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Synthesis of 8-Carbamoyl- and 8-Carboxyadenosine 3',5'-Cyclic Phosphates

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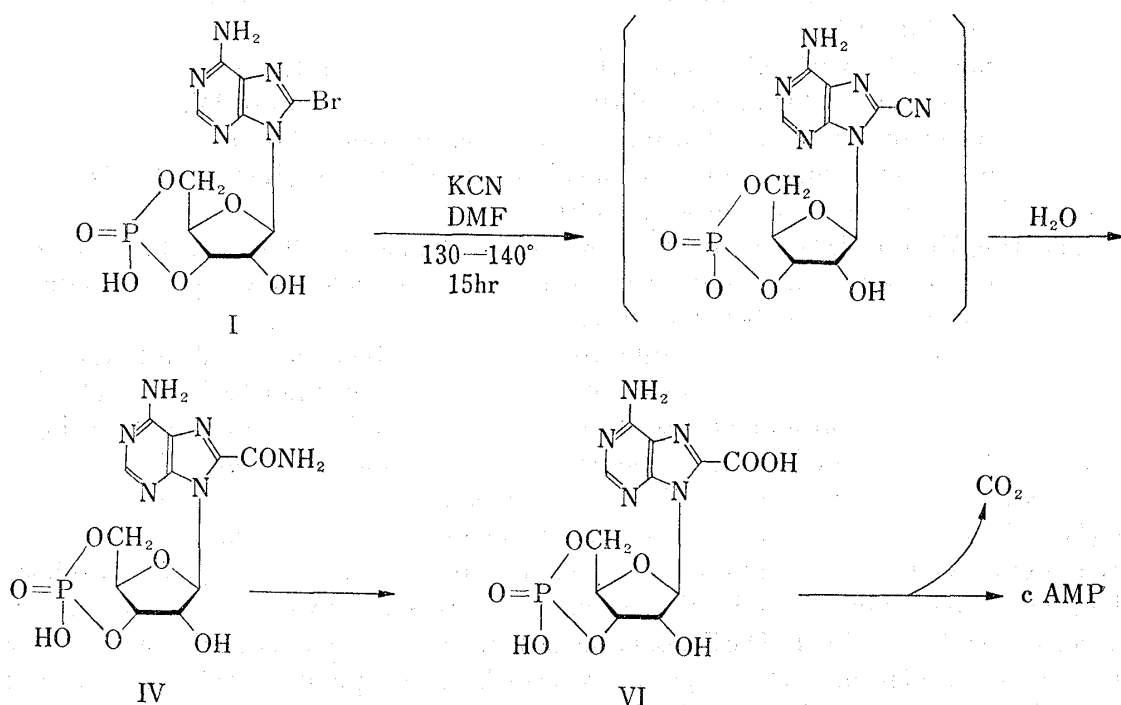
The reaction of 8-bromo-cAMP (cAMP: adenosine 3',5'-cyclic phosphate) (I) with potassium cyanide in hot dimethylformamide (DMF) afforded 8-carbamoyl-cAMP (IV). Compound IV was hydrolyzed with aqueous sodium hydroxide to 8-carboxy-cAMP (VI), which was converted to cAMP by heating in dimethylsulfoxide. A similar reaction of 8-bromo-5'-AMP (II) or 8-bromo-2',3'-O-isopropylideneadenosine (VII) with potassium cyanide in DMF yielded 8-bromoadenosine or 8,5'-anhydro-2',3'-O-isopropylidene-8-oxadenosine (VIII) respectively. The treatment of 5'-nucleotides with hot aqueous DMF afforded the corresponding nucleosides in high yields.

Since the discovery of adenosine 3',5'-cyclic phosphate (cAMP)²⁾ and subsequent establishment of its vital role in all stages of evolution of life, a large number of cAMP-analogs have been synthesized to obtain substances that might have a better biological action than the original nucleotide (cAMP) and to find substances that might act as antagonists to the original nucleotide.³⁾ Out of these compounds, 8-mercapto- and 8-bromo-cAMP showed remarkable activating effect on protein kinase or on the growth-hormone production.⁴⁾ A series of analogs bearing halogen or nitrogen-, oxygen- or sulfur-containing functionalities at the 8 position have been prepared, but the corresponding carbon-substituted cAMP derivatives have not yet been synthesized. As for the formation of carbon-carbon bond at the 8 position of purine base, the homolytic alkylation has been described in recent years.⁵⁾ To our best knowledge, however, nucleophilic displacement involving carbon nucleophiles and 8-halogenopurine derivatives has not been reported, except for a few examples of caffeine derivatives (8-carbamoylcaffeine⁶⁾ and caffeine-8-malonic acid⁷⁾).

