15] On treatment with conc. H_2SO_4 at 35 °C in the presence of toluene for 3 h, **7**·H_2O furnished the desired compound, adenine 7-oxide (**8**), in 55% yield. Characterization of **8** as the N(7)-oxide was readily achieved by measurement of its UV spectrum, which was different from those of the three known isomeric *N*-oxides [adenine 1-oxide (**2**),[3] adenine 3-oxide,[16] and 9-hydroxyadenine[17]], and by the examination of its chemical

properties, as described below.

Catalytic reduction (Raney Ni/H_2, H_2O, 1 atm, 50 °C, 4 h) of **8** was found to provide adenine (**1**) in 92% yield. Treatment of **8** with boiling AcOH for 5 h gave, after recrystallization of the product from 5% aqueous H_2SO_4, known[18] 8-oxoadenine sulfate (**4**·1/2H_2SO_4) in 69% yield. This is indicative of the N(7)-oxide structure of **8**, since the apparent migration of the oxygen function from N(7) to C(8) under acidic conditions has been observed for guanine 7-oxide[19] and hypoxanthine 7-*N*-oxide (**9**).[15d,20] Deamination of **8** with $NaNO_2$ in aqueous HCl at 90 °C for 30 min afforded **9** and its N(7)→C(8) O-migration product (**5**), both of which were already known,[15d] in 4% and 45% yields, respectively.

In addition, methylation of **8** with MeI in $AcNMe_2$ at 25 °C for 20 h gave 3-methyladenine 7-oxide (**11**) in 25% yield and 7-methoxy-3-methyladenine (**13**), which was isolated in 17% yield in the form of the perchlorate salt (**13**·$HClO_4$). The same perchlorate salt was obtained from **11** in 89% yield by a similar methylation (25 °C, 18 h) and work-up. The UV spectrum of **13**·$HClO_4$ was similar to those[10a] of 3,7-dialkyladenine salts (type **3**). On catalytic reduction (Raney Ni/H_2, H_2O, 1 atm, 40 °C, 4 h), **11** and **13**·$HClO_4$ separately gave 3-methyladenine (**12**) in 90% and 73% yields, respectively. On the other hand, treatment of **8** with dimethyl sulfate in a mixture of 1 N aqueous NaOH and MeOH at room temperature for 1 h produced **11** and 7-methoxyadenine (**10**) in 23% and 22% yields, respectively. Characterization of **10** as the 7-methoxy derivative was accomplished by catalytic hydrogenolysis (Raney Ni/H_2, H_2O, 1 atm, 40 °C, 4 h), which led to the formation of adenine (**1**) in 81% yield.

Further interest in the *N*-oxidation stems from that of **6** with peroxycarboximidic acid.[21] Treatment of **6** with a large excess of 30% aqueous H_2O_2 in MeOH in the presence of MeCN and $KHCO_3$[21] at 25 °C for 22 h afforded the N(7)-oxide **7** and 7-acetamido-3-benzyladenine (**15**) in 12% and 1% yields, respectively, together with 28% recovery of **6**. For the minor product **15**, the gain of a C_2H_3NO portion during the reaction was suggested by elemental analysis and MS determination. Its UV spectra in H_2O at pH 1 and 13 were similar to those[10a] of 3,7-dialkyladenines (type **3**), and its ^{1}H-NMR spectrum in Me_2SO-d_6 showed a three-proton singlet at δ 1.84 attributable to a methyl group that would have originated from the MeCN used. Final identification as 7-acetamido-3-benzyladenine rested on its X-ray molecular structure (*vide infra*). As shown in Chart 3, the formations of **7** and **15** from **6** may be explained in terms of separate attacks of the N(7) atom in **6** on the hydroxy and imino groups of the peroxyacetimidic acid intermediate (**14**)[21] generated from MeCN and H_2O_2 under basic conditions. Replacement of MeCN by PhCN in this oxidation did not improve the yield of **7**.

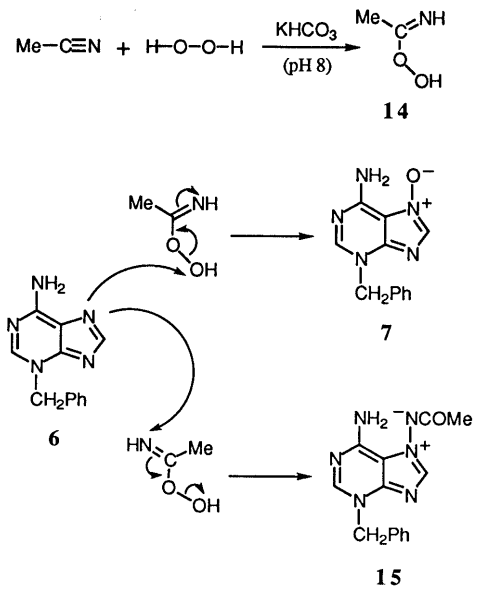


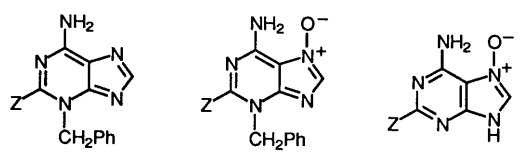
Chart 3

The N(7)-oxides **7** and **8** thus synthesized were awaiting unambiguous assignments of the purine-ring proton signals in their ^{1}H-NMR spectra. We therefore decided to prepare their C(2)-deuterated species (**17** and **18**) by following a parallel synthetic route starting from 3-benzyladenine-*2-d* (**16**). Thus, 3-benzyladenine (**6**) was treated with NaOMe in boiling CD_3OD for 30 h, according to the method of Maki *et al.*,[22] and the C(2)-deuterated species **16** of 85% isotopic purity was obtained in *ca.* 95% yield. Oxidation of **16** with MMPP·$6H_2O$ in MeOH at 30 °C for 18 h gave **17** (of 79% isotopic purity) in *ca.* 32% yield. Nonreductive debenzylation of **17** was effected in a mixture of conc. H_2SO_4 and toluene at 35 °C for 3 h, affording **18** (of 78% isotopic purity) in *ca.* 46% yield. Table II assembles the chemical shifts for the purine-ring protons of the C(2)-deuterated species **16**, **17**, and **18**, together with those of the isotopically unmodified counterparts (**6**, **7**, and **8**). It may be seen that in Me_2SO-d_6 the C(2)-proton of 3-benzyladenine 7-oxide (**7**) resonates at lower field than does the C(8)-proton, standing in the same relationship to that observed for the corresponding protons of the parent base **6**. On the other hand, the C(8)-proton of the debenzylated derivative **8** resonates at

Table II. Selected ^{1}H-NMR Data for C(2)-Deuterated and Unlabeled Adenine Derivatives

Compound			Chemical shift (δ)[a] in Me_2SO-d_6		
No.	N(3)-R	Label at C(2)	C(2)-H	C(8)-H	$\delta_{C(2)-H}-\delta_{C(8)-H}$
16	$PhCH_2$	D	(8.55)[b]	7.75	0.80
6	$PhCH_2$	None	8.55	7.76	0.79
17	$PhCH_2$	D	(8.64)[b]	7.90	0.74
7	$PhCH_2$	None	8.64	7.90	0.74
18	—	D	(8.16)[b]	8.34	−0.18
8	—	None	8.17	8.35	−0.18

a) Expressed in ppm downfield from internal Me_4Si. *b*) Appeared as a small singlet (0.15—0.22 H).



6: Z = H **16**: Z = D

7: Z = H **17**: Z = D

8: Z = H **18**: Z = D

slightly lower field than does the C(2)-proton. When **8** was heated in D_2O for a while, the relative integral intensity of the C(8)-proton signal at δ 8.29 (in D_2O) decreased. This isotopic exchange at C(8)-H is in general agreement with that[23] reported for adenine (**1**) and 9-substituted adenines. As in the case of pyridine 1-oxide,[24] the *N*-oxide oxygen atom in **8** would release electrons to the C(8) atom, thereby promoting the exchange reaction.

