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Synthesis of 1-N-Oxides of Adenosine and 2-Alkyladenosine and Inosine 1-N-Oxide 5'-Phosphate

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Reaction of 5-amino-4-cyano-1-(2',3'-O-isopropylidene-β-D-ribofuranosyl)imidazole (I) with ethyl orthoformate followed by treatment with hydroxylamine afforded 2',3'-O-isopropylideneadenosine 1-N-oxide (IVa), from which adenosine 1-N-oxide (Va) was obtained by removal of the isopropylidene group. Similarly, 2-methyladenosine 1-N-oxide (Vb) and 2-ethyladenosine 1-N-oxide (Vc) were prepared. When O-methylhydroxylamine was substituted for hydroxylamine in the ring closure reaction of IIa, 9-(2',3'-O-isopropylidene-β-D-ribofuranosyl)-6-methoxyaminopurine (VIII) was obtained by ring opening and subsequent recyclization of the 1-methoxyadenosine derivative (VII).

Deamination of IVa with nitrous acid resulted in the formation of inosine 1-N-oxide (X). Its isopropylidene derivative (XI) was phosphorylated with phosphoryl chloride to give, after acidic treatment, inosine 1-N-oxide 5'-phosphate (XII), whose flavoring activity was examined.

A chemistry of purine N-oxides has been studied by several investigators and much attensions are attracted to some of them from biological point of view. For instance, 7-N-oxides of guanine and xanthine are reported by Sugiura and Brown[2] to exhibit carcinogenic activity. Our interest in such purine N-oxides has led to undertake the synthesis of purine nuceloside N-oxides. Although a number of purine N-oxides have been prepared in some different ways, very little synthetic work in the nucleoside level has been reported in the literatures.[3]

Recent work[4] from our laboratories has dealt with the preparation of 2',3'-O-isopropylideneadenosine (IX) by cyclization of 5-amino-4-cyano-1-(2',3'-O-isopropylidene-β-D-ribofuranosyl)imidazole (I) with ethyl orthoformate followed by treatment with ammonia.

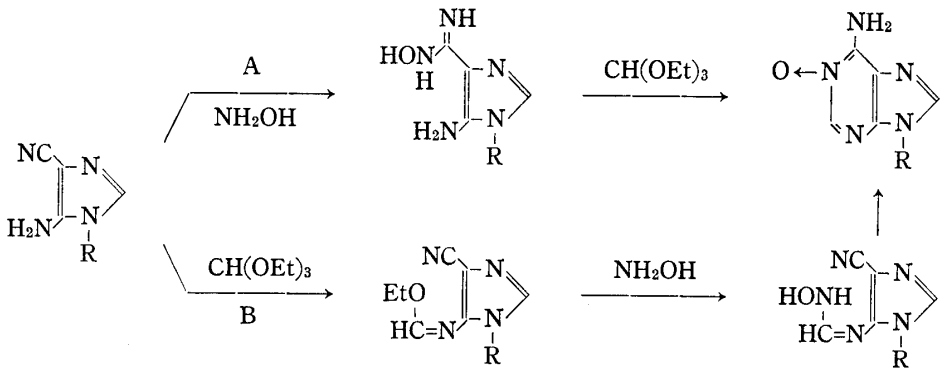


Chart 1

1) Location: *Suzuki-cho, Kawasaki.*
2) K. Sugiura and G.B. Brown, *Cancer Res.*, **27**, 925 (1967).
3) *a*) J.C. Parham, J. Fissekis, and G.B. Brown, *J. Org. Chem.*, **31**, 966 (1966); *b*) H. Kawashima and I. Kumashiro, *Bull. Chem. Soc. Japan*, **40**, 639 (1967); *c*) M.A. Stevens, D.I. Magrath, H.W. Smith, and G.B. Brown, *J. Am. Chem. Soc.*, **80**, 2755 (1958).
4) K. Suzuki, T. Meguro, I. Kumashiro, and T. Takenishi, Abstract of Papers, 21th Annual Meeting of the Chemical Society of Japan, Osaka, March 1968, No. 3, p. 1775.

