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Transformation of Adenosine into N_3,3′- and N_3,5′-Cycloadenosines *via* the Reactions with Sulfuryl Chloride and Thionyl Chloride

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Reaction of adenosine with sulfuryl chloride gave two compounds, 5′-chloro-5′-deoxyadenosine 2′,3′-cyclic sulfate (I) and N_3,3′-cycloadenosine derivative (II). I was converted into 2′-sulfate (III) and 3′-sulfate (IV) by treatment with aqueous alkali and into N_4,3′-cyclo-4-aminoimidazole 5-carboxamidine nucleoside (V) by treatment in aqueous ethanol at an elevated temperature. 5′-Chloro-5′-deoxyadenosine (VI) derived from adenosine by reaction with thionyl chloride was readily converted into N_3,5′-cycloadenosine chloride (VIII). VIII was transformed into N_4,5′-cyclo-4-aminoimidazole 5-carboxamidine nucleoside (XII) *via* the unidentified characteristic compound (X) by reaction with diluted alkali. Characteristic changes of ultraviolet spectra of VIII were described.

Keywords——adenosine; thionyl chloride; sulfuryl chloride; N_3,3′-cycloadenosine; N_3,5′-cycloadenosine; 5′-chloro-5′-deoxyadenosine 2′,3′-cyclic sulfate; 5′-chloro-5′-deoxyadenosine; N_4,3′-cyclo-4-aminoimidazole 5-carboxamidine nucleoside; N_4,5′-cyclo-4-aminoimidazole 5-carboxamidine nucleoside

Reaction of nucleosides with acid halides such as sulfonyl chlorides,[2] thionyl chloride,[3–7] phosphorus oxychloride,[8–11] acyl halides[12,13] and sulfuryl chloride[14] has afforded useful nucleoside derivatives. Adenosine and its analogs have been converted into 5′-chlorinated derivatives[5] and 2′,3′-cyclic sulfinate derivatives[6] by the reaction with thionyl chloride. They have been also transformed into 2′- and 3′- halogenated derivatives by the reaction with acyl halides[13].

The present paper deals with the reaction of adenosine with sulfuryl chloride and thionyl chloride and the properties of the reaction products.

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13) A.F. Russel, S. Greenberg, and J.G. Moffatt, *J. Am. Chem. Soc.*, **95**, 4025 (1973); J.C. Jain, A.F. Russel, and J.G. Moffatt, *J. Org. Chem.*, **38**, 3179 (1973).

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Treatment of adenosine with sulfuryl chloride in the presence of acetonitrile and trietylamine at room temperature gave water-insoluble compound (I) in a yield of 53%. Elemental analysis and high resolution mass spectrophotometry showed the empirical formula $C_{10}H_{10}ClN_5O_5S$. Fragmentation pattern in a low resolution mass spectroscopy is presented in Fig. 1