TABLE III. Selected Bond Lengths in $7 \cdot 2H_2O$

Bond	Length[a] (Å)	Bond	Length[a] (Å)	Bond	Length[a] (Å)
C(1)–N(2)	1.350 (12)	N(5)–C(6)	1.359 (12)	C(13)–C(14)	1.364 (20)
C(1)–C(8)	1.418 (13)	N(5)–C(9)	1.338 (12)	C(13)–C(18)	1.359 (20)
C(1)–N(10)	1.323 (11)	C(6)–N(7)	1.344 (13)	C(14)–C(15)	1.432 (26)
N(2)–C(3)	1.325 (13)	N(7)–C(8)	1.360 (12)	C(15)–C(16)	1.342 (31)
C(3)–N(4)	1.337 (13)	N(7)–O(11)	1.363 (9)	C(16)–C(17)	1.385 (28)
N(4)–C(9)	1.362 (12)	C(8)–C(9)	1.370 (12)	C(17)–C(18)	1.385 (23)
N(4)–C(12)	1.480 (12)	C(12)–C(13)	1.522 (17)		

a) Estimated S.D.'s are given in parentheses for the least significant digits.

TABLE IV. Selected Bond Angles in $7 \cdot 2H_2O$

Bond	Angle[a] (°)	Bond	Angle[a] (°)	Bond	Angle[a] (°)	Bond	Angle[a] (°)
N(2)–C(1)–C(8)	117.6 (8)	C(9)–N(4)–C(12)	121.3 (8)	C(1)–C(8)–C(9)	121.2 (8)	C(12)–C(13)–C(18)	119.5 (11)
N(2)–C(1)–N(10)	119.9 (9)	C(6)–N(5)–C(9)	103.9 (8)	N(7)–C(8)–C(9)	105.3 (8)	C(14)–C(13)–C(18)	121.9 (13)
C(8)–C(1)–N(10)	122.6 (8)	N(5)–C(6)–N(7)	111.4 (8)	N(4)–C(9)–N(5)	128.9 (8)	C(13)–C(14)–C(15)	116.4 (15)
C(1)–N(2)–C(3)	118.5 (8)	C(6)–N(7)–C(8)	107.4 (7)	N(4)–C(9)–C(8)	119.0 (8)	C(14)–C(15)–C(16)	120.9 (18)
N(2)–C(3)–N(4)	126.5 (9)	C(6)–N(7)–O(11)	128.3 (8)	N(5)–C(9)–C(8)	112.1 (8)	C(15)–C(16)–C(17)	122.0 (17)
C(3)–N(4)–C(9)	117.3 (8)	C(8)–N(7)–O(11)	124.2 (7)	N(4)–C(12)–C(13)	113.4 (7)	C(16)–C(17)–C(18)	116.7 (17)
C(3)–N(4)–C(12)	121.4 (8)	C(1)–C(8)–N(7)	133.5 (7)	C(12)–C(13)–C(14)	118.6 (12)	C(13)–C(18)–C(17)	122.0 (14)

a) Estimated S.D.'s are given in parentheses and denote the least significant digits.

TABLE V. Selected Bond Lengths in $8 \cdot H_2O$

Bond	Length[a] (Å)	Bond	Length[a] (Å)	Bond	Length[a] (Å)
C(1)–N(2)	1.350 (2)	C(3)–N(4)	1.324 (2)	C(6)–N(7)	1.325 (2)
C(1)–C(8)	1.402 (2)	N(4)–C(9)	1.346 (2)	N(7)–C(8)	1.396 (2)
C(1)–N(10)	1.322 (2)	N(5)–C(6)	1.352 (2)	N(7)–O(11)	1.332 (2)
N(2)–C(3)	1.341 (2)	N(5)–C(9)	1.368 (2)	C(8)–C(9)	1.376 (2)

a) Estimated S.D.'s are given in parentheses for the last digits.

TABLE VI. Selected Bond Angles in $8 \cdot H_2O$

Bond	Angle[a] (°)	Bond	Angle[a] (°)	Bond	Angle[a] (°)	Bond	Angle[a] (°)
N(2)–C(1)–C(8)	115.7 (1)	C(3)–N(4)–C(9)	111.1 (1)	C(8)–N(7)–O(11)	125.0 (1)	N(4)–C(9)–N(5)	127.4 (1)
N(2)–C(1)–N(10)	120.1 (1)	C(6)–N(5)–C(9)	108.0 (1)	C(1)–C(8)–N(7)	133.8 (1)	N(4)–C(9)–C(8)	125.1 (1)
C(8)–C(1)–N(10)	124.2 (2)	N(5)–C(6)–N(7)	109.8 (1)	C(1)–C(8)–C(9)	119.5 (1)	N(5)–C(9)–C(8)	107.5 (1)
C(1)–N(2)–C(3)	119.3 (1)	C(6)–N(7)–C(8)	108.0 (1)	N(7)–C(8)–C(9)	106.7 (1)		
N(2)–C(3)–N(4)	129.2 (2)	C(6)–N(7)–O(11)	127.0 (1)				

a) Estimated S.D.'s are given in parentheses for the last digits.

TABLE VII. Selected Bond Lengths in **15**

Bond	Length[a] (Å)	Bond	Length[a] (Å)	Bond	Length[a] (Å)
C(1)–N(2)	1.367 (4)	C(5)–N(9)	1.353 (7)	C(13)–C(14)	1.387 (8)
C(1)–C(6)	1.401 (6)	C(6)–N(7)	1.375 (5)	C(14)–C(15)	1.362 (8)
C(1)–N(10)	1.321 (5)	N(7)–C(8)	1.366 (5)	C(15)–C(16)	1.364 (6)
N(2)–C(3)	1.306 (5)	N(7)–N(18)	1.416 (7)	C(16)–C(17)	1.392 (7)
C(3)–N(4)	1.348 (6)	C(8)–N(9)	1.340 (5)	N(18)–C(19)	1.322 (12)
N(4)–C(5)	1.373 (5)	C(11)–C(12)	1.502 (7)	C(19)–O(20)	1.252 (14)
N(4)–C(11)	1.485 (6)	C(12)–C(13)	1.381 (5)	C(19)–C(21)	1.507 (10)
C(5)–C(6)	1.374 (6)	C(12)–C(17)	1.372 (6)		

a) Estimated S.D.'s are given in parentheses and denote the least significant digits.

TABLE VIII. Selected Bond Angles in **15**

Bond	Angle[a] (°)	Bond	Angle[a] (°)	Bond	Angle[a] (°)	Bond	Angle[a] (°)
N(2)–C(1)–C(6)	117.0 (3)	N(4)–C(5)–C(6)	119.4 (4)	C(8)–N(7)–N(18)	134.6 (4)	C(13)–C(14)–C(15)	119.6 (5)
N(2)–C(1)–N(10)	119.1 (4)	N(4)–C(5)–N(9)	127.9 (4)	N(7)–C(8)–N(9)	113.2 (4)	C(14)–C(15)–C(16)	120.6 (5)
C(6)–C(1)–N(10)	124.0 (3)	C(6)–C(5)–N(9)	112.7 (4)	C(5)–N(9)–C(8)	103.1 (4)	C(15)–C(16)–C(17)	119.9 (4)
C(1)–N(2)–C(3)	119.1 (3)	C(1)–C(6)–C(5)	121.5 (3)	N(4)–C(11)–C(12)	112.1 (4)	C(12)–C(17)–C(16)	120.3 (4)
N(2)–C(3)–N(4)	126.7 (3)	C(1)–C(6)–N(7)	133.2 (4)	C(11)–C(12)–C(13)	120.7 (4)	N(7)–N(18)–C(19)	114.3 (7)
C(3)–N(4)–C(5)	116.1 (4)	C(5)–C(6)–N(7)	105.3 (4)	C(11)–C(12)–C(17)	120.5 (4)	N(18)–C(19)–O(20)	127.5 (7)
C(3)–N(4)–C(11)	122.1 (3)	C(6)–N(7)–C(8)	105.8 (3)	C(13)–C(12)–C(17)	118.8 (4)	N(18)–C(19)–C(21)	113.0 (9)
C(5)–N(4)–C(11)	121.7 (4)	C(6)–N(7)–N(18)	119.4 (4)	C(12)–C(13)–C(14)	120.8 (4)	O(20)–C(19)–C(21)	119.5 (8)

a) Estimated S.D.'s are given in parentheses for the last digits.

