Anal. Calcd for $C_{14}H_{14}N_5O_7P \cdot H_2O$: C, 40.68; H, 3.90; N, 16.95. Found: C, 40.87; H, 3.85; N, 16.96.

2-Ferrocenyladenosine Cyclic 3′,5′-Phosphate (13). An open flask containing a solution of 1.0 g (3.1 mmol) of **2**, 1.5 ml of 2 *N* NaOH, 5 ml of H_2O, 20 ml of MeOH, and 0.70 g (3.3 mmol) of ferrocenecarboxaldehyde was vigorously stirred for 3 days. The evaporated mixture was taken up in 30 ml of 50% aqueous EtOH and warmed to *ca.* 80°. Addition of 1.5 ml of 2 *N* HCl and cooling gave a solid which was further purified on an Avicel (Brinkman) microcrystalline cellulose column (2.5 × 20 cm), packed in and eluted with *i*-PrOH/concd NH_4OH/H_2O, 8:1:1. Evaporation of the appropriate fractions and acidification of an aqueous solution of the residue gave 0.30 g (19%).

Anal. Calcd for $C_{20}H_{20}FeN_5O_6P$: C, 46.81; H, 3.93; N, 13.65. Found: C, 46.89; H, 4.06; N, 13.49.

2-Thioadenosine Cyclic 3′,5′-Phosphate (14). A mixture of 3.5 g (11.3 mmol) of **2**, 1.60 g (10.5 mmol) of DBU, and 50 ml of Me_2SO was brought to solution by heating then was cooled to 0°. With stirring, 2.0 g (11.3 mmol) of 1,1′-thiocarbonyldiimidazole was added. After 10 min of stirring, the solution was stored at −20° for 20 hr, then an additional 1.0 g of 1,1′-thiocarbonyldiimidazole was added. After 30 min of stirring at ambient temperature, the solution was diluted with 100 ml of H_2O and 1 ml of formic acid, then passed through a 2.6 × 20 cm column of Dowex 1-X2 (formate form, 100–200 mesh). The column was washed with H_2O, then eluted with a gradient of 900 ml of 1 *N* formic acid in the mixing chamber and 900 ml of 1 *N* formic acid + 1 *N* ammonium formate in the reservoir. The product began to appear near the end of the gradient, and elution of the product was completed with 1 *N* formic acid + 2 *N* ammonium formate. Fractions containing product were passed through a column containing 1 l. of Dowex 50-X8 (H^+, 100–200 mesh). Evaporation of the eluate to dryness gave 2.05 g (48%) of product.

Anal. Calcd for $C_{10}H_{12}N_5O_6PS \cdot H_2O$: C, 31.58; H, 3.71; N, 18.42. Found: C, 31.87; H, 3.59; N, 18.58.

2-Hydroxyadenosine Cyclic 3′,5′-Phosphate (15). A mixture of 2.0 g (6.3 mmol) of **2**, 0.80 g (6.45 mmol) of DBN, and 10 ml of Me_2SO was brought to solution by heating. To this was added, with stirring at 25°, 1.0 g (6.2 mmol) of 1,1′-carbonyldiimidazole. After 30 min of stirring another 1.0 g of 1,1′-carbonyldiimidazole was added, and stirring was continued an additional 30 min. The solution was diluted with 50 ml of H_2O and 1 ml of formic acid and passed through a 2.6 × 10 cm column of Dowex 1-X2 (formate form, 100–200 mesh). After washing with H_2O, the column was eluted with a gradient of 1 l. of H_2O in the mixing chamber and 1 l. of 5 *N* formic acid in the reservoir. Evaporation of fractions containing product, which appeared between 750 and 1250 ml of eluate, gave 0.79 g (33%).

Anal. Calcd for $C_{10}H_{12}N_5O_7P \cdot 2H_2O$: C, 31.50; H, 4.23; N, 18.37. Found: C, 31.42; H, 4.29; N, 18.44.

2-Methylthioadenosine 3′,5′-Cyclic Phosphate (16). A mixture of 1.8 g (4.5 mmol) of **14**, 5 ml of 2 *N* NaOH, 2 ml of MeI (32 mmol), 20 ml of H_2O, and 20 ml of MeOH was stirred 2 hr. The solution was evaporated *in vacuo*, taken up in 100 ml of H_2O, and passed through a column of 50 ml of Dowex 1-X2 (formate, 100–200 mesh). The product appeared as the major component upon elution with a gradient of 1 l. of 1 *N* formic acid in the mixing chamber and 1 l. of 5 *N* formic acid in the reservoir. To remove a trace of impurity, the product, isolated after evaporation of the above fractions, was taken up in water and passed onto a 200 ml column of Dowex 50-X8 (H^+, 100–200 mesh), which was washed in the 500 ml of H_2O then 1 l. of 0.5 *N* formic acid. Fractions containing product were evaporated, giving 0.64 g (38%).

Anal. Calcd for $C_{11}H_{14}N_5O_6PS \cdot 1.5H_2O$: C, 32.84; H, 4.26; N, 17.41. Found: C, 32.98; H, 4.57; N, 17.49.

4-Amino-7-β-D-ribofuranosylimidazo[4,5-*d*]-*v*-triazine Cyclic 3′,5′-Phosphate (17, 2-Azaadenosine Cyclic 3′,5′-Phosphate). Compound **2** (1.5 g 4.7 mmol) was dissolved in 92 ml of 6 *N* HCl, precooled to −25°, and a solution of $NaNO_2$ (370 mg, 6.2 mmol) in 14 ml of H_2O was added dropwise with stirring over 25 min. The temperature was maintained at −25°, stirring was continued an additional 40 min, and 30 ml of EtOH was added. With cooling, the solution was neutralized with concentrated NH_4OH. The filtered solution was passed through a 5.5 × 46 cm column of Dowex 50-X8 (H^+, 100–200 mesh) and the column was eluted with H_2O. The appropriate fractions were combined and evaporated to dryness, giving, after trituration with EtOH, 1.19 g (76%).

Anal. Calcd for $C_9H_{11}N_6O_6P$: C, 32.73; H, 3.35; N, 25.45. Found: C, 32.54; H, 3.47; N, 25.23.

Acknowledgments. We wish to thank Mr. Ed Banta and Ms. Milda Strikaitas for the nmr and uv spectra, Mr. O. P. Crews and his staff for the large scale preparation of intermediates, and Dr. Jon Miller and Ms. Mieka Scholten for the biochemical data.

