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Studies on the Synthesis of Compounds related to Adenosine 3′,5′-Cyclic Phosphate. IV.[1] The Synthesis of 2-Sulfo- and 2-Carboxy-adenosine 3′,5′-Cyclic Phosphate

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The reactions of 2-mercapto-1,N^6-etheno c-AMP (c-AMP: adenosine 3′,5′-cyclic phosphate) (V) and 2-mercapto c-AMP (III) with hydrogen peroxide afforded 2-sulfo-1,N^6-etheno c-AMP (VIII) and 2-sulfo c-AMP (IX), respectively. Deblocking of the etheno residue of VIII afforded IX. Potassium cyanide reacted with 2-bromo-1,N^6-etheno c-AMP (VI) in dimethylformamide (DMF) at room temperature to yield 2-cyano-1,N^6-etheno c-AMP (X), which was isolated by Dowex 50-X8 (H^+) column chromatography. Compound (X) was hydrolyzed to 2-carbamoyl-1,N^6-etheno c-AMP (XI), then to 2-carboxy-1,N^6-etheno c-AMP (XII) by aqueous sodium hydroxide. Compound XII was converted to 1,N^6-etheno c-AMP (IV) by heating in dimethylsulfoxide (DMSO). Deblocking of the etheno residue of XI and XII afforded 2-carbamoyl c-AMP (XIII) and 2-carboxy c-AMP (XIV), respectively. Compound XIII was hydrolyzed by aqueous sodium hydroxide to yield XIV.

Keywords——cyclic AMP derivative; etheno group; N-bromosuccinimide; mass spectrum; UV; liquid chromatography

Adenosine 2-sulfonic acid and 2-carbamoyladenosine have been synthesized from 5-amino-4-cyano-1-β-D-ribofuranosylimidazole (AICN-riboside)[3] and 2-methylmercaptoadenosine,[4] but no derivatives of adenosine 3′,5′-cyclic phosphate (c-AMP) have been reported. The object of this work was to synthesize new c-AMP derivatives by utilizing our deblocking reaction of the etheno group for 1,N^6-etheno c-AMPs having an electron-withdrawing group at the 2 position.

During studies of the synthesis of 2-substituted 1,N^6-etheno c-AMPs, we have observed high reactivity of the bromine atom of 2-bromo-1,N^6-etheno c-AMP. By utilizing this reactivity of the bromo function, we have now found a new and general preparative method for 2-cyano-1,N^6-etheno c-AMP, 2-carbamoyl-1,N^6-etheno c-AMP, and 2-carboxy-1,N^6-etheno c-AMP. 2-Carbamoyl c-AMP and 2-carboxy c-AMP were then synthesized by the deblocking reaction of the corresponding 2-substituted 1,N^6-etheno c-AMPs with N-bromosuccinimide (NBS). In a similar manner, 2-sulfo c-AMP was also synthesized from 2-sulfo 1,N^6-etheno c-AMP.

Hence, it was confirmed that the deblocking reaction of the etheno function could be applied successfully to 1,N^6-etheno c-AMPs having an electron-withdrawing group in the same way as to previously reported compounds.[5] Thus we have established convenient synthetic routes to 2-carbamoyl c-AMP, 2-carboxy c-AMP, and 2-sulfo c-AMP, all of which are potentially valuable for obtaining further information on the unique properties of c-AMP

