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# Nucleotides. III.[1] Syntheses of Deazaadenosine 3',5'-Cyclic Phosphates and Related Nucleotides of Biological Interest

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1-Deaza- (I) and 3-deazaadenosine 3',5'-cyclic phosphate (II) (analogs of adenosine 3',5'-cyclic phosphate) were synthesized from $N^6$-acetyl-1-deazaadenosine (III) and 3-deazaadenosine (VI), respectively. In addition, a number of 1- and 3-deazaadenosine phosphates including 1- (XII) and 3-deazaadenosine 2',3'-cyclic phosphate (XIII) were also prepared. Both VII and XIII were found to be hydrolyzed to the corresponding nucleoside 3'-phosphate by ribonuclease M. Both 1-deaza- (V) and 3-deazaadenosine 5'-phosphate (XI) were hydrolyzed by snake venom 5'-nucleotidase to the respective deazaadenosine and inorganic phosphate; the latter, however, was found to be hydrolyzed with much reduced rate.

During the past few years, adenosine 3',5'-cyclic phosphate (cAMP) has been recognized as being one of the most important low-molecular weight regulation molecules in all stages of evolution.[3]

A large number of cAMP analogs have been prepared to obtain substances having specific biological activity and to elucidate molecular interaction with the receptor.[4] Kuo and Greengard have reported on the activity of 7-deazaadenosine 3',5'-cyclic phosphate which was shown to be about 50% as active as the parent nucleotide using a protein kinase system.[5] The crystal structures of 5'-methylene 3',5'-cyclic phosphate as well as cAMP have been examined by X-ray analysis.[6,7]

It was felt desirable for us to prepare additional deaza analogs of cAMP: 1-deaza- (I) and 3-deazaadenosine 3',5'-cyclic phosphate (II) and to compare their enzymatic and crystalographic properties with those of the parent nucleotide and the above-mentioned analogs.

The present paper deals with chemical synthesis of a variety of phosphates of 1- and 3-deazaadenosines which might be useful for the analysis of reactions of adenine nucleotide-utilizing enzymes. Emphasis is laid upon the synthesis of cAMP analogs (I and II).

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2) Location: *Kita-12 Nishi-6 Kitaku, Sapporo.*
3) *a*) J.P. Jost and H.V. Rickenberg, *Ann. Rev. Biochem.*, **40**, 741 (1971); *b*) B. Jastorff and W. Freist, *Angew. Chem. Intern. Ed. Engl.*, **11**, 713 (1972).
4) *a*) G.H. Jones, H.P. Albrecht, N.P. Damodaran, and J.G. Moffatt, *J. Am. Chem. Soc.*, **92**, 5510 (1970); *b*) K. Muneyama, R.J. Bauer, D.A. Shuman, R.K. Robins, and L.N. Simon, *Biochemistry*, **10**, 2390 (1971); *c*) A. Murayama, B. Jastorff, F. Ramer, and H. Hettler, *J. Org. Chem.*, **36**, 3029 (1971); *d*) G. Michael, V. Nelbrock, and G. Weinman, *Z. Anal. Chem.*, **252**, 189 (1970); *e*) T. Posternack, I. Marcus, and G. Cehovic, *C. R. Acad. Sci., Ser. D*, **272**, 622 (1971); *f*) T.A. Khawaja, R. Harris, and R.K. Robins, *Tetrahedron Letters*, **1972**, 4681.
5) J.F. Kuo and P. Greengard, *Biochim. Biophys. Res. Comm.*, **40**, 1032 (1970).
6) C.L. Coutler, *Acta Crystallogr. Sec. B*, **25**, 2055 (1969).
7) M. Sundaraligam and J. Abola, *J. Am. Chem. Soc.*, **94**, 5070 (1972).

## Materials and Methods[8]

1-Deaza- and 3-deazaadenosine were prepared by reported procedures.[9,10] Ribonuclease M having a base specificity similar to that of Ribonuclease $T_2$ was prepared from *Aspergillus saitoi* according to a reported method[11] and was a gift from Dr. Masachika Irie of Hoshi College of Pharmacy, Tokyo. Snake venom 5′-nucleotidase (*Crotalus adamanteus*) was obtained from Sigma Chemical Co. DEAE-cellulose (which was used in the bicarbonate form) and phospho-cellulose were the products of Jujo Paper Co. and gifts therefrom.