This paper deals with some reactions of 8-bromo-cAMP (I), 8-bromo-5'-AMP (II) and 8-bromoadenosine (III) with potassium cyanide in DMF, emphasis being laid upon the preparation of 8-carbamoyl- and 8-carboxyadenosine 3',5'-cyclic phosphates starting from 8-bromopurine derivative. Interesting observation obtained during the course of this work will be also touched on.

Potassium cyanide reacted with I in DMF at 130—140°. Monitoring of the reaction by paper electrophoresis (PE) revealed formation of a blue fluorescent compound⁸⁾ together

- 1) Location: Jusohommachi, Yodogawa-ku, Osaka, 532, Japan.
- 2) Abbreviations used are: cAMP, adenosine 3',5'-cyclic phosphate; 5'-AMP, adenosine 5'-monophosphate; 5'-CMP, cytidine 5'-monophosphate; TMS, trimethylsilyl; DMF, dimethylformamide; DMA, dimethylacetamide; DMSO, dimethylsulfoxide; HMPA, hexamethylphosphoric triamide.
- 3) R.B. Meyer and J.P. Miller, *Life Sciences*, **14**, 1019 (1974); J.P. Miller, K.H. Boswell, K. Muneyama, L.N. Simon, R.K. Robins and D.A. Shuman, *Biochemistry*, **12**, 5310 (1973).
- 4) Th. Posternak, *Ann. N.Y. Acad. Sci.*, **185**, 42 (1971); G. Cehonic, Th. Posternak and E. Charollais, "Advances in Cyclic Nucleotide Research," Vol. 1, ed. by P. Greengard and G.A. Robison, Raven Press, New York, N.Y., 1972, p. 521.
- 5) H. Stainmaus, I. Rosenthal and D. Elad, *J. Am. Chem. Soc.*, **91**, 4921 (1969); D. Elad and J. Salomon, *Tetrahedron Letters*, **1971**, 4783; J. Salomon and D. Elad, *J. Org. Chem.*, **38**, 3420 (1973); D. Lenov and D. Elad, *J. Org. Chem.*, **39**, 1470 (1974); Y. Kawazoe, M. Maeda and K. Nishi, *Chem. Pharm. Bull.* (Tokyo), **20**, 1341 (1972); M. Maeda, K. Nishi and Y. Kawazoe, *Tetrahedron*, **30**, 2677 (1974).
- 6) M. Gombery, *Am. J. Chem.*, **17**, 403 (1895).
- 7) H. Bargioni, *Boll. Chim. Far.*, **64**, 869 (1935); G. Ehrhart, *Arch. Pharm.*, **290**, 16 (1957).
- 8) It was reported that 2',3'-O-isopropylidene-5'-keto-8,5'-cycloadenosine was blue fluorescent in ultraviolet light.⁹⁾
- 9) P.J. Harper and A. Hampton, *J. Org. Chem.*, **37**, 795 (1972).



with unreacted I. After a period of 15 hr, a product (colorless needles, IV, $mp > 300^\circ$) was isolated by charcoal treatment and subsequent diethylaminoethyl (DEAE) cellulose column chromatography. The 8-carbamoyl-cAMP structure was assigned to compound IV on the basis of three lines of evidence (spectra, elemental analytical data and chemical conversions). The nuclear magnetic resonance (NMR) spectrum of IV showed the presence of one-proton singlet at δ 8.35 ppm (attributable to H-2) and four-protons (which were exchangeable with deuterium) signal at δ 8.00 ppm. Two protons of the latter are attributable to the N-6 protons which appear significantly downfield compared with those of cAMP (δ ca. 7.00 ppm) and the remaining two protons could be assigned to the protons in the substituent introduced to C-8. A significant downfield shift of the signal due to the anomeric proton (δ 6.97 ppm) relative to that (δ 5.95 ppm) of cAMP was also observed. This downfield shift might be taken as a reflection of the anisotropic effect of the purine ring bearing a bulky group at C-8 and suggests that IV might adopt the *syn*-conformation.¹⁰⁾ Compound IV had a chemical formula of $C_{11}H_{13}O_7N_6P \cdot H_2O$ and no cyano group could be detected in its infrared (IR) spectrum. The above assignment was confirmed by mass spectro-