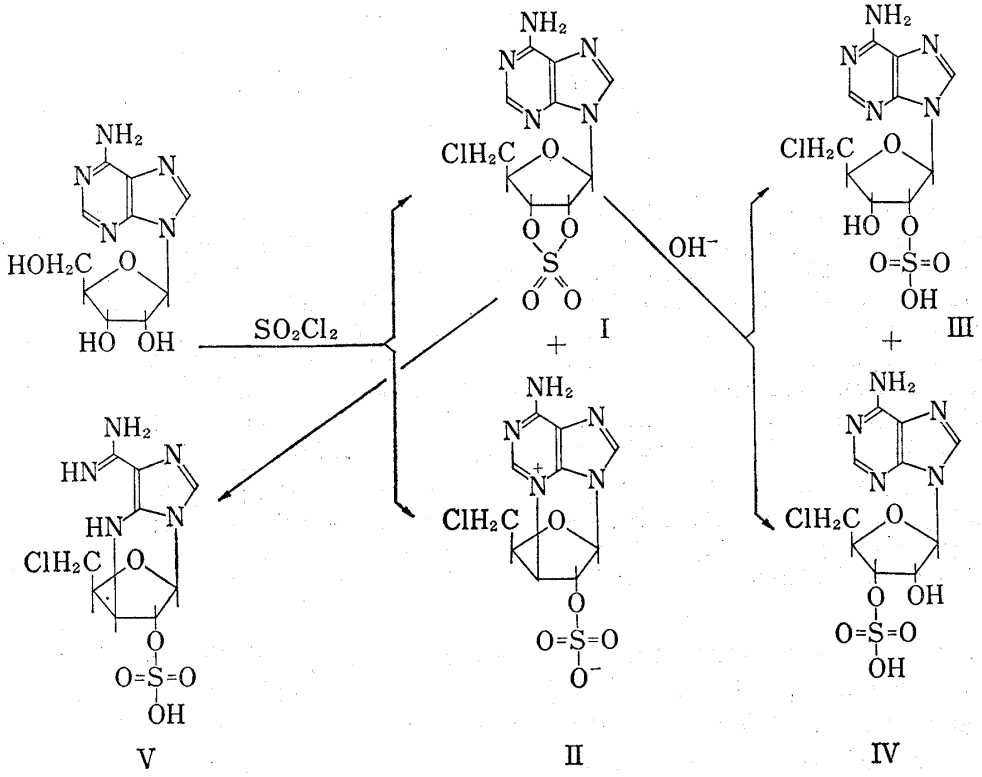

Chart 1

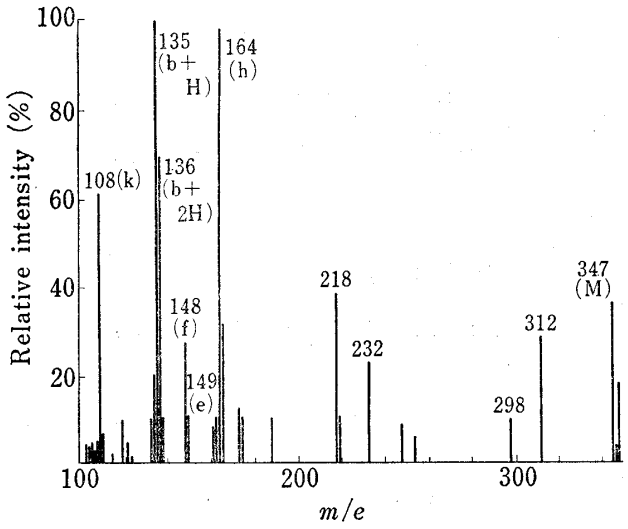

Fig. 1. Mass Spectrum of Compound (I)

Fragment ion peaks (k, b+H, b+2H, f, e and h) were identical with those of adenosine described by Shaw, *et al.* (*J. Am. Chem. Soc.*, **92**, 2510 (1970)).

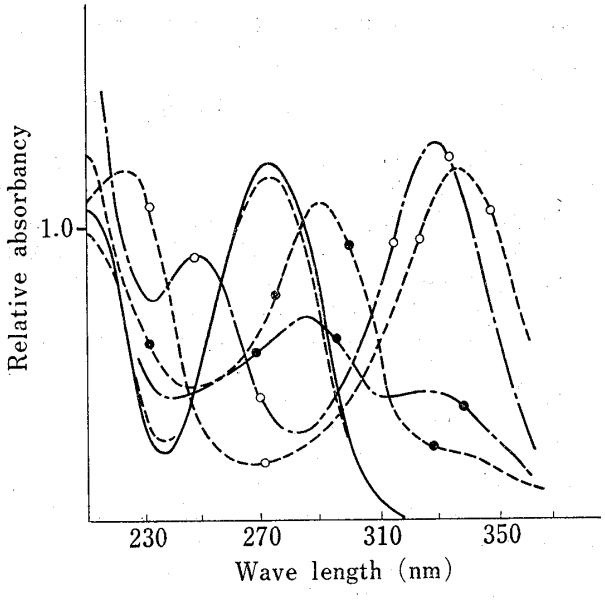

Fig. 2. UV Spectra of Compound (II)

Spectra were taken in ——, H_2O; ------, 0.1 N HCl: and ———, 0.01 N NaOH. Treatment of II with 0.01 N NaOH at 22° for: ○, 1 min; and ●, 20 min gave different spectrum.

and molecular ion peak of I appeared at *m/e* 347. No consumption of metaperiodate and negative reaction against pyridine-aniline reagent[15] proved the absence of 2′,3′-*cis*-glycol function and chlorosulfonyl group, respectively. Nuclear magnetic resonance (NMR) spectrum measured in d_6-dimethylsulfoxide (d_6-DMSO) revealed no proton signals derived from sugar hydroxyl functions. The assignment of other sugar proton resonances were confirmed by spin-decoupling experiments. The down-field shifts of signals of $C_{2'}H$ and $C_{3'}H$, compared to those of 5′-chloro-5′-deoxyadenosine (VI), indicated that the substitution occurred on both $C_{2'}$- and $C_{3'}$-positions. These results and the transformation reaction of I described below established the structure of I to be 5′-chloro-5′-deoxyadenosine 2′,3′-cyclic sulfate.

The water-soluble by-product (II)was obtained from the reaction. Although the structure of II could not be rigidly confirmed, it must be 5′-chloro-5′–deoxy-N_3,3′-cycloadenosine 2′-sulfate from the following lines of evidence. It had a covalently bound chlorine atom and thus was not a N_3,5′-cycloadenosine chloride type. It was unstable in an alkaline medium; ultraviolet (UV) absorption maximum, $\lambda_{max}^{pH\,1}$ 272 nm, was changed to $\lambda_{max}^{pH\,1}$ 338 nm after 1 min-treatment with 0.01 N NaOH and finally to $\lambda_{max}^{pH\,1}$ 292 nm after 20 min-treatment (Fig. 2), which was quite similar to that of N_4,3′-cyclo-3-(5-chloro-5-deoxy-β-D-xylofuranosyl)-4-aminoimidazole 5-carboxamidine 2′-sulfate (V).

Treatment of the major product (I) with aqueous ammonia gave negatively charged products, III (yield, 15.4%) and IV (31.2%), whose structures were confirmed to be 5′-chloro-5′-deoxyadenosine 2′-sulfate and 5′-chloro-5′-deoxyadenosine 3′-sulfate, respectively. NMR studies revealed that $C_{2'}H$ proton signals of III appeared in rather lower field than that of VI. Paper chromatographic *Rf* value of III was higher than that of IV in the solvent system which can characterize the position of the sulfate group of nucleosides: 2′-sulfate moves faster than 3′-sulfate.[16]

