**Studies of Nucleosides and Nucleotides. XXXVIII.¹⁾
Synthesis of 8-Bromoadenosine Nucleotides²⁾**

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Direct bromination of adenine nucleotides was achieved by the use of bromine-water as brominating agent in the alkaline or buffer solution. By this method 8-bromoadenosine 2',3'- and 5'-monophosphate, 5'-di- and tri-phosphate, and 3',5'-cyclic phosphate were synthesized in satisfactory yield. Mechanism of bromination of adenine nucleotides was discussed.

Halogenation of nucleic acid and its components, nucleosides and nucleotides, has been investigated extensively by a number of researchers.⁴⁻¹⁴⁾ Among these studies, bromination reaction especially draw their attention, because introduction of bromine on the nucleobase may cause mutation in several viral systems.¹⁵⁻¹⁷⁾

Before our investigation of the bromination of adenine nucleoside and nucleotide²⁾ appeared, it has been generally accepted that halogenation of adenine moiety was difficult.^{4,18)} Recently, bromination of purine nucleosides,¹⁹⁻²¹⁾ especially of adenosine,²²⁻²⁴⁾ was successively achieved. However, the procedure involving the use of organic solvent could not be applied to the nucleotides. Accordingly, it is of worthwhile to investigate the bromination of adenine nucleotides, which are in the central role in metabolic pathways of living systems.

Since we have found a new method²⁾ for the bromination of adenosine and adenine nucleotides using bromine water as reagent, we describe in this paper synthesis of 8-bromoadenosine 2', 3'- and 5'-monophosphate, 5'-di- and tri-phosphate, and 3',5'-cyclic phosphate.

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Experimental²⁵⁾

Search for the Optimal Condition of Bromination of A5'P²⁶⁾—i) Variation of the Amount of Sodium Hydroxide: A5'P.Na₂·4.5H₂O²⁷⁾ (24 mg, 0.05 mmole) was dissolved in 0.1–0.4 ml of 1N NaOH. Into this solution was added 0.4 ml of bromine–water (containing 0.5 mmole/ml). The volume of each mixture was adjusted to 1 ml by the addition of water (0.2–0.5 ml). The reaction mixture was tightly stoppered and allowed for standing at room temperature in the dark. An aliquot (0.1 ml) was withdrawn from the reaction mixture at the time indicated in Table I. Paper chromatography (solvent A) of the aliquot showed each 3 spots having *R_f* 0.31, 0.19 and 0.05 corresponding to BrAMP, AMP and a degradation product (presumably 8-oxy-AMP). Spots corresponding to BrAMP and HOAMP were cut out, extracted with water (5 ml) and optical density at 260 mμ was measured. Results were summarized in Table I.

TABLE I. Bromination of AMP in Alkaline Solution

Experiment	1N NaOH (ml)	Br-H ₂ O (ml)	H ₂ O (ml)	1.5 hr	BrAMP (%)			X ^{a)} (%)
					3	8	24	
1	0.1	0.4	0.5	0	0	0	0	0.5
2	0.2	0.4	0.4	16	18	18	16	5.8
3	0.3	0.4	0.3	53	59	51	51	13
4	0.4	0.4	0.2	38	37	34	35	30

Reaction conditions were as in Experimental.

a) byproduct remaining at the origin of paper chromatography at 24 hrs' reaction

ii) Variation of pH: A5'P. Na₂ (20 mg) was dissolved in phosphate or acetate buffer (pH as indicated in Table II, 5 ml), followed by the addition of bromine–water (containing 0.21 mmole bromine/ml, 0.2 ml). The mixture was allowed to stand for 24 hr at room temperature. At the end of the reaction pH of the mixture decreased about 0.2–0.8 unit. The reaction mixture was applied to column chromatography of Dowex IX8 (formate form) resin, which was eluted with 0.1N formic acid. Peaks corresponding to AMP and BrAMP were separated and characterized with the authentic samples. The extent of the reaction was estimated either by TOD₂₆₅²⁸⁾ or by OD unit of the extract from the spot on paper chromatography performed in solvent A. The results were summarized in Table II.