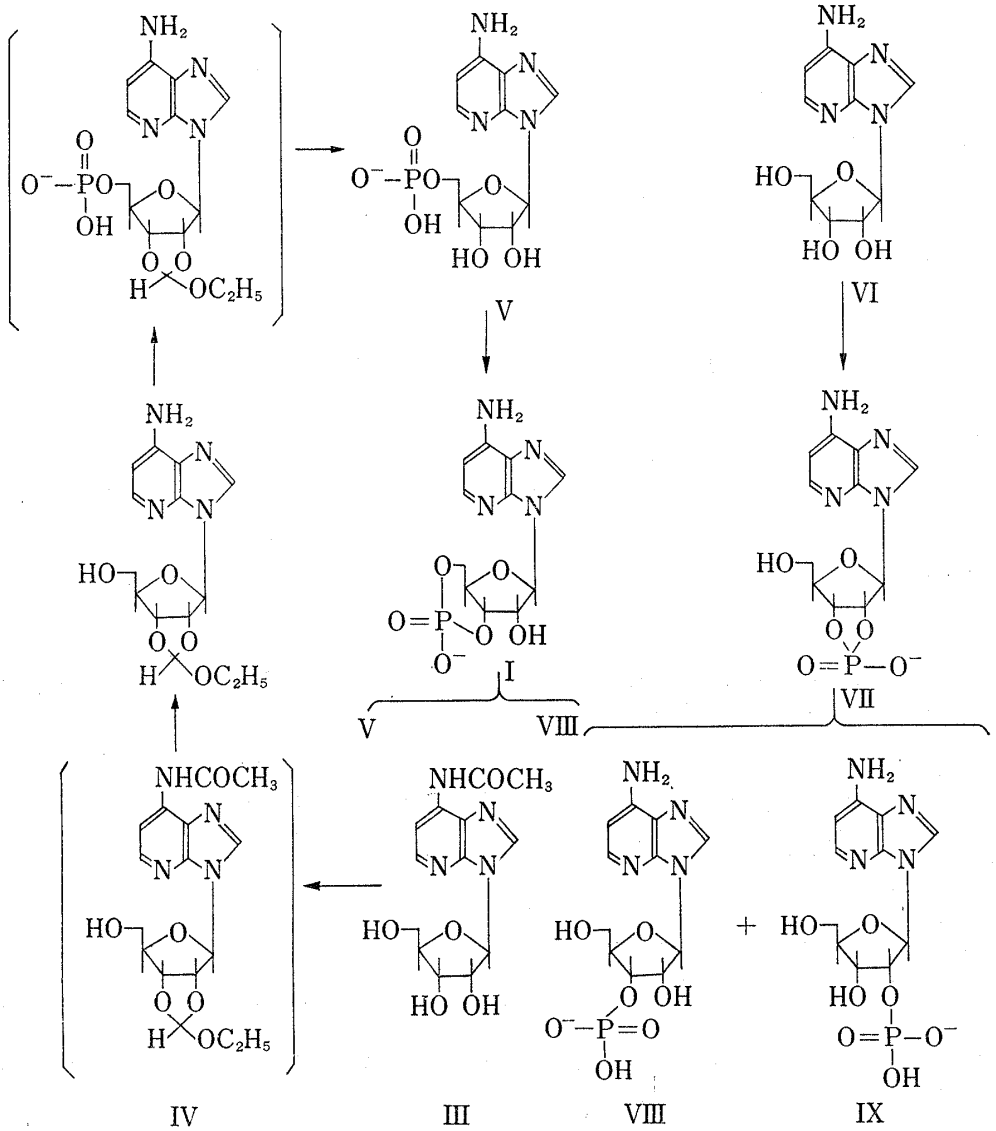


Chart 1

8) Abbreviations: $^1$Ado, $^3$Ado, c$^1$AMP, c$^3$AMP, $^1$A>p, $^3$A>p, pN, Np, and N$^p$ (N: nucleoside) stand for 1-deazaadenosine, 3-deazaadenosine, 1-deazaadenosine 3′,5′-cyclic phosphate, 3-deazaadenosine 3′,5′-cyclic phosphate, 1-deazaadenosine 2′,3′-cyclic phosphate, 3-deazaadenosine 2′,3′-cyclic phosphate, nucleoside 5′-phosphate, nucleoside 3′-phosphate, and nucleoside 2′-phosphate, respectively. Pep, TLC, PPC, DMF, TEAB, and DCC stand for paper electrophoresis, thin layer-, paper chromatography, N,N-dimethylformamide, triethylammonium bicarbonate and dicyclohexylcarbodiimide, respectively.

9) *a*) T. Itoh, S. Kitano, and Y. Mizuno, *J. Heterocyclic Chem.*, **9**, 465 (1972); *b*) P.C. Jain, S.K. Chatterjee, and N. Anand, *Ind. J. Chem.*, **4**, 403 (1966); *c*) K.B. de Roos and C.A. Salemink, *Recueil*, **90**, 654 (1971).

10) *a*) R.J. Rousseau, L.B. Townsend, and R.K. Robins, *Biochemistry*, **5**, 756 (1971); *b*) Y. Mizuno, S. Tazawa, and K. Kageura, *Chem. Pharm. Bull.* (Tokyo), **16**, 2011 (1968); *c*) J.A. May and L.B. Townsend, *Chem. Commun.*, **1973**, 64.