The present paper is concerned with the extension of the above method to adenosine 1-N-oxide (Va) and its analogs, and moreover, the phosphorylation of inosine 1-N-oxide derivative is described.

For the synthesis of 2′,3′-O-isopropylideneadenosine 1-N-oxide (IVa)[3c] from I, two cyclization routes appear to be possible as shown in Chart 1.

A successful preparation of IVa was achieved by route B involving condensation[5] of I with ethyl orthoformate and subsequent reaction of the resulting 5-ethoxymethylenamino derivative (IIa) with hydroxylamine, in which ring closure reaction proceeds *via* the hydroxyaminomethylene derivative (IIIa). The yield of IVa based on I was 35%. When IVa was heated in acidic solution (pH 1.5) for 40 min, compound Va was obtained, which was identical with an authentic sample.[3c]

Reaction of I with ethyl orthoacetate followed by treatment of the resulting 5-(1-ethoxyethylidenamino)-4-cyano-1-(2′,3′-O-isopropylidene-β-D-ribofuranosyl)imidazole (IIb) with hydroxylamine caused ring closure of IIb to 2′,3′-O-isopropylidene-2-methyladenosine 1-N-oxide (IVb) *via* the corresponding hydroxyamino derivative (IIIb). Hydrolysis of IVb in acidic solution gave 2-methyladenosine 1-N-oxide (Vb). The structure of this compound was supported by elementary analysis and catalytic hydrogenation of Vb to the known 2-methyladenosine.[6] Similar treatment of I with ethyl orthopropionate followed by cyclization of the resulting ethoxypropylidene derivative (IIc) afforded 2′,3′-O-isopropylidene-2-ethyladenosine 1-N-oxide (IVc), which was hydrolyzed as usual to 2-ethyladenosine 1-N-oxide (Vc). The structure of Vc was assigned on the basis of elementary analysis and the NMR spectrum.

Methylation of adenine 1-N-oxide with methyl iodide was reported by Fujii, *et al.*[7] to give 1-methoxyadenine. The preparation of its nucleoside, 2′,3′-O-isopropylidene-1-methoxyadenosine (VII), was tried by reaction of IIa with O-methylhydroxylamine *via* the methoxyamino derivative (VI). However, the obtained compound was found to be 9-(2′,3′-O-isopropylidene-β-D-ribofuranosyl)-6-methoxyaminopurine (VIII) on the basis of its catalytic hydrogenation to yield IX and similarity of ultraviolet absorption spectra to those of 6-ethoxyamino-9-ethylpurine[8] but no 1-methoxy-9-methyladenine.[7] The formation of VIII from VII in absolute ethanol can be rationalized by the mechanism of ring opening and sub-