TABLE II. Bromination of AMP in Buffer Solution of Various pH

pH	Buffer	TOD ₂₆₀ ^{a)}		Yield (%)			pH after reaction
		Start	End	BrAMP	AMP	X ^{b)}	
3	acetate	737	652	69			3.6
4	acetate	659	625	89			3.8
5	acetate	641	482	66			4.5
6	phosphate	745	401	22	20	12	
7	phosphate	630 ^{c)}	561	24	55		6.4
8	phosphate	630 ^{c)}	540	19	69		6.8
9	borate	630 ^{c)}	400	0 ^{d)}	81		9.1

Reaction conditions were as in Experimental.

a) Optical density units measured at 260 mμ multiplied by volume (ml) of the solution

b) byproduct remaining at the origin of paper chromatography

c) TOD was calculated on the weight basis assuming $\epsilon_{260} = 15000$.

d) By the column chromatography no BrAMP was obtained.

- 25) Ultraviolet absorption was taken by Shimadzu QR-50 or QV-50 spectrophotometer. Paper chromatography was performed by descending technique in the following solvent: A, 1-butanol–acetic acid–water, 5:2:3; B, 2-propanol–conc. ammonia–water, 7:1:2; C, 1-propanol–conc. ammonia–water, 55:10:35; and D, water adjusted at pH 7.0.
- 26) Abbreviations used were: A5'P, adenosine 5'-monophosphate; AMP, adenosine monophosphate; BrA, 8-bromoadenosine; HOA, 8-hydroxyadenosine; ADP, adenosine 5'-diphosphate; ATP, adenosine 5'-triphosphate; A3',5'MP, adenosine 3',5'-cyclic phosphate.
- 27) Estimated ultraviolet photometrically on the basis of $\epsilon_{260} = 15400$.
- 28) TOD₂₆₅ stands for optical density units measured at 265 mμ multiplied by volume (ml) of the solution.

Large Scale Preparation of BrA5'P—i) Using NaOH: Bromine-water (containing 0.3 mmole Br/ml, 6.6 ml, 2 equivalent) was combined with 1N NaOH (2 ml). Into the mixture was added AMP.Na₂·4.6 H₂O (476 mg, 1 mmole). After complete dissolution by brief shaking, the reaction mixture was allowed to stand for 3 days at room temperature. Solvent was evaporated *in vacuo*, the residue was taken up in 25 ml of water and adjusted to pH 8.0 with ammonia. The solution was applied to a column (1.5 × 17 cm) of Dowex I × 8 (formate form, 100–200 mesh). After the water wash, the elution was performed with 0.1N formic acid. Fractions were collected in 12 ml and the flow rate was 36 ml/hr. The largest peak (fraction No. 74–156 in Fig. 1) was pooled (TOD₂₆₀ 8520), adsorbed on activated charcoal (1 g × 3), and the charcoal was eluted with 50% ethanol containing 2% ammonia (150 ml × 3). Solvent was evaporated and the residue was dissolved in a small amount of water. The solution was filtered when necessary, concentrated to a small bulk, and to it was added methanol–water mixture (total *ca.* 5 ml). Addition of 10 vol of acetone and ether gave 239 mg of BrA5'P.(NH₄)₂ (yield 50%). *Anal.* Calcd. for C₁₀H₁₉O₇N₇PBr·H₂O: C, 25.12; H, 4.42; P, 6.48. Found: C, 25.23; H, 4.84; P, 6.48. Ultraviolet absorption properties: $\lambda_{\max}^{H^+}$ 262.5 m μ (ϵ 16400), $\lambda_{\min}^{H^+}$ 231 m μ ; $\lambda_{\max}^{H_2O, OH^-}$ 264.8 m μ (ϵ 15100), $\lambda_{\min}^{H_2O, OH^-}$ 232 m μ . A280/A260 (at pH 1) = 0.513 (A5'P 0.19), A280/A260 (at pH 11) = 0.454. Paper chromatography: *Rf*(A) 0.31 (A5'P 0.19), *Rf*(B) 0.11 (A5'P 0.11).

Fig. 1. Ion-exchange Column Chromatography of 8-Bromoadenosine 5'-Monophosphate

Conditions were as in experimental.