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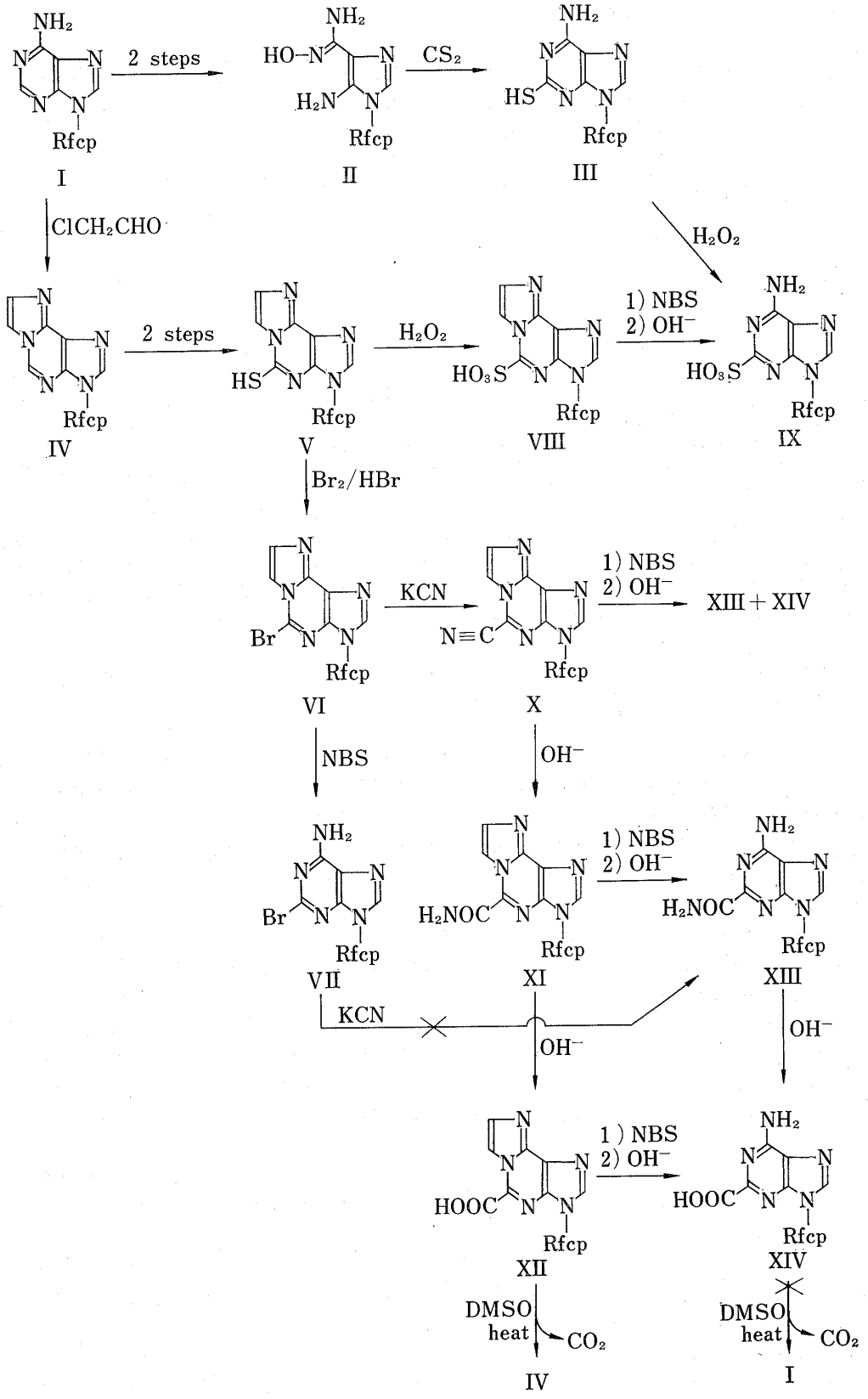

Rfcp: β-D-ribofuranosyl cyclic 3′,5′-phosphate

Chart 1

analogs in biological systems. The details of the present procedure are shown schematically in Chart 1.