11) M. Irie, *J. Biochem.*, **62**, 509 (1967).

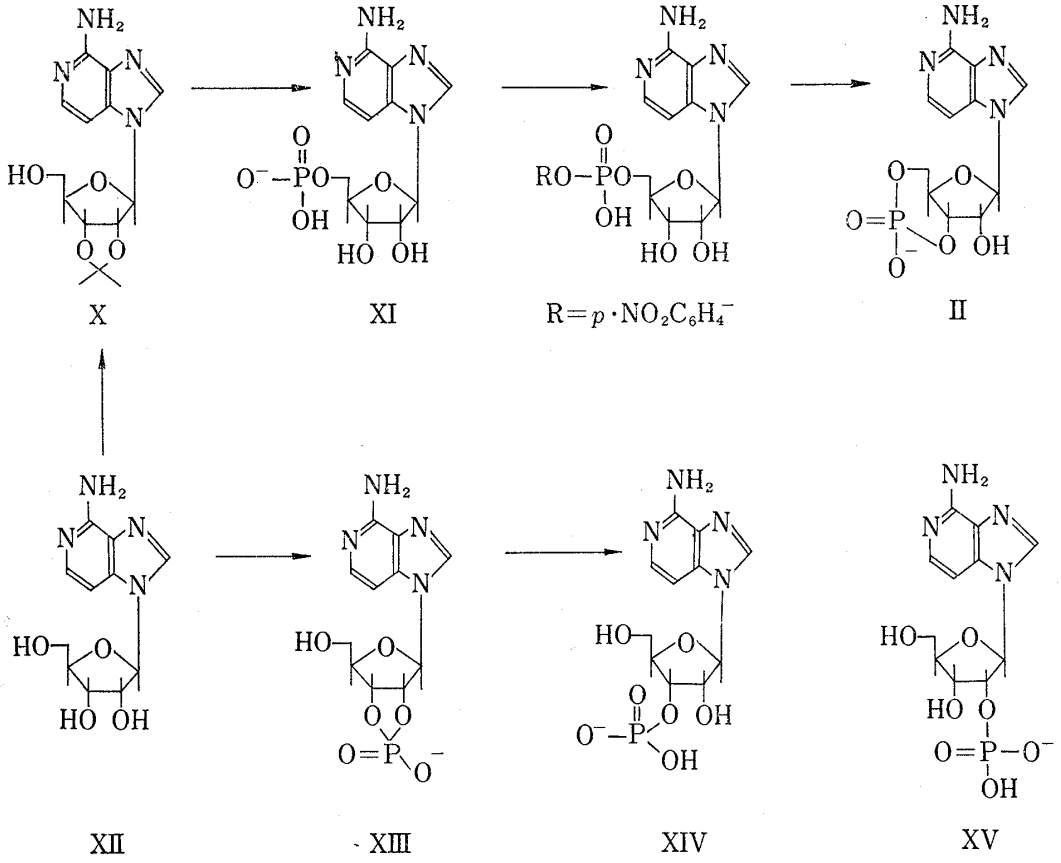


Chart 2

UV spectra were recorded on a Hitachi Recording Spectrometer Model 3T. Inorganic phosphate released by acid hydrolysis (60% $HClO_4$, 110°, 10 min) or 5′-nucleotidase digestion of the nucleotides was determined by Allen's method.[12]

Chromatographic separation on paper was carried out on a Toyo-Roshi No 51A paper in solvent systems, A: $isoC_3H_7OH$–$concNH_4OH$–$H_2O$ (7:1:2), B: saturated $(NH_4)_2SO_4$–1 M AcONa–$isoC_3H_7OH$ (80:18:2), C: isobutylic acid–0.5 N $NH_4OH$ (5:3), D: EtOH–$concNH_4OH$–$H_2O$ (80:4:16) either by the ascending technique or by the descending technique. Spots were usually detected on paper chromatograms under an ultraviolet lamp at 2537 A. However, for the detection of nucleosides and nucleotides having *cis*-glycol system, a metaperiodate-benzidine spray reagent was employed.[13] The detection of phosphorous in the nucleotides on paper chromatogram was performed as reported.[14] For the assay of hydrolysates of 1- (I) and 3-deazaadenosine 3′,5′-cyclic phosphate (II), either a Varian Aerograph LCS-1000 (in the case of I) or a paper chromatography (solvent system B) (in the case of II) was employed. Paper electrophoresis was performed on Toyo-Roshi paper No 51A (size A, 10×40 cm or size B, 15×40 cm) at pH 7.5 in 0.05 M triethylammonium bicarbonate (700 volts) and at pH 9.5 in 0.05 M borate buffer. The following molar extinction coefficients were used: $^1$Ado: 14000 (262 mμ, pH 7); $^3$Ado: 8730 (262 mμ, pH 7).