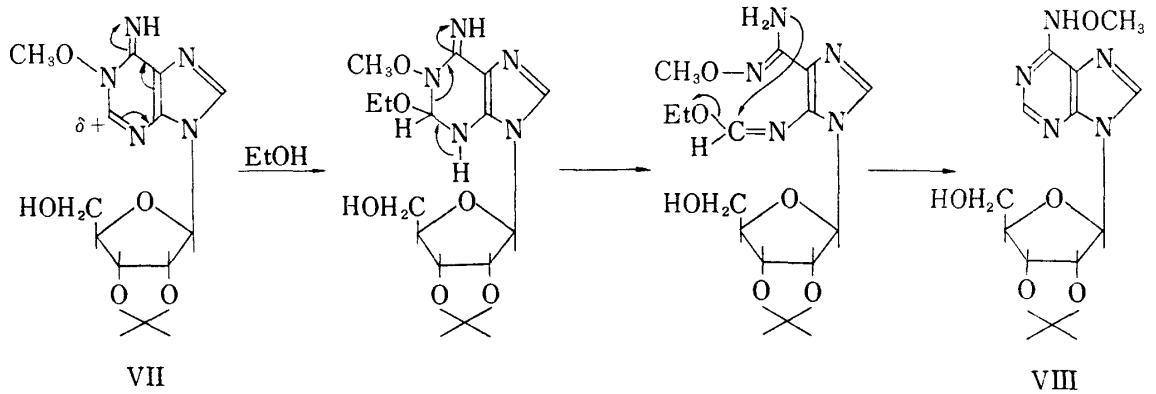


Chart 2

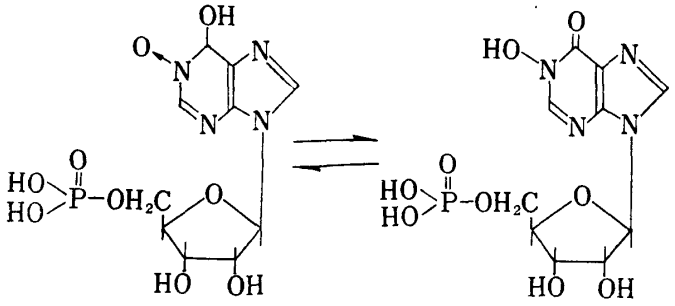
5) It was found by Suzuki, *et al.*[4] that the reaction of I with ethyl orthoformate is accompanied with the formation of 5′-diethoxymethyl derivative and that the diethoxymethyl group was readily removed by mild acidic or alkaline treatment.

6) *a*) A. Yamazaki, I. Kumashiro, and T. Takenishi, *J. Org. Chem.*, **33**, 2583 (1968); *b*) J. Davoll and B.A. Lowy, *J. Am. Chem. Soc.*, **74**, 1563 (1952).

7) T. Fujii, T. Itaya, and S. Yamada, *Chem. Pharm. Bull.* (Tokyo), **13**, 1017 (1965); T. Fujii, C.C. Wu, T. Itaya, and S. Yamada, *Chem. Ind.* (London), **1966**, 1598.

sequent recyclization as shown in Chart 2. This facil rearrangement is entirely consistent with a finding of Fujii and co-workers.[8]

As a part of our continuing studies on the flavoring activity of 5'-nucleotide, it seemed interest to compare the activity of inosine 1-N-oxide 5'-phosphate (XII) with that of 5'-inosinic acid, considering tautomeric forms as shown in Chart 3. Previously, the former was pre-



XIIa XIIb

Chart 3

pared by deamination of adenosine 1-N-oxide 5'-phosphate with nitrosyl chloride[9] or sodium nitrite in acetic acid.[10] Alternatively, the authors have now synthesized XII by phosphorylation[11] of 2',3'-O-isopropylideneinosine 1-N-oxide (XI) followed by removal of the protecting group and have isolated it by column chromatography on decolorizing resin and Dowex 1-XI ($HCOO^-$ form). The isopropylidene derivative XI[12] was obtained by treatment of inosine 1-N-oxide (X) with acetone in the presence of 2,2-dimethoxypropane and hydrochloric acid, according to the convenient method developed in our laboratories.[13] The structural assignment of XII was supported by the fact that XII was similar to X in ultraviolet absorption spectrum and gave a purple-red color with ferric chloride test. On the basis of infrared spectral data, it seems reasonable to assume that XII exists predominantly as the 1-hydroxy derivative (XIIb) of inosine 5'-phosphate in neutral solution as in the case of hypoxanthine 1-N-oxide[3a,14] and X[3b]; XII exhibited a strong absorption band at 1699 cm^{-1}, which was almost equal to the 6-carbonyl absorption (1700 cm^{-1}) of sodium salt of 1-methylinosine 5'-phosphate,[15] whereas, in the sodium salt of inosine 5'-phosphate, the 6-carbonyl absorption was at 1680 cm^{-1}. This suggests that XII posesses carbonyl character at 6-position.