Compound (I) was converted into V in a yield of 54% by heating in aqueous ethanol. The product (V) was intact in aqueous ammonia, indicating the absence of cyclic sulfarte function. NMR studies showed the presence of the signal of C_2H of imidazole ring and the absence of that of C_2H of pyrimidine ring of I. Signal of $C_{1'}H$ appeared as a singlet characteristic to the cyclonucleoside series. UV spectrum of V was characteristic. Robins, *et al.*[17] have recently established the structure of the product derived from 2′,3′-ribo-epoxy-adenosine to be N_4,3′-cyclo-3-β-D-xylofuranosyl-4-aminoimidazole 5-carboxamidine hydroformate from UV spectrum and optical rotation, while Goodman and his coworkers[18–20] had suggested the compounds derived from 5′-deoxy-2′,3′-ribo-epoxyadenosine and 2′,3′-episulfonium derivative of adenosine to be 5′-deoxy-N_3,3′-cycloadenosine and N_3,3′-cyclo-1-(2-benzylthio-2-deoxy-β-D-xylofuranosyl)adenine, respectively. UV spectrum of the compound (V) having an absorption maximum at 292 nm (H^+) resembled those of the compounds reported.[17–20] Hence the structure of V must be N_4,3′-cyclo-3-(5-chloro-5-deoxy-β-D-xylofuranosyl)-4-aminoimidazole 5-carboxamidine 2′-sulfate.

N_3,5′-cycloadenosine and its isopropylidene derivative have been prepared through 5′-O-*p*-toluenesulfonyladenosine derivatives,[21–23] or from the reaction of methyltriphenoxy-

15) H.J. Jennings and J.K.N. Jones, *Can. J. Chem.*, **40**, 1408 (1962).
16) J. Arnold and T.D. Price, *J. Am. Chem. Soc.*, **84**, 1406 (1962).
17) M.J. Robins, Y. Fouron, and R. Mengel, *J. Org. Chem.*, **39**, 1564 (1974); UV: $\lambda_{max}^{pH\,0}$ (ε) nm, 292 (13100), $\lambda_{max}^{pH\,7}$ 293 (14500), $\lambda_{max}^{pH\,13}$ 278 (9600), 222 (8400).
18) E.J. Reist, V.J. Bartuska, D.F. Calkins, and L. Goodman, *J. Org. Chem.*, **30**, 3401 (1965); UV: λ_{max} 293 nm.
19) E.J. Reist, D.F. Calkins, and L. Goodman, *J. Org. Chem.*, **32**, 2538 (1967).
20) A.P. Martinez, W.W. Lee, and L. Goodman, *J. Org. Chem.*, **31**, 3263 (1966); UV: $\lambda_{max}^{pH\,1}$ (ε) nm, 293 (13300), $\lambda_{max}^{pH\,7}$ 293 (13100), $\lambda_{max}^{pH\,13}$ 275 (9000), 334 (3200).
21) V.M. Clarke, A.R. Todd, and J. Zussman, *J. Chem. Soc.*, **1951**, 2952.
22) S.M. Mudd, G.A. Jamieson, and G.L. Cantoni, *Biochim. Biophys. Acta.*, **38**, 164 (1960).
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phosphonium iodide with adenosine.[24] The present author treated 5′-chloro-5′-deoxyadenosine (VI) in dimethylformamide at an elevated temperature to obtain N_3,5′-cycloadenosine chloride (VIII) in a good yield of 56%. 5′-Chloro-5′-deoxyadenosine (VI), which had been prepared from a 5′-O-*p*-toluenesulfonyl derivative,[25,26] was readily prepared from the reaction