## Synthetic Methods

**1-Deazaadenosine 5′-Phosphate (V)**[15]——To a solution of $N^6$-acetyl-1-deazaadenosine (III, 616 mg, 2 mmoles) in DMF (22.5 ml) and ethyl orthoformate (4.0 ml) was added a solution of hydrogen chloride (1.51 g) in DMF (2.5 ml). The solution was stirred for 1 hr at room temperature and then neutralized with triethylamine at 0° and the stirring was continued at the same temperature for 1 hr. The triethylamine hydrochloride

12) R.J. Allen, *Biochem. J.*, **34**, 858 (1940).
13) Y. Mizuno, M. Ikehara, K.A. Watanabe, S. Suzaki, and T. Itoh, *J. Org. Chem.*, **28**, 3329 (1963), footnote (6).
14) R.S. Bandurski and B. Axelrod, *J. Biol. Chem.*, **193**, 405 (1951).
15) Chemical or enzymatic preparation of V has been briefly reported by M. Ikehara, T. Fukui, and S. Uesugi, [*J. Biochem.*, **76**, 107 (1974)] and L. Hagenberg, H.G. Gassen, and H. Mathaei, [*Biochem. Biophys. Res. Commun.*, **50**, 1104 (1973)].

was filtered off, washed with DMF (10 ml). The combined filtrate and washing were concentrated to dryness. TLC (solvent system, benzene–AcOEt–$CH_3OH$ 6: 3: 1, silica gel) showed the absence of the starting material in the residue. But there appeared a faint spot (*Rf* 0.63) in addition to a main spot (*Rf* 0.54) corresponding to $N^6$-acetyl-2′,3′-O-ethoxymethylene-1-deazaadenosine (IV). The residue was partitioned between $CHCl_3$ (20 ml) and water (10 ml). The organic layer was separated and dried over $Na_2SO_4$. The salt was filtered off. The filtrate was concentrated to dryness (978 mg). The residue was purified by silica gel column chromatography (24×2.0 cm, benzene–AcOEt 1: 1). Fractions containing IV were pooled. Removal of the solvent left colorless gum (237 mg, 32.5%). Similar experiments were repeated and the combined products were used for the subsequent experiment. This nucleoside (IV, 2.0 g, 6.21 mmoles) was dissolved in absolute methanol containing 2 N methanolic methoxide (0.5 ml) and the resulting solution was refluxed for 9 hr. The cooled solution was neutralized with a resin (IRC-50 $H^+$ form). The resin was filtered off and the filtrate was concentrated to dryness. The residue which weighed 1.37 g was applied to a silica gel column. The column was washed with chloroform. Fractions containing deaceylated product were pooled and concentrated to dryness. Yield of 2′,3′-O-ethoxymethylene-1-deazaadenosine, 1.09 g (62%). The structure was confirmed by spectral (UV and NMR) data and the negative test with a metaperiodate-benzidine test. To a cooled solution of the nucleoside (573 mg, 1.78 mmoles) in acetonitrile (20 ml) was added with stirring 1.5 ml of pyrophosphoryl tetrachloride[16] at below 0°. The stirring was continued for 1 hr at the same temperature. The reaction mixture was then poured into ice water (20 ml). The mixture was extracted with ether (20×2 ml). The aq. layer was separated, adjusted to pH 4 with 1 N NaOH and allowed to stand at room temperature for 22 hr. Paper chromatographic examination (solvent A) showed that a complete deblocking took place. The aq. solution was treated with activated charcoal (15 g). The charcoal was washed with water and then eluted with EtOH–conc $NH_4OH$–$H_2O$ (50: 2: 48). The eluate was concentrated to dryness. The residue was dissolved in water and passed through a DEAE-cellulose column (column size 43×3.4 cm). The column was washed with a linear gradient system (2 l. of $H_2O$ in the mixing chamber and 2 l. of 0.2 M TEAB in the reservoir). Fraction size was 16 ml. Nucleotide (V) was eluted in fractions 84—188. These fractions were concentrated to dryness *in vacuo* and the residue was dissolved in a small amount of water and applied to a phosphocellulose column, $H^+$ form (13×3.0 cm). The column was washed with water. Eluate was concentrated to dryness to give colorless powder. Yield, 310 mg (49%). UV $\lambda_{max}^{H_2O}$ mμ: 263, 282. *Anal.* Calcd for $C_{11}H_{15}O_7N_4Pl/2H_2O$: C, 37.19; H, 4.54; N, 15.77. Found: C, 36.86; H, 4.60; N, 15.64.