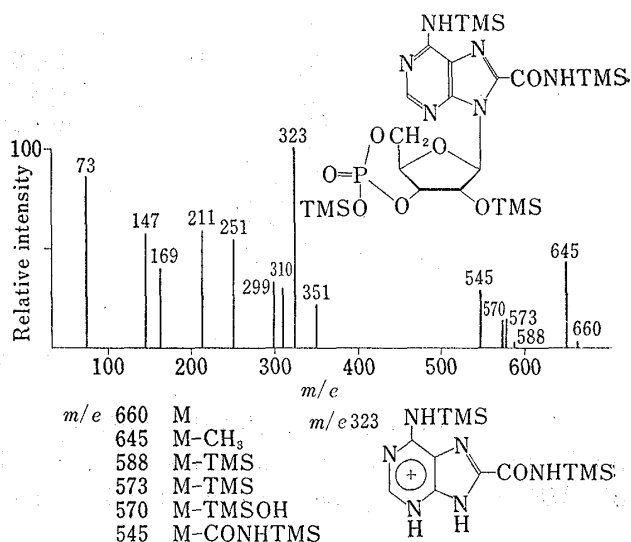


Fig. 1. Mass Spectrum of 8-CONH₂-cAMP(TMS)₄

10) D.W. Miles, L.B. Townsend, M.J. Robins, R.K. Robins, H. Inskeep and W.H. Eyring, *J. Am. Chem. Soc.*, **93**, 1600 (1971).

metry of trimethylsilylated derivative¹¹⁾ [V, m/e 660 (M^+), Fig. 1] along with the chemical conversion of IV into VI.

The reaction of IV with 0.5*N* sodium hydroxide at 100° for 1.5 hr afforded a compound lacking blue fluorescence, which migrated the double distance as that of IV, on PE, suggesting the presence of carboxyl group. Neutralization of the reaction solution with acid gave white powder (VI, $mp > 300^\circ$) which had a chemical composition of $C_{11}H_{12}O_8N_5 \cdot 3H_2O$ (elemental analysis) and characterized as 8-carboxy-cAMP (VI).

On heating VI in DMSO decarboxylation easily took place to afford cAMP. The structure of the latter was determined, based on its NMR and ultraviolet (UV) spectral data as well as on its paper electrophoretic behavior (Chart 1).

This is the first example of successful introduction of carbon nucleophile into the 8 position of adenine derivatives. However, attempts at isolating 8-cyano-cAMP as such from the reaction mixture failed, presumably because its hydrolysis might occur rapidly owing to the presence of a small amount of water in potassium cyanide and DMF, used.

The above reaction was also applied to 8-bromoadenosine (III) as well as to 8-bromo-5'-AMP (II). The reaction of II with potassium cyanide in hot DMF failed to yield the expected 8-carbamoyl-5'-AMP and instead the formation of 8-bromoadenosine (VII, a dephosphorylated product) was detected by PE and UV spectrometry. It is worthy of note that compound VII was formed even by heating in DMF alone, suggesting that potassium cyanide was not essential to the dephosphorylation. The reaction proceeded almost quantitatively in aqueous DMF. A mechanism proposed is given in Chart 2.

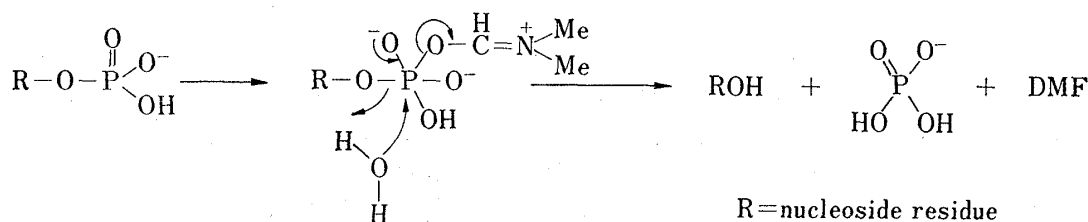


Chart 2

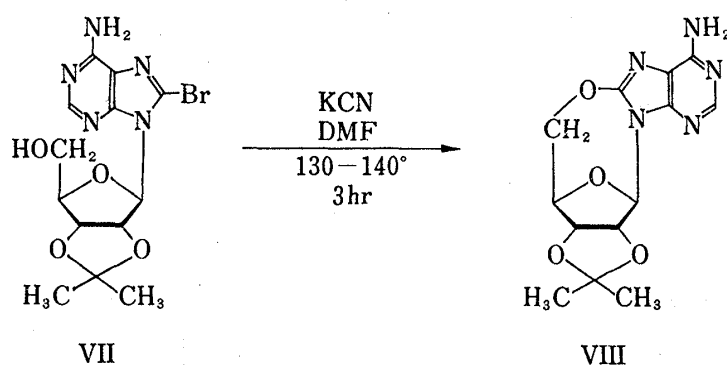


Chart 3

A similar reaction of 5'-AMP with aqueous DMF yielded adenosine quantitatively and no formation of adenine was observed.¹²⁾ This reaction could be successfully applied to 5'-CMP to obtain pure cytidine in 65% yield. The conventional dephosphorylation with