Polynucleotides. XXIV.[1] Synthesis and Properties of a Dinucleoside Monophosphate Derived from 8,2′-*O*-Cycloadenosine

Morio Ikehara,* Seiichi Uesugi, and Junichi Yano

Contribution from the Faculty of Pharmaceutical Sciences, Osaka University, Tayonaka, Osaku-fu, Japan. *Received February 22, 1974*

Abstract: A dinucleoside monophosphate of *O*-cycloadenosine, 8,2′-anhydro-8-oxy-9-β-D-arabinofuranosyladenine phosphoryl-(3′-5′)-8,2′-anhydro-8-oxy-9-β-D-arabinofuranosyladenine (Ib), was synthesized from 5′-monomethoxytrityl-8,2′-anhydro-8-oxy-9-β-D-arabinofuranosyladenine (VI) and N^6-3′-diacetyl-8,2-′anhydro 8-oxy-9-β-D-arabinofuranosyladenine (VIII) using dicyclohexylcarbodiimide as the condensing reagent. From the uv spectra taken under various conditions of temperature and salt concentration, it was deduced that compound I had a well-stacked thermally stable conformation. CD spectra of I, which have curves symmetrically reversed from those of usual ApA, suggest a stacking of two adenine moieties along the left-handed screw axis, as has been previously observed in *S*-cyclonucleoside oligomers. It is suggested that direction of the rotation of the screw axis and the conformational stability of cyclonucleoside oligomers are dependent on the torsion angle of bases in the component nucleosides.

We have synthesized previously a dinucleoside monophosphate[2,3] and oligomers[4] of 8,2′-anhydro-8-mercapto-9-β-D-arabinofuranosyladenine (8,2′-*S*-cycloadenosine, A^s) (Ia) (Chart I). Compared with

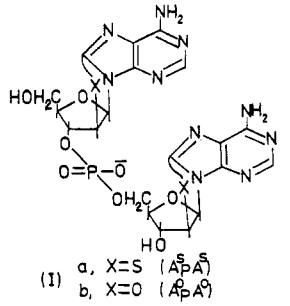
(1) Part XXIII of this series: E. Ohtsuka, S. Nakamura, M. Yoneda, and M. Ikehara, *Nucleic Acid Res.*, 1, 323 (1974).

(2) M. Ikehara, S. Uesugi, and M. Yasumoto, *J. Amer. Chem. Soc.*, 92, 4735 (1970).

(3) S. Uesugi, M. Yasumoto, M. Ikehara, K. N. Fang, and P. O. P. Ts'o, *J. Amer. Chem. Soc.*, 94, 5480 (1972).

(4) M. Ikehara and S. Uesugi, *J. Amer. Chem. Soc.*, 94, 9189 (1972).

Chart I. Dinucleoside Monophosphate of Cycloadenosine



(I) a, X=S ($A^s_pA^s$)
b, X=O ($A^o_pA^o$)

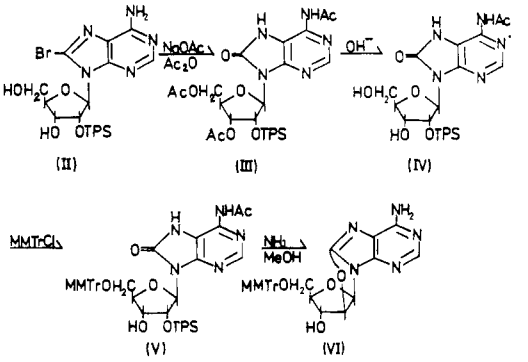
oligonucleotides of natural nucleosides, these oligonucleotides of cyclonucleosides have unique features. A left-handed stacked conformation, which is very stable against thermal perturbation, is assigned to them. These properties were confirmed by uv, CD, nmr, and T_m measurements.

Recent finding[5] that poly(A^s) forms a 1:1 and probably left-handed complex with polymers of 6,2′-anhydro-6-oxy-1-β-D-arabinofuranosyluracil (U^o) led us to consider the synthesis of a dinucleoside monophosphate (A^opA^o) (Ib) of 8,2′-anhydro-8-oxy-9-β-D-arabinofuranosyladenine (8,2′-*O*-cycloadenosine, A^o). Since A^s and A^o have almost the same torsion angle of the base fixed at ϕ_{CN}[6] nearly equal to −120°,[7] we can probably know whether unusual properties of A^s originated from S atoms in the anhydro linkage or from its unique torsion angle different from the natural nucleotides. Previous observations of some of the properties of A^opA^o have appeared.[8]

Results

Synthesis of the Dinucleoside Monophosphate. In order to synthesize dinucleoside phosphate A^opA^o we chose a route to condense a nucleoside component having free 3′-OH and a 5′-phosphate of A^o. In the case of A^spA^s an alternate route using a 5′-OH nucleoside and a 3′-phosphate[2,3] was employed. However, the latter route caused a ring opening of cyclonucleoside A^o [9] and was thought to be inapplicable to the present case. For obtaining the 3′-OH free nucleoside component, tritylation of A^o in DMF was attempted first. However, as experienced in the case of A^s,[3] the reaction proceeded very slowly and the yield of the desired product was extremely low. When pyridine was used as the solvent, N^6,5′-dityrityl derivative was obtained and the protection of the 6-NH_2 group with dimethylaminomethylene[10] or benzoyl group gave poor yields. To circumvent these difficulties, 8-bromo-2′-*O*-triisopropylbenzenesulfonyladenosine[11] (II) was treated with acetic anhydride in acetic acid in the presence of sodium acetate (Chart II).[12] Resulting N^6,3′,5′-*O*-triacetyl-2′-triisopropylbenzenesulfonyl-8-oxyadenosine (III) was treated with 2 *N* NaOH[13] at 0° for 5 min to give N^6-acetyl derivative (IV) having uv $\lambda_{max}^{H_2O}$ 288.5 nm. The overall

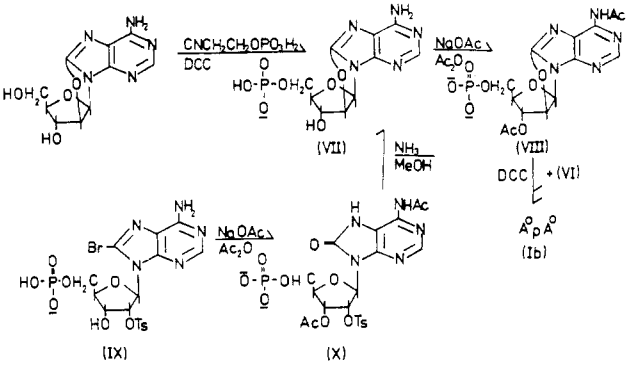
Chart II. Synthesis of the Protected Nucleoside Component



yield from II was 75%. Compound IV was then allowed to react with monomethoxytrityl chloride in pyridine to give N^6-acetyl-2′-triisopropylbenzenesulfonyl-5′-monomethoxytrityl-8-oxyadenosine (V) in a yield of 95%. Heating compound V with anhydrous methanol, which was previously saturated with NH_3 gas at 0°, at 80° for 6 hr gave 5′-monomethoxytrityl-8,2′-anhydro-8-oxy-9-β-D-arabinofuranosyladenine (MMTrA^o) (VI). Since 6-NH_2 group of 8-cyclonucleoside was found to be inert in the next condensation step, reprotection of this group was not attempted.