I: adenosine 5'-monophosphate,

II: 8-bromoadenosine 5'-monophosphate

Paper electrophoresis:²⁹⁾ at pH 3.5 *R_{A5'P}* 1.0, at pH 7.5 *R_{A5'P}* 1.0. Base: total P: labile P = 1.00: 1.01: 0.00. *pK_a*³⁰⁾ = 3.6.

ii) Using CaCO₃³⁰⁾: A5'P. Na₂ (476 mg, 1 mole) was dissolved in dioxane–water (50 ml, 1:4, vol/vol). To the mixture was added CaCO₃ (150 mg) to afford a suspension. Bromine (1.5 mmole) dissolved in water (20 ml) was added dropwise to the mixture under stirring. The stirring was continued overnight at room temperature. The orange–yellow color at the end of the reaction was discharged with NaHSO₃ (52 mg). Solvent was evaporated *in vacuo* and the residue was dissolved in water (25 ml). The solution was applied to a column (1.5 × 15 cm) of Dowex I × 8 (formate form) resin. After the water wash, the column was eluted with 0.1N formic acid and fractions were collected in 50 ml. The largest peak (fraction No. 21–42, TOD₂₆₀ 10200) was pooled, treated with charcoal as described above, and dissolved in a small amount of methanol–water mixture. Precipitate, which was caused by the addition of acetone (10 volume), was collected by centrifugation, washed with methanol, acetone and ether, and dried over P₂O₅ at 3 mm for 5 hr. BrAMP. (NH₄)₂·H₂O was obtained in the yield of 64% (305 mg). This sample was identical with that obtained in i).

iii) Using Acetate Buffer: A5'P.Na₂ (476 mg, 1 mole) was dissolved in 1M acetate buffer (pH 4.0, 40 ml), followed by the addition of bromine–water (7.15 ml, 1.5 equiv.). The reaction mixture was allowed to stand for 12 hr at room temperature. The color of the solution was discharged by the addition of NaHSO₃ (50 mg) and the solvent was evaporated by azeotropic distillation with ethanol. The residue was dissolved in water (50 ml) and applied to a column (1.5 × 15.5 cm) of Dowex I × 8 (formate) resin. After the water wash (300 ml), the column was eluted with 0.1N formic acid. The largest peak (fraction No. 30–56, TOD₂₆₀ 13000) was pooled, treated with charcoal, and evaporated to a small bulk. Precipitation with ethanol–acetone and drying as described above gave 332 mg (69%) of A5'P. (NH₄)₂·H₂O. The sample was identical with those obtained above.

The same experiment using 2–3 equivalents of bromine gave BrA5'P in the yield of 75–78%.

Chemical Reactivity of BrA5'P—i) In 1N NaOH: BrA5'P (5 mg) was dissolved in 1N NaOH and kept at room temperature for 3 days. No change was observed by paper chromatography. Upon heating at 100° in a sealed tube for 15 min BrA5'P disappeared completely. Major products were found to be bromoadenine³¹⁾ (*Rf*(A) 0.72) and 8-oxy-AMP (*Rf*(A) 0.20) in the ratio of 14:10.

ii) In 1N HCl: BrA5'P (5 mg) was dissolved in 1N HCl (0.5 ml) and stored at room temperature. Examination of the aliquots by paper chromatography in solvent A showed the spot corresponding to BrA5'P

29) Solvent of pH 3.5 was 0.05M ammonium acetate and pH 7.5 was 0.05M triethylammonium bicarbonate. Electrophoresis was carried out at 20 v/cm for 1 hr on Toyo filter paper No. 51A.

30) Measured photometrically (unpublished experiment by S. Uesugi).

31) Kruger, Z. *physiol. Chem.*, 16, 5 (1892).

estimated as 94, 37 and 29% at 5, 24 and 240 hrs' reaction period, respectively. The major product at the end of the reaction was 8-bromoadenosine.

iii) In NaOBr Solution: BrA5'P was dissolved in 0.1N NaOH and 0.2 ml of water. On addition of bromine-water (0.1 ml), TOD_{260} of the mixture did not change even after 11 days at room temperature. Spot other than BrA5'P was found in amount less than 5% of BrA5'P by paper chromatography.