11) A.M. Lawson, R.N. Stillwell, M.M. Tracker, K. Tsuboyama and J.A. McClosky, *J. Am. Chem. Soc.*, **93**, 1014 (1971).

12) Tri-*n*-butylammonium salt of 5'-AMP was refluxed in DMF for 2 hr to give adenosine, adenine, and a complex mixture of nucleotides.¹³⁾

13) T. Ueda and I. Kawai, *Chem. Pharm. Bull.* (Tokyo), **18**, 2303 (1970).

hot formic acid-ammonium formate¹⁴) gave cytidine in 54% yield, accompanying the formation of a considerable amount (17%) of uridine. Solvent such as formamide, DMA, DMSO or HMPA having the group $>C=O$, $>S=O$ or $-P=O$ was also effective for the dephosphorylation. In the case of 5'-AMP, DMA or formamide gave the same result as DMF, whereas DMSO or HMPA yielded adenosine (from 5'-AMP), even under the milder conditions (100—120°), with a concomitant formation of a small amount of by-products.

The reaction of 8-bromoadenosine (III) with potassium cyanide in hot DMF gave no isolable products except unreacted III. A similar reaction of 8-bromo-2',3'-O-isopropylideneadenosine (VII) with potassium cyanide, however, afforded colorless needles (VIII, mp 225—226°) which was identified as 8,5'-anhydro-2',3'-O-isopropylidene-8-oxyadenosine¹⁵) based on its UV ($\lambda_{\text{max}}^{\text{MeOH}}$ 260 nm), IR, NMR and mass (m/e 305, molecular ion peak) spectral data.

The reaction of 8-bromo-2',3',5'-tri-O-substituted adenosine with potassium cyanide in hot DMF gave several degradation products and out of the products we failed to isolate the expected 8-carbamoyl compound. The successful nucleophilic substitution of I with cyano group might be a reflection of its more stable glycosyl bond than that of III in addition to the protection of both 3'- and 5'-hydroxyl groups.

Two cAMP analogs (IV and VI) were capable of activating the protein kinases of rat brain and liver more strongly than the original nucleotide.¹⁶) Compound IV was a very poor substrate for the phosphodiesterase of rat heart.¹⁷)

Experimental

Melting points were determined on a Yanagimoto micro melting point apparatus and are uncorrected. NMR spectra were recorded on a Hitachi Model R-24 spectrometer using tetramethylsilane as an internal standard. Ultraviolet, infrared and mass spectra were measured on a Hitachi Model EPS-3T, a Hitachi Model 215 (Nujol mull) and a Hitachi Model RMS-4, respectively. Paper electrophoresis was run on Whatman No. 1 paper at a constant voltage of 22 V/cm in 0.05 M borate buffer (pH 9.2) for 1 hr.

8-Carbamoyladenosine 3',5'-Cyclic Phosphate (I)—To a solution of the triethylammonium salt of 8-bromoadenosine 3',5'-cyclic phosphate (I) (3.0 g) in DMF (30 ml) was added KCN (1.5 g) and the suspension was stirred at 130—140° for 10 hr. Another crop of KCN (0.5 g) was added and the mixture was stirred at the same temperature for 5 hr. After the evaporation of the solvent, the resulting brown syrup was dissolved in water (500 ml) and the solution was passed through a column of charcoal (30 g). The column was washed with water and eluted with EtOH—BuOH—H₂O—conc. NH₄OH (50:5:40:5). The eluate was evaporated to a syrup, which was taken up in water and passed through a column of DEAE cellulose (HCO₃⁻, 100 g). After washing with water, the column was eluted with 0.05 M NH₄HCO₃. Evaporation of the appropriate fractions gave a syrup (500 mg), which was dissolved in water (10 ml) and the solution was acidified with 1 N HCl to give a precipitate. A similar precipitation was repeated to afford colorless needles (300 mg), mp >300°. UV $\lambda_{\text{max}}^{\text{H}_2\text{O}}$ nm (ϵ): 288 (10200), 224 (19100). NMR (d_6 -DMSO) δ : 8.35 (1H, s, H₂), 8.00 (4H, m, 6-NH₂ and 8-CONH₂), 6.79 (1H, s, H_{1'}). Anal. Calcd. for C₁₁H₁₃O₇N₆P·H₂O: C, 33.76; H, 3.86; N, 21.47; P, 7.92. Found: C, 33.67; H, 4.24; N, 21.63; P, 7.86.