The compounds IV, V, VI and VII were synthesized from c-AMP by the method described in a previous paper.[6] The oxidation of V with hydrogen peroxide in a neutral solution afforded 2-sulfo-1,N^6-etheno c-AMP (VIII). This compound was converted to 2-hydroxy-1, N^6-etheno c-AMP in an alkaline solution. The removal of the etheno group from VIII by treatment with NBS in dilute hydrochloric acid solution afforded 2-sulfo c-AMP (IX). To confirm the structure of this compound (IX), we synthesized 2-mercapto c-AMP (III) from 5-amino-1-β-D-ribofuranosylimidazole-4-carboxamidoxime cyclic 3′,5′-phosphate (II) by treatment with carbon disulfide.[7,8] The reaction of III with hydrogen peroxide afforded 2-sulfo c-AMP which was identical in all respects with the product (IX) obtained as described above. Potassium cyanide reacted with 2-bromo-1,N^6-etheno c-AMP (VI) in dimethylformamide (DMF) solution at room temperature, and 2-cyano-1,N^6-etheno c-AMP (X) was isolated by Dowex 50-X8 (H^+, 100—200 mesh) column chromatography. The structure (X) was determined on the basis of the mass spectrum, elemental analytical data, and its chemical conversion as described below. The absorption of the cyano group was observed at 2240 cm^{-1} in the infrared spectrum, but the intensity was very low. Treatment of X with 0.7 N sodium hydroxide solution afforded 2-carbamoyl-1,N^6-etheno c-AMP (XI) in 84% yield. The structure of XI was indicated by the presence of a carbamoyl group in the IR (1690 cm^{-1}), and by the mass spectrum and elemental analytical data. Further hydrolysis of XI under more severe alkaline conditions (1.3 N NaOH) afforded 2-carboxy-1,N^6-etheno c-AMP (XII) in 64% yield. The structure of XII was deduced from the presence of a carboxy group in the IR (1680 cm^{-1}), the mass spectrum, and elemental analytical data. We also obtained 2-carbamoyl c-AMP (XIII) and 2-carboxy c-AMP (XIV) from the corresponding 2-substituted 1,N^6-etheno c-AMP by deblocking of the etheno group with NBS in dilute hydrochloric acid solution and leaving the reaction mixture to stand under alkaline conditions. The cyano

Table I. Physical Constants of the c-AMP Derivatives

Compound No.	Residue	*Rf*[a] A	*Rf*[a] B	Retention time (min)	λ max, (nm), ($\varepsilon \times 10^{-3}$)[b] 0.1 N HCl	λ max, (nm), ($\varepsilon \times 10^{-3}$)[b] 0.1 N NaOH
III	SH	0.33	0.12	13.5[c]	232.5 (14.5), 290.5 (20.9)	241.5 (20.7), 283.5 (16.3)
VIII	SO_3H 1,N^6-etheno	0.18	0.28	6.5[d]	227.0sh (27.6), 232.5 (28.9), 272.5sh (8.8), 280.0 (9.1)	
IX	SO_3H	0.17	0.11	6.5[d]	261.5 (13.4)	262.0 (13.6)
X	CN 1,N^6-etheno	0.33	0.40	20.0[c]	242.5 (34.0), 249.0sh (30.8), 273.0sh (5.2), 281.0 (5.5), 294.0 (5.2), 323.5 (5.8)	
XI	$CONH_2$ 1,N^6-etheno	0.22	0.26	6.0[c]	239.5 (30.7), 278.0 (7.2), 308.5 (6.1)	
XII	COOH 1,N^6-etheno	0.14	0.17	10.5[c]	237.5 (28.7), 276.5 (8.2), 284.0sh (8.2), 306.0sh (5.4)	237.5 (32.2), 270.0sh (5.9), 322.0 (4.0)
XIII	$CONH_2$	0.31	0.19	13.5[c]	258.5sh (12.5), 265.0 (13.3), 295.0 (6.4)	
XIV	COOH	0.22	0.07	9.5[c]	258.0sh (10.1), 264.5 (10.8), 289.5 (4.9)	258.0sh (8.5), 263.5 (12.9), 290.0sh (5.7)

a) *Rf* on Toyo No. 51A paper in solvent system A (*n*-butanol/acetic acid/water, 5: 2: 3) or B (0.5 M ammonium acetate/ethanol, 2: 5).
b) sh indicates a shoulder.
c) Retention time on high-pressure liquid chromatography eluting with 0.2 M NaCl/0.013 N HCl.
d) Retention time on high-pressure liquid chromatography eluting with 1 M NaCl/0.013 N HCl.