For the synthesis of nucleotide component VIII two routes were investigated (Chart III). The first method

Chart III. Synthesis of the Protected Nucleotide Component



consists of a selective phosphorylation of A^o with cyanoethyl phosphate and dicyclohexylcarbodiimide (DCC).[14] 8,2′-*O*-Cycloadenosine and cyanoethyl phosphate were rendered anhydrous by repeated evaporations with added pyridine and condensed by using DCC at 25° for 5 days. After the appropriate work-up and deprotection with methanolic ammonia, the nucleotide was chromatographed on a Dowex (chloride form) column. 5′-Monophosphate VII appeared in the first peak and the yield was 40%. 3′-Monophosphate and 3,5′-diphosphate were obtained from second and third peaks, respectively. The 5′-phosphate VII was characterized by the dephosphorylation with snake venom 5′-nucleotidase.[15] Compound VII was dephosphorylated to an extent of 27% with 10 μl of enzyme solution in 4 hr and was hydrolyzed to 75% with 30 μl of enzyme solution in 12 hr. Under similar condition, 5′-AMP was completely hydrolyzed with 5 μl of enzyme solution in 4 hr. Compound VII seems to be much more resistant to 5′-nucleotidase than corresponding *S*-cycloadenosine 5′-phosphate. The 3′-phosphate synthesized

(5) M. Ikehara and T. Tezuka, *J. Amer. Chem. Soc.*, **95**, 4054 (1973).
(6) J. Donohue and K. N. Trueblood, *J. Mol. Biol.*, **2**, 363 (1960).
(7) K. Tomita, T. Tanaka, M. Yoneda, T. Fujiwara, and M. Ikehara, *Acta Crystallogr., Sect. A*, **28**, 45 (1972).
(8) M. Ikehara S. Uesugi and J. Yano *Nature* (*London*), *New Biol.*, **240**, 16 (1972).
(9) M. Ikehara and Y. Ogiso, *Tetrahedron*, **28**, 3695 (1972).
(10) J. Zémlička, *Collect. Czech. Chem. Commun.*, **28**, 1060 (1963).
(11) M. Ikehara and M. Kaneko, *Tetrahedron*, **26**, 4251 (1970).
(12) M. Ikehara and M. Kaneko, *Chem. Pharm. Bull.*, **18**, 2401 (1970).
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(14) G. M. Tener, *J. Amer. Chem. Soc.*, **83**, 159 (1961).
(15) Y. Mizuno, M. Ikehara, T. Ueda, A. Nomura, E. Ohtsuka, F. Ishikawa, and Y. Kanai, *Chem. Pharm. Bull.*, **9**, 338 (1961).

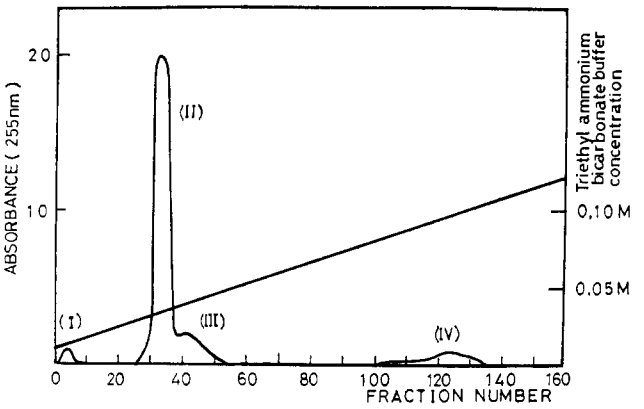

Figure 1. Chromatographic separation of A°pA° on a column of DEAE-Sephadex A-25 (bicarbonate form, 1 × 35 cm). Elution was carried out with a linear gradient from 0.02 *M* triethylammonium bicarbonate (TEAB) buffer (pH 7.5) (1 l.) to 0.15 *M* TEAB buffer (pH 7.5) (1 l.). Fractions of 10 ml each were collected.

separately resisted completely hydrolysis catalyzed by this enzyme. The structure of VII was further confirmed by a synthesis starting from 5′-AMP. Previously, Ikehara and Uesugi[16] showed that 8,2′-*S*-cAMP could be synthesized from 5′-AMP by a selective tosylation of the 2′-OH, followed by bromination and cyclization with NaSH. We attempted O-cyclization from a common intermediate, 8-bromo-2′-*O*-tosyladenosine 5′-phosphate (IX). The compound IX sodium salt was heated with sodium acetate in an acetic acid–acetic anhydride (1 : 1) mixture at reflux temperature for 2 hr. Uv $\lambda_{max}^{H_2O}$ changed from 262 to 289.5 nm suggesting the conversion of compound VIII to an 8-oxy derivative (X). The product was then rendered anhydrous and heated with methanolic ammonia at 60° for 6 hr. Purification of the cyclization product on a column of Dowex (formate) and treatment with charcoal gave 8,2′-*O*-cAMP (VII) in a good yield. This sample was completely identical with that obtained by the former procedure and the position of phosphate in VII was thus established as on 5′-OH. For condensation of the nucleotide VII with nucleoside component VI, 6-NH_2 and 3′-OH groups of VII were protected with acetyl group. Diacetyl-pA° (VIII), thus obtained, was rendered strictly anhydrous by repeated evaporations with pyridine and condensed with compound VI in the presence of DCC. After the reaction at room temperature for 2 days, the reaction product was treated successively with 50% pyridine, methanolic ammonia, and 80% acetic acid. Powdered nucleotidyl materials were applied to a column of DEAE-Sephadex and eluted with a linear gradient of 0–0.15 *M* triethylammonium bicarbonate buffer. The elution pattern is shown in Figure 1. The desired dinucleoside monophosphate, A°pA° (Ib), was obtained from peak II in a yield of 87%. R_f values of A°pA° on paper chromatography are shown in Table I.