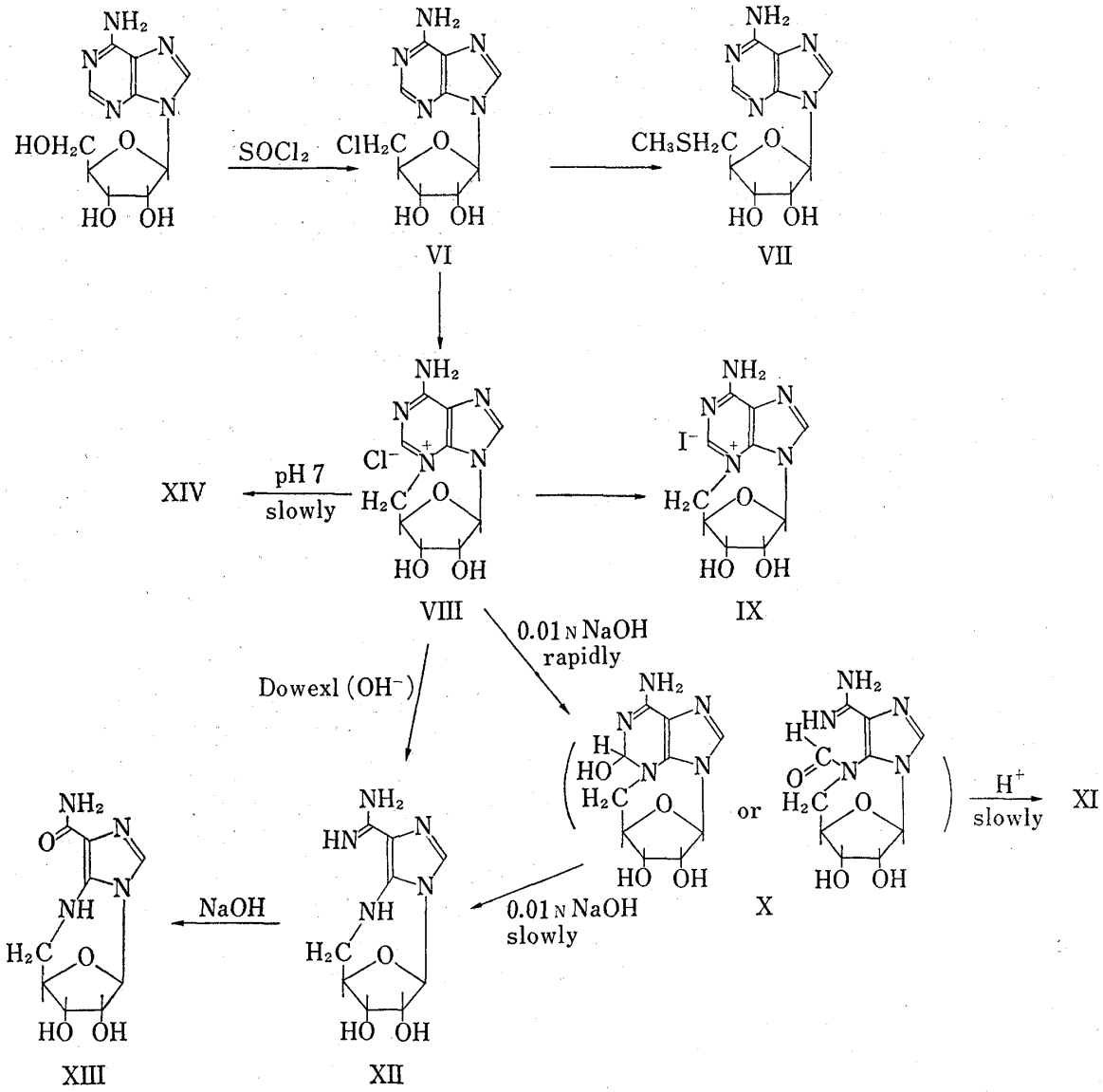

Chart 2

of adenosine with thionyl chloride-hexamethylphosphoroamide complex[5] in a yield of 73%. Melting points of VI reported are not always identical,[5,25,26] which must be due to the partial transformation into VIII during the measurement as has been suggested by Verhyden and Moffatt.[26] The structure of VI obtained here was rigidly confirmed by the conversion into 5′-methylthio-5′-deoxyadenosine (VII).[27] The compound (VIII), UV spectrum of which was identical with that of the product,[22] was converted into the iodide (IX) which was found identical with that prepared by Verhyden, and Moffatt.[24] Whereas the compounds which had been determined to be 2′, 3′-O-isopropylidene N_3,5′-cycloinosine hydrate

24) J.P.H. Verhyden and J.G. Moffatt, *J. Org. Chem.*, **35**, 2319 (1970).
25) W. Jahn, *Chem. Ber.*, **98**, 1705 (1965).
26) J.P.H. Verhyden and J.G. Moffatt, *J. Org. Chem.*, **37**, 2289 (1972).
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and 2′,3′-O-isopropylidene N_6-benzoyl N_3,5′-cycloadenosine hydrate were corrected to be of N_1–C_2 splitted N-formyl structure,[28,29] the compound (VIII) obtained here was not such a degradation product because the empirical formula found was identical with the calculated value without the addition of any solvent.

N_3,5′-cycloadenosine derivatives suffer cleavage of the glycosidic linkage under strong acidic conditions,[30] and C_2-elimination in an alkaline medium,[2b,31,32] but the detailed studies on the properties of N_3,5′-cycloadenosine itself has not yet been done.

UV spectrum of N_3,5′-cycloadenosine chloride (VIII) measured under several conditions is shown in Fig. 3. The spectra of VIII having an absorption maximum at 272.5 nm in H_2O or in 0.1 N HCl rapidly changed by treatment in diluted alkali (Fig. 3A). Thus, after

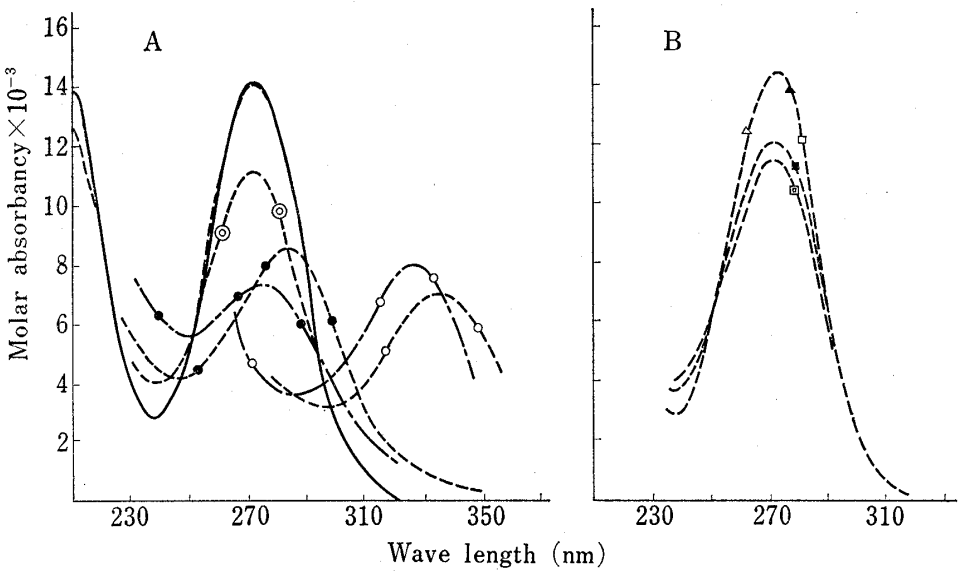

Fig. 3. UV Spectra of N_3,5′-Cycloadenosine (VIII)