The compound XII was found to have a flavoring activity and to show higher flavoring strength in synergistic effect with monosodium L-glutamate than that of its original nucleotide, inosine 5'-phosphate, or 1-methylinosine 5'-phosphate. The synergistic flavoring strength of XII was found to be comparable to that[16] of guanosine 5'-phosphate. It is concluded, therefore, that the relative effects of the 1-N-substituents on the flavoring activities decrease in the order HO>H>CH_3 and that the active hydrogen such as hydroxyl group may increase

8) T. Fujii, T. Itaya, C.C. Wu, and S. Yamada, *Chem. Ind.* (London), **1966**, 1967.
9) H. Sigel and H. Brintzinger, *Helv. Chim. Acta*, **48**, 433 (1965).
10) D.B. McCormick, *Biochemistry*, **5**, 746 (1966).
11) M. Yoshikawa, T. Kato, and T. Takenishi, *Tetrahedron Letters*, **1967**, 5065.
12) Deamination[3b] of IVa with nitrous acid in aqueous acetic acid gave X in which the isopropylidene group was removed.
13) Y. Tsuchiya, T. Takenishi, T. Kato, I. Kumashiro, and T. Mori, Japan. Patent 12345 (1964).
14) *a*) H. Kawashima and I. Kumashiro, *Bull. Chem. Soc. Japan*, **39**, 633 (1966); *b*) E. C. Taylor, C. C. Cheng, and O. Vogl, *J. Org. Chem.*, **24**, 2019 (1959).
15) A. Yamazaki, I. Kumashiro, and T. Takenishi, *Chem. Pharm. Bull.* (Tokyo), **16**, 1561 (1968).
16) A. Yamazaki, I. Kumashiro, and T. Takenishi, *Chem. Pharm. Bull.* (Tokyo), **16**, 338 (1968); M. Ohara, T. Ninomiya, S. Ikeda, S. Yamaguchi, and T. Yoshikawa, *J. Agr. Chem. Soc. Japan*, **40**, 169 (1966).

the flavoring intensity. The measurement of the flavoring strength was performed by Mr. Ninomiya, *et al.* in our laboratories and the detailes will be reported later.

The testing for biological activities of Vb and Vc is now under investigation.