8-Carboxyadenosine 3',5'-Cyclic Phosphate (VI)—A solution of IV (200 mg) in 0.5 N NaOH (5 ml) was heated at 100° for 1.5 hr. The resulting solution was neutralized with 1 N HCl to give a yellow precipitate. The similar precipitation was repeated to afford pale yellow needles (100 mg), mp >300°. UV $\lambda_{\text{max}}^{\text{H}_2\text{O}}$ nm (ϵ): 262 (13000). Anal. Calcd. for C₁₁H₁₂O₈N₅P·3H₂O: C, 30.84; H, 4.23; N, 16.35. Found: C, 30.95; H, 4.02; N, 16.02.

Decarboxylation of VI—A suspension of VI (30 mg) in DMSO (0.4 ml) was heated at 60—70° for 15 min, followed by the addition of water (1.0 ml) to give a crystalline product, cAMP (25 mg).

8,5'-Anhydro-2',3'-O-isopropylidene-8-oxyadenosine (VIII)—A suspension of 8-bromo-2',3'-O-isopropylideneadenosine (VII, 800 mg) and KCN (800 mg) in DMF (20 ml) was stirred at 130—140° for 3 hr. The

14) A. Takami, M. Imazawa, M. Irie and C. Ukita, *Yakugaku Zasshi*, **85**, 658 (1965).

15) M. Ikehara and M. Kaneko, *J. Am. Chem. Soc.*, **90**, 497 (1968); K. Nagpal and M.M. Dhar, *Tetrahedron Letters*, **1968**, 47.

16) M. Takeda and R. Shimomura, personal communication.

17) Y. Maki and T. Suzuki, personal communication.

resulting brown mixture was evaporated to dryness. The residue was dissolved in water (200 ml). The solution was passed through a column of charcoal (8 g), washed with water and eluted with CHCl_3 -MeOH (1:1). The eluate was evaporated and the residue was recrystallized from EtOH to give colorless crystals (400 mg), mp 224—225°. UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm: 260. Mass Spectrum m/e : 305 (M^+). NMR (d_6 -DMSO) δ : 8.19 (1H, s, H_2), 7.09 (2H, s, $-\text{NH}_2$), 6.13 (1H, s, H_1').

Dephosphorylation of 8-Bromoadenosine 5'-Phosphate (II)—A solution of the triethylammonium salt of II (100 mg) in water (2.0 ml) and DMF (8.0 ml) was heated at 130—135° for 15 hr. The electrophoresis showed the formation of 8-bromoadenosine in 90% yield, whose UV spectra and mobility in PE were in good accord with those of an authentic sample.

Dephosphorylation of 5'-AMP—A solution of the triethylammonium salt of 5'-AMP (5.0 g) in water (100 ml) and DMF (400 ml) was heated at 130—135° for 15 hr. The resulting solution was adjusted to pH 5 with HCl and passed through a column of charcoal (50 g). The column was washed with water and eluted with BuOH-EtOH- H_2O -conc. NH_4OH (5:50:40:5). The eluate was evaporated to dryness and the resulting syrup was triturated with EtOH to give a crystalline product, which was recrystallized from 90% EtOH to give colorless crystals (2.8 g, 85%), mp 234—236°. UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm: 260.

Dephosphorylation of 5'-CMP—A solution of the triethylammonium salt of 5'-CMP (5.0 g) in water (100 ml) and DMF (400 ml) was heated at 130—135° for 15 hr. The resulting solution contained cytidine (92%), uridine (7%) and unreacted 5'-CMP (1%). The solution was adjusted to pH 5 with 1 N HCl and passed through a column of charcoal (50 g). The column was washed with water and eluted with BuOH-EtOH- H_2O -conc. NH_4OH (5:50:40:5). The eluate was evaporated to dryness and the residue was triturated with EtOH to give a crystalline product, which was recrystallized from 90% EtOH to give colorless crystals (1.9 g, 65%), mp 214—216°. UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm: 272.

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