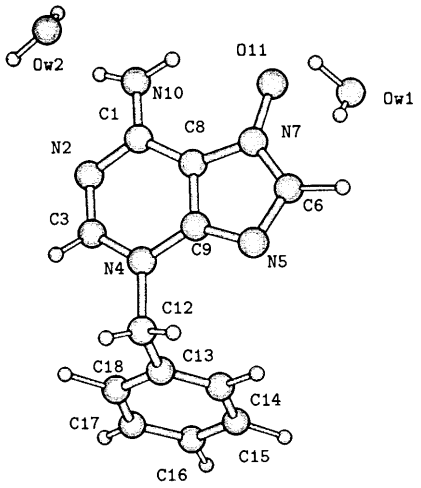


Fig. 1. A Perspective View of the Molecular Structure of $7 \cdot 2H_2O$ and the Numbering Scheme Employed for X-ray Crystallographic Data

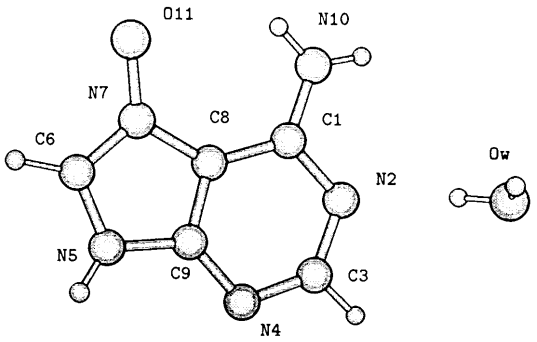


Fig. 2. A Perspective View of the Molecular Structure of $8 \cdot H_2O$ and the Numbering of Non-hydrogen Atoms

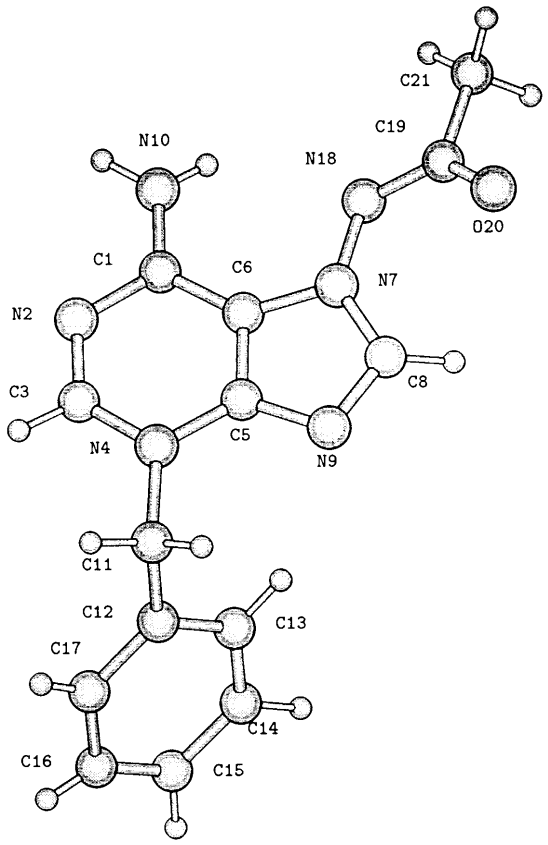


Fig. 3. A Parallel View of the Molecular Structure of **15** and the Numbering Scheme Adopted for X-ray Crystallographic Data

We next investigated the X-ray molecular structures of **7**, **8**, and **15** in order to reach a definitive identification and to examine their tautomeric forms in the solid state. Tables III and IV, V and VI, and VII and VIII list selected bond lengths and selected bond angles obtained for $7 \cdot 2H_2O$, $8 \cdot H_2O$, and **15**, respectively. Computer-generated drawings of the final X-ray models, together with the atomic numbering schemes employed for the X-ray crystallographic data, are presented in Figs. 1—3. Thus, it became clear that in the solid state both $7 \cdot 2H_2O$ and $8 \cdot H_2O$ exist in the N(7)-oxide form rather than the N(7)-OH form and that **15** is 7-acetamido-3-benzyladenine, which exists in a zwitterionic form in the solid state.

In approaching the problem of the tautomeric form of adenine 7-oxide (**8**) in solution, we determined its pK_a values spectrophotometrically in H_2O at 30 °C, obtaining two values of 3.4 (basic) [for protonated form (**19**)⇌ neutral form] and 5.75 (acidic) [for neutral form⇌ monoanion (**20**)] (Chart 4). The strong UV absorption of purine *N*-oxides in the 215—240 nm region is considered to be due to $\geq N \rightarrow O$ or the enol anion $\geq N\text{–}O^-$.[17] Although a fairly strong absorption band (ε 12400) was observed at 235 nm in the UV spectrum of **8** in H_2O at pH 7, it may be regarded as that arising from the monoanionic species (**20**) in view of the above pK_a values. The spectrum in H_2O at pH 4.1 should reflect that of the neutral species, and it exhibited two absorption bands at 243 nm (ε 8200) and 267 nm (ε 8900). On the other hand, the neutral species spectrum of 7-methoxyadenine (**10**), a fixed model for the N(7)-OH form (**21**), showed a fairly strong absorption at

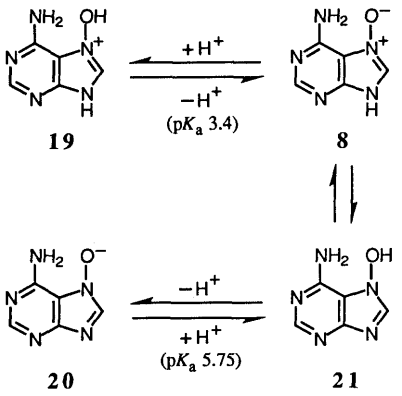


Chart 4

271 nm (ε 10200). This led us to consider that the neutral species of adenine 7-oxide exists in H_2O as an equilibrated mixture of the N(7)-oxide (**8**) and N(7)-OH (**21**) tautomers.

As regards 3-benzyladenine 7-oxide (**7**) in H_2O, its pK_a values in H_2O at 30°C were spectrophotometrically determined to be 3.42 (basic) [for protonated form (**22**)⇌ neutral form] and >11 (acidic) (for neutral form⇌ monoanion). Therefore, the observed absorption bands at 262 nm (ε 12300) and 307 nm (ε 4000) in the UV spectrum of **7** in H_2O at pH 7 may be regarded as arising from the neutral species. The absence of a strong absorption in the 215—240 nm region may suggest the predominance of the N(7)-OH tautomer (**23**) in the neutral species in H_2O. However, the nonavailability of the neutral species spectrum of 7-methoxy-3-methyladenine (**13**),[25] a fixed model for the N(7)-OH form (**23**), renders this discussion inconclusive at present.

In a preliminary test for antileukemic activity against murine L5178Y cells, the N(7)-oxides **7** and **8** were found to be only very weakly cytotoxic at a concentration of 50 μg/ml.[26] Interestingly, this presents a contrast to the significant and weak antileukemic activities of guanine 7-oxide[15b,19] and hypoxanthine 7-*N*-oxide (**9**),[15d,20] respectively.

In conclusion, the present work has established a three-step synthetic route to adenine 7-oxide (**8**) from adenine (**1**) through 3-benzyladenine (**6**) and its 7-oxide (**7**). The route features the use of the readily removable benzyl group at the 3-position of **1** as a control synthon for *N*-oxidation. Interestingly, the regioselectivity in this *N*-oxidation has been found to be parallel to that in *N*-alkylation[9,10] of **6**. Since the chemical, chromatographic, and spectroscopic data obtained with our synthetic **8** do not accord with those reported first by Rhaese[6] and then by Yamamoto,[7] we consider their descriptions of adenine 7-oxide to be invalid. Thus, this paper is considered to be the first report dealing with the unequivocal synthesis and full characterization of hitherto unknown adenine 7-oxide (**8**).