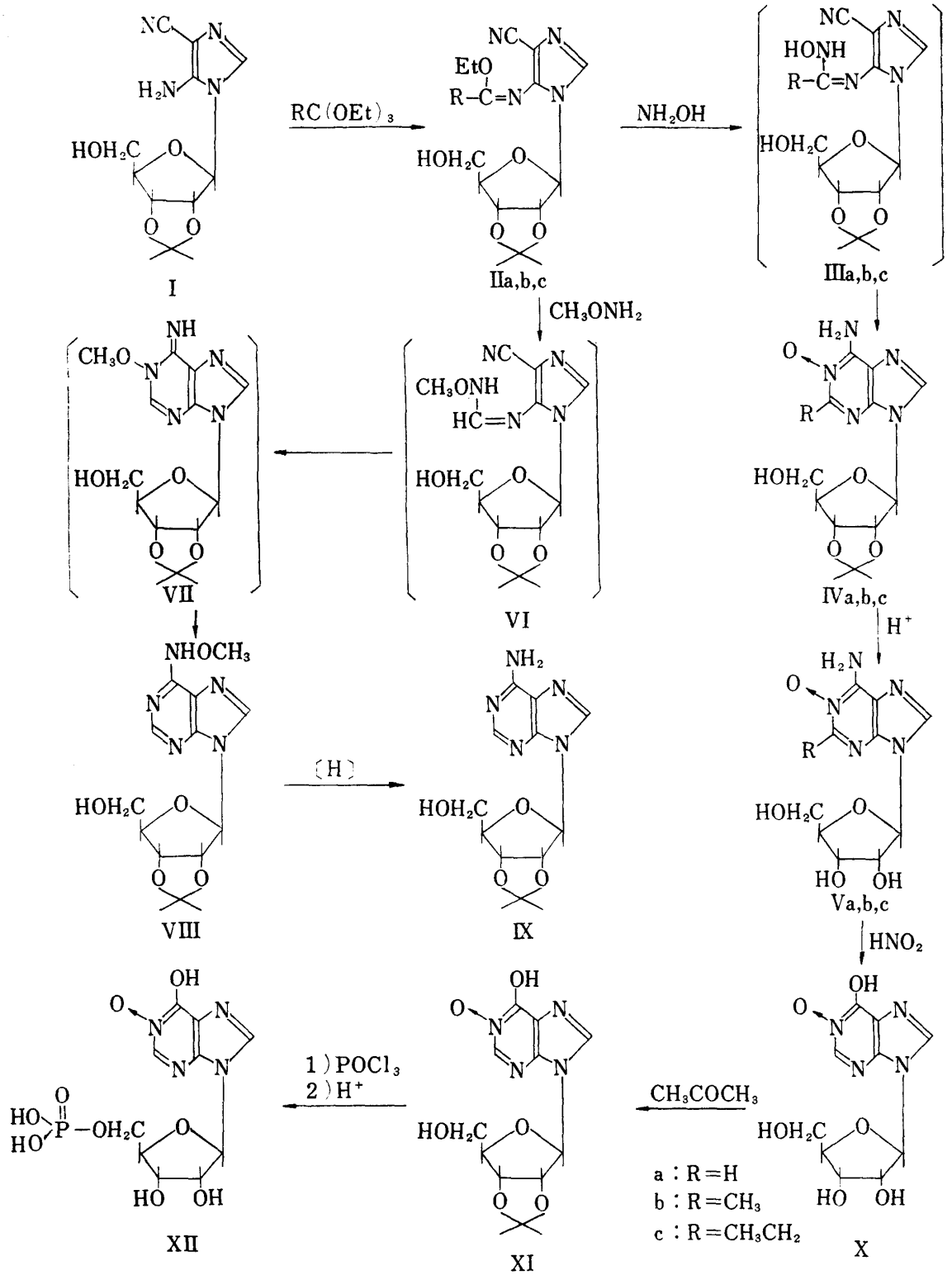


Chart 4

Experimental[17]

2′,3′-O-Isopropylideneadenosine 1-N-Oxide (IVa)——A solution of 5-amino-4-cyano-1-(2′,3′-O-isopropylidene-β-D-ribofuranosyl)imidazole[4] (I, 5 g, 17.8 mmoles) in 50 ml of $CH(OEt)_3$ was refluxed for 3 hr. The reaction mixture was concentrated *in vacuo* to give a gummy product, the ethoxymethylene derivative (IIa), in quantitative yield, which was used in the next reaction without purification. The compound was dissolved in 50 ml of EtOH containing 2.95 g (89.5 mmoles) of hydroxylamine and the solution was heated to reflux for 2 hr. After the reaction, the solvent was removed under reduced pressure and the residual crude IVa was crystallized from EtOH and ether. Recrystallization from EtOH afforded analytically pure white needles; yield, 2.04 g (35%). This compound was identical with an authentic sample previously reported.[3c] *Anal.* Calcd. for $C_{13}H_{17}O_5N_5$: C, 48.30; H, 5.26; N, 21.67. Found: C, 48.15; H, 5.26; N, 21.71.

2′,3′-O-Isopropylidene-2-methyladenosine 1-N-Oxide (IVb)——A solution of I (5 g) in 50 ml of ethyl orthoacetate was refluxed for 3 hr and the reaction mixture was evaporated *in vacuo* to dryness. The resulting ethoxyethylidene derivative (IIb) was treated with NH_2OH as described for IVa. After the reaction, removal of the solvent gave a gummy product (IVa), which was suspended in 50 ml of H_2O and extracted five times with 50 ml-portions of $CHCl_3$. The combined extracts were dried with anhyd. Na_2SO_4 and evaporated to dryness. The residue was dissolved in a small amount of EtOH and the solution was allowed to stand at room temperature overnight. The resulting precipitate was collected by filtration and recrystallized from H_2O to give 1.1 g (18.5%) of pure, white crystals. mp 178° (decomp.). $[\alpha]_D^{25}$ −55.2° (c=0.4, 0.1 N NaOH). *Rf*: 0.80 (solvent A), 0.70 (solvent B). UV $\lambda_{max}^{pH\,1}$ mμ (ε): 260 (11800); $\lambda_{max}^{pH\,7}$ mμ (ε): 233 (40600), 264 (8000); $\lambda_{max}^{pH\,13}$ mμ (ε): 232 (32200), 269 (8700). *Anal.* Calcd. for $C_{14}H_{19}O_5N_5 \cdot \frac{3}{4}H_2O$: C, 47.92; H, 5.89; N, 19.97. Found: C, 48.06; H, 6.10; N, 19.88.