Properties of A°pA° as the Substrate of Enzymes. The properties of A^{s}pA^s toward enzymatic hydrolysis were investigated previously.[2] In the present study the properties of A°pA° were compared with ApA, TpT, and A^{s}pA^s by the incubation with crude snake venom, purified venom phosphodiesterase, and spleen phosphodiesterase. As summarized in Table II, compound

(16) M. Ikehara and S. Uesugi, *Tetrahedron*, **28**, 3687 (1972).

Table I. Paper Chromatographic Properties of *O*-Cycloadenosine Derivatives[a]

Compd	R_f in solvent A	B	C
A°pA°	0.16	0.22	0.22
pA°	0.08	0.15	0.21
A°	0.49	0.58	0.46
pA	0.10	0.20	0.19
<pA[b]	0.37	0.46	0.32
A	0.52	0.61	0.43

[a] Descending technique was used with Toyo filter paper No. 51A. For composition of the solvent system, see the Experimental Section. [b] Adenosine cyclic 3′,5′-monophosphate.

Table II. Susceptibility of A°pA° to Phosphodiesterases[a]

Compd	% hydrolysis[b] Spleen phosphodiesterase	Venom phosphodiesterase	Crude snake venom
A°pA°	0	0	8
A^{s}pA^s	0	0	17[c]
ApA	100	100	100
TpT	100	100	100

[a] Conditions for enzyme reaction are given in the Experimental Section. [b] % hydrolysis in 12 hr. It was examined by paper electrophoresis. [c] % hydrolysis in 18 hr.

Ib completely resisted hydrolysis by venom and spleen phosphodiesterase, which completely hydrolyzed ApA and TpT. The crude venom hydrolyzed Ib to an extent of 8%. A^{s}pA^s also behaved like compound Ib and was hydrolyzed by the crude venom to 17%. Analysis of hydrolyzed products of A°pA° (Ib) showed the existence of a nucleoside (A°) and a nucleotide (pA°). The behaviors of A°pA° toward hydrolytic enzymes closely resembled those observed in the case of A^{s}pA^s and the following points may be emphasized.

(i) Dinucleoside monophosphate having bases fixed at $\phi_{CN} \sim 120°$ is extremely resistant to the hydrolysis with phosphodiesterase, though the internucleotidyl linkage is the usual 3′,5′-phosphodiester. This may be interpreted by the difficulties of binding of the substrate to the enzyme active site because of the unusual conformation. Recently, the dinucleoside monophosphate of 6,2′-anhydro-6-oxy-1-β-D-arabinofuranosyluracil (U°pU°) was also shown to be highly resistant to the hydrolysis by venom phosphodiesterase.[17]

(ii) A°pA° and A^{s}pA^s can be hydrolyzed by the use of a large amount of snake venom phosphodiesterase (in crude snake venom) to some extent. A°pA° seems to be more resistant to this enzyme than A^{s}pA^s. Higher resistance of the nucleotide derivatives containing A° to 5′-nucleotidase and phosphodiesterase could be due to more constrained structure of the O-cyclonucleoside moiety.

Uv Absorption Properties of A°pA°. The uv spectra of A°pA° are shown in Figure 2. As $\lambda_{max}^{pH\ 7.0}$ of the monomer A° is 257 nm,[12] a hypsochromic shift of 2–3 nm was found. This shift is somewhat larger than that found between A and ApA (1 nm).[18] The ε(p) value determined from the phosphate analysis and uv absorbance for A°pA° was 2.54×10^4 in the neutral condition. Since ε(p) of pA° is 1.5×10^4, ε(p) of A°pA° is about

(17) M. Ikehara and T. Tezuka, *Nucleic Acid Res.*, **1**, 479 (1974).
(18) I. Tazawa, S. Tazawa, L. M. Stempel, and P. O. P. Ts'o, *Biochemistry*, **9**, 3499 (1970).

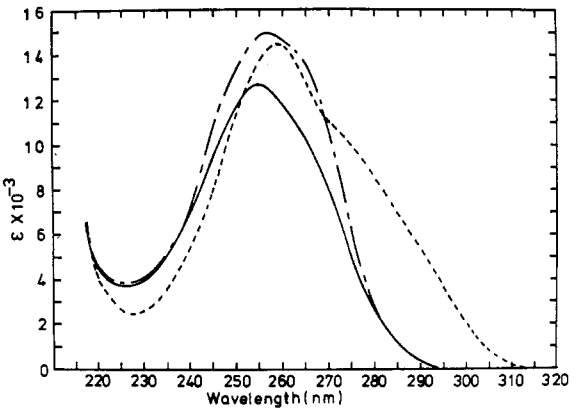

Figure 2. Ultraviolet absorption spectra of A°pA° at pH 7.0 (0.05 *M* phosphate buffer; —), at pH 2 (0.01 *N* HCl; - - -), and pA° at pH 7.0 (0.05 *M* phosphate buffer; -·-·-). All samples were measured at room temperature. ε is expressed in terms of the per residue value.

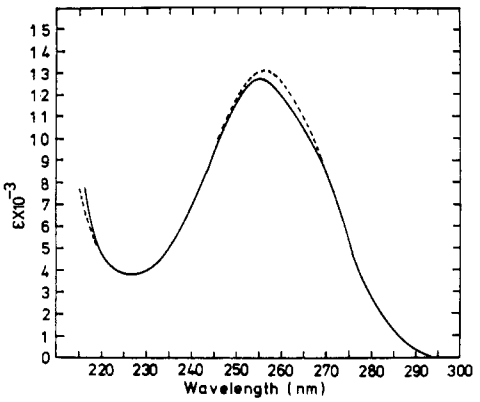

Figure 3. Ultraviolet absorption spectra of A°pA° at 0° (—) and 70° (- - -) in 0.05 *M* phosphate buffer (pH 7.0)–0.5 *M* KF.

twice as large as that of the monomer, thus confirming the presence of two bases per phosphorus atom in the dimer. ε per residue of A°pA° is equal to 1.27×10^4. Hypochromicity was calculated as in Table III together

Table III. Ultraviolet Spectral Properties of A°pA°

Compd	$\lambda_{max}^{pH\ 7}$, nm	ε at λ_{max}[a] ($\times 10^3$)	Hypochromicity,[b] %
A°pA°	255	12.7	15
ApA[c]	258	13.6	12
A[s]pA[s] [d]	271	22.5	15

[a] Expressed in terms of the per residue value. [b] Calculated from ϵ_{max} of the dimer and ϵ_{max} of the monomer. [c] Taken from ref 19. [d] Taken from ref 3.

with results obtained from calculations on ApA and A[s]pA[s]. Hypochromicity of A°pA° (15%) at λ_{max} is larger than that of ApA (12%)[19] and comparable to that of A[s]pA[s] (15%).[3] These features suggest that the stacking tendency in cyclonucleoside dimers is distinctly higher than that in the natural nucleoside dimer.