Experimental

General Notes All melting points were determined by using a Yamato MP-1 or a Büchi model 530 capillary melting point apparatus and are corrected. TLC was run on Merck silica gel 60 F_{254} plates (0.25-mm thickness), Merck aluminum oxide F_{254} (type E) plates (0.25 mm), or Funakoshi Avicel SF-2020F plates, and spots were detected by means of UV absorbance measurement at 254 nm. Flash chromatography[27] was carried out by using Merck silica gel 60 (No. 9385). Spectra reported herein were recorded on a Hitachi M-80 mass spectrometer, a Hitachi model 320 UV spectrophotometer [on solutions in 95% (v/v) aqueous EtOH, 0.1 N aqueous HCl (pH 1), 0.005 M phosphate buffer (pH 7), and 0.1 N aqueous NaOH (pH 13)], a JASCO A-202 or a Shimadzu FTIR-8100 IR spectrophotometer, or a JEOL JNM-EX-270 or a JEOL JNM-GSX-500 NMR instrument. Internal standards used for the measurements of ^{1}H-NMR spectra were Me_4Si (for Me_2SO-d_6 solutions) and sodium 3-(trimethylsilyl)-1-propanesulfonate (for D_2O solutions). For the measurements of pH values, a Toa HM-18ET pH meter equipped with a Toa type GST-5211C glass electrode was employed. Spectrophotometric determination of acid dissociation constants was carried out in a manner similar to that described previously.[28] Elemental analyses and MS measurements were performed by Mr. Y. Itatani and his associates at Kanazawa University. The following abbreviations are used: br = broad, m = multiplet, s = singlet, sh = shoulder.

3-Benzyladenine 7-Oxide (7) i) By Oxidation of **6** with MMPP·$6H_2O$: 3-Benzyladenine (**6**)[9] (2.26 g, 10 mmol) was dissolved in MeOH (240 ml) with application of heat. After the methanolic solution had cooled to room temperature, MMPP·$6H_2O$[11] (purchased from Tokyo Kasei Kogyo Co.) (3.71 g, 7.5 mmol)[12] was added in one portion. The resulting methanolic solution was then stirred at 30 °C for 20 h. The colorless crystals that deposited were collected by filtration and triturated with saturated aqueous $NaHCO_3$ (7 ml). The resulting aqueous mixture was combined with H_2O (50 ml), heated to reflux, and filtered while hot in order to remove the insoluble material. The filtrate was cooled in a refrigerator, and the pale yellow needles that deposited were filtered off, washed successively with EtOH (1 ml) and ether (2 × 1 ml), and dried to give a first crop (853 mg, 33%) of **7**·H_2O, mp 250—254 °C (dec.). The last aqueous filtrate and ethanolic washings were combined with the first methanolic filtrate and concentrated *in vacuo* to leave a pale yellow oil, which was purified by flash chromatography[27] [silica gel, $CHCl_3$–MeOH (5 : 1; 3 : 1, v/v)]. An oil obtained from earlier fractions was triturated with a small amount of conc. aqueous NH_3, and the colorless solid that resulted was filtered off, washed successively with EtOH (2 × 1 ml) and ether (2 × 1 ml), and dried to recover **6** (1.15 g, 51%). This sample was identical (by comparison of the IR spectrum and TLC mobility) with authentic **6**.

Later fractions of the above chromatography gave a colorless solid, which was triturated with saturated aqueous $NaHCO_3$ (2 ml). The aqueous mixture was combined with H_2O (10 ml), heated to reflux, and filtered while hot. On cooling, the filtrate deposited pale yellowish crystals, which were collected by filtration, washed successively with EtOH (1 ml) and ether (1 ml), and dried to give a second crop (184 mg, 7%) of **7**·H_2O, mp 248—250 °C (dec.). The total yield of **7**·H_2O was 1.037 g (40%). Recrystallization of the crude **7**·H_2O from H_2O and drying over P_2O_5 at 2 mmHg and room temperature for 24 h furnished an analytical sample of **7**·H_2O as yellowish needles, mp 262—265 °C (dec.); pK_a (in H_2O at 30 °C and ionic strength 1.0): 3.42, >11; MS *m/z*: 241 (M^+); UV $\lambda_{max}^{95\%\,aq.EtOH}$ 265 nm (ε 12200), 322 (2200); $\lambda_{max}^{H_2O}$ (pH 1) 280 (14700); $\lambda_{max}^{H_2O}$ (pH 7) 262 (12300), 307 (4000); $\lambda_{max}^{H_2O}$ (pH 13), 265 (10700), 296 (sh) (5800); ^{1}H-NMR (Me_2SO-d_6) δ: 5.44 [2H, s, N(3)-C$\underline{H}_2$Ph], 7.29—7.38 (3H, m) and 7.42—7.46 (2H, m) [N(3)-CH$_2$$\underline{Ph}$], 7.90 [1H, s, C(8)-H], 8.13 and 8.81 (1H each, br, NH's), 8.64 [1H, s, C(2)-H].[29] *Anal.* Calcd for $C_{12}H_{11}N_5O \cdot H_2O$: C, 55.59; H, 5.05; N, 27.01. Found: C, 55.72; H, 4.92; N, 27.00.

Table I summarizes the above result (entry 2) and those (entries 1 and 3—5) of runs carried out under different reaction conditions, but with similar work-up.

ii) By Oxidation of **6** with MCPBA: A suspension of **6**[9] (2.25 g, 9.99 mmol) in a mixture of MeOH (120 ml) and 1 M AcOH–AcONa buffer (pH 5.0) (120 ml) was stirred at 30°C, and a solution of MCPBA (of *ca.* 80% purity) (3.24 g, 15 mmol) in MeOH (60 ml) was added dropwise

over a period of 5 h. The resulting mixture was further stirred at 30 °C for 15 h and then concentrated *in vacuo* to leave a pale yellowish solid. The residue was triturated with a mixture of 10% aqueous HCl (40 ml) and ether (20 ml), and the insoluble material that resulted was removed by filtration. The aqueous layer in the filtrate was separated from the ethereal layer, washed with ether (2 × 20 ml), neutralized with saturated aqueous $NaHCO_3$, and then filtered in order to remove the insoluble solid. The aqueous filtrate was concentrated *in vacuo*, and the residue was extracted with MeOH (150 ml). The methanolic extract was concentrated *in vacuo* and then subjected to flash chromatography[27] [silica gel, $CHCl_3$–MeOH (5 : 1; 3 : 1, v/v)]. A solid obtained from earlier fractions was recrystallized from EtOH to recover **6** (655 mg, 29%). This sample was identical (by comparison of the IR spectrum and TLC mobility) with authentic **6**.

Later fractions of the above chromatography yielded a colorless solid, which was recrystallized from H_2O to afford **7**·H_2O (621 mg, 24%) as yellowish needles, mp 250—254 °C (dec.). This sample was identical (by comparison of the IR spectrum and TLC mobility) with the one prepared by method (i).

Table I includes the above result (entry 6) and those (entries 7—11) of runs performed under different reaction conditions, but with similar work-up.

Adenine 7-Oxide (8) A suspension of **7**·H_2O (1.815 g, 7 mmol) in toluene (14 ml) was stirred at room temperature, and conc. H_2SO_4 (7 g, 70 mmol) was added dropwise. The resulting mixture was stirred vigorously at 35 °C for 3 h and then poured onto ice (30 g). The aqueous mixture was washed with toluene (2 × 10 ml), diluted with H_2O to a volume of *ca.* 60 ml, and then passed through a column of Amberlite IRA-402 (HCO_3^-) (150 ml). The column was eluted first with H_2O (60 ml) and then with H_2O (1000 ml) containing AcOH (22 g). The acidic eluates containing **8** were combined and concentrated *in vacuo* to leave a colorless solid. The solid was triturated with a little MeOH, and the insoluble solid that resulted was filtered off and recrystallized from H_2O at pH 4 (adjusted by addition of a few drops of saturated aqueous $NaHCO_3$) to provide **8** (578 mg, 55%) as colorless needles, mp >300 °C. Further recrystallization from H_2O yielded an analytical sample as colorless needles, mp >300 °C; pK_a (in H_2O at 30 °C and ionic strength 1.0): 3.4, 5.75; MS *m/z*: 151 (M^+), 135 (M^+-16); UV $\lambda_{max}^{95\%\ aq.\ EtOH}$ 246 nm (sh) (ε 5400), 271 (9100); $\lambda_{max}^{H_2O}$ (pH 1) 274 (11500); $\lambda_{max}^{H_2O}$ [pH 4.1 (in 0.005 M acetate buffer (ionic strength 1.0))] 243 (8200), 267 (8900); $\lambda_{max}^{H_2O}$ (pH 7) 235 (12400), 284 (6100); $\lambda_{max}^{H_2O}$ (pH 13) 235 (13000), 285 (6200); ^{1}H-NMR (Me_2SO-d_6) δ: 7.01 (2H, dull s, NH_2), 8.17 [1H, s, C(2)-H], 8.35 [1H, s, C(8)-H], 12.0—13.0 (1H, br, NH); ^{1}H-NMR (D_2O) δ: 8.26 [1H, s, C(2)-H], 8.29 [1H, s, C(8)-H]. *Anal.* Calcd for $C_5H_5N_5O$: C, 39.74; H, 3.33; N, 46.34. Found: C, 39.88; H, 3.26; N, 46.41.