2′,3′-O-Isopropylidene-2-ethyladenosine 1-N-Oxide (IVc)——A solution of I (5 g) in 50 ml of ethyl orthopropionate was refluxed for 3 hr and the reaction mixture was treated with hydroxylamine in a manner similar to that described for IVa to give a gummy product. This was triturated with a small amount of H_2O and the resulting crystals were collected by filtration and recrystallized from H_2O, giving 1.2 g (19%) of colorless crystals. mp 193° (decomp.). $[\alpha]_D^{25}$ −21.9° (c=0.07, 0.1 N NaOH). *Rf*: 0.80 (solvent A), 0.70 (solvent B). UV $\lambda_{max}^{pH\,1}$ mμ (ε): 260 (11500); $\lambda_{max}^{pH\,7}$ mμ (ε): 233 (31800), 264 (7600); $\lambda_{max}^{pH\,13}$ mμ (ε): 232 (32400), 268 (7700). *Anal.* Calcd. for $C_{15}H_{21}O_5N_5 \cdot H_2O$: C, 48.82; H, 6.28; N, 18.98. Found: C, 49.16; H, 6.52; N, 19.11.

Adenosine 1-N-Oxide (Va)——After IVa (1 g) was dissolved in 50 ml of H_2O, the solution was brought to pH 1.5 with 0.5 N HCl, heated at 70° for 40 min with stirring, and neutralized by adding portionwise Amberlite IRA-410 (OH^- form) to pH 7. The resin was removed by filtration and the filtrate was evaporated under reduced pressure to dryness. The residue was crystallized from water to yield 0.42 g (48%) of pure crystals, identical with an authentic sample.[3c]

2-Methyladenosine 1-N-Oxide (Vb)——A) This compound was prepared from IVb (1 g) by the same procedure as described for Va and crystallized from water to give a pure sample. Yield, 0.57 g (63.5%).

B) In another run, crude IVb, obtained from 5 g of I, was hydrolyzed as described above to afford 1.1 g (20.8%) of Vb. mp 252° (decomp.). $[\alpha]_D^{25}$ −73.7° (c=0.9, 0.1 N NaOH). *Rf*: 0.50 (solvent A), 0.23 (solvent B). NMR (in DMSO-d_6) ppm: 2.59 (3H, singlet, $C\underline{H}_3$). UV $\lambda_{max}^{pH\,1}$ mμ (ε): 260 (13100); $\lambda_{max}^{pH\,7}$ mμ (ε): 233 (38000), 264 (8100); $\lambda_{max}^{pH\,13}$ mμ (ε): 233 (31200), 268 (8700). *Anal.* Calcd. for $C_{11}H_{15}O_5N_5$: C, 44.44; H, 5.09; N, 23.56. Found: C, 44.44; H, 5.36; N, 23.82.