In the acidic condition (pH 2), ϵ_{max} increased by 14% and λ_{max} shifted to 260 nm. In other words, ϵ_{max} (per residue) and λ_{max} became close to those of the monomer.

(19) N. S. Kondo, H. M. Holmes, L. M. Stempel, and P. O. P. Ts'o, *Biochemistry*, **9**, 3479 (1970).

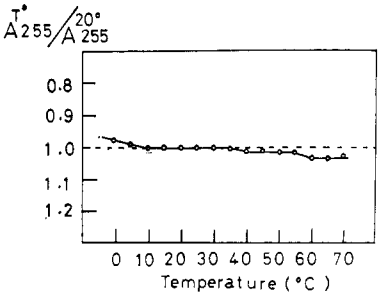

Figure 4. Effect of temperature on ultraviolet absorption of A°pA° at 255 nm in 0.05 *M* phosphate buffer (pH 7.0)–0.5 *M* KF. The observed absorbance is normalized to that measured at 20°. The ratio of ε of pA° to that of A°pA°, at 255 nm and 20°, is 1.18.

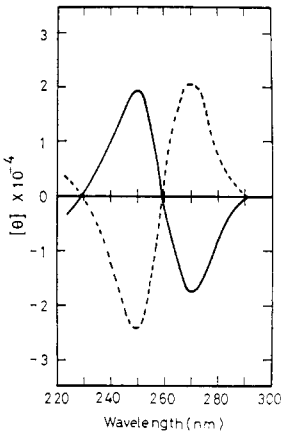

Figure 5. CD spectra of A°pA° (—) in 0.01 *M* phosphate buffer (pH 7.0)–0.1 *M* KF at 21.5° and ApA (- - - -) in 0.1 *M* Tris-HCl buffer (pH 7.4)–0.1 *M* NaCl at 25°. The latter is taken from ref 21.

These results indicate that the stacking conformation of A°pA° is decreased in an acidic medium by protonation of both adenine rings.

The temperature dependency of the uv absorbance of A°pA° was investigated using 0.05 *M* phosphate buffer at pH 7.0 in the presence of 0.1–1.0 *M* KF. The temperature was raised from 0 to 70°. In Figure 3 are shown the results in the presence of 0.5 *M* KF. A hyperchromicity of *ca.* 4% and a bathochromic shift (2 mm) of the absorption maximum were observed. Therefore, even at 70°, some stacked conformers were left and a relatively thermostable property of A°pA° was suggested. A plot of A_t/A_{25} against temperature is shown in Figure 4. Average increase of absorbance per degree is nearly the same as that of ApA.[19]

From the uv study we can assume a well-stacked, thermostable conformation for A°pA°.

Circular Dichroism of A°pA°. The CD spectrum of A°pA° was taken at pH 7.0 and 21.5° in the presence of 0.1 *M* KF. As shown in Figure 5 (solid line), A°pA° has a negative Cotton band at 272 nm and a positive band at 248 nm. The crossing-over point is at 258 nm. The Cotton curve is almost rotationally symmetrical around the crossing-over point and no big shoulder is observed. Since the uv absorption maximum of A°pA° was at 255 nm, this CD curve may be explained by the exciton splitting band[20] arising from the coupling of two transitions situated in close proximity. The CD curve

(20) C. A. Bush and I. Tinoco, Jr., *J. Mol. Biol.*, **23**, 601 (1967).

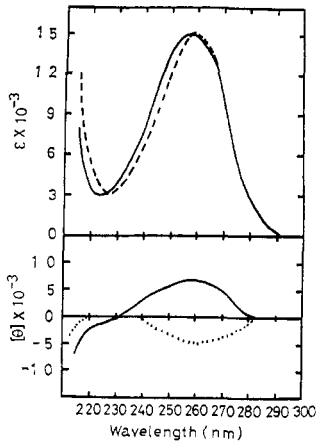

Figure 6. Ultraviolet absorption and CD spectra of A° (—) and A (- - - -) at pH 7.0 and room temperature.

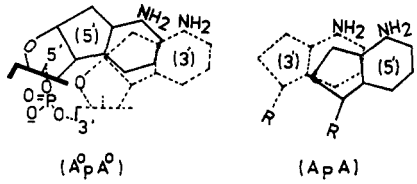

Figure 7. Schematic presentation of the conformational models for A°pA° and ApA. Solid line shows 5′-linked nuleoside and dotted line shows 3′-linked nucleoside. All nucleoside units have an anti conformation.

of ApA[21] is also plotted in Figure 5 (dotted line) for comparison. This curve has its maximum at 271 nm and minimum at 247 nm resulting in a curve of opposite signs in each wavelength to the curve of A°pA°. In the curve of A°pA°, the crossing-over point is again exactly at 258 nm, which is the maximum wavelength in uv absorption. As discussed by Tinoco, *et al.*,[20] two transition moments crossing each other in a certain angle will split into a positive and a negative band. In ApA, the splitting showed (+) and (−) bands from the long wavelength and, in contrast, A°pA° showed a (−) and (+) splitting. As was observed in the uv spectra, both compounds had almost similar λ_{max} at 255–258 nm having ϵ of 12.7–13.6 × 10^3 (see Figure 6). We can predict, therefore, that the coupling in A°pA° and ApA is in the opposite way, presumably as shown in Figure 7. Since it was found[22] by nmr study that in ApA the two adenine rings are stacked around a right-handed screw axis, the stacking of A°pA° could be in the left-handed fashion. A left-handed type stacking has been previously suggested in A^{s}pA^s from the CD spectrum, which shows the same splitting pattern as that of A°pA°, and was confirmed by pmr study.[3] Since A° and A^s have anhydro linkage between the 8- and 2′-carbon atoms, the conformation must be similar to have ϕ_{CN} −122° which was determined exactly by X-ray crystallography.[7] Both adenines in ApA were shown[22] to have anti conformation (ϕ_{CN} −60°) and to stack in a right-handed fashion. By contrast, in A°pA°, the bases are at the syn–anti boundary region and they may be stacked along a left-handed screw axis. The previous prediction[3,4] that fixing the torsion angle in the syn–anti region leads to the conclusion that the left-handed screw axis may be also relevant in the present case.

(21) K. E. Van Holde, J. Brahms, and A. M. Michelson, *J. Mol. Biol.*, 12, 726 (1965).
(22) P. O. P. Ts'o, N. S. Kondo, M. P. Schweizer, and P. Hollis, *Biochemistry*, 8, 997 (1969).