A: VIII (10^{-4} M) was treated in 0.01 N NaOH at 22° for: ○, 0.5—1 min; ●, 10 min; and in 0.01 N NaOH for: ◎, 0.5 min and subsequently in 0.1 N HCl for 24 hr.
B: VIII (10^{-2} M) was treated in 0.1 N HCl at 22° for: △, 0 hr; ▲, 24 hr; and in 0.1 M phoshate buffer (pH 7.2) for: □, 0 hr; ■, 24 hr; ⧈, 6 days.
Spectra were taken in ——, H_2O; – – –, 0.1 N HCl; and ·—·—·. 0.01 N NaOH. The molar absorbancy was calculated and determined on the basis of the initial concentration of VIII.

treatment in 0.01 N NaOH at 22° for 1 min absorption maxima of VIII appeared at 338 nm (0.1 N HCl) and 328 nm (0.01 N NaOH), indicating the formation of previously unreported compound (X). The unstable compound (X) was converted rather slowly into the another compound (XI) having absorption maximum at 273 nm (0.1 N HCl) by treatment in 0.1 N HCl at 22° for 24 hr, and rapidly into the compound (XII) having absorption maximum at 285 nm (0.1 N HCl) and 277 nm (0.01 N NaOH) by treatment in 0.01 N NaOH at 22° for 10 min. Fig. 4 shows the time course of the conversion of VIII into XII *via* the intermediate compound (X) in 0.01 N NaOH. This indicated the formation of X was maximum within 1 min at 22°. Several efforts to isolate the intermediate (X) failed. The final compound (XII) was isolated in a yield of 79.6% by passing the aqueous solution of VIII through a column of Dowex 1 (OH^-). The compound (XII) was proved to be N_4,5′-cyclo-3-β-D-ribofur-

28) J.T. Witkowski, G.P. Kreishman, M.P. Schweizer, and R.K. Robins, *J. Org. Chem.*, **38**, 130 (1973).
29) K. Anzai and M. Matsui, *Agr. Biol. Chem.*, **37**, 301 (1973); *idem*, *ibid.*, **37**, 2431 (1973).
30) R.E. Holmes and R.K. Robins, *J. Org. Chem.*, **28**, 3483 (1963).
31) B.R. Baker and J.P. Joseph, *J. Am. Chem. Soc.*, **77**, 15 (1955).
32) K. Anzai, 2nd Symposium on Nucleic Acid Chemistry held in Tokyo, October, 1974, Abstract, p. 10.

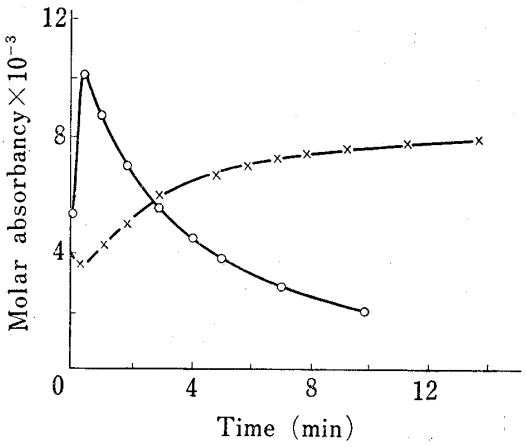

Fig. 4. Time Course of the Changes of the Absorbancy of N_3,5′-Cycloadenosine Chloride (VIII) in 0.01 N NaOH at 22°

Spectra were taken in 0.01 N NaOH at ○, 330 nm; and ×, 280 nm. The molar absorbancy was calculated on the basis of the initial concentration of VIII.

anosyl-4-aminoimidazole 5-carboxamidine. UV spectrum having $\lambda_{max}^{pH\,1}$ 287 nm was similar to that of N_4,3′-cyclo derivative (V). NMR studies revealed the absence of C_2H proton of the pyrimidine ring of VIII. A signal due to $C_1'H$ proton appeared as a singlet. The assignment of sugar proton resonances were confirmed by spin-decoupling experiments. The structure of the intermediate compound (X) must be C_2-hydrated or N-formyl derivative as shown in the Chart.

Treatment of XII in 0.1 N NaOH at an elevated temperature gave N_4,5′-cyclo-3-β-D-ribofuranosyl-4-aminoimidazole 5-carboxamide (XIII), whose structure was confirmed by UV spectrum, NMR spectrum and infrared (IR) absorption spectrum. UV spectrum of XIII was similar to the previously reported analog.[33]

Alteration of UV spectrum of VIII in neutral conditions was also noted when it was treated for a longer period (Fig. 3B). Although treatment of VIII in 0.1 N HCl for 24 hr did not show any changes in absorbancy, treatment in phosphate buffer (pH 7.2) for 24 hr and 6 days showed 16% and 20% decreases in absorbancy, respectively. These changes in absorbancy must reflect the transformation of VIII into another type of compound (XIV) under the conditions. The compound (XIV) could not be isolated because the physicochemical properties were similar to those of VIII.