**3-Deazaadenosine 5′-Phosphate (XI)**——This nucleotide was prepared by a modification of a reported method.[10b] To a cooled solution of 2′,3′-O-isopropylidene-3-deazaadenosine[10b] (X, 133 mg, 0.43 mmole) in acetonitrile (10 ml) was added 0.8 ml of pyrophosphoryl tetrachloride[16] (6 mmole) with stirring at below 0°. The mixture was kept under stirring for 1 hr at the same temperature and then poured into ice water (50 ml). The mixture was treated with ether (20 ml). The aq. layer was separated and concentrated to dryness. The residue was dissolved in 85% formic acid (20 ml). The solution was allowed to stand at room temperature for 8 hr. After checking by PPC that the deblocking was complete (the presence of a single spot, *Rf* 0.1 in the solvent system A), the solution was concentrated to dryness. Remaining formic acid was completely removed by three codistillations with water. The final residue was dissolved in a small volume of water and applied to a DEAE-cellulose column (column size: 32×1.4 cm; fraction volume: 15 ml). The column was washed with a linear gradient of 0.2 M TEAB (2 l.) and $H_2O$ (2 l.). Fractions 46—64 were concentrated to dryness. Yield of XI, 37 mmoles (86%). UV $\lambda_{max}^{pH\ 7.0}$ mμ: 262, ε(p)[17] 9240. This nucleotide (IX) was completely hydrolyzed to XII and inorganic phosphate by snake venom 5′-nucleotidase.

**3-Deazaadenosine 5′-*p*-Nitrophenylphosphate**——To a suspension of XI (triethylammonium salt, 2520 $A_{263\ m\mu}$ units) in pyridine (2 ml) and DMF (2 ml) was added *p*-nitrophenol (403 mg, 2.90 mmoles) and DCC (611 mg, 2.96 mmoles). The mixture was stirred at room temperature for 7 days. The reaction mixture was then concentrated to dryness and the residue was partitioned between water and ether. The aq. layer was washed several times with ether to remove *p*-nitrophenol, and then applied to a DEAE-cellulose column (45× 1.8 cm). The column was washed with a linear gradient system (2 l. of $H_2O$ in the mixing chamber and 2 l. of 0.15 M TEAB in the reservoir, fraction size, 15 ml). Fractions 34—80 were pooled and concentrated to dryness. Yield, 1900 $A_{260\ m\mu}$ units (triethylammonium salt). UV $\lambda_{max}^{H_2O}$ (ε): 239 (4400); $\lambda_{max}^{pH\ 1.4}$ mμ (ε): 279 (14300), 260 (13200), 280 (14200), 310 (5750); $\lambda_{max}^{pH\ 1.40}$ mμ (ε): 239 (4500); $\lambda_{max}^{pH\ 12.7}$ mμ (ε): 272 (14900), 260 (13300), 280 (1310), 310 (6270); $\lambda_{max}^{pH\ 12.7}$ mμ (ε): 239 (5650), pep: *Rf* in solvent A, 0.44; pep: R(5′AMP) 0.37.

**3-Deazaadenosine 3′,5′-Cyclic Phosphate (II)**——II was prepared by application of the method developed by Borden and Smith.[18] 3-Deazaadenosine-5′ *p*-nitrophenylphosphate (triethylammonium salt, 1830 $A_{260\ m\mu}$ units) in DMSO (10 ml) was treated with 1.5 ml of 1 M potassium *t*-butoxide in *t*-butyl alcohol for 30 min at 20°. The reaction mixture was charged on a phospho-cellulose column, $H^+$ form (24×1.4 cm). The column was washed with $H_2O$. The eluate was applied after concentration to a DEAE-cellulose column (56×2.2). Elution (one fraction, 15 ml) was performed with a linear gradient of $H_2O$ (2 l.) and 0.05 M TEAB (2 l.), and

16) P.C. Crofts, I.M. Downie, and R.B. Heslop, *J. Chem. Soc.*, **1960**, 3673.
17) ε(p) stand for extinction coefficient per mole of phosphoric acid.
18) K. Borden and M. Smith, *J. Org. Chem.*, **31**, 3247 (1966).

monitored by PPC (solvent A). II (*Rf* 0.34) was eluted in fractions 116—141 which was contaminated with a small amount of the starting material (*Rf* 0.51). These fractions were further purified by a Dowex (1×2) column. For this purpose, the above experiment was twice repeated and combined crude product ($A_{263\,m\mu}^{0.1N\ HCl}$ 3310) was applied to a Dowex column ($Cl^-$, 100—200 mesh, 43×1.8 cm). Elution was performed with a linear gradient of $H_2O$ (2 l.) and 0.005 N HCl (2 l.). One fraction was 15 ml. Fractions 69—98 (*Rf* 0.34, $A_{263m\mu}$ 2200) were pooled and concentrated to dryness. The residue was triturated with methanol to afford an analytical sample, after being dried over $P_4O_{10}$ *in vacuo* for two days. UV $\lambda_{max}^{pH\ 2.2}$ mμ (ε): 263 (10200). Upon electrophoresis at pH 8.5. the product had a mobility of 4.9 cm compared to 9.5 cm for pA. Yield, 42 mg. *Anal.* Calcd for $C_{11}H_{13}O_6N_4P3/2H_2O$: C, 37.19; H, 4.54; N, 15.77; P, 8.72. Found: C, 36.65; H, 4.76; N, 15.73; P, 8.79.