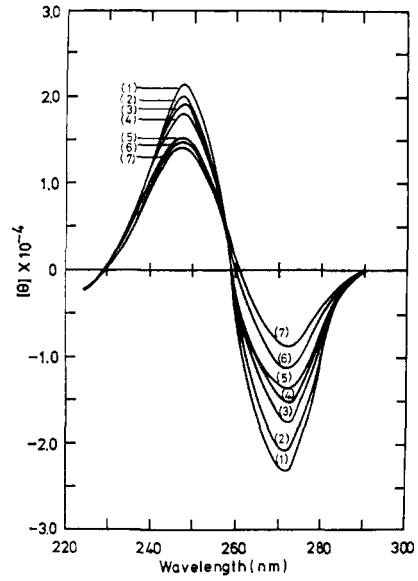

Figure 8. Circular dichroic melting of A°pA° in 0.05 *M* phosphate buffer (pH 7.0)–4.7 *M* KF. Temperatures (°C) are: (1) 0, (2) 10, (3) 21.5, (4) 28, (5) 40, (6) 50, (7) 60.5.

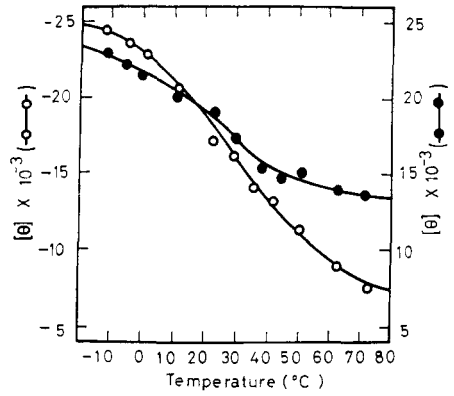

Figure 9. Effect of temperature on [θ] of A°pA° at 248 nm (●, maximum) and at 272 nm (○, minimum) in 0.05 *M* phosphate buffer (pH 7.0) at 26°.

The change of CD spectra of A°pA° at pH 7.0 in 4.7 *M* KF with increasing temperature from 0 to 60° is shown in Figure 8. The temperature dependencies of the absolute [θ] values at two extrema (248 and 272 nm) in 4.7 *M* KF–0.05 *M* phosphate buffer (pH 7.0) are shown in Figure 9. When the temperature is raised over the range 0–60°, the magnitude of the extrema decreases almost linearly. The decreases in the absolute values of $[\theta]_{272}$ and $[\theta]_{248}$ are 65 and 38%, respectively. This difference in decrease may be caused by different contributions from the monomer bands at 272 and 248 nm. For ApA, the [θ] value of the first positive extrema also decreased linearly over the same temperature range, by 70–80%.[17,19] Comparing the decrease in the absolute value of [θ] at the first extrema, where interference by the monomer Cotton effect is small, the CD of A°pA° is slightly less sensitive to the temperature perturbation than ApA, but more sensitive than A^{s}pA^s.[3] In other words, we can say that the stacking conformation

of A°pA° is slightly more stable than that of ApA, but less stable than that of A^{s}pA^s. As discussed in the case of A^{s}pA^s, the cause of the stability may be attributed to the rigidity of the monomer, which had bases fixed around the glycosidic linkage by means of the anhydro bond. The additional stability in the case of A^{s}pA^s could be due to an attracting force between two S atoms and also to less strained and more favorable conformation of the monomer unit for stacking interaction. A further study with other cyclonucleosides having NH or N–CH_3 cyclo bonds[23] must be investigated to clarify this point. In comparison of results shown in Figure 4 with results shown in Figure 9, it appears that the CD spectrum of A°pA° is far more sensitive to temperature than the uv absorbance of this molecule. It is possible that the left- to right-handed transformation of A°pA° is reflected in the CD measurement but not in the uv measurement.

The dependence of $[\theta]$ values on the salt concentration (0.1–3.0 *M* KF) was almost negligible. Since this molecule, a dinucleoside monophosphate, contains only one charge group, it is not surprising that the conformation of the molecule is not sensitive to salt concentration. The uv absorption spectrum was not affected by various salt concentrations either.

Interaction of A°pA° with Poly(U). Since it was shown by Tazawa, *et al.*,[18] that L-ApA, in spite of the left-handed stacked conformation, formed a complex of 1:2 ratio with poly(U), the complex formation of A°pA° with poly(U) was investigated. When A°pA° (0.5×10^{-4} *M*/residue) was mixed with poly(U) (1×10^{-4} *M*) at 0° in the presence of 0.05 *M* phosphate buffer (pH 7.0), no differences in the CD and uv spectra were observed when compared with the curve obtained by calculation (Figure 10). The same result has previously been observed with A^{s}pA^s and poly(U).[3] These results may be interpreted by a different torsion angle fixed at around −120° of A°pA° from that of poly(U) inhibiting the formation of hydrogen bondings between adenine and uracil moieties and a stable left-handed conformation of A°pA°, which is resistant to winding up along the right-handed poly(U) helix.

Concluding Remarks

From the results described above on the properties of A°pA°, the following points may be emphasized. As shown previously in the case of *S*-cycloadenosine oligomers,[3,4] the fixation of base moieties at $\phi_{CN} - 120°$ in nucleosides of a dinucleoside monophosphate, A°pA°, gave a highly stacked, thermally stable conformation compared to the naturally occurring ApA. Furthermore, the two bases in A°pA° may be stacked along a left-handed screw axis opposite to the right-handedness of ApA.

This conformation may cause resistance of A°pA° to the digestion with nucleolytic enzyme phosphodiesterase and to the complex formation with poly(U). Compared with the similar nature of A^{s}pA^s, these properties may not solely be due to S or O atoms in anhydro linkages, but to the unusual conformation of A°pA°.

Further studies of oligonucleotides involving cyclonucleoside monomers are in progress in our laboratory.

(23) M. Kaneko, B. Shimizu, and M. Ikehara, *Tetrahedron Lett.*, 3113 (1971).

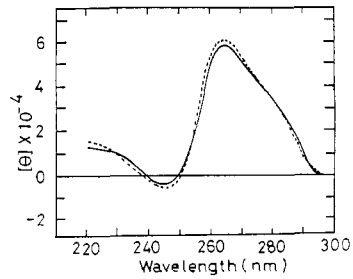

Figure 10. CD spectrum (—) of a 1:2 mixture of A°pA° (0.5×10^{-4} *M*) and poly(U) (1×10^{-4} *M*) in 0.05 *M* phosphate buffer (pH 7.0) at 0°. Addition spectrum (- - - - -) is also shown.