Adenine 7-Oxide Hydrochloride (8·HCl) A solution of **8** (70.0 mg, 0.463 mmol) in 1 N aqueous HCl (2 ml) was concentrated to dryness *in vacuo*, leaving a colorless solid. The solid was washed with MeOH (1 ml) and dried to furnish **8**·HCl (81 mg, 93%), mp >300 °C (dec.). Recrystallization from 75% (v/v) aqueous MeOH yielded an analytical sample as colorless prisms, mp >300 °C (dec.); UV $\lambda_{max}^{95\%\ aq.\ EtOH}$ 272 nm (ε 9700); $\lambda_{max}^{H_2O}$ (pH 1) 274 (12100); $\lambda_{max}^{H_2O}$ (pH 7) 235 (13100), 284 (6400); $\lambda_{max}^{H_2O}$ (pH 13) 235 (13400), 285 (6400); ^{1}H-NMR (D_2O) δ: 8.42 and 8.46 (1H each, s, purine protons). *Anal.* Calcd for $C_5H_5N_5O$·HCl: C, 32.01; H, 3.22; N, 37.33. Found: C, 32.22; H, 3.22; N, 37.57.

Deoxygenation of 8 Leading to Adenine (1) A solution of **8** (15 mg, 0.099 mmol) in H_2O (10 ml) was hydrogenated over Raney Ni W-2 catalyst[30] (0.2 ml) at atmospheric pressure and 50 °C for 4 h. After cooling, the catalyst was removed by filtration and washed with H_2O (20 ml). The filtrate and washings were combined and concentrated *in vacuo* to leave, after drying over P_2O_5 at 2 mmHg and 100 °C for 3 h, adenine (**1**) (12 mg, 92%) as a colorless solid, mp >300 °C. This sample was identical (by comparison of the IR spectrum and TLC behavior) with authentic adenine.

Conversion of 8 into 8-Oxoadenine (4) A stirred mixture of **8** (10 mg, 0.066 mmol) and AcOH (1 ml) was heated under reflux for 5 h. The reaction mixture was concentrated *in vacuo* to leave **4** as a pale yellow solid. Recrystallization of the solid from 5% aqueous H_2SO_4 gave **4**·$1/2H_2SO_4$ (9 mg, 69%) as yellowish prisms, mp >300 °C. This sample was identical (by comparison of the IR spectrum and TLC mobility) with authentic **4**·$1/2H_2SO_4$.[18]

Deamination of 8 Leading to 6,8-Dioxopurine (5) and Hypoxanthine 7-*N*-Oxide (9) A solution of **8** (227 mg, 1.5 mmol) in 0.8 N aqueous HCl (7 ml) was heated to 90 °C, and a solution of $NaNO_2$ (124 mg, 1.8 mmol) in H_2O (4 ml) was added dropwise over a period of 30 min. After the addition had been completed, the temperature was maintained at 90 °C for 30 min. After cooling, the pale yellow solid that deposited was collected by filtration and dried to yield a first crop (93 mg, 41%) of **5**, mp >300 °C, which was identical (by comparison of the IR spectrum and TLC mobility) with authentic **5**.[15d] The filtrate was concentrated *in vacuo*, and the residue was triturated with H_2O (3 ml). The insoluble green solid that resulted was filtered off and recrystallized from H_2O to give a second crop (9 mg, 4%) of **5**, mp >300 °C. The total yield of **5** was 102 mg (45%). The filtrate, obtained when the green solid was isolated, was kept standing at room temperature, and the brownish solid that deposited was collected by filtration to obtain **9** (10 mg, 4%), mp >300 °C. This sample was identical (by comparison of the IR spectrum and TLC mobility) with authentic **9**.[15d]

Methylation of 8 Leading to 7-Methoxyadenine (10) and 3-Methyladenine 7-Oxide (11) A solution of **8** (302 mg, 2 mmol) in a mixture of 1 N aqueous NaOH (2 ml) and MeOH (2 ml) was stirred under ice-cooling, and dimethyl sulfate (of 95% purity) (266 mg, 2 mmol) was added. The resulting mixture was stirred at room temperature for 1 h and then concentrated *in vacuo*. The residual oil was co-evaporated with MeOH (10 ml), and the residue was subjected to flash chromatography[27] [silica gel, $CHCl_3$–MeOH (4 : 1, v/v)]. Earlier fractions gave **10** (74 mg, 22%) as a colorless solid, mp 200—205 °C (dec.). Recrystallization from MeOH yielded an analytical sample of **10** as colorless needles, mp >300 °C (darkened at *ca.* 200 °C); MS *m/z*: 165 (M^+), 134 (M^+-OMe); UV $\lambda_{max}^{95\%\ aq.\ EtOH}$ 273 nm (ε 9400); $\lambda_{max}^{H_2O}$ (pH 1) 274 (13400); $\lambda_{max}^{H_2O}$ (pH 7) 271 (10200); $\lambda_{max}^{H_2O}$ (pH 13) 273 (10600); ^{1}H-NMR (Me_2SO-d_6) δ: 4.13 (3H, s, OMe), 7.11 (2H, br, NH_2), 8.21 and 8.64 (1H each, s, purine protons). *Anal.* Calcd for $C_6H_7N_5O$: C, 43.63; H, 4.27; N, 42.40. Found: C, 43.49; H, 4.33; N, 42.15.

Later fractions, eluted with $CHCl_3$–MeOH (1 : 1, v/v), of the above chromatography gave a yellowish solid, which was triturated with H_2O (1 ml). The resulting insoluble solid was filtered off and dried to furnish **11**·$2H_2O$ (92 mg, 23%), mp 255—260 °C (dec.). Recrystallization from H_2O, drying over P_2O_5 at 2 mmHg and room temperature for 24 h, and subsequent moisturizing (by exposure to air until a constant weight was reached) provided an analytical ample of **11**·$2H_2O$ as pale yellow needles, mp 255—265 °C (dec.); MS *m/z*: 165 (M^+), 149 (M^+-16); UV $\lambda_{max}^{95\%\ aq.\ EtOH}$ 263 nm (ε 13100), 316 (3200); $\lambda_{max}^{H_2O}$ (pH 1) 278 (14400); $\lambda_{max}^{H_2O}$ (pH 7) 259 (12100), 303 (4100); $\lambda_{max}^{H_2O}$ (pH 13) 262 (11000), 295 (sh) (5400); ^{1}H-NMR (Me_2SO-d_6) δ: 3.81 [3H, s, N(3)-Me], 7.90 [1H, s, C(8)-H], 8.05 and 8.69 (1H each, br, NH's), 8.38 [1H, s, C(2)-H]. *Anal.* Calcd for $C_6H_7N_5O$·$2H_2O$: C, 35.82; H, 5.51; N, 34.81. Found: C, 35.67; H, 5.43; N, 34.68.

Hydrogenolysis of 10 Leading to Adenine (1) A solution of **10** (26 mg, 0.16 mmol) in H_2O (10 ml) was hydrogenated over Raney Ni W-2 catalyst[30] (0.1 ml) at atmospheric pressure and 40 °C for 4 h. The catalyst was removed by filtration and washed with hot H_2O (2 × 5 ml). The filtrate and washings were combined and concentrated *in vacuo*. The residue was dried over P_2O_5 at 2 mmHg and 100 °C for 3 h to yield adenine (**1**) (17 mg, 81%) as a colorless solid, mp >300 °C. This sample was identical (by comparison of the IR spectrum and TLC mobility) with authentic adenine.

Methylation of 8 Leading to 3-Methyladenine 7-Oxide (11) and 7-Methoxy-3-methyladenine (13) A mixture of **8** (529 mg, 3.50 mmol) and MeI (2.48 g, 17.5 mmol) in $AcNMe_2$ (53 ml) was stirred at 25 °C for 20 h. The reaction mixture was concentrated *in vacuo* to leave a pale yellow solid, which was dissolved in H_2O (5 ml). The aqueous solution was combined with a solution of $NaClO_4$·H_2O (590 mg) in H_2O (2 ml) and kept in a refrigerator for 2 h. The colorless prisms that deposited were filtered off, washed successively with H_2O and MeOH, and dried to give **13**·$HClO_4$ (163 mg, 17%), mp 225—253 °C (dec.). Recrystallization from H_2O yielded an analytical sample of **13**·$HClO_4$ as colorless prisms, mp 251—253 °C (dec.); UV $\lambda_{max}^{95\%\ aq.\ EtOH}$ 281 nm (ε 16200); $\lambda_{max}^{H_2O}$ (pH 1) 278 (15600); $\lambda_{max}^{H_2O}$ (pH 7) 278 (15600); $\lambda_{max}^{H_2O}$ (pH 13) unstable; ^{1}H-NMR (Me_2SO-d_6) δ: 3.94 [3H, s, N(3)-Me], 4.24 (3H, s, OMe), 8.69 and 9.49 (1H each, br, NH_2), 8.77 and 9.11 (1H each, s, purine protons). *Anal.* Calcd for $C_7H_9N_5O$·$HClO_4$: C, 30.07; H, 3.60; N, 25.04. Found: C, 29.82; H, 3.62; N, 24.89.

The aqueous filtrate and washings, both of which were obtained when the crude **13**·$HClO_4$ was isolated, were combined, brought to pH 8 by addition of saturated aqueous $NaHCO_3$, and concentrated *in vacuo*. The residue was triturated with H_2O (5 ml), and the insoluble material was filtered off. The resulting filtrate was concentrated *in vacuo*, and the

residue was triturated with H_2O (2 ml). The insoluble yellow solid that resulted was filtered off and recrystallized from H_2O to afford **11**·$2H_2O$ (175 mg, 25%), mp 255—265 °C (dec.). This sample was identical (by comparison of the IR spectrum and TLC behavior) with the one obtained from **8** by methylation with dimethyl sulfate under alkaline conditions (*vide supra*).

Conversion of 11 into 13 A mixture of **11**·$2H_2O$ (92 mg, 0.46 mmol) and MeI (355 mg, 2.50 mmol) in $AcNMe_2$ (9 ml) was stirred at 25 °C for 18 h. The reaction mixture was concentrated *in vacuo*, and the residue was dissolved in H_2O (1 ml). The resulting aqueous solution was combined with a solution of $NaClO_4 \cdot H_2O$ (83 mg) in H_2O (0.5 ml) and cooled in a refrigerator for 3 h. The pale yellowish prisms that deposited were collected by filtration, washed with a few drops of H_2O, and dried to give **13**·$HClO_4$ (114 mg, 89%), mp 249—253 °C (dec.). This sample was identical (by comparison of the IR spectrum and TLC behavior) with the one obtained directly from **8** by methylation with MeI (*vide supra*).

Hydrogenolysis of 11 Leading to 3-Methyladenine (12) A solution of **11**·$2H_2O$ (55 mg, 0.27 mmol) in H_2O (10 ml) was hydrogenated over Raney Ni W-2 catalyst[30] (0.1 ml) at atmospheric pressure and 40 °C for 4 h. The catalyst was removed by filtration and washed with hot H_2O (2 × 10 ml). The filtrate and washings were combined and concentrated to dryness *in vacuo* to leave **12** (37 mg, 90%) as a colorless solid, mp 270—280 °C (dec.). This sample was identical (by comparison of the IR spectrum and TLC mobility) with authentic **12**.[31]

Hydrogenolysis of 13 Leading to 3-Methyladenine (12) A solution of **13**·$HClO_4$ (28 mg, 0.1 mmol) in H_2O (10 ml) was hydrogenated over Raney Ni W-2 catalyst[30] (0.1 ml) at atmospheric pressure and 40 °C for 4 h. The catalyst was removed by filtration and washed with hot H_2O (2 × 10 ml). The filtrate and washings were combined and passed through a column of Amberlite IRA-402 (HCO_3^-) (2 ml), and the column was eluted with H_2O (10 ml). The eluates were combined and concentrated to dryness *in vacuo*, leaving **12** (11 mg, 73%) as a colorless solid, mp 270—280 °C (dec.). This sample was identical (by comparison of the IR spectrum and TLC mobility) with authentic **12**.[31]

Formation of 7-Acetamido-3-benzyladenine (15) and 7 from 6 3-Benzyladenine (**6**)[9] (2.25 g, 9.99 mmol) was dissolved in MeOH (240 ml) with application of heat. After the methanolic solution had cooled to 25 °C, MeCN (41.1 g, 1.00 mol), $KHCO_3$ (30 g, 0.30 mol), and 30% aqueous H_2O_2 (34 g, 0.3 mol) were added in that order. The resulting mixture was stirred at 25 °C for 22 h and then filtered in order to remove the insoluble material. The filtrate was concentrated *in vacuo* to a volume of *ca.* 180 ml, diluted with H_2O (300 ml), and stirred at 25 °C for a further 2 h after addition of a catalase (from bovine liver) suspension (Boehringer Mannheim Co., 20 mg/ml) (0.1 ml). The resulting mixture was concentrated *in vacuo* to obtain 100 ml of distillate and again stirred at 25 °C for 3.5 h after addition of more catalase suspension (0.05 ml). Stirring was further continued for 30 min after addition of more catalase suspension (0.05 ml) in order to destroy the excess of H_2O_2. The pale yellowish crystals that deposited were collected by filtration and recrystallized from EtOH to recover **6** (636 mg, 28%), which was identical (by comparison of the IR spectrum and TLC mobility) with an authentic sample.

The filtrate, obtained when the crude **6** was isolated, was concentrated *in vacuo*, and the residue was dried and then triturated with MeOH (80 ml). The colorless solid that resulted was filtered off, and the filtrate was concentrated *in vacuo* after addition of silica gel (10 g). The residue was then subjected to flash chromatography[27] [silica gel, $CHCl_3$–MeOH (5 : 1, v/v)]. Earlier fractions gave acetamide (formed from the MeCN and H_2O_2 through the Radziszewski reaction[32]) as colorless needles, and the last fractions yielded a pale yellowish solid, which was recrystallized from H_2O to afford **7**·H_2O (300 mg, 12%) as yellowish needles, mp 249—250 °C (dec.). This sample was identical (by comparison of the IR spectrum and TLC mobility) with the one prepared by the peroxycarboxylic acid oxidation of **6** (*vide supra*).

Middle fractions of the above chromatography afforded crude **15**, which was purified by recrystallization from EtOH as well as by preparative TLC [silica gel, $CHCl_3$–MeOH (5 : 1, v/v)] to yield **15** (32 mg, 1%), mp 244—247.5 °C. Further recrystallization from EtOH produced an analytical sample of **15** as colorless prisms, mp 246.5—250 °C; MS *m/z*: 282 (M^+); UV $\lambda_{max}^{95\%\ aq.\ EtOH}$ 269 nm (ε 14100); $\lambda_{max}^{H_2O}$ (pH 1) 279 (17700); $\lambda_{max}^{H_2O}$ (pH 7) 267 (12100), 280 (sh) (10600); $\lambda_{max}^{H_2O}$ (pH 13) 283 (12000); IR ν_{max}^{Nujol} 1671 cm^{-1} (amide CO); ^{1}H-NMR (Me_2SO-d_6) δ: 1.84 (3H, s, Me), 5.55 [2H, s, N(3)-C<u>H</u>$_2$Ph], 7.28—7.41 (3H, m) and 7.42—7.49 (2H, m) [N(3)-CH_2<u>Ph</u>], 8.85 [1H, s, C(8)-H or C(2)-H], 9.23 (br, NH's), 9.26 [1H, s, C(2)-H or C(8)-H]. *Anal.* Calcd for $C_{14}H_{14}N_6O$: C, 59.56; H, 5.00; N, 29.77. Found: C, 59.77; H, 5.05; N, 29.73.

The result of the above oxidation is included in Table I (entry 12).

3-Benzyladenine-*2-d* (16) A stirred suspension of **6**[9] (1.00 g, 4.44 mmol) in CD_3OD (of 99% isotopic purity) (9 ml) containing MeONa (71 mg, 1.3 mmol) was heated under reflux for 30 h. After cooling, the colorless prisms that deposited were collected by filtration, washed with MeOH, and dried over P_2O_5 at 2 mmHg and 50 °C for 8 h, giving **16** (958 mg, *ca.* 95%), ^{1}H-NMR (Me_2SO-d_6) δ: 5.51 [2H, s, N(3)-C<u>H</u>$_2$Ph], 7.24—7.40 (3H, m) and 7.41—7.52 (2H, m) [N(3)-CH_2<u>Ph</u>], 7.75 [1H, s, C(8)-H], 7.92 (*ca.* 1.5H, br, NH_2), 8.55 [0.15H, s, C(2)-H]. On the basis of the relative integral intensity of the C(2)-proton signal, the isotopic purity of this sample was estimated to be 85%.

3-Benzyladenine-*2-d* 7-Oxide (17) A solution of **16** (of 85% isotopic purity) (679 mg, 3 mmol) in MeOH (72 ml) containing MMPP·$6H_2O$ (1.15 g, 2.32 mmol) was stirred at 30 °C for 18 h. Work-up of the reaction mixture was carried out in a manner similar to that described above for the MMPP oxidation of **6**, yielding **17** (231 mg, *ca.* 32%) as yellowish needles, ^{1}H-NMR (Me_2SO-d_6) δ: 5.44 [2H, s, N(3)-C<u>H</u>$_2$Ph], 7.25—7.35 (3H, m) and 7.40—7.45 (2H, m) [N(3)-CH_2<u>Ph</u>], 7.90 [1H, s, C(8)-H], 8.13 and 8.82 (1H each, br, NH's), 8.64 [0.21H, s, C(2)-H]. The relative integral intensity of the C(2)-proton signal indicated that the isotopic purity of this sample was 79%. The recovery of **16** was 321 mg (47%).

Adenine-*2-d* 7-Oxide (18) A suspension of **17** (of 79% isotopic purity) (121 mg, 0.499 mmol) in a mixture of toluene (1 ml) and conc. H_2SO_4 (0.5 g) was stirred at 35 °C for 3 h. The reaction mixture was worked up in a manner similar to that described above for the debenzylation of **7**, giving **18** (35 mg, *ca.* 46%) as colorless prisms, mp >300 °C; ^{1}H-NMR (Me_2SO-d_6) δ: 6.95 (2H, dull s, NH_2), 8.16 [0.22H, s, C(2)-H], 8.34 [1H, s, C(8)-H], 12.0—13.0 (1H, br, NH); ^{1}H-NMR (D_2O) δ: 8.27

TABLE IX. Final Atomic Coordinates and Equivalent Isotropic or Isotropic Thermal Parameters for Atoms of **7**·$2H_2O$ with Estimated S.D.'s in Parentheses

Atom	*x*	*y*	*z*	B_{eq} (Å^2)
C(1)	0.6286 (4)	0.1244 (4)	0.9718 (68)	3.04 (27)
N(2)	0.6037 (4)	0.0652 (4)	0.8497 (69)	3.48 (24)
C(3)	0.5803 (5)	−0.0145 (5)	0.8891 (69)	3.97 (35)
N(4)	0.5778 (4)	−0.0449 (3)	1.0434 (68)	3.57 (25)
N(5)	0.6069 (4)	−0.0006 (4)	1.3357 (69)	3.93 (27)
C(6)	0.6369 (5)	0.0794 (5)	1.4033 (69)	3.94 (33)
N(7)	0.6497 (4)	0.1377 (3)	1.2884 (68)	3.30 (23)
C(8)	0.6270 (4)	0.0968 (4)	1.1372 (69)	2.77 (25)
C(9)	0.6024 (4)	0.0124 (4)	1.1727 (69)	3.16 (27)
N(10)	0.6525 (4)	0.2048 (4)	0.9336 (69)	3.54 (26)
O(11)	0.6823 (3)	0.2240 (3)	1.3088 (68)	3.68 (19)
C(12)	0.5521 (5)	−0.1366 (5)	1.0769 (70)	4.26 (36)
C(13)	0.6260 (5)	−0.1528 (5)	1.0685 (69)	5.03 (38)
C(14)	0.6692 (7)	−0.1482 (7)	1.2103 (70)	7.04 (53)
C(15)	0.7373 (10)	−0.1636 (9)	1.2072 (68)	10.50 (94)
C(16)	0.7601 (8)	−0.1842 (7)	1.0580 (73)	9.85 (86)
C(17)	0.7166 (9)	−0.1901 (7)	0.9084 (66)	9.51 (78)
C(18)	0.6486 (6)	−0.1733 (5)	0.9174 (71)	6.49 (51)
O(w1)	0.8597 (5)	0.3113 (7)	1.4112 (69)	6.37 (34)
O(w2)	0.6800 (4)	0.2804 (4)	0.6215 (69)	4.06 (20)
H(3)	0.554 (4)	−0.055 (4)	0.809 (12)	0.7
H(6)	0.636 (4)	0.101 (4)	1.531 (12)	0.8
H(10a)	0.650 (5)	0.211 (5)	0.825 (15)	3.3
H(10b)	0.668 (5)	0.242 (5)	1.024 (13)	1.6
H(12a)	0.519 (4)	−0.149 (4)	1.194 (12)	1.4
H(12b)	0.519 (5)	−0.163 (5)	1.004 (13)	0.7
H(14)	0.643 (5)	−0.138 (5)	1.333 (13)	2.2
H(18)	0.610 (6)	−0.175 (6)	0.795 (18)	6.4
H(w1a)	0.827 (10)	0.329 (10)	1.324 (26)	15.4
H(w1b)	0.836 (12)	0.270 (10)	1.399 (30)	16.3
H(w2a)	0.722 (5)	0.326	0.611 (12)	1.2
H(w2b)	0.676 (6)	0.241	0.520 (15)	4.7
H(15)	0.776	−0.162	1.324	
H(16)	0.810	−0.198	1.065	
H(17)	0.738	−0.203	0.804	

TABLE X. Final Atomic Coordinates and Equivalent Isotropic or Isotropic Thermal Parameters for Atoms of **8**·H_2O with Estimated S.D.'s in Parentheses

Atom	x	y	z	B_{eq} (Å^2)
C(1)	0.5799 (2)	0.0648 (1)	0.7072 (2)	2.29 (6)
N(2)	0.6928 (2)	0.1362 (1)	0.6433 (2)	2.61 (6)
C(3)	0.6290 (3)	0.2287 (1)	0.6466 (2)	2.69 (7)
N(4)	0.4641 (2)	0.2641 (1)	0.7070 (2)	2.67 (6)
N(5)	0.1717 (2)	0.2016 (1)	0.8406 (2)	2.62 (6)
C(6)	0.1119 (3)	0.1119 (1)	0.8883 (2)	2.70 (7)
N(7)	0.2461 (2)	0.0463 (1)	0.8494 (2)	2.32 (6)
C(8)	0.4013 (2)	0.0951 (1)	0.7741 (2)	2.24 (6)
C(9)	0.3521 (2)	0.1925 (1)	0.7702 (2)	2.25 (6)
N(10)	0.6392 (2)	−0.0269 (1)	0.7027 (2)	2.80 (7)
O(11)	0.2372 (2)	−0.0497 (1)	0.8744 (2)	3.11 (5)
O(w)	0.9901 (2)	0.1232 (1)	0.3978 (2)	3.03 (6)
H(3)	0.717 (3)	0.277 (1)	0.599 (2)	0.8
H(5)	0.110 (3)	0.257 (2)	0.854 (3)	1.9
H(6)	−0.016 (3)	0.099 (1)	0.953 (2)	1.3
H(10a)	0.565 (3)	−0.072 (1)	0.745 (2)	0.7
H(10b)	0.746 (4)	−0.040 (2)	0.660 (3)	2.0
H(wb)	0.916 (4)	0.124 (2)	0.489 (3)	2.7
H(wa)	0.925 (4)	0.098 (2)	0.313 (4)	3.2

[0.22H, s, C(2)-H], 8.30 [1H, s, C(8)-H]. On the basis of the relative integral intensity of the C(2)-proton signal, the isotopic purity of this sample was estimated to be 78%.

X-ray Structure Determination of 7 For X-ray analysis, colorless transparent prisms of **7**·2H_2O were grown from H_2O–tetrahydrofuran. A crystal measuring 0.25 × 0.25 × 0.15 mm was selected from among them and used for all data collection. Unit cell constants and intensity data were obtained with a Rigaku AFC-5R automatic diffractometer using graphite-monochromated Cu $K\alpha$ radiation (λ = 1.5418 Å). The unit cell dimensions were determined from angular settings of 20 2θ-values in the range of 50—65°, giving the following data: a = 17.911(2) Å; b = 17.911(2) Å; c = 8.017(4) Å; α = 90.00(0)°; β = 90.00(0)°; γ = 120.00(0)°; U = 2227.3(1.1) Å^3; space group $P6_1$; Z = 6; D_x = 1.240 g/cm^3; $F(000)$ = 876; μ(Cu $K\alpha$) = 7.820 cm^{-1}. Out of 1189 unique reflections ($0° \leq 2\theta \leq 120°$) measured by using the $\omega/2\theta$ scan technique at a rate of 8°/min, 995 without $|F_{obs}| = 0$ were considered unique and observed. No absorption corrections were applied.

The structure was solved by a direct method using the program SIR-88[33] and the difference Fourier method. Refinement of atomic parameters was carried out using the full-matrix least-squares method with anisotropic temperature factors. All hydrogen atoms were clearly located on difference Fourier maps and refined with isotropic temperature factors. Throughout the refinement, the function $\Sigma w(|F_O| - |F_C|)^2$ was minimized, and the weight used during the final refinement stage was $\sqrt{w} = 1/\sigma(F_O)$; the final R value was 0.0570 (R_w = 0.0690). The atomic scattering factors were taken from the literature.[34] The final atomic positions and equivalent isotropic or isotropic thermal parameters for all atoms are listed in Table IX. The selected bond lengths and angles are shown in Tables III and IV, respectively. A computer-generated,[35] perspective view of the structure of **7**·2H_2O is presented in Fig. 1.

X-ray Structure Determination of 8 Colorless transparent prisms of **8**·H_2O were grown from H_2O–MeCN. A crystal measuring 0.50 × 0.20 × 0.20 mm was selected from among them, and unit cell constants and intensity data were obtained as described above for **7**. The unit cell dimensions were determined from angular settings of 25 2θ-values in the range of 40—60°, yielding the following data: a = 6.755(2) Å; b = 13.722(3) Å; c = 7.460(1) Å; α = 90.00(0)°; β = 94.20(2)°; γ = 90.00(0)°; U = 689.6(3) Å^3; space group $P2_1/c$; Z = 4; D_x = 1.629 g/cm^3; $F(000)$ = 352; μ(Cu $K\alpha$) = 11.306 cm^{-1}. Out of 1025 unique reflections ($0° \leq 2\theta \leq 120°$) measured by using the $\omega/2\theta$ scan technique at a rate of 8°/min, 962 without $|F_{obs}| = 0$ were considered reliable.

The structure was solved in a manner similar to that described above for **7**, but by a direct method using the program MULTAN-80.[36] Refinement of atomic parameters was carried out using the block-diagonal least-squares method with anisotropic and isotropic temperature factors. The final R value was 0.0330 (R_w = 0.0420). The final atomic positions and equivalent isotropic or isotropic thermal parameters for all atoms are listed in Table X. Selected bond lengths and angles are shown in Tables V and VI, respectively. A computer-generated,[35] perspective view of the structure of **8**·H_2O is presented in Fig. 2.

TABLE XI. Final Atomic Coordinates and Equivalent Isotropic or Isotropic Thermal Parameters for Atoms of **15** with Estimated S.D.'s in Parentheses

Atom	x	y	z	B_{eq} (Å^2)
C(1)	0.2084 (5)	0.5657 (3)	0.1549 (5)	2.66 (1)
N(2)	0.3075 (4)	0.5028 (3)	0.3125 (5)	3.17 (1)
C(3)	0.4029 (4)	0.4081 (3)	0.2987 (4)	3.38 (1)
N(4)	0.4186 (5)	0.3642 (3)	0.1417 (6)	2.90 (1)
C(5)	0.3272 (5)	0.4276 (4)	−0.0188 (6)	2.71 (1)
C(6)	0.2240 (5)	0.5275 (3)	−0.0120 (5)	2.46 (1)
N(7)	0.1519 (4)	0.5699 (3)	−0.1920 (5)	2.67 (1)
C(8)	0.2162 (4)	0.4935 (3)	−0.2943 (4)	3.21 (1)
N(9)	0.3241 (6)	0.4048 (4)	−0.1925 (7)	3.11 (2)
N(10)	0.1065 (5)	0.6585 (3)	0.1681 (5)	3.18 (1)
C(11)	0.5247 (5)	0.2521 (4)	0.1409 (5)	3.40 (1)
C(12)	0.4253 (6)	0.1452 (4)	0.1196 (6)	3.04 (2)
C(13)	0.2738 (5)	0.1353 (4)	−0.0312 (5)	3.87 (2)
C(14)	0.1841 (8)	0.0353 (6)	−0.0537 (8)	5.48 (3)
C(15)	0.2472 (6)	−0.0547 (4)	0.0735 (7)	6.90 (3)
C(16)	0.3974 (6)	−0.0472 (4)	0.2226 (6)	6.58 (2)
C(17)	0.4870 (6)	0.0535 (4)	0.2462 (6)	4.70 (2)
N(18)	0.0272 (8)	0.6686 (5)	−0.2352 (9)	3.08 (4)
C(19)	0.0119 (11)	0.7283 (6)	−0.3860 (14)	3.01 (10)
O(20)	0.0978 (8)	0.7092 (6)	−0.4875 (10)	4.06 (5)
C(21)	−0.1265 (12)	0.8298 (6)	−0.4324 (13)	4.65 (9)
H(3)	0.488 (6)	0.369 (4)	0.413 (6)	2.3
H(8)	0.169 (6)	0.494 (4)	−0.431 (6)	2.2
H(10a)	0.108 (5)	0.687 (4)	0.280 (6)	3.7
H(10b)	0.043 (6)	0.691 (4)	0.075 (6)	0.8
H(11a)	0.635 (8)	0.252 (6)	0.261 (8)	1.5
H(11b)	0.571 (8)	0.258 (5)	0.044 (8)	1.3
H(13)	0.242 (6)	0.199 (4)	−0.117 (6)	1.4
H(14)	0.067 (7)	0.028 (5)	−0.171 (8)	4.6
H(15)	0.189 (6)	−0.130 (4)	0.059 (6)	6.1
H(16)	0.448 (6)	−0.092 (4)	0.316 (6)	4.0
H(17)	0.574 (7)	0.063 (5)	0.341 (8)	0.9
H(21b)	−0.171 (8)	0.845 (6)	−0.346 (9)	3.5
H(21c)	−0.074 (8)	0.906 (5)	−0.454 (8)	6.8
H(21a)	−0.237 (8)	0.802 (5)	−0.550 (8)	5.3

X-ray Structure Determination of 15 Colorless, transparent prisms of **15** were grown from MeOH. A crystal measuring 0.25 × 0.25 × 0.05 mm was selected from among them, and unit cell constants and intensity data were obtained as described above for **7**. The unit cell dimensions were determined from angular settings of 46 2θ-values in the range of 85—90°, furnishing the following data: a = 8.390(2) Å; b = 11.323(1) Å; c = 7.739(1) Å; α = 91.41(1)°; β = 111.81(1)°; γ = 84.89(1)°; U = 679.8(2) Å^3; space group $P\bar{1}$; Z = 2; D_x = 1.379 g/cm^3; $F(000)$ = 296; μ(Cu $K\alpha$) = 0.779 mm^{-1}. Out of 2023 unique reflections ($0° \leq 2\theta \leq 120°$) measured by using the $\omega/2\theta$ scan technique at a rate of 16°/min, 1956 without $|F_{obs}| = 0$ were considered reliable.

The structure was solved and refined as in the case of **7**, but by a direct method using the program SHELXS-86.[37] The final R value was 0.0540 (R_w = 0.0710). Table XI lists the final atomic positions and equivalent isotropic or isotropic thermal parameters for all atoms. Tables VII and VIII assemble the selected bond lengths and angles, respectively. Figure 3 presents a computer-generated,[35] parallel view of the structure of **15**.

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