Catalytic Hydrogenation of Vb——The compound Vb (100 mg) was dissolved in 25 ml of H_2O and 5% Pd–C (100 mg) was added. The mixture was shaken with hydrogen until no more hydrogen was absorbed. After the catalyst was filtered off, the filtrate was concentrated to afford 2-methyladenosine, which was identified by comparison of ultraviolet spectrum with that of an authentic sample.[6]

2-Ethyladenosine 1-N-Oxide (Vc)——Compound IVc (1 g) was deacetonated in the usual manner and a crude product that obtained was crystallized from H_2O to afford an analytically pure sample. Yield, 0.6 g (68%). mp 238° (decomp.). $[\alpha]_D^{25}$ −69.5° (c=1, 0.1 N NaOH). *Rf*: 0.50 (solvent A), 0.23 (solvent B). NMR (in DMSO-d_6) ppm: 1.23 (3H, triplet, $CH_2C\underline{H}_3$), 3.03 (2H, quartet, $C\underline{H}_2CH_3$). UV $\lambda_{max}^{pH\,1}$ mμ (ε): 260 (12300); $\lambda_{max}^{pH\,7}$ mμ (ε): 233 (44200), 264 (8700); $\lambda_{max}^{pH\,13}$ mμ (ε): 232 (38300), 268 (9100). *Anal.* Calcd. for $C_{12}H_{17}O_5N_5 \cdot H_2O$: C, 43.77; H, 5.78; N, 21.28. Found: C, 44.39; H, 6.31; N, 21.13.

9-(2′,3′-O-Isopropylidene-β-D-ribofuranosyl)-6-methoxyaminopurine (VIII)——A mixture of crude IIa, prepared by reaction of I (5 g, 17.8 mmoles) with $CH(OEt)_3$, and O-methylhydroxylamine (4.2 g, 89.5 mmoles) was heated to reflux in 50 ml of absolute EtOH for 2 hr. The solvent was then removed under reduced

17) All melting points are uncorrected. Ultraviolet absorption spectra were taken with a Hitachi EPS-2 automatic recording spectrophotometer, and infrared absorption spectra were measured with a Jasco Model IR-S spectrophotometer. The nuclear magnetic resonance (NMR) spectra were measured with a Varian A-60 using tetramethylsilane as internal standard. All chromatographies were performed on Toyo No. 51 filter paper by the ascending technique. Solvent systems: A, *n*-PrOH–NH_3(28%)–H_2O (20:12:3, v/v); B, *n*-BuOH–AcOH–H_2O (4:1:1, v/v); C, iso-PrOH–sat. $(NH_4)_2SO_4$–H_2O (2:79:19, v/v).

pressure and the resulting gummy product was crystallized from a mixture of EtOH and ether. After recrystallization from EtOH, 0.8 g (13.3%) of pure crystals was obtained. mp 179° (decomp.). $[\alpha]_D^{25}$ −66.5° (c= 0.08, 0.1N NaOH). *Rf*: 0.85 (solvent A), 0.75 (solvent B). NMR (in pyridine) ppm: 4.0 (3H, singlet, $OC\underline{H}_3$). UV $\lambda_{max}^{pH\,1}$ mμ (ε): 268 (15400); $\lambda_{max}^{pH\,7}$ mμ (ε): 268.5 (14600); $\lambda_{max}^{pH\,13}$ mμ (ε): 284 (11200). *Anal.* Calcd. for $C_{14}H_{19}O_5N_5$: C, 49.84; H, 5,68; N, 20.77. Found: C, 50.24; H, 6.29; N, 21.11.

Catalytic Hydrogenation of VIII——The compound VIII (100 mg) was hydrogenated over 5% Pd-C (50 mg) in 300 ml of EtOH to afford 2′,3′-O-isopropylideneadenosine, whose physical properties were identical with those of an authentic sample.

2′,3′-O-Isopropylideneinosine 1-N-Oxide (XI)——To a suspension of X (5 g, 15.8 mmoles) in a mixture of EtOH (10 ml), CH_3COCH_3 (10 ml), and 2,2-dimethoxypropane (17 g, 163 mmoles), 10 ml of dry 10N HCl (MeOH) was added with stirring at room temperature. Within 30 min, the reaction mixture became clear and it was then poured into 200 ml of cold 5N NH_4OH with stirring. The solution was evaporated *in vacuo* to dryness and the residue was crystallized from H_2O. Yield, 3 g (52.5%). mp 198° (decomp.); $[\alpha]_D^{25}$ −57.8° (c=0.9, 0.1N NaOH). *Rf*: 0.58 (solvent A), 0.62 (solvent B). UV $\lambda_{max}^{pH\,1}$ mμ (ε): 252 (7200); $\lambda_{max}^{pH\,7}$ mμ (ε): 228 (43500), 252 (7400); $\lambda_{max}^{pH\,13}$ mμ (ε) : 257 (6900), 295 (4300). *Anal.* Calcd. for $C_{13}H_{16}O_6N_4\cdot\frac{1}{2}H_2O$: C, 46.84; H, 5.14; N, 16.81. Found: C, 46.81; H, 5.00; N, 17.16.