iv) With Thiourea: BrA5'P (0.01 mmole) was refluxed in 2-propanol-water (1 ml, 3:1, vol/vol) in the presence of thiourea (0.1 mmol) for 2 hr. Ultraviolet absorption maxima of the reaction mixture changed to those reported for 8-mercaptadenosine²²⁾ ($\lambda_{\text{max}}^{\text{H}^+}$ 310 m μ ; $\lambda_{\text{max}}^{\text{H}_2\text{O}}$ 300, 307 m μ ; $\lambda_{\text{max}}^{\text{OH}^-}$ 297 m μ). On the paper chromatogram spot having $R_f(\text{B})$ 0.03 revealed by Haines-Isherwood spray³²⁾ and corresponding to 8-mercapto-AMP was found.

v) With Sodium Benzoate in Acetic Acid: BrAMP (5 mg) was dissolved in a mixture of glacial acetic acid (1 ml) and acetic anhydride (0.1 ml). After the addition of sodium benzoate (14 mg) into mixture, the solution was heated to 50° for 3 hr. Solvent was evaporated *in vacuo*, 1N NaOH (1 ml) was added, and the mixture was allowed to stand for 2 hr at 40–50°. On the neutralization with IRC G-50 (H^+ form) resin, the reaction mixture was examined by paper chromatography. Spots having $R_f(\text{A})$ 0.88, 0.33 and 0.21 were revealed. Since the spot having R_f 0.21 was BrA5'P, the other two spots were corresponding to 8-HOAMP (R_f 0.31) and 8-HO-adenine³³⁾ (R_f 0.88), respectively. Ultraviolet absorption properties of substance at R_f 0.33: $\lambda_{\text{max}}^{\text{H}^+}$ 264 m μ , $\lambda_{\text{max}}^{\text{H}_2\text{O}}$ 270 m μ , $\lambda_{\text{max}}^{\text{OH}^-}$ 280 m μ . These properties were similar to those reported for 8-oxyadenosine.³⁴⁾

Hydrolysis of BrA5'P catalyzed by Snake Venom 5' Nucleotidase³⁵⁾—BrA5'P (5 OD units) was incubated with 1M $(\text{NH}_4)_2\text{CO}_3$ 20 μl , 0.05M Tris-HCl buffer (pH 8.0) 10 μl and crude snake venom (10 mg/ml) 20 μl at 37° for 4 hr. In the same condition A5'P was incubated separately. Whereas A5'P was hydrolyzed completely to adenosine and inorganic phosphate, BrA5'P was hydrolyzed to the extent of 29% and gave bromoadenosine and inorganic phosphate.

8-Bromoadenosine 5'-Diphosphate—ADP.Li₃³⁶⁾ (23 mg) was dissolved in a mixture of 1M acetate buffer (pH 4.0, 2 ml) and water (2.5 ml). Into the mixture was added bromine-water (0.47 ml) and the reaction mixture was allowed to stand at room temperature overnight. After the addition of NaHSO₃ (5 mg), the solvent was evaporated by azeotropic distillation with ethanol. The residue was taken up in water (50 ml) and applied to a column (0.7 $\times$ 15.5 cm) of Dowex 1 $\times$ 8 (chloride form) resin. After the water wash, elution was carried out stepwise with 0.05M NaCl+0.001N HCl and 0.08M NaCl+0.001N HCl. Fractions were collected in 10 ml and the flow rate was 25 ml/hr. The elution

Fig. 2. Ion-exchange Chromatography of 8-Bromoadenosine 5'-Diphosphate

Conditions were as in experimental.

Fig. 3. Ion-exchange Chromatography of 8-Bromoadenosine 5'-Triphosphate

Conditions were as in experimental.

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33) L.F. Cavalieri and A. Bendich, *J. Am. Chem. Soc.*, **72**, 2587 (1950).

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35) Y. Mizuno, M. Ikehara and A. Nomura, *Chem. Pharm. Bull.* (Tokyo), **9**, 238 (1961).

36) Purchased from Sigma Chemical Co.

pattern was as shown in Fig. 2. The third peak (TOD₂₆₀ 304) was pooled, neutralized with 1 N NaOH and evaporated to a small bulk. By the charcoal treatment and precipitation as described in the case of BrAMP, BrADP was obtained in 46% yield (calculated from $\epsilon_{260}^{H^+} = 14400$ for BrADP). Ultraviolet absorption properties: $\lambda_{max}^{H^+}$ 263 m μ , $\lambda_{max}^{H_2O}$ 265 m μ were same with those of BrAMP. Paper chromatography: *Rf*(A) 0.14 (ADP 0.07), *Rf*(C) 0.51 (ADP 0.51). Paper electrophoresis: pH 3.5, *R*_{ADP} 1.0; *R*_{AMP} 1.42. Base-total P-labile P=1.00: 1.98: 1.02 (theoretical, 1:2:1).