Fractions 99—160 ($A_{260m\mu}$ 642 units) were a mixture of II (*Rf* 0.34, 66%) and 3-deazaadenosine-5′ *p*-nitrophenylphosphate (*Rf* 0.51, 34%).

**1-Deazaadenosine 3′,5′-Cyclic Phosphate (I)**——(I) was prepared by application of the method developed by Smith and his coworkers.[19] V($1/2H_2O$, 1.0 mmole) was dissolved in a mixture of pyridine (20 ml), $H_2O$ (5 ml), and N,N′-dicyclohexyl-4-morpholine-carboxamidine (297 mg, 1.0 mmole). The solution was concentrated to dryness and the residue was azeotropically dried by codistillations with pyridine (10 ml×3). The final residue was dissolved in pyridine (100 ml). The solution was added dropwise through a condenser into a refluxing pyridine (100 ml) containing DCC (419 mg) over the period of 1 hr. The mixture was refluxed for additional 3 hr. The progress of the reaction was followed by pep. After the reaction was complete, the solvent was evaporated to dryness *in vacuo*. The residue was partitioned between $H_2O$ (20 ml) and ether (20 ml). The aq. layer was separated and filtered. The filtrate was washed three times with ether (20 ml×3) and then applied to DEAE-cellulose column (82×3.0 cm). Elution was performed with a linear gradient of $H_2O$ (5 l.) and 0.15 M TEAB (5 l.). One fraction was 19 ml. Fractions 115—164 were pooled, concentrated, lyophilized. Yield, 8080 $A_{262m\mu}$ units (58%). UV $\lambda_{max}^{H_2O}$ mμ (ε): 262 (13900). *Rf* in PCC (solvent A): 0.66. Upon electrophoresis (pH 7.5), the product had a mobility of 9.8 cm compared to 20 cm for pA. In order to obtain a sample for elemental analysis an aq. solution of I was passed through a phospho-cellulose column, $H^+$ form and the eluate was lyophilized to give a white powder (167 mg). Application of the sample (116 mg) to a Dowex (formate form, 34×1.7 cm, 100—200 mesh) and elution with a linear gradient of $H_2O$ (4 l.) and 0.1 M formic acid (4 l.) afforded an analytical sample in fractions 291—358 (fraction size, 18 ml). These fractions were concentrated to dryness and dried. Yield, 76 mg. *Anal.* Calcd for $C_{11}H_{13}O_6N_4P5/2H_2O$: C, 35.40; H, 4.86; N, 15.01; P, 8.30. Found: C, 35.31; H, 4.17; N, 15.25; P, 8.53.

**Hydrolysis of 1- (I) and 3-Deazaadenosine 3′,5′-Cyclic Phosphate (II) with Barium Hydroxide**——Hydrolysis was carried out essentially according to a reported procedure.[19] (a) A solution of I (109 $A_{262m\mu}$ units) in 0.2 M $Ba(OH)_2$ (0.33 ml) was heated in a boiling water-bath for 1 hr. The cooled hydrolysate was subjected to pep (paper size, 40×15 cm, two sheets). A band ($R_{pA}$ 1.0) was extracted with water. The extract was examined by a Varian LCS-1000.[20] It was found that the hydrolysate contained V (27%) and 1-deazaadenosine 3′-phosphate (VIII, 73%). (b) Hydrolysis of II and preparation of an isomeric mixture were performed as in (a). The mixture was subjected to ppc (solvent B). Each spot was excised, cut into small pieces, transferred to a test tube and eluted with $H_2O$ (5 ml) at 30° overnight. Estimation of the extract was performed as reported.[21] It was found that the hydrolysate contained XI (16%) and 3-deazaadenosine 3′-phosphate (84%).

**1-Deazaadenosine 2′,3′-Cyclic Phosphate (VII)**——The following procedure was essentially that of Ueda and Kawai.[22] A solution of tri-*n*-butylammonium pyrophosphate (1.0 mmole) in DMF (50 ml) was reacted with VI (135 mg, 0.50 mmole) at refluxing temperature for 2 hr and then cooled and filtered. The filtrate was concentrated to dryness and the residue was dissolved in 1 N $NH_4OH$ (1 ml). The solution was shaken with ether (2 ml). The aq. layer was separated and concentrated to dryness. The residue was dissolved in $H_2O$ and the solution was applied to a DEAE-cellulose column (54×2.4 cm). A linear gradient elution was performed.[22b] One fraction was 15 ml. Fractions 1—221 were discarded and fractions 222—238 were pooled and lyophilized. Yield, 1610 $A_{262.5m\mu}$ units (23%, triethylammonium salt). UV $\lambda_{max}^{H_2O}$ mμ (ε): 262.5 (15600); *Rf* in solvent system B: 0.34 ($R_{A>P}$ 1.03), *Rf* in solvent system D: 0.56 ($R_{A>P}$ 1.30). Upon Electrophoresis (pH 7.5) the product had a mobility of 5.3 cm compared to 10 cm for adenosine 5′-phosphate.