Experimental[34]

5′-Chloro-5′-deoxyadenosine 2′,3′-Cyclic Sulfate (I)——Adenosine (2.0 g, 7.5 mmol) was suspended in 8 ml of acetonitrile and was cooled in an ice-water bath with stirring. To this were added 2.0 ml (25 mmol) of sulfuryl chloride and subsequently 0.20 ml (1.45 mmol) of triethylamine under the anhydrous conditions. The mixture was stirred at room temperature for 5 hr. It was evaporated *in vacuo* to dryness below 30° and the residue was dissolved in a mixture of ethyl acetate (100 ml) and H_2O (50 ml). The organic layer was separated and allowed to stand at room temperature overnight which was then evaporated *in vacuo*. The residue was dissolved in a mixture of ethanol (30 ml) and H_2O (10 ml), and it was adjusted to pH 3 with diluted aqueous ammonia. Granules separated were collected by filtration, 1.375 g (yield, 53%), mp 227—

33) UV of N_4,5′-cyclo-3-(2,3-carbonyl-3-amino-3-deoxy-β-D-ribo-furanosyl)-4-aminoimidazole 5-carboxamide; $\lambda_{max}^{pH\,1}$ (ε) nm, 259 (12200), $\lambda_{max}^{H_2O}$ 271 (10600), $\lambda_{max}^{pH\,14}$ 272 (12300), (Ref. 31).

34) Melting points were determined on a Buchi melting point apparatus and were uncorrected. UV spectra were taken with a Hitachi Recording Spectrophotometer EPS-3T. NMR spectra were taken with a Varian T-60 spectrometer with tetramethylsilane as an internal standard, and the assignment of sugar proton resonances were confirmed by spin-decoupling experiments. Measurements of NMR spectra were greatly acknowledged to Mr. T. Kawashima of the laboratories. Optical rotation and IR spectrum were measured with a JASCO automatic polarimeter DIP-SL and a Hitachi Grating Infrared Spectrophotometer, respectively. Mass spectroscopy was measured with a CEC 110B double-focus mass spectrometer (the National Chemical Laboratory for Industry). Paper chromatography was carried out with solvent 1: *n*-BuOH–H_2O (84: 16), solvent 2: *n*-BuOH–CH_3COOH–H_2O (2: 1: 1) and solvent 3: $(NH_4)_2SO_4$ saturated H_2O–H_2O-iso-PrOH (79: 19: 2) (Ref. 16). Paper electrophoresis was performed with system A: 0.05 M phosphate buffer (pH 7.4) and system B: 0.02 M acetate buffer (pH 4.5) at 1000 V/25 cm for 1 hr. Metaperiodate consumption test was done by spraying a 0.5% $NaIO_4$ solution and subsequently a 5% KI-starch solution. Chlorosulfate group was detected with a spray reagent made of a *n*-BuOH solution of aniline and pyridine (Ref. 15). Cellulose column was prepared by use of cellulose powder A (Toyo Roshi Kaisha, Ltd.). Elemental analysis was carried out in the laboratories (C, H, N) and at Shonan Analysis Center (Cl, S).

228° (dec.). $[\alpha]_D^{25°} = -22.7°$ (c; 0.39, DMSO). UV: $\lambda_{max}^{H^+}$ nm, 258, $\lambda_{max}^{H_2O}$ 260. NMR (d_6-DMSO), ppm: 8.43 (1H, s, C_2H or C_8H), 8.27 (1H, s, C_2H or C_8H), 7.50 (2H, broad s, NH_2), 6.72 (1H, d, $C_{1'}H$, $J_{1',2'}=3$ Hz), 6.57 (1H, dd, $C_{2'}H$, $J_{1',2'}=3$ Hz, $J_{2',3'}=8$ Hz), 6.10 (1H, dd, $C_{3'}H$, $J_{2',3'}=8$ Hz, $J_{3',4'}=4$ Hz), 4.78 (1H, m, $C_{4'}H$, $J_{3',4'}=4$ Hz, $J_{4',5'}=6$ Hz), 3.97 (2H, d, $C_{5'}H$, $J_{4',5'}=6$ Hz). *Rf*: 0.80 (solvent 1). High resolution mass spectroscopy showed the empirical formula of $C_{10}H_{10}ClN_5O_5S$ and a molecular ion peak at *m/e* 347. Low resolution spectrum is shown in Fig. 1. Negative reaction to metaperiodate test indicated the absence of 2′,3′-*cis*-glycol function. Chlorosulfate group was also absent. *Anal.* Calcd. for $C_{10}H_{10}ClN_5O_5S$; C, 34.54; H, 2.90; Cl, 10.20; N, 20.14; S, 9.22. Found: C, 34.59; H, 2.92; Cl, 10.32; N, 20.38; S, 9.22.

Compound (II)——The aqueous layer from the ethyl acetate-H_2O mixture of the above reaction mixture was evaporated *in vacuo* to dryness. The residue was crystallized from ethanol–H_2O to yield small amount of II in a crystalline form, which darkened at 208° and melted at 220° with decomposition. Full UV spectrum is shown in Fig. 2 and the spectrum is similar to that of N_3,5′-cycloadenosine chloride (VIII). NMR (d_6-DMSO), ppm: 8.73 (1H, s, C_2H or C_8H), 8.43 (1H, s, C_2H or C_8H), 6.63 (1H, s, $C_{1'}H$). *Rf*: 0.25 (solvent 1). Qualitative tests of *cis*-glycol function and chloride ion were negative and those of covalently bound chlorine atom and sulfur atom were positive.