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group of 2-cyano-1,N^6-etheno c-AMP and 2-cyano c-AMP was so reactive under these conditions that no 2-cyano derivative could be isolated, but 2-carbamoyl c-AMP and 2-carboxy c-AMP were observed on high-pressure liquid chromatography. 2-Carboxy c-AMP was also obatined from 2-carbamoyl c-AMP under the conditions used for the synthesis of 2-carboxy-1,N^6-etheno c-AMP from 2-carbamoyl-1,N^6-etheno c-AMP. The structures of 2-carbamoyl c-AMP and 2-carboxy c-AMP were confirmed by their mass spectra, IR spectra (XIII; 1690 cm^{-1} (C=O), XIV; 1680 cm^{-1} (C=O)), and elemental analytical data. The NMR data of these compounds (XIII and XIV) were very similar to those of 2-carbamoyl-1,N^6-etheno c-AMP and 2-carboxy-1,N^6-etheno c-AMP except for the absence of the etheno proton signals.

On heating XII in dimethylsulfoxide (DMSO), decarboxylation occured readily to afford 1,N^6-etheno c-AMP (IV). 2-Carboxy c-AMP (XIV), however, was found to be stable on heating in DMSO and the starting material was recovered. In the synthesis of 8-carboxy c-AMP, potassium cyanide was reacted with 8-bromo c-AMP in DMF at 130—140° to afford 8-carbamoyl c-AMP. The presumed intermediate, 8-cyano c-AMP, however, could not be isolated in the above reaction.[9] 2-Cyano-1,N^6-etheno c-AMP could be isolated, because the reaction conditions used to effect the substitution of the corresponding 2-bromo-1,N^6-etheno c-AMP were so mild (DMF, room temperature) that the hydrolysis did not proceed. As potassium cyanide did not react with 2-bromo c-AMP (VII) under any of the above conditions, it was concluded that the reactivity of the bromine substituent decreased in the following order: 2-bromo-1,N^6-etheno c-AMP>8-bromo c-AMP>2-bromo c-AMP. Therefore it is very convenient to use the etheno group for the synthesis of 2-carbamoyl and 2-carboxy c-AMP from c-AMP.

Experimental

Melting points were determined on a Yanagimoto micro melting point apparatus and are uncorrected. UV absorption spectra were determined on a Hitachi R-323 spectrometer. Mass spectra were measured by the method of Lawson *et al.* on a Hitachi RMU-7M mass spectrometer.[10] Nuclear magnetic resonance (NMR) spectra were determined on a Hitachi R-24A spectrometer in DMSO-d_6. Paper chromatograms were run on Toyo No. 51A papers, developing with solvent system A (*n*-butanol/acetic acid/water, 5: 2: 3) or B (0.5 M ammonium acetate/ethanol, 2: 5). The retention times of c-AMP derivatives were estimated on HPLC using a Hitachi 634 high-pressure liquid chromatograph; packing, 2632; column, 2.1 mm I.D.×500 mL; flow rate, 1.1 ml/min; pressure, 50 kg/cm^2; temperature, 70°; detector, 254 nm filter; and eluent, system C (0.2 M NaCl/0.013 N HCl) or D (1.0 M NaCl/0.013 N HCl).

2-Mercapto-adenosine 3′,5′-Cyclic Phosphate (III)——A solution of 1.0 g of 5-amino-1-β-D-ribofuranosylimidazole-4-carboxamidoxime cyclic 3′,5′-phosphate (II) in 1.2 ml of 2 N NaOH, 20 ml of ethanol, 20 ml of pyridine, and 6 ml of carbon disulfide was heated in an autoclave at 120° for 5 hr. The reaction mixture was concentrated and neutralized with 2 N NaOH. The solution was applied to a 1.4×17 cm column of Dowex 50-X8 (H^+, 100—200 mesh) and eluted with water. Concentration of the appropriate fractions gave crystals (230 mg, 20%) of III. These crystals was filtered off and dissolved in 1 N NH_4OH. Adjustment of the pH of the solution to 2.0 with 2 N HCl induced the crystallization of III. The product was filtered off and dried over P_2O_5 at 30° and 3 mmHg for 16 hr, mp 198—200° (dec.). NMR (in DMSO-d_6) δ: 5.90 (1H, singlet, C'_1H), 8.27 (1H, singlet, C_8H). *Anal.* Calcd for $C_{10}H_{12}N_5O_6PS \cdot H_2O$: C, 31.66; H, 3.69; N, 18.47. Found: C, 31.62; H, 3.61; N, 18.38.