8-Bromoadenosine 5'-Triphosphate—ATP. Na₂·2.5H₂O³⁷⁾ (600 mg) was dissolved in 1 M acetate buffer (pH 4.0, 40 ml). To this mixture was added saturated bromine-water (9.4 ml). After it was kept at room temperature overnight, color of the solution was discharged by the addition of NaHSO₃ (104 mg). Solvent was evaporated *in vacuo* with several additions of ethanol. The residue was dissolved in water (100 ml) and applied to a column (1.2×20 cm) of Dowex I×8 (chloride form) resin. After the water wash, the column was eluted with 0.13–0.25M LiCl+0.001N HCl (21+21) by the linear gradient elution technique. Fractions were collected in 50 ml and the flow rate was 150 ml/hr. BrATP was eluted in the fourth peak (TOD₂₆₀ 9040, 56%, Fig. 3). The fractions corresponding to BrATP was pooled and evaporated *in vacuo*. The residue was dissolved in methanol-water (3 ml, 1:1, vol/vol) and the precipitates caused by the addition of acetone (40 ml) were collected by centrifugation. Washing with acetone and ether, followed by the drying over P₂O₅ gave 351 mg of BrATP. Li₄ (yield calculated from $\epsilon_{260}^{H^+} = 16100$ for BrATP was 50%). Ultraviolet absorption properties: $\lambda_{max}^{H^+}$ 263 m μ , $\lambda_{min}^{H^+}$ 232 m μ ; $\lambda_{max}^{H_2O}$ 265 m μ , $\lambda_{min}^{H_2O}$ 231 m μ (same as those of BrAMP).

8-Bromoadenosine 3',5'-Cyclic Phosphate³⁸⁾—Adenosine 3',5'-cyclic phosphate³⁹⁾ (104 mg, 0.3 mmole) was dissolved in water (6.9 ml) and 1N NaOH (0.3 ml). Into this mixture was added 1M sodium acetate buffer (pH 4, 10 ml), followed by the addition of bromine-water (2.8 ml). Reaction mixture was allowed to stand at room temperature overnight. Solvent was evaporated with the repeated additions of ethanol and the residue was dissolved in water (100 ml). Chromatography of this solution on a column (1.1×17 cm) of Dowex I×8 (chloride form) resin was performed by the elution with 0.02M LiCl+0.001N HCl and 0.06M LiCl+0.001N HCl. Fractions were collected in 50 ml at the flow rate of 60 ml/hr. The main peak (TOD₂₆₀ 3820) was pooled, treated with activated charcoal as described above. Evaporation of the solvent and precipitation with methanol-acetone gave a powder (TOD₂₆₀ 3100, yield 72% based on $\epsilon_{260}^{H_2O} = 14400$ for BrA-3',5'-P). Ultraviolet absorption properties: $\lambda_{max}^{H^+}$ 263 m μ , $\lambda_{max}^{H_2O, OH^-}$ 265 m μ . Paper chromatography: *Rf*(B) 0.48 (A-3',5'-P 0.38), *Rf*(A) 0.37 (A-3',5'-P 0.28). Paper electrophoresis: *R*_{A-3',5'-P} 1.00 (at pH 7.5).

8-Bromoadenosine 2'- and 3'-Phosphate—2'(or 3')-AMP⁴⁰⁾ (365 mg, 1 mmole, containing 21.5% A2'P and 78.5% A3'P) was dissolved in 1N KOH (2 ml) and 1M acetate buffer (pH 4.0, 40 ml). Into the mixture was added bromine-water (9.4 ml) and the solution was allowed to stand at room temperature overnight. Solvent was evaporated azeotropically with ethanol and the residue was dissolved in water (100 ml). Chromatography of this solution on a column (1.2×19 cm) of Dowex I×8 (formate form) was performed with elution with 0.05M ammonium formate+0.1N formic acid (1.81), 0.1M ammonium formate+0.1N formic acid (21) and 0.2M ammonium formate+0.1N formic acid (31). Fractions were collected in 50 ml at the flow rate of 150 ml/hr. BrA2'P and BrA3'P were obtained in peak I (TOD₂₆₀ 2160) and II (TOD₂₆₀ 10624), respectively. Fractions corresponding to peak I and II were pooled and treated with charcoal. Recovery at this stage were TOD₂₆₀ 1977 (15%) for BrA2'P and TOD₂₆₀ 8098 (56%) for BrA3'P. Solvent was evaporated and the residue was taken up into methanol-water. Precipitation with acetone and drying over P₂O₅