**3-Deazaadenosine 2′,3′-Cyclic Phosphate (XIII)**——3-Deazaadenosine (XII) (12.9 mg, 0.0485 mmole) was dissolved in 0.2 M DMF solution of tri-*n*-butylammonium pyrophosphate (0.5 ml). The solution was concentrated to dryness. The residue was dissolved in DMF (5 ml). The solution was refluxed for 2 hr.

19) M. Smith, G.I. Drummond, and H.G. K-orana, *J. Am. Chem. Soc.*, **83**, 698 (1961).

20) A column used was packed with PA 38 pellicular anion exchange resin (column size 300 cm×1 mm); temperature: 70°; flow rate 10/hr; elution was carried out by the linear gradient from 0.02 M $KH_2PO_4$ (pH 3.25) to 1.0 M $KH_2PO_4$ (pH 3.85); initial gradient chamber volume: 40 ml; gradient delay: 10 min. Under these conditions, retention times of $p^1Ado$, $^1Ado^p$, and $^1Adop$ were 25, 23, and 45 min, respectively.

21) F. Harada, F. Kimura, and S. Nishimura, *Biochemistry*, **10**, 3269 (1971).

22) *a*) T. Ueda and I. Kawamura, *Chem. Pharm. Bull.* (Tokyo), **18**, 2303 (1970); *b*) $H_2O$ (21.) and 0.2 M TEAB (21.).

After work-up as in the synthesis of VII, the reaction mixture was applied to DEAE-cellulose column, (bicarbonate form, column size; 54×2.4 cm). Linear gradient elution was carried out using 2 l. of water and 2 l. of 0.2 M TEAB. Fraction volume, 15 ml. Peaks containing XIII were pooled and concentrated to dryness. The residue was dissolved in a minimal volume of water. The solution was streaked on a sheet of paper (40×40 cm) and developed by solvent system A. Faster travelling band was excised and extracted with water (5 ml). Yield, 17.9 $A_{262m\mu}$ untis (3%). UV $\lambda_{max}^{pH\ 7}$ mμ (ε): 262 (9100).

**Enzymatic Hydrolysis of Deazaadenosine 2′,3′-Cyclic Phosphates (VII and XIII) with Ribonuclease M**——The incubation mixture contained 5 mM acetate buffer (pH 5.0), RNase M in 100 μl and each substrate at the concentrations shown in Table I. The temperature was 37°, length of incubation, 24 hr. Conditions for separation of the products and estimation of each product in hydrolysates were as described in a literature.[21]

Digestion of deazaadenosine 5′-phosphates (V and XI) was performed as given in the legend of Table II.

## Results and Discussion

1-Deazaadenosine 5′-phosphate (V) which was prerequisite for the synthesis of the corresponding 3′,5′-cyclic phosphate (I) was prepared by treatment of 2′,3′-O-ethoxymethylene 1-deazaadenosine with pyrophosphoryl tetrachloride in 49% yield. When 2′,3′-O-isopropylidene 1-deazaadenosine was used in place of 2′,3′-O-ethoxymethylene derivative for the synthesis of V, removal of the protecting group by acidic treatment was accompanied by hydrolysis of the N-glycosyl bond.[23] Another prerequisite starting material, 3-deazaadenosine 5′-phosphate (XI) was prepared by treatment of 2′,3′-O-isopropylidene 3-deazaadenosine[23] with pyrophosphoryl tetrachloride in acetonitrile, followed by hydrolysis in 86% yield. Both nucleotides (V and XI) were completely hydrolysable by snake venom 5′-nucleotidase, but with each different rate (see Table II). It is to be noted that XI was found to be a comparatively poor substrate for the nucleotidase under conditions used.

By the use of a general procedure for the synthesis of nucleoside 3′,5′-cyclic phosphates from 5′-phosphates developed by Smith, *et al.*,[19] 1-deazaadenosine 3′,5′-cyclic phosphate (I) could be prepared from V in 58% yield. Unexpectedly, however, this general procedure failed to give satisfactory result when applied to 3-deazaadenosine 5′-phosphate (XI). By this approach, yield of the required 3′,5′-cyclic phosphate (II) was very poor (*ca.* several per cent) and the product was found to be contaminated with 3-deazaadenosine 2′,3′-cyclic phosphate (XIII).