Experimental Section

Uv spectra were taken with a Hitachi EPS-3T or 124 spectrophotometer; CD spectra were taken with a JASCO ORD/UV-5 spectropolarimeter equipped with a CD attachment in 10-mm light path. Concentration of nucleotides was 1–2 OD_{max}/ml. In order to obtain accurate curves all runs were made at least twice and calibrated with *d*-10-camphorsulfonic acid. The temperature of the cuvettes in uv or CD measurements was controlled by a Komatsu Solidate thermostat within ±0.1° and measured with a constantan–Cu thermocouple connected to a Takara thermister. ϵ and $[\theta]$ were presented as per residue value. Paper chromatography was performed on Toyo filter paper No. 51A in solvent A (2-propanol–concentrated ammonia–water, 7:1:2), B (ethanol–1 *M* ammonium acetate, 7:3), or C (1-butanol–acetic acid–water, 5:2:3). Paper electrophoresis was performed at pH 7.5 (0.05 *M* triethylammonium bicarbonate buffer) under 35 V/cm for 1 hr. Tlc was performed on Kieselgel HF 254 in solvent of $CHCl_3$–ethanol mixture (ratio as shown in the text). All crystalline compounds gave satisfactory elemental analysis results.

***N*^{6}-Acetyl-2′-*O*-triisopropylbenzenesulfonyladenosine (IV).** 8-Bromo-2′-*O*-triisopropylbenzenesulfonyladenosine[11] (II) (612.2 mg, 1 mmol) was dissolved in a mixture of anhydrous sodium acetate (612.2 mg), glacial acetic acid (15 ml), and acetic anhydride (15 ml). The solution was heated at refluxing temperature for 3 hr. After confirmation of total conversion of II by tlc, the solvent was evaporated *in vacuo*. Residual caramel (III) was dissolved in 70% pyridine (50 ml) and treated with 2 *N* NaOH[13] (50 ml) under cooling to 0° with an ice–water bath. After 5 min, the reaction mixture was poured into ice–water (300 ml) and neutralized with 1 *N* HCl. At pH 7 a white powder precipitated out. The powder was collected by filtration, washed with water, and dried. The yield was 414 mg (75%): uv $\lambda_{max}^{H^+}$ 288.5 nm, $\lambda_{max}^{50\%\ EtOH}$ 288.5 nm, $\lambda_{max}^{OH^-}$ 309 nm: tlc ($CHCl_3$–EtOH, 19:1) R_f 0.16, compound III, R_f 0.34.

***N*^{6}-Acetyl-2′-*O*-triisopropylbenzenesulfonyl-5′-*O*-monomethoxytrityl-8-oxyadenosine (V).** Compound IV (682 mg, 1.15 mmol) was dissolved in anhydrous pyridine (10 ml) and monomethoxytrityl chloride (372 mg, 1.2 mmol) was added. The reaction mixture was kept at room temperature for 4 hr under stirring and poured into ice–water (100 ml) containing 2% ammonia. The precipitating powder was collected by centrifugation, washed with water, and dried, yield was 95%: uv $\lambda_{max}^{H^+}$ 288 nm, $\lambda_{max}^{50\%\ EtOH}$ 288 nm $\lambda_{max}^{OH^-}$ 288–290 nm; tlc ($CHCl_3$–EtOH, 19:1) R_f 0.67.

5′-*O*-Monomethoxytrityl-8,2′-*O*-cycloadenosine (VI). Compound V (3.6 mmol) was dissolved in absolute methanol (50 ml), which was cooled to 0° in an ice–water bath and saturated with dry ammonia gas. The solution was sealed in a steel tube and heated at 80° for 6 hr. The reaction mixture was carefully evaporated after cooling and residual oil was crystallized from methanol or ethyl acetate. The yield was 1.29 g (62%): mp 184–186°; uv $\lambda_{max}^{H^+}$ 236 and 266 nm; $\lambda_{max}^{50\%\ EtOH}$ 234 and 257 nm; $\lambda_{max}^{OH^-}$ 258 nm; tlc ($CHCl_3$–EtOH, 1:1) R_f 0.22.

Compound VI gave 8,2′-*O*-cycloadenosine by treatment with acetic acid at room temperature for 5 hr.

***N*^{6},3′-*O*-Diacetyl-2′-*O*-tosyl-8-oxyadenosine 5′-Monophosphate (X).** 2′-*O*-Tosyl-8-bromoadenosine 5′-monophosphate (IX) (0.5 mmol) was dissolved in a mixture of anhydrous sodium acetate (310 mg), glacial acetic acid (15 ml), and acetic anhydride (15 ml). The solution was heated at refluxing temperature for 2 hr under exclusion of the moisture. Examination by paper chromatography in solvent A showed almost quantitative conversion of compound IX to X: uv $\lambda_{max}^{H^+}$ 233 (sh) and 289.5 nm; $\lambda_{max}^{50\%\ EtOH}$ 233 (sh) and

289.5 nm; $\lambda_{max}^{OH^-}$ 305 nm; paper chromatography R_f 0.70 (solvent A), compound VIII R_f 0.73, 5′-AMP R_f 0.33.

8,2′-*O*-Cycloadenosine 5′-Monophosphate (VII). (i) A pyridine solution of cyanoethyl phosphate (pyridinium form, 2 mmol) was evaporated *in vacuo*. To the residue was added 8,2′-*O*-cycloadenosine (135 mg, 0.5 mmol). The whole was rendered anhydrous by coevaporation several times with pyridine. Final volume was *ca.* 2 ml. After addition of DCC (850 mg, *ca.* 5 mmol), the reaction mixture was kept at 25° for 10 hr. The reaction was stopped by adding 50% pyridine–water (2 ml) and the solution was kept at room temperature overnight. Dicyclohexylurea was filtered off and unreacted DCC was extracted with *n*-pentane. The pyridine–water layer was separated and evaporated *in vacuo*. To the residue was added methanol saturated with ammonia at 0° (10 ml). After keeping the solution at room temperature for 5 days, deprotection of the phosphate was confirmed by paper electrophoresis. Methanol–ammonia was evaporated carefully and the residue was dissolved in anhydrous pyridine. The pyridine solution was dropped into an ether–pentane (1:1) mixture to cause precipitation. The precipitates were dissolved in water, made slightly alkaline with ammonia, and applied to a Dowex 1-X2 column (1.1 × 10 cm, chloride form, 100–200 mesh). Elution was carried out with a linear gradient from 0.003 *N* HCl (1 l.) to 0.15 *M* NaCl–0.003 *N* HCl (1 l.). Fractions of 13 ml each were collected. Fractions (No. 50–75) containing 5′-phosphate (VII) were eluted around 0.07 *M* NaCl concentration and collected. After desalting through a charcoal column, this fraction was further purified with a Dowex 1-X2 column (1.1 × 10 cm, chloride form). Elution was carried out with 0.1 *M* NaCl–0.003 *N* HCl solution. A single peak of 5′-phosphate (VII) was obtained. The yield was 3720 OD_{260} (50%). Fractions of this peak were desalted by adsorption on a short column of activated charcoal, which was washed with water and eluted with 50% ethanol containing 2% concentrated ammonia. Effluents were evaporated and the residue was dissolved in anhydrous pyridine. Dropping the pyridine solution into anhydrous ether gave a powder of compound VII: uv $\lambda_{max}^{pH\,2}$ 260 nm (ϵ 15,000); $\lambda_{max}^{pH\,7}$ 257.5 nm (ϵ 15,000); $\lambda_{max}^{pH\,9}$ 258 nm (ϵ 14,900); R_f values on paper chromatography are shown in Table I; Paper electrophoresis R_{AMP} 0.98.