TABLE III. *Rf* Value of BrA2'P and BrA3'P in Paper Chromatography and Paper Electrophoresis

Solvent	BrA2'P	BrA3'P	A2'P	A3'P	A5'P
A	0.45	0.45			0.26
C	0.25	0.30			0.16
D	0.36	0.24	0.57	0.48	
pH 7.5 ^{a)}	0.99 ^{b)}	0.96 ^{b)}			1.00

a) Performed in 0.05 M triethylammonium bicarbonate at 20 v/cm for 1 hr.

b) *R*_{A5'P} was shown.

37) Purchased from Schwarz Bioresearch Inc.

38) Purchased from Sigma Chemical Co.

39) Bromination of adenosine 3',5'-cyclic phosphate using N-bromacetamide²²⁾ in DMF solution gave 8-BrA-3',5'-cyclic P in the yield of 15–20% (unpublished experiment by S. Uesugi).

40) Obtained from Kokoku Jinken Co. and recrystallized from water.

41) A. Nomura and H. Suno, Abstract of Papers presented at 20th Symposium of Enzyme Research, Kana-zawa, 1968.

gave BrA2'P (44 mg) and BrA3'P (210 mg), respectively. Ultraviolet absorption properties of BrA2'P: $\lambda_{\text{max}}^{\text{H}^+}$ 263 m μ , $\lambda_{\text{min}}^{\text{H}^+}$ 232 m μ ; $\lambda_{\text{max}}^{\text{H}_2\text{O}}$ 265.5 m μ , $\lambda_{\text{min}}^{\text{H}_2\text{O}}$ 232 m μ ; $\lambda_{\text{max}}^{\text{OH}^-}$ 265.5 m μ , $\lambda_{\text{min}}^{\text{OH}^-}$ 233 m μ . BrA3'P: $\lambda_{\text{max}}^{\text{H}^+}$ 263.5 m μ , $\lambda_{\text{min}}^{\text{H}^+}$ 232 m μ ; $\lambda_{\text{max}}^{\text{H}_2\text{O}}$ 265.5 m μ , $\lambda_{\text{min}}^{\text{H}_2\text{O}}$ 233 m μ ; $\lambda_{\text{max}}^{\text{OH}^-}$ 266.5 m μ , $\lambda_{\text{min}}^{\text{OH}^-}$ 233.5 m μ . Paper chromatography and paper electrophoresis were summarized in Table III.

Hydrolysis of BrA2'P and BrA3'P by the Catalysis of Potato 3'-Nucleotidase—BrA2'P or 3'P (0.5 μ mole) was incubated with 3'-nucleotidase⁴¹⁾ (containing 10 mg/ml protein) 100 μ l and 0.5 M Tris-HCl buffer (pH 8.1) 50 μ l in the total volume 250 μ l at 37° for 1 hr. Paper chromatography in solvent A of the reaction mixture showed no hydrolysis of BrA2'P and 30.1% hydrolysis of BrA3'P. Since in the same condition A3'P was hydrolyzed as much as 90%, the rate of hydrolysis of BrA3'P was about 1/3 of A3'P. Resulted nucleoside was characterized as 8-bromoadenosine by the paper chromatography and ultraviolet absorption properties compared with those of an authentic sample³⁾ ($\lambda_{\text{max}}^{\text{H}^+}$ 263 m μ , $\lambda_{\text{max}}^{\text{H}_2\text{O}}$ 265 m μ).