We adopted, therefore, another procedure developed by Smith and his coworkers.[18] 3-Deazaadenosine 5′-phosphate (XI) was converted to the corresponding *p*-nitrophenyl ester which in turn was treated with potassium *tert*-butyl alkoxide in DMSO to afford II.

Hydrolysis of 1-deazaadenosine 3′,5′-cyclic phosphate (II) with $Ba(OH)_2$ afforded 1-deazaadenosine 5′-phosphate (V) and 3′-phosphate (VIII) in a ratio 27:73, A parallel experiment with 3-deazaadenosine 3′,5′-cyclic phosphate (II) afforded a similar result.

For the synthesis of the nucleoside 2′,3′-cyclic phosphate a number of methods have been developed among which pyrophosphate-DMF method[22a] appeared the most attractive when started from a nucleoside. By this procedure, 1-deazaadenosine 2′,3′-cyclic phosphate (VII) could be prepared in 23% yield, whereas 3-deazaadenosine 2′,3′-cyclic phosphate was obtained by this approach only in 3% yield. These nucleoside 2′,3′-cyclic phosphates (VII and XIII) were hydrolyzable to the corresponding nucleoside 3′-phosphate by RNase M.

Analogs of adenosine or adenosine phosphates have been found to be very useful for the analysis of the reactions of adenosine nucleotide-utilizing enzymes or nucleotidic-allosteric effectors.[3,24–26] However, information available from the use of deaza-analogs is comparatively

23) We have observed that 1-deazaadenosine was more acid-labile than 3-deazaadenosine with respect to the N-glycosyl bond cleavage. Relative stability of 1- (VI) and 3-deazaadenosine (XII) toward aqueous acid treatment will be the subject of a separate paper.

24) L. Hagenberg, H.G. Gassen, and H. Mathaei, *Biochem. Biophys. Res. Commun.*, **50**, 1104 (1973).

25) S. Minato, *J. Biochem.*, **64**, 815 (1968).

26) G. Acs, E. Reich, and M. Mori, *Proc. Natl. Acad. Sci. U. S.*, **52**, 493 (1964).

TABLE I. RNase M Digestion of 2′,3′-Cyclic Phosphates

| | Percentage of hydrolysis (%) | *Rf* values of paper chromatography[a] Starting material Solvent A | B | Hydrolysis product system A | B |
|---|---|---|---|---|---|
| A>P (3.33 μmole) RNase M 25 μg | 93 | 0.42 | | 0.22 | 0.39[b] |
| $^{1}$A>P (9.71 νmole) RNase M 25 μg | 97 | 0.63 | 0.34 | 0.28 | 0.36[c] |
| $^{3}$A>P (0.802 μmole) RNase M 67 μg | 57 | 0.43 | 0.33 | | 0.46[d] |

*a*) Paper chromatography was performed by the ascending technique.
*b*) A: 0.52, *c*) $^{1}$A: 0.49, *d*) $^{3}$A: 0.55; p$^{3}$A: 0.66.

TABLE II. Relative Rates of Hydrolysis of Nucleoside 5′-Phosphates with Venom 5′-Nucleotidase

| pN | Conc. μmole/ml | Relative rate (%) |
|---|---|---|
| Adenosine | 3.2 | 100 |
| 1-Deazaadenosine | 3.4 | 90 |
| 3-Deazaadenosine | 3.0 | 42 |
| 7-Deazaadenosine | 2.7 | 97 |

digestion conditions:
Digestion conditions: the incubation mixture contained 3 μmols of substrate, 10 μmoles of $MgCl_2$, 100 μmoles of Tris-HCl buffer (pH) and 39 μg of the enzyme in a total volume of 1.0 ml. Incubation temperature: 37°, time: 15 min. An aliquot (200 μl) was withdrawn from the reaction and subjected to determination of inorganic phosphate liberated (Allen's procedure).

limited with the exception of a naturally-occurring and commercially available 7-deaza-analog (Tubercidin), because other deaza-analogs were quite inaccessible.

We were able to prepare a number of 1- and 3-deazaadenosine phosphate (I, II, VII, and XIII) and to improve the synthesis of V[15] and XI.[10b] These deazaanalogs (I and II) might be useful for the analysis of the action of cAMP, and also deazaadenosine monophosphates (V and XI) might be useful for the understanding or clarification of a possible role of $N^3$ of purine-ring in the intramolecular interaction.[27]

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27) For example, it is well known that preferential conformations in solution of adenosine, its 2′-, and 3′-phosphate are *syn*, whereas that of adenosine 5′-phosphate is *anti*.[28] These facts strongly suggest that interaction between $N^3$ of purine ring and 5′-hydroxyl group might have something to do with the preferential conformation of adenosine 5′-phosphate. It appears interesting to determine the conformation of V and XI in order to obtain information on the possible role of $N^3$ in the intramolecular interaction.
28) T.-D. Dinh and C. Chachaty, *Biochim. Biophys. Acta*, **335**, 1 (1974).