2-Sulfo-1,N^6-etheno Adenosine 3′,5′-Cyclic Phosphate (VIII)——An ice-cooled and stirred solution of V (2.0 g, 4.8 mmol) in 0.5 N NaOH (6.0 ml) was treated with 30% H_2O_2 (3.0 ml). After 2 hr, the reaction mixture was applied to a 1.4×15 cm column of Dowex 50-X8 (H^+, 100—200 mesh) and eluted with water. Concentration of the appropriate fractions gave crystals (1.11 g, 49%) of VIII. This material was recrystallized and dried as described for the synthesis of III, mp 176—178° (dec.). NMR (in DMSO-d_6) δ: 6.22 (1H, singlet, C'_1H), 8.06 and 8.54 (2H, doublets, etheno protons), 8.91 (1H, singlet, C_8H). *Anal.* Calcd for $C_{12}H_{12}N_5O_9PS \cdot 0.5H_2O$: C, 32.58; H, 2.94; N, 15.84. Found: C, 32.87; H, 3.04; N, 15.99.

2-Sulfo-adenosine 3′,5′-Cyclic Phosphate (IX)——i) A suspension of VIII (200 mg, 0.45 mmol) in 0.5 N HCl (5.0 ml) was treated with added NBS (100 mg, 0.56 mmol) and the reaction mixture was stirred at room

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10) A.M. Lawson, R.N. Stillwell, M.M. Tacker, K. Tsuboyama, and J.A. McCloskey, *J. Am. Chem. Soc.*, **93**, 1014 (1971).

temperature for 5 min. The solution was made alkaline (pH≒13) with 6 N NaOH and stirred for 4 hr. Finally, the pH of the solution was adjusted to 2.0 with conc. HCl and the whole was passed through a column of charcoal (1.4×14 cm). The column was washed with water and the nucleotide (IX) was eluted with water–ethanol–28% NH_4OH (10: 10: 1 v/v). The appropriate fractions were concentrated, and adjustment of the pH of the solution to 2.0 with 2 N HCl induced the crystallization of IX. The crystals were filtered off, washed with water, and dried to give 144 mg (73%) of chromatographically pure IX. This material was recrystallized and dried as described for the synthesis of III, mp 185—188° (dec.). NMR (in DMSO-d_6) δ: 6.02 (1H, singlet, C′$_1$H), 8.46 (1H, singlet, C_8H). *Anal.* Calcd for $C_{10}H_{12}N_5O_9PS \cdot 1.5H_2O$: C, 27.52; H, 3.44; N, 16.06. Found: C, 27.81; H, 3.54; N, 15.89.

ii) An ice-cooled and stirred solution of III (200 mg) in 0.5 N NaOH (13 ml) was treated dropwise with 30% H_2O_2 (0.8 ml) and the mixture was kept in a refrigerator overnight. The reaction mixture was concentrated, and adjustment of the pH of the solution to 1.0 with conc. HCl gave crystals (60 mg, 25%) of IX. This sample was identical with the product obtained in i).

2-Cyano-1,N^6-etheno Adenosine 3′,5′-Cyclic Phosphate (X)——A suspension of VI (2.0 g, 4.43 mmol) in dimethylformamide (500 ml) was treated with KCN (1.0 g), and the mixture was stirred at room temperature for 16 hr. The solution was neutralized with Dowex 50-X8 (H^+) and filtered. The filtrate was concentrated and dissolved in a minimum volume of water. The solution was applied to a 1.4×30 cm column of Dowex 50-X8 (H^+, 100—200 mesh) and eluted with water. Concentration of the appropriate fractions gave crystals (1.11 g, 65%) of X. This material was recrystallized and dried as described for the synthesis of III, mp 191—193° (dec.). Mass Spectrum *m/e*; 522 (M^+, (X) $(TMS)_2$). *Anal.* Calcd for $C_{13}H_{11}N_6O_6P \cdot 0.5H_2O$: C, 40.31; H, 3.10; N, 21.71. Found: C, 40.37; H, 3.03; N, 21.66.