(ii) N^6,3′-*O*-Diacetyl-2′-*O*-tosyl-8-oxyadenosine 5′-phosphate (X) (0.5 mmol) was rendered anhydrous by evaporation several times with pyridine and dissolved in methanol previously saturated with ammonia at 0° (30 ml). The solution was heated at 60° in a steel tube for 6 hr. After the solution was cooled, compound VII partly precipitated out. The solvent was removed by vacuum evaporation. The residue was dissolved in water and applied to a column of Dowex 1-X2 (formate form). Elution with 0.1 *N* formic acid gave cycloadenosine 5′-phosphate in a major peak. Eluents were neutralized with 1 *N* NaOH and adsorbed on a charcoal column, which was eluted with 50% ethanol containing 2% concentrated ammonia. The desalted solution was evaporated *in vacuo* and the residue was dissolved in anhydrous pyridine. Precipitation with ether–pentane (2:1, v/v) gave a powder. This sample was identical with that obtained in (i) by criteria of paper chromatography, paper electrophoresis, and uv absorption properties.

Dephosphorylation of pA° by 5′-Nucleotidase in Crude Snake Venom.[15] The incubation mixture (100 μl) contained 0.05 *M* Tris buffer (pH 8.7), 0.01 *M* $MgCl_2$, and crude snake venom (an appropriate volume was taken from 20 mg/ml of solution). Incubation was carried out at 37°. pA° was dephosphorylated to an extent of 27% with 10 μl of venom solution in 4 hr and to 75% with 30 μl of venom solution in 12 hr. 5′-AMP was hydrolyzed completely with 5 μl of venom solution in 4 hr under the same condition.

N^6,3′-*O*-Diacetyl-8,2′-*O*-cycloadenosine 5′-Phosphate (VIII). 8,2′-*O*-Cycloadenosine 5′-monophosphate(VII) (1050 OD_{260}, 0.07 mmol) was dissolved in *ca.* 10 ml of an acetic anhydride–pyridine mixture (1:2, v/v) and the resulting solution was kept at room temperature for 2 days, 5 volumes of 50% pyridine–water was added to the reaction mixture. After the mixture was kept at room temperature for 6 hr, the solvent was removed *in vacuo*. The residue was dissolved in anhydrous pyridine and dropped into an ether–pentane (2:1) mixture to bring about precipitation: uv $\lambda_{max}^{H^+}$ 285 nm; $\lambda_{max}^{H_2O}$ 282 nm; $\lambda_{max}^{OH^-}$ 293 nm.

8,2′-*O*-Cycloadenylyl-(3′,5′)-8,2′-*O*-cycloadenosine (A°pA°) (Ib). 5′-*O*-Monomethoxytrityl-8,2′-*O*-cycloadenosine (VI) (53 mg, 0.1 mmol) and N^6,2′-*O*-diacetyl-8,2′-*O*-cycloadenosine 5′-phosphate (VIII) (obtained above from 1050 OD_{260} of VII) were rendered anhydrous by coevaporation with pyridine several times and finally dissolved in 1 ml of pyridine. DCC (175 mg, 12 equiv to VIII) was added and the reaction mixture was kept at 32° for 2 days. The reaction was stopped by addition of 50% pyridine (3 ml). After the mixture was kept at room temperature for 36 hr, dicyclohexylurea was removed by filtration. The pyridine solution was evaporated *in vacuo;* the residue was dissolved in methanolic ammonia and kept at 32° for 16 hr. Solvent was evaporated *in vacuo* and the residue was treated with 80% AcOH (5 ml) at room temperature for 1.5 hr. Completely deprotected dinucleoside monophosphate was dissolved in anhydrous pyridine and dropped into an ether–pentane (1:1) mixture to bring about precipitation. The precipitating powder was dissolved in water, made weakly alkaline with ammonia, and applied to a column of DEAE-Sephadex A-25 (bicarbonate form, 1 × 35 cm). Elution was carried out with a linear gradient from 0.02 *M* triethylammonium bicarbonate (TEAB) buffer (1 l.) to 0.15 *M* TEAB buffer, pH 7.5 (1 l.). Fractions of 10 ml each were collected. A major peak containing A°pA° was eluted around 0.04 *M* TEAB buffer concentration. Appropriate fractions (31–35) were pooled and desalted by repeated evaporation with water. The yield was 770 OD_{255} (87%): uv $\lambda_{max}^{pH\,2}$ 260 nm (ϵ 14,500); $\lambda_{max}^{pH\,7}$ 255 nm (ϵ 12,700); R_f values on paper chromatography are shown in Table I; paper electrophoresis R_{AMP} 0.51, 2′,3′-cyclic AMP R_{AMP} 0.69.

Enzymatic Hydrolysis of A°pA°. (i) Crude Snake Venom.[15] An incubation mixture (230 μl) containing A°pA° (5 OD_{260}), 1 *M* ammonium carbonate (40 μl), and snake venom (20 mg/ml, 100 μl) was incubated at 37° for 12 hr.

(ii) Purified Venom Phosphodiesterase.[24] An incubation mixture (200 μl) containing A°pA° (10 OD_{260}), 1 *M* ammonium carbonate (40 μl), and enzyme (1 mg/ml, 40 μl) was incubated at 37° for 12 hr.

(iii) Spleen Phosphodiesterase.[24] An incubation mixture (200 μl) containing A°pA° (10 OD_{260}), 1 *M* ammonium acetate buffer (pH 5.7) (40 μl), and enzyme (20 units/ml, 40 μl) was incubated at 37° for 12 hr.

The results of enzymatic hydrolysis are summarized in Table II.

(24) Purchased from Worthington Biochemical Corp.