Discussion

As judged from the experimental data presented here, adenine nucleotides, such as adenosine 2', 3'- and 5'-monophosphate, adenosine 5'-di- and triphosphate, as well as adenosine 3',5'-cyclic phosphate could be effectively brominated by the use of bromine-water in the presence of alkali or in buffered solution. As shown in Table I, the use of limited amount of alkali for the acceptor of hydrogen bromide formed during the course of the reaction is necessary. The use of large excess alkali caused the decrease in ultraviolet absorption of the reaction mixture.⁴²⁾ Since alkaline treatment of the final product 8-bromo-AMP at room temperature did not show the degradation, the intermediary formed complex should be affected by the excess alkali in the presence of bromine. Recent report of Shapiro²¹⁾ on the degradation of guanosine during the course of bromination would provide an interpretation for this reaction.

From the experiments in buffer solution of various pH, it could be deduced that pH lower than 3 and higher than 7 should be avoided. In the former case, the protonation on N¹⁴³⁾ of adenine moiety decreased nucleophilic susceptibility of C⁸ position against the attack of bromonium cation. The pK_a value reported for AMP (3.80)⁴⁴⁾ supports this explanation. On the other hand, in the bromination at pH higher than 7 the resulting bromination intermediate might be decomposed by hydrolysis and/or oxydation as in the case of excess alkali. In general, pH of the reaction mixture shifted significantly toward lower region in due course of the bromination reaction. This may have caused failure of the previous experiments^{7,9,12,18)} using dilute buffer solution for bromination.

The bromination of AMP in the preparative scale using NaOH, CaCO₃ and sodium acetate buffer as acid acceptor showed the effectiveness of use of buffer solution taking the work-up procedure after the reaction into account. In the case of NaOH, the yield was the worst of three (40%). This may be caused by the undesired degradation of the intermediate in alkaline solution, because of difficulty in stirring large volume of reaction mixture homogeneously. Although the use of CaCO₃, which has been shown to be suitable for the bromination of guanosine in dioxane solution,²⁰⁾ raised the yield as high as 64%, separation of contaminating calcium salt from the product is cumbersome. So far the most excellent procedure was the use of buffer of pH 4.0. In this case almost complete bromination occurred and the isolated yield of BrAMP was 69–78%. The ultraviolet absorption properties closely resembled to those of 8-bromoadenosine,²²⁾

42) In the case of adenosine, 48–50% loss of total optical density after the bromination with bromine-water in the presence of excess alkali was observed (Unpublished experiment of S. Uesugi).

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which showed that the bromination had occurred at 8-position. The pK_a value, slightly lower than that of AMP also supported this structure. Phosphate analysis and the mobility in paper chromatography and paper electrophoresis suggested the structure to be correct. 8-Bromo-AMP, thus obtained, was hydrolyzed easily by dilute acid to afford 8-bromoadenine and relatively stable against alkaline hydrolysis. The reaction with thiourea⁴⁵⁾ and sodium benzoate⁴⁶⁾ also proceeded as expected and gave 8-mercaptopadenosine 5'-phosphate and 8-oxyadenosine 5'-phosphate as the product. The fact that BrA5'P was relatively stable against hydrolysis catalyzed by snake venom 5'-nucleotidase and BrA3'P was stable against potato 3'-nucleotidase, suggested the unfavorable configuration of the nucleotides towards these enzymes. These points together with the fact that BrADP could not extensively polymerized by the catalysis of polynucleotide phosphorylase,⁴⁷⁾ should be clarified by further investigations. ADP and ATP could be brominated in good yield by this method. Since pyrophosphate linkage was thought to be labile for the acidic condition, the present results were rather surprising. It should be emphasized that these brominated polyphosphates could be utilized as the starting material for the 8-substituted adenosine polyphosphates including 8-tritiated compounds.⁴⁸⁾

In the bromination of A-3',5'-P, which could be solubilized in the organic solvent such as DMF by the use of tri-*n*-butylammonium salt, the use of N-bromoacetamide²²⁾ was first attempted. However, yield of bromo compound was not satisfactory. By the use of buffered solution and bromine-water, 8-bromoadenosine 3',5'-cyclic phosphate was obtained in the yield of 72%.

Thus far the present method is quite satisfactory for the bromination of adenine nucleotides and the synthesis of adenine nucleotides having various substituents became easily accessible. The studies along this line is in progress in our laboratory and will be reported elsewhere.

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