2-Carbamoyl-1,N^6-etheno Adenosine 3′,5′-Cyclic Phosphate (XI)——A solution of X (1.0 g, 2.58 mmol) in 0.7 N NaOH (10 ml) was stirred at room temperature for 5 min. Adjustment of the pH of the solution to 1.0 with conc. HCl induced the crystallization of XI. The crystals were filtered off, washed with water, and dried to give 793 mg (74%) of chromatographically pure XI. This material was recrystallized and dried as described for the synthesis of III, mp 196—199° (dec.). Mass Spectrum *m/e*; 612 (M^+, (XI) $(TMS)_3$). NMR (in DMSO-d_6) δ: 6.34 (1H, singlet, C′$_1$H), 7.75 and 8.95 (2H, doublets, etheno protons), 8.72 (1H, singlet, C_8H). *Anal.* Calcd for $C_{13}H_{13}N_6O_7P \cdot H_2O$: C, 37.68; H, 3.62; N, 20.29. Found: C, 37.65; H, 3.34; N, 20.12.

2-Carboxy-1,N^6-etheno Adenosine 3′,5′-Cyclic Phosphate (XII)——A solution of XI (1.0 g, 2.42 mmol) in 1.3 N NaOH (10 ml) was stirred at room temperature for 20 min. Treatment of the reaction mixture as described for the synthesis of XI gave 843 mg (82%) of XII. This material was recrystallized and dried as described for the synthesis of III, mp 200—202° (dec.). Mass Spectrum *m/e*; 613 (M^+, (XII) $(TMS)_3$). NMR (in DMSO-d_6) δ: 6.23 (1H, singlet, C′$_1$H), 7.76 and 8.76 (2H, doublets, etheno protons), 8.79 (1H, singlet, C_8H). *Anal.* Calcd for $C_{13}H_{12}N_5O_8P \cdot 1.5H_2O$: C, 36.79; H, 3.54; N, 16.51. Found: C, 36.57; H, 3.47; N, 16.66.

2-Carbamoyl-adenosine 3′,5′-Cyclic Phosphate (XIII)——A solution of XI (500 mg, 1.21 mmol) in 0.1 N HCl (500 ml) was treated with NBS (250 mg 1.40 mmol), and the reaction mixture was stirred at room temperature for 5 min. The solution was made alkaline (pH≒12) with 6 N NaOH and stirred for 1 hr. Finally, the pH of the solution was adjusted to 2.0 with conc. HCl and the whole was passed through a column of charcoal (1.6×30 cm). The column was washed with water and the nucleotide (XIII) was eluted with water–ethanol–28% NH_4OH (10: 10: 1 v/v). The eluate was concentrated, applied to a 1.2×20 cm column of Dowex 50-X8 (H^+, 100—200 mesh) and eluted with water. Concentration of the appropriate fractions gave crystals (140 mg, 30%) of XIII. This material was recrystallized and dried as described for the synthesis of III, mp 213—216° (dec.). Mass Spectrum *m/e*; 660 (M^+, (XIII) $(TMS)_4$). NMR (in DMSO-d_6) δ: 6.11 (1H, singlet, C′$_1$H), 8.55 (1H, singlet, C_8H). *Anal.* Calcd for $C_{11}H_{13}N_6O_7P \cdot H_2O$: C, 33.85; H, 3.85; N, 21.54. Found: C, 33.82; H, 3.65; N, 21.50.

2-Carboxy-adenosine 3′,5′-Cyclic Phosphate (XIV)——A solution of XII (500 mg, 1.18 mmol) in 0.1 N HCl (500 ml) was treated with NBS (250 mg, 1.40 mmol), and the reaction mixture was stirred at room temperature for 5 min. Treatment of the solution as described for the synthesis of XIII gave 108 mg (23%) of XIV. This material was recrystallized and dried as described for the synthesis of III, mp 190—193° (dec.). Mass Spectrum *m/e*; 661 (M^+, (XIV) $(TMS)_4$). NMR (in DMSO-d_6) δ: 6.05 (1H, singlet, C′$_1$H), 8.52 (1H, singlet, C_8H). *Anal.* Calcd for $C_{11}H_{12}N_5O_8P \cdot 1.5H_2O$: C, 33.00; H, 3.75; N, 17.50. Found: C, 33.02; H, 3.75; N, 17.54.