5′-Chloro-5′-deoxyadenosine 2′-Sulfate (III) and 5′-Chloro-5′-deoxyadenosine 3′-Sulfate (IV)——A mixture of 5′-chloro-5′-deoxyadenosine 2′,3′-cyclic sulfate (I), 0.98 g (2.8 mmol) in 50 ml of ethanol and 150 ml of concentrated aqueous ammonia was allowed to stand at room temperature for 4 hr. It was then evaporated *in vacuo* to dryness and the residue was dissolved in 50 ml of H_2O, which was passed through a column of Dowex 50×4 (H^+) (50 ml). The column was eluted with H_2O and the UV-absorbing fraction (250 ml, the recovery the column was 70% estimated by the total optical density at 260 nm and pH 1) was evaporated *in vacuo* to dryness. The residue was applied onto a column of cellulose (1.7×60 cm) which was eluted with *n*-BuOH–H_2O (84: 16). Two UV-absorbing fractions were obtained. The first fraction was evaporated *in vacuo* to dryness and the residue was crystallized from *n*-BuOH–H_2O (84: 16) to afford leaflets of 2′-sulfate (III), 163 mg (yield 15.4%), mp 169—171° (dec.). Recrystallization from the same solvent gave a pure sample, mp 175—179° (dec.). $[\alpha]_D^{22°}$ −0.4° (c; 0.45, H_2O). UV: $\lambda_{max}^{pH\,1}$ (ε) nm, 257 (15000), $\lambda_{max}^{H_2O}$ 259.5 (15000), $\lambda_{max}^{pH\,13}$ 260 (15600). NMR (d_6-DMSO), ppm: 8.70 (1H, s, C_2H or C_8H), 8.53 (1H, s, C_2H or C_8H), 6.30 (1H, d, $C_{1'}H$, $J_{1',2'}=6$ Hz), 5.28 (1H, t, $C_{2'}H$, $J_{1',2'}=6$ Hz). *Rf*: 0.15 (solvent 1) and 0.19 (solvent 3). Paper electrophoretic mobility: +6.5 cm (system A) and +5.8 cm (system B). *Anal.* Calcd. for $C_{10}H_{12}ClN_5O_6S\cdot 2/3H_2O$; C, 31.79; H, 3.56; Cl, 9.39; N, 18.54; S, 8.49. Found: C, 31.54; H, 3.54; Cl, 10.09; N, 18.75; S, 8.84.

The second fraction from the cellulose column was evaporated *in vacuo* to dryness and the residue was crystallized from *n*-BuOH saturated H_2O to yield 320 mg (yield 31.2%) of granules of 3′-sulfate (IV), mp 186—214° (dec.). Recrystallization from the same solvent gave a pure sample, mp 188—198° (dec.). $[\alpha]_D^{22°}$ −26.3° (c: 0.46, H_2O). UV: $\lambda_{max}^{pH\,1}$ (ε) nm, 259.5 (15200), $\lambda_{max}^{H_2O}$ 257 (14900), $\lambda_{max}^{pH\,13}$ 260 (15600). NMR (d_6-DMSO), ppm: 8.73 (1H, s, C_2H or C_8H), 8.57 (1H, s, C_2H or C_8H), 6.05 (1H, d, $C_{1'}H$, $J_{1',2'}=5$ Hz). *Rf*: 0.12 (solvent 1) and 0.11 (solvent 3). Paper electrophoretic mobility: +5.3 cm (system A) and +5.2 cm (system B). *Anal.* Calcd. for $C_{10}H_{12}ClN_5O_6S$; C, 32.84; H, 3.31; Cl, 9.69; N, 19.15; S, 8.77. Found: C, 32.95; H, 3.44; Cl, 9.67; N, 18.91; S, 8.70.

N_4,3′-Cyclo-3-(5-chloro-5-deoxy-β-D-xylofuranosyl)-4-aminoimidazole 5-Carboxamidine 2′-Sulfate (V)——The compound (I), 1.0 g (2.9 mmol), was dissolved in a mixture of 100 ml of H_2O and 100 ml of ethanol and refluxed for 5 hr. It was evaporated *in vacuo* to dryness and the residue was applied onto a cellulose column (1.7×50 cm). The column was eluted with *n*-BuOH–H_2O (84: 16) and the main UV-absorbing fraction was collected. It was evaporated *in vacuo* to dryness and crystallized from ethanol–H_2O, 560 mg (yield 54%). Recrystallization from ethanol–H_2O gave pure needles of V, mp 217—225° (dec.). $[\alpha]_D^{22°}$ +98° (c: 0.3, H_2O). UV: $\lambda_{max}^{pH\,1}$ (ε) nm, 292 (14500), $\lambda_{min}^{pH\,1}$ 253 (2600), $\lambda_{max}^{pH\,7}$ 292.5 (14100), $\lambda_{min}^{pH\,7}$ 245 (2700), $\lambda_{max}^{pH\,13}$ 273 (8200), 335 (6000), $\lambda_{min}^{pH\,13}$ 243 (5100), 304 (2800). NMR (d_6-DMSO), ppm: 7.68 (1H, s, C_2H), 6.07 (1H, s, $C_{1'}H$), 4.97 (1H, broad s, $C_{2'}H$). *Rf*: 0.25 (solvent 1). Paper electrophoretic mobilty: 0 (system A). A qualitative test of chloride ion was negative. *Anal.* Calcd. for $C_9H_{12}ClN_5O_5S\cdot H_2O$; C, 30.38; H, 3.97; Cl, 9.97; N, 19.69; S, 9.01. Found: C, 30.64; H, 3.59; Cl, 9.91; N, 19.34; S, 9.19.

5′-Chloro-5′-deoxyadenosine (VI)——Thionyl chloride (7.5 ml) was dissolved in 50 ml of hexamethylphophoramide and to the solution was added 5.0 g (18.7 mmol) of adenosine under cooling. The mixture was stirred at room temperature for 20 hr. It was then poured into 500 ml of H_2O and the mixture was stirred for about 3 hr in order to destroy the reagent and remove sulfur dioxide evolved. The solution was applied to a column of Diaion SK-1B (H^+) (1500 ml), and the column which had been previously washed well with H_2O was eluted with 7.5 l of 1 N NH_4OH and then 5 l of 5 N NH_4OH. The effluent which had 80% of the initial total optical density at 260 nm and pH 1 was evaporated to about 150 ml. Colorless needles separated were collected by filtration. A crude sample of I was obtained in a yield of 73% (4.00 g), mp 188° (dec.). Recrystallization from H_2O gave pure needles which partially melted at 79—90° and decomposed at 187—191° after drying at 50° for 6 hr over P_2O_5 *in vacuo*. $[\alpha]_D^{30°}$ −17.6° (c: 0.62, H_2O). UV: $\lambda_{max}^{pH\,1}$ (ε) nm, 257 (15400), $\lambda_{max}^{H_2O}$ 260 (15400), $\lambda_{max}^{pH\,13}$ 260 (16000). NMR (d_6-DMSO), ppm: 8.30 (1H, s, C_2H or C_8H), 8.14 (1H, s, C_2H or C_8H), 7.24 (2H, broad s, NH_2), 5.92 (1H, d, $C_{1'}H$, $J_{1',2'}=5$ Hz), 4.6—4.9 (2H, unresolved m, $C_{2'}H$ and $C_{3'}H$), 3.8—4.4 (3H, unresolved m, $C_{4'}H$ and $C_{5'}H$). *Rf*: 0.47 (solvent 1) and 0.61

(solvent 2). *Anal.* Calcd. for $C_{10}H_{12}ClN_5O_3 \cdot 1/2H_2O$; C, 40.75; H, 4.45; Cl, 12.03. N, 23.77; Found: C, 40.89; H, 4.34; Cl, 12.03; N, 23.90.

When the semi-hydrate obtained above was further dried at 90° for 6 hr over P_2O_5 *in vacuo*, hygroscopic crystals which sintered at 130—145° and decomposed at 189—194° were obtained. When the semi-hydrate was recrystallized from methanol and dried at 50° for 5 hr over P_2O_5, colorless needles which partially melted at 158—161° and decomposed at 190—195° were obtained. (lit.[25] as a hydrate, melted at 78—85° resolidified again and decomposed at 180°. lit.[26] mp 140—165° from methanol).

N_3,5′-Cycloadenosine (VIII and IX)——5′-Chloro-5′-deoxyadenosin (VI) (3.0 g, 10.1 mmol) was dissolved in 30 ml of anhydrous dimethylformamide and the mixture was heated at 145° in an oil bath for 2 hr. Crystals separated were collected by filtration and dried, 1.68 g (yield 56%). Recrystallization from ethanol–H_2O gave pure needles of N_3,5′-cycloadenosine chloride (VIII) which darkened at 225° and decomposed at above 280°. $[\alpha]_D^{24°}$ −24.7° (*c*: 0.56, H_2O). UV: $\lambda_{max}^{pH\,1}$ (ε) nm, 272.5 (14300), $\lambda_{min}^{pH\,1}$ 237.5 (2900), $\lambda_{max}^{H_2O}$ 272.5 (14300), $\lambda_{min}^{H_2O}$ 238 (2600). Full spectra are shown in Fig. 3. NMR (d_6-DMSO), ppm: 9.33 (2H, s, NH_2), 8.78 (1H, s, C_2H or C_8H), 8.53 (1H, s, C_2H or C_8H), 6.44 (1H, s, $C_{1'}H$), 4.99 (1H, broad s, $C_{4'}H$), 4.70 (2H, broad s, $C_{5'}H$), 4.27 (1H, m, $C_{3'}H$), 4.03 (1H, d, $C_{2'}H$). *Rf*: 0.27 (solvent 2). Paper electrophoretic mobility: −8.1 cm (system A). *Anal.* Calcd. for $C_{10}H_{12}ClN_5O_3$; C, 42.04; H, 4.23; Cl, 12.41. N, 24.51; Found: C, 41.80; H, 4.20; Cl, 12.54; N, 24.38. (lit.[22] mp above 230° with decomposition and UV: λ_{max} 272 nm).

The chloride (VIII) (200 mg) was dissolved in 5 ml of H_2O and passed through a column of 5 ml of Dowex 1×2 (I^-). The effluent and washings were combined and evaporated to dryness. The residue was crystallized and recrystallized from methanol–H_2O to afford yellow granules, 150 mg, of N_3,5′-cycloadenosine iodide (IX), mp 213—215° (dec.). Comparisons of IR spectrum (KBr), UV spectrum and NMR spectrum of the sample with those of an authentic sample[24] kindly supplied by Dr. Verhyden showed that both were identical. (lit.[24] mp 215—230° (dec.). UV: $\lambda_{max}^{H_2O}$ (ε) nm, 220 (21700), 273 (13500). NMR (d_6-DMSO), ppm; 8.72 (1H, s, C_2H or C_8H), 8.49 (1H, s, C_2H or C_8H), 6.41 (1H, s, $C_{1'}H$), 4.90 (1H, broad s, $C_{4'}H$), 4.69 (2H, broad s, $C_{5'}H$), 4.30 (1H, m, $C_{3'}H$), 3.99 (1H, d, $C_{2'}H$).)

N_4,5′-Cyclo-3-β-D-