Inosine 1-N-Oxide 5′-Phosphate (XII)——To a stirred solution of trimethyl phosphate (20 ml) and phosphoryl chloride (10.8 g, 70.5 mmoles), cooled at −10° in a three-necked flask equipped with a thermometer and a silica gel drying tube, compound XI (2 g, 6 mmoles) was added portionwise. The mixture was stirred for 3 hr at 0° and the excess of phosphoryl chloride and the solvent was extracted with ether. After the oily residue was dissolved in 200 ml of ice water, the solution was brought to pH 1.5 with 0.5N NaOH and heated at 70° for 40 min with stirring to remove the isopropylidene group. Phosphorylation was shown to take place at 5′-position of XI by ultraviolet absorption spectra and color reaction. After being cooled, the solution was brought to pH 2 and passed through a column (2.5×120 cm) of decolorizing resin.[18] The column was washed with H_2O, the nucleotide was eluted with 0.5N NH_4OH, and the eluate was concentrated under reduced pressure to dryness. The residue was dissolved in 100 ml of H_2O and then applied to a column (1.5×80cm) of Dowex 1-XI ($HCOO^-$ form, 50—100 mesh). The column was washed with H_2O and the desired product was eluted with 1M HCOOH. The eluate was concentrated *in vacuo* at 30—40° with a rotary evaporator and the residue was dissolved in 50 ml of H_2O. After the pH of the solution was adjusted to 7.8 with 0.5N NaOH, the solution was refluxed for 5 min and the pH was readjusted to 7.8. This procedure was repeated until the pH became constant. The solvent was removed *in vacuo* and the residue crystallized on trituration with 50% aqueous EtOH. Recrystallization from the same solvent with charcoal afforded pure crystals, which were dried over P_2O_5 at room temperature for 3 hr. Yield, 580 mg (23%). mp 127°. $[\alpha]_D^{25}$ −27.0° (c=0.08, 0.1N NaOH). *Rf*: 0.08 (solvent A), 0.65 (solvent C). UV $\lambda_{max}^{pH\,1}$ mμ (ε): 252 (8400); $\lambda_{max}^{pH\,7}$ mμ (ε): 228 (44800), 254 (5500); $\lambda_{max}^{pH\,13}$ mμ (ε): 258 (6500), 295 (4000). *Anal.* Calcd. for $C_{10}H_{11}O_9N_4PNa_2\cdot 4.5H_2O$: C, 24.55; H, 4.09; N, 11.45; P, 6.34. Found: C, 25.06; H, 4.17; N, 10.92; P, 6.29.

Acknowledgement The authors wish to thank professor T. Fujii of the Kanazawa University for his helpful suggestions relating to the determination of 9-(2′,3′-O-isopropylidene-β-D-ribofuranosyl)-6-methoxyaminopurine and Professor M. Ikehara of the Osaka University and Dr. H. Oeda of Ajinomoto Co., Inc. for their encouragement throughout the course of this work. Our thanks are also due to Dr. T. Meguro for many helpful discussions and to Messrs. M. Okutsu and M. Akiyama for their skillful technical assistance.

18) This decolorizing resin was prepared in our Laboratories by copolymerization of metaphenylenediamine, resorcin, and formalin.[19]

19) Y. Tsuchiya, I. Hayashi, T. Kato, M. Yoshikawa, T. Mori, and Y. Miyasaka, Japan. Patent 12343 (1964).