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Studies of Nucleosides and Nucleotides. LXIX.[1] Purine Cyclonucleosides. (30). Elimination of the 8-Oxy Function of Purine Nucleosides

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Triacetyl-8-oxyinosine (Ib) was treated with phosphoryl chloride in diethylaniline or thionyl chloride in dimethyl formamide (DMF) to give only 6-chloro-8-oxy compound (IV). Treating (Ib) with phosphoryl chloride in tri-*n*-butylamine gave 6,8-dichloro compound (VII). Compound (VII) was converted to bis(methylmercapto) compound (VIII) and treated successively with dimethylamine and Raney nickel to give N^6-dimethyladenosine. Thus, a procedure for eliminating 8-oxy function of purine nucleosides has been substantiated.

Purine O-cyclonucleosides gave 8-substituted nucleosides by the attack of various nucleophiles.[3–7] In many cases the 8-oxy function remained to be eliminated for obtaining analogs of naturally occuring nucleosides. For this purpose we attempted to chlorinate 8-oxyadenosine[4] by using phosphoryl chloride in pyridine or thionyl chloride in dimethyl formamide (DMF), but no 8-chloro compound has been isolated so far. N^6-Acylated 8-oxyadenosine,[8] which was treated analogously, also failed to give the 8-chloro compound. Separate attempt to thiolate the 8-oxy group by using phosphorus pentasulfide in pyridine[9] also gave no 8-thio compound. We therefore changed the substrate of the chlorination to 8-oxyinosine (Ia), which was easily obtainable from 8-bromoadenosine[10] (II). 8-Bromoadenosine (II) was deaminated by sodium nitrite in acetic acid to give 8-bromoinosine (III) in a yield of 72%. The compound III was then treated with sodium acetate in acetic acid-acetic anhydride mixture for 3 hr at reflux temperature. 2′,3′,5′-Tri-O-acetyl-8-oxyinosine (Ib) was obtained in a yield of 77%. Elemental analysis and other physical data showed the structure to be correct. When Ib was treated with Vilsmeier reagent freshly prepared from thionyl chloride and DMF,[11] a nucleoside (IV) having a 6-chloro-8-oxypurine chromophore was obtained. Treatment of this compound (IV) with dimethylamine gave N^6-dimethyl-8-oxyadenosine (V), which was identical to a sample obtained from 8-bromo-N^6-dimethyladenosine (VI).[12] Changing the chlorinating reagent to phosphoryl chloride in diethylaniline which was found to be effective

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3) M. Ikehara, *Accounts of Chem. Res.*, **2**, 47 (1969).
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5) M. Ikehara and M. Kaneko, *J. Amer. Chem. Soc.*, **90**, 497 (1968); M. Ikehara, H. Kaneko, and R. Okano, *Tetrahedron*, **26**, 5675 (1970).
6) M. Ikehara and K. Muneyama, *Chem. Pharm. Bull.* (Tokyo), **18**, 1196 (1970).
7) M. Ikehara, M. Kaneko, and Y. Ogiso, *Tet. Lett.*, **1970**, 4673; M. Ikehara and Y. Ogiso, *Tetrahedron*, **28**, 3695 (1972).
8) M. Ikehara and M. Kaneko, *Chem. Pharm. Bull.* (Tokyo), **18**, 2401 (1970).
9) J.J. Fox, I. Wempen, A. Hampton, and I.L. Doerr, *J. Amer. Chem. Soc.*, **80**, 1669 (1959).
10) M. Ikehara and M. Kaneko, *Tetrahedron*, **26**, 4251 (1970).
11) M. Ikehara, H. Uno, and F. Ishikawa, *Chem. Pharm. Bull.* (Tokyo), **10**, 665 (1962); *idem, ibid.*, **13**, 947 (1965).
12) M. Ikehara and H. Morisawa, *Chem. Pharm. Bull.* (Tokyo), **19**, 2543 (1971).

in chlorinating 6,8-dioxy purine to dichloropurine,[13,14] also gave the 6-chloro-8-oxy nucleoside (IV). Finally, we used phosphoryl chloride in tri-*n*-butylamine, which had a lower p*K* and higher boiling point 217° than diethylaniline. When triacetyl-8-oxyinosine (Ib) was heated for 3 hr at reflux temperature with phosphoryl chloride in tri-*n*-butylamine, two spots appeared at *Rf*s 0.56 and 0.81 on a thin-layer chromatogram (TLC) developed in chloroform–ethanol (20: 1). The former spot corresponded to the 6-chloro-8-oxy compound (IV) and the latter showed ultraviolet spectrum (UV) absorption maxima at 251 and 268 nm. Addition of more reagents and prolongation of refluxing for 7 hr gave a further increase of the latter compound. The appropriate work up gave 6,8-dichloro-9-*β*-(2,3,5-tri-O-acetyl-D-ribofuranosyl)purine (VII) in a yield of 38%. Although attempts to crystallize (VII) failed, the structure was proved to be correct by the following evidence. The compound showed UV absorption spectra resembling those of 6,8-dichloropurine[14] except at alkaline pH. The mass spectrum showed a molecular ion peak at *m/e* 259 together with peaks at 189 and 191 corresponding to 6,8-dichloropurine. The nuclear magnetic resonance (NMR) spectrum showed signals at 8.76 *δ* corresponding to H-2, which was shifted significantly to low field relative to that of inosine. Signals corresponding to H-1′,2′,3′,4′, and 5′ appeared at 6.27—4.40 *δ* and the coupling constant $J_{2'-3'}$ and $J_{3'-4'}$ were equal to 5 Hz. Three signals having intensity of 3Hs showed the presence of acetyl groups. Faster migrating tendency in TLC also supported the introduction of two chloro atoms. Further support of the structure was obtained by the conversion of VII to the bis(methylthio) compound (VIII) as described later.

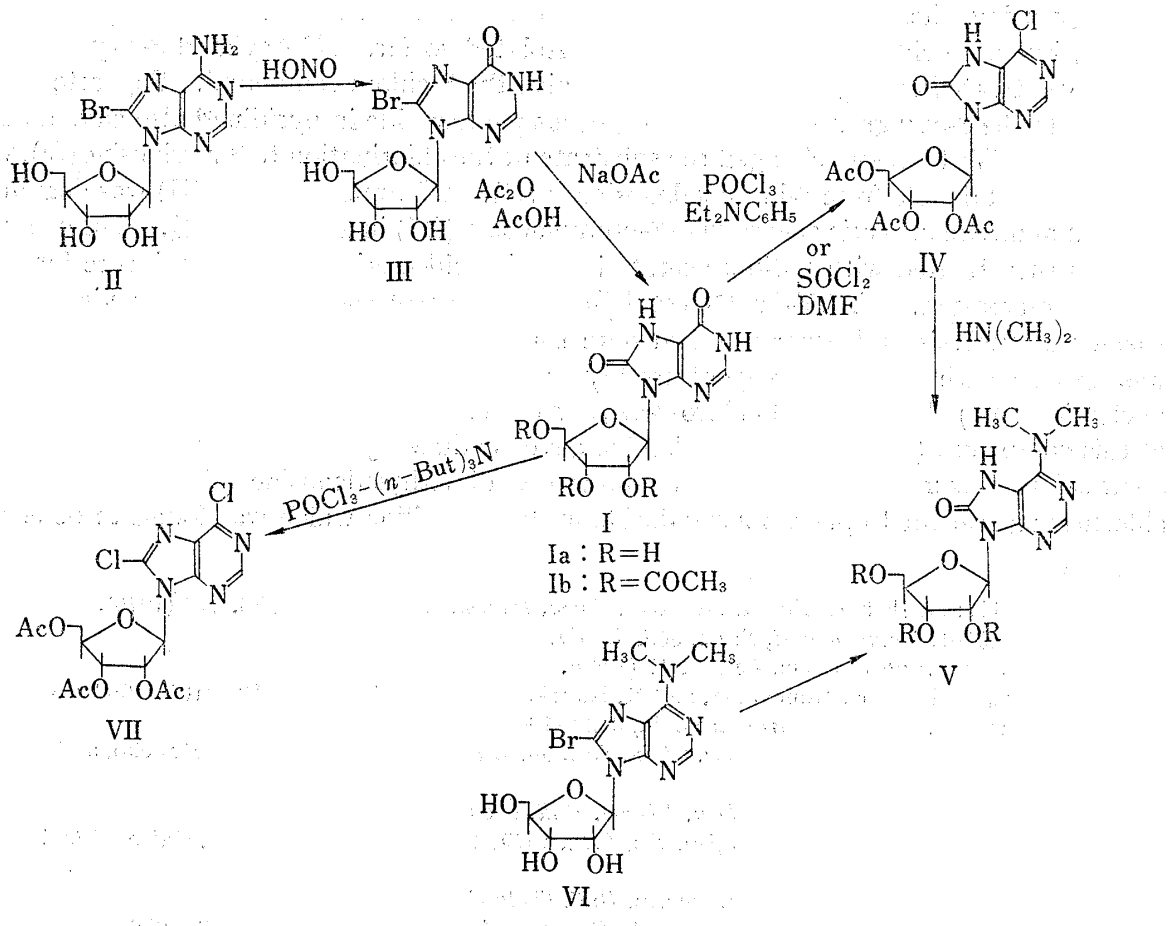

Chart 1

13) E. Fischer and L. Ach, *Ber.*, **30**, 2208 (1897).
14) R.K. Robins, *J. Amer. Chem. Soc.*, **80**, 6671 (1958).

If the 6-Cl atom of the 6,8-dichloro compound (VII) was replaced by NH_3 or methylamines, the 8-chloro function must be easily eliminated by reduction. However, when compound (VII) was treated with aqueous dimethylamine either at room temperature or 80°, only 8-dimethylamino-N^6-dimethyladenosine (IX) was obtained. A weaker nucleophile, methanolic ammonia, is known to give the 8-amino compound[15] from VII. Compound (VII) was then allowed to react with sodium methylmercaptide in aqueous dioxane at room temperature overnight. 6,8-Bis(methylthio)-9-β-D-ribofuranosylpurine (VIII) was obtained in a yield of 58%. The structure of VIII was elucidated by elemental analysis, UV absorption properties resembling those of 6,8-dimethylmercaptopurine[14] and NMR spectra showing two CH_3 signals at 2.68 and 2.78 and a H-2 signals at 8.63 δ. Deacetylation of compound (VII) with sodium methylmercaptide is noteworthy.

When the compound (VIII) was treated with 40% aqueous dimethylamine at 100° for 14 hr, followed by acetylation with acetic anhydride in pyridine, it gave N^6-dimethyl-8-methylmercapto-2',3',5'-tri-O-acetyladenosine (X) in a yield of 47%. Elemental analytical data, UV absorption properties showing λ_{max}^{EtOH} 231 and 292 nm, and NMR signals appearing at 8.23 δ for H-2, 3.48 δ for $N(CH_3)_2$, and 2.71 δ for SCH_3 groups comfirmed the structure of X.

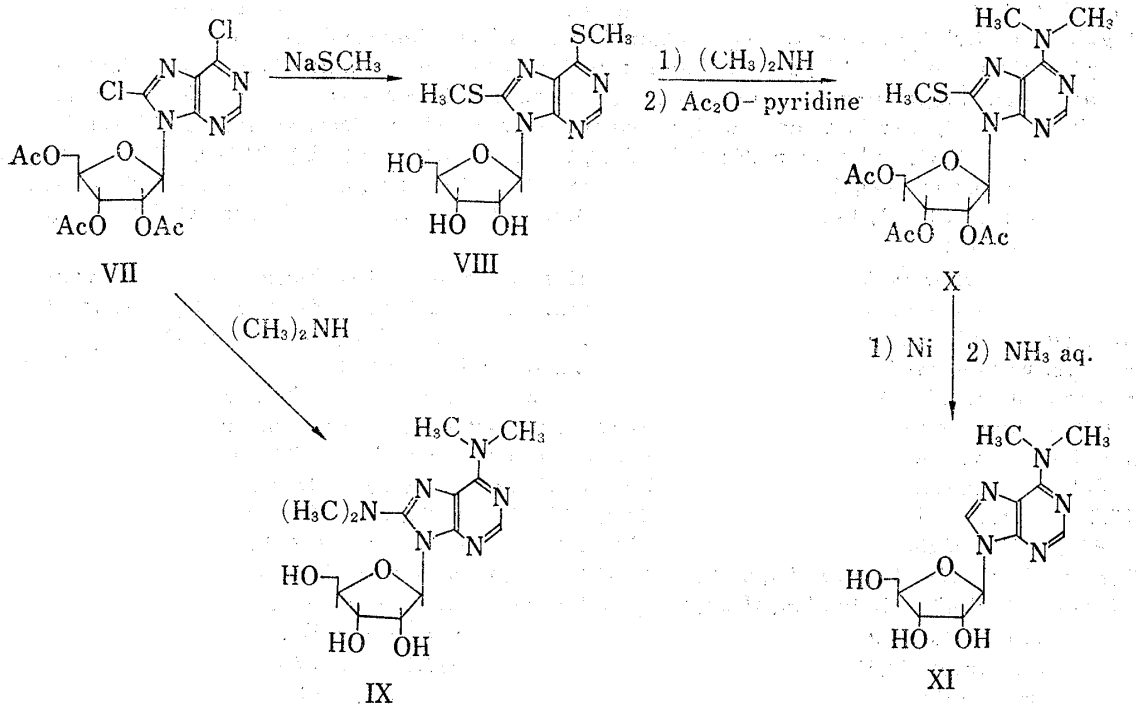

Chart 2

Compound (X) was then dethiolated by refluxing in ethanol with Raney nickel for 1 hr. After removal of acetyl groups with aqueous ammonia, N^6-dimethyladenosine (X) was obtained in a yield of 35%. The product was identical with an authentic sample of N^6-dimethyl adenosine[12,16] by paper chromatography, thin-layer chromatography and UV absorption properties.

By this experiment it became possible to eliminate the 8-oxy function of a purine nucleoside and the present method might be applicable to the cleavage product of purine O-cyclonucleosides. Work along these lines will be reported in subsequent papers.

15) G.L. Syekers, R.A. Long, and R.K. Robins, Abstracts of IVth International Congress of Heterocyclic Chemistry, 1973, p. 5.
16) M. Ikehara, E. Ohtsuka, and F. Ishikawa, *Chem. Pharm. Bull.* (Tokyo), 9, 173 (1961).

Experimental[17]

8-Oxy-2′,3′,5′-tri-O-acetylinosine (Ib)——Anhydrous sodium acetate (2.92 g, 45.3 mmoles) was dissolved in a mixture of acetic acid (35 ml) and acetic anhydride (35 ml) with heating. Well-dried 8-bromoinosine[18] (2.75 g, 8 mmoles) was added into the solution and heated at reflux temperature for 3 hr. The extent of reaction was examined by TLC and the solvent was evaporated *in vacuo*. EtOH was added to the residue to decompose acetic anhydride and the whole was evaporated. The residue was dissolved in H_2O–$CHCl_3$. The chloroform layer was washed with saturated $NaHCO_3$ aq and H_2O and dried over $MgSO_4$. Chloroform was evaporated to give a residue, which was recrystallized from benzene. 8-Oxy-triacetylinosine was obtained in a yield of 2.48 g (0.05 mmoles, 77%). mp 114—116°. *Anal.* Calcd. for $C_{18}H_{18}O_9N_4 \cdot 1/3C_6H_6$: C, 49.54; H, 4.62; N, 12.84. Found: C, 49.19; H, 4.59; N, 12.56. UV: $\lambda_{max}^{50\%\ EtOH}$ 256.5 nm (ε 12100), 260 (sh, 6500); $\lambda_{max}^{0.1N\ HCl}$ 256.5 (12300), 280 (6500); $\lambda_{max}^{0.1N\ NaOH}$ 273.5 (15300). IR: ν_{max}^{KBr} 1740 cm^{-1} (8–C=O). Mass Spectrum: M^+ 410, *m/e* 259 (M-8-oxyhypoxanthine), 152 (8-oxyhypoxanthine). NMR: ( ) 12.50 (b, 1H, N_7-H), 7.25 (b, 1H, N_1-H), 8.10 (s, 1H, H-2), 6.15 (m, 2H, H-1′ and 2′), 5.74 (m, 1H, H-3′), 4.40 (m, 3H, H-4′ and 5′), 2.13 (s, 6H, acetyl-CH_3), 2.08 (s, 3H, acetyl-CH_3), $J_{H_2'-H_3'}$=5.0 Hz.

6,8-Dichloro-9-β-(2,3,5-tri-O-acetyl-D-ribofuranosyl)purine (VII)——Freshly distilled phosphoryl chloride (12 ml) and tri-*n*-butylamine (1.2 ml) were combined and kept for 10 min in a stoppered flask. Into the solution was added 8-oxy-triacetylinosine (600 mg, 1.47 mmole). The whole was refluxed in an oil bath with exclusion of moisture. After 3 hr and 5 hr tri-*n*-butylamine (0.6 ml each) was added again. After 7 hr refluxing, the reaction mixture was poured into saturated $NaHCO_3$ aq with vigorous stirring. The mixture was stirred for 30 min at room temperature and extracted with $CHCl_3$ (200 ml). The $CHCl_3$ layer was separated and washed with H_2O (100 ml), 0.1N HCl aq (100 ml × 2), and finally with water (100 ml). After drying on Mg_2SO_4, the $CHCl_3$ solution was concentrated to a half its volume and applied to a column (1.8 × 5 cm) of silica gel. Elution with $CHCl_3$ gave a fraction containing the 6,8-dichloro compound, which was concentrated and applied again to a silica gel column (1.8 × 5 cm). Elution with $CHCl_3$–cyclohexane (1: 1, vol/vol) gave a fraction of 6,8-dichloro compound, which was evaporated to a hard syrup. Yield was 175 mg (0.39 mmole, 38%). UV $\lambda_{max}^{50\%\ EtOH}$ 268 nm, 251, 275 (sh), $\lambda_{max}^{0.1N\ HCl}$ 268, 251, 275 (sh), $\lambda_{max}^{0.1N\ NaOH}$ 270, 250. Mass Spectrum: *m/e* 259 (M-dichloropurine), 189, 191 (dichloropurine+H). NMR: (δ) 8.76 (s, 1H, H-2), 6.27 (m, 2H, H-1′ and 2′), 5.83 (t, 1H, H-3′), 4.40 (m, 3H, H-4′ and 5′), 2.17, 2.09, 2.01 (each s, 3H, acetyl-CH_3). $J_{H_2'-H_3'}$=5.0 Hz, $J_{H_3'-H_4'}$=5.0 Hz.

6,8-Bis(methylmercapto)-9-β-D-ribofuranosylpurine (VIII)——6,8-Dichloro-triacetylribofuranosylpurine (298 mg, 0.64 mmole) was dissolved in dioxane (30 ml). To the solution was added 20% sodium methylmercaptane aq (4.3 ml, 15.9 mmoles). The reaction mixture was stirred at room temperature overnight. The water-layer was removed and the dioxane solution was neutralized with 1N HCl. Evaporation of the solvent with a trap of conc. NaOH to absorb methyl mercaptane gave a residue. The residue was triturated with H_2O (30 ml) and the insoluble material was collected by filtration. Recrystallization of this material from EtOH (5 ml) gave the 6,8-bis(methylmercapto) compound in a yield of 126 mg (0.37 mmole, 58%). mp 200—202°. *Anal.* Calcd. for $C_{12}H_{16}O_4N_4S_2$: C, 41.86: H, 4.68; N, 16.28; S, 18.09. Found: C, 41.75; H, 4.69; N, 16.29; S, 18.16. UV: λ_{max}^{EtOH} 248 nm (ε, 16600), 305 (sh, 22200), 311 (23000); $\lambda_{max}^{0.1N\ HCl}$ 248.5 (15,500), 305 (sh, 18800), 312 (20200), 332 (sh, 6400); $\lambda_{max}^{0.1N\ NaOH}$ 248 (16800), 305 (sh, 22300), 311.5 (23000). NMR: (δ) 8.63 (s, 1H, H-2), 5.78 (d, 1H, H-1′), 5.41 (d, 1H, 2′-OH), 5.05 (m, 2H, H-2′ and 5′-OH), 4.28 (hexa, 1H, H-3′), 3.98 (hexa, 1H, H-4′), 3.62 (m, 2H, H-5′), 2.78, 2.68 (each s, 3H, S-CH_3); $J_{H_1'-H_2'}$=6.0 Hz, $J_{H_2'-H_3'}$=6.0 Hz, $J_{H_3'-H_4'}$=3.0 Hz, $J_{H_4'-H_5'}$=5.0 Hz, $J_{H_2'-2'-OH}$=5.0 Hz, $J_{H_3'-3'-OH}$=5.0 Hz.

8-Methylmercapto-2′,3′,5′-tri-O-acetyl-N^6-dimethyladenosine (X)——6,8-Bis(methylmercapto)-9-β-D-ribofuranosylpurine (300 mg) was dissolved in 40% dimethylamine aq (30 ml) and heated at 100° for 14 hr in a sealed tube. The solution was evaporated and pyridine (10 ml) was added to the residue. After evaporation of pyridine *in vacuo* for removing traces of water, pyridine (10 ml) and acetic anhydride (5 ml) were added. The mixture was kept at room temperature for 2 hr with stirring. The solvent was removed by vacuum evaporation and EtOH was added to the residue. After standing at room temperature for 1 hr, the solution was evaporated *in vacuo* with added toluene to remove traces of pyridine. The residue was dissolved in $CHCl_3$ and washed twice each with $NaHCO_3$ aq and water. The $CHCl_3$ solution was dried over Mg_2SO_4 and evaporated *in vacuo*. The residue was recrystallized from EtOH at −15—20° to give a white solid in a yield of 213 mg (47%). The solid melted at room temperature. *Anal.* Calcd. for $C_{19}H_{25}O_7N_5S$: C, 48.81; H, 5.39; N, 14.98; S, 6.85. Found: C, 49.60; H, 5.63; N, 14.61; S, 7.11. UV: $\lambda_{max}^{50\%\ EtOH}$ 231 nm (ε 17200), 292

17) UV absorption spectra were taken with a Hitachi EPS-3T spectrophotometer, infrared (IR) spectra were taken with a Hitachi EPI-G3 spectrophotometer, and NMR spectra were taken with a Hitachi R-22 spectrometer (90 HC) using tetramethylsilane as external standard in the solvent d_6-DMSO. Paper chromatography was performed on Toyo filter paper No 51A in the following solvent systems: B, *n*–butanol–H_2O (84: 16); C, isopropanol–conc. ammonia–H_2O (7: 1: 2), G: *n*–butanol–acetic acid–H_2O (5: 2: 3). TLC was performed on Kieselgel HF 254 plates using $CHCl_3$–EtOH development system.

18) R.E. Holmes and R.K. Robins, *J. Amer. Chem. Soc.*, **86**, 1242 (1964).

(19500); $\lambda_{max}^{0.1N\ HCl}$ 290 (21400); $\lambda_{max}^{0.1N\ NaOH}$ 294 (20500). NMR: (δ) 8.23 (s, 1H, H-2), 6.27 (t, 1H, H-2'), 6.10 (d, 1H, H-1'), 5.84 (t, 1H, H-3'), 4.40 (m, 3H, H-4'), 3.48 (3H, acetyl-CH_3), $J_{H_1'-H_2'}$=5.0 Hz, $J_{H_2-H_3}$=6.0 Hz, $J_{H_3'-H_4'}$=6.0 Hz.

N^6-Dimethyladenosine (XI)——8-Methylmercapto-N^6-dimethyladenosine (40 mg) was dissolved in EtOH (3 ml). After the addition of Raney Ni (0.4 ml), the mixture was heated at reflux temperature for 1 hr with stirring. The catalyst was removed by filtration and washed thoroughly with hot alcohol. The filtrate was evaporated and the residue was dissolved in $CDCl_3$ (0.4 ml). Examination by NMR spectroscopy showed that the SCH_3 group was totally removed and a signal of H-8 appeared. $CDCl_3$ was evaporated *in vacuo* and the residue was dissolved in EtOH (3 ml). To the solution was added 28% NH_3 aq (1 ml) and the whole was kept at room temperature overnight. N^6-Dimethyladenosine thus obtained showed UV: $\lambda_{max}^{50\%\ EtOH}$ 277 nm, $\lambda_{max}^{0.1N\ HCl}$ 270, $\lambda_{max}^{0.1N\ NaOH}$ 277. TLC ($CHCl_3$–EtOH, 8: 1, vol/vol) *Rf* 0.26. Paper chromatography: *Rf* (B), 0.58, *Rf*(C) 0.75. These properties were identical with an authentic sample.

6-Chloro-8-oxy-9-β-(2,3,5-tri-O-acetyl-D-ribofuranosyl)purine (IV)——i) Triacetyl-8-oxyinosine (300 mg, 0.74 mmole) was dissolved in $CHCl_3$ (6 ml) and combined with Vilsmeier reagent, which was prepared by the addition of $SOCl_2$ (0.72 g) to anhydrous DMF (0.2 ml) under ice-cooling. The reaction mixture was kept at room temperature for 30 min. Refluxing of the reaction mixture for 2 hr gave two spots at *Rf* 0.33 and *Rf* 0.57 on TLC ($CHCl_3$–EtOH, 10: 1). The former corresponded to the starting material and the latter spot showed UV absorption: λ_{max}^{EtOH} 245 (sh), 283 nm; $\lambda_{max}^{0.1N\ HCl}$ 243.5, 280; $\lambda_{max}^{0.1N\ NaOH}$ 263, 298. Yield, estimated from the UV absorption intensity, was 15.8%. Since this material was converted to N^6-dimethyl-8-oxyadenosine by treatment with dimethylamine, the structure could be assigned as the 6-chloro-8-oxy compound. ii) When the same reaction was performed without $CHCl_3$ and using DMF (6 ml) as the solvent, the reaction proceeded faster and the yield of 6-chloro-8-oxy compound increased to 27%. However, the colour of the reaction mixture darkened considerably. iii) Triacetyl-8-oxyinosine (260 mg, 0.64 mmole) was dissolved in a mixture of $POCl_3$ (2 ml) and diethylaniline (0.3 ml). Refluxing the mixture for 3.5 hr produced the 6-chloro-8-oxy compound in a yield of 43%.

8-Oxy-2',3',5'-tri-O-acetyl-N^6-dimethyladenosine (Va)——Anhydrous sodium acetate (2.05 g, 24 mmoles) was dissolved in a mixture of acetic acid (20 ml) and acetic anhydride (20 ml). To the mixture was added 8-bromo-N^6-dimethyladenosine (1 g). The solution was refluxed for 10 hr with exclusion of the moisture. The solvent was removed *in vacuo* and traces of acetic anhydride were removed by repeated addition of EtOH (50 ml) and evaporation. The residue was equilibrated in H_2O (50 ml) and $CHCl_3$ (50 ml). The $CHCl_3$–layer was separated, washed with $NaHCO_3$ aq (50 ml), and dried over Mg_2SO_4. The solvent was evaporated and the residue was recrystallized from 99% EtOH. 8-Oxy-triacetyl-N^6-dimethyladenosine, mp 165—170°, was obtained in a yield of 0.82 g (42.3%). *Anal.* Calcd. for $C_{18}H_{23}O_8N_5$: C, 49.52; H, 5.03; N, 16.01. Found: C, 49.33; H, 5.36; N, 15.87. UV: $\lambda_{max}^{50\%\ EtOH}$ 282 nm (ε 15300), $\lambda_{max}^{0.1N\ HCl}$ 277 (13800), 305 (sh, 9200); $\lambda_{max}^{0.1N\ NaOH}$ 295 (18900). IR: ν_{max}^{KBr} 1735 cm^{-1} (acetyl), 1700 (8-C=O).

8-Oxy-N^6-dimethyladenosine (Vb)——i) 8-Oxy-triacetyl-N^6-dimethyladenosine (5 mg) was dissolved in conc. NH_3 aq (0.5 ml). After 3 hr, the whole was examined by paper chromatography: *Rf* (B) 0.40, *Rf* (C) 0.85, *Rf* (G) 0.68. UV: $\lambda_{max}^{50\%\ EtOH}$ 281 nm; $\lambda_{max}^{0.1N\ HCl}$ 277, 305; $\lambda_{max}^{0.1N\ NaOH}$ 294.

ii) 6-Chloro-8-oxy-9-β-(2,3,5-tri-O-acetyl-D-ribofuranosyl)purine extracted from the spot on paper chromatography was heated with aqueous dimethylamine at 80° for 3 hr. UV λ_{max}^{EtOH} 280 nm; $\lambda_{max}^{0.1N\ HCl}$ 276, 305; $\lambda_{max}^{0.1N\ NaOH}$ 275. Paper chromatography: *Rf* (B) 0.40, *Rf* (C) 0.84; *Rf* (G) 0.68. These properties were identical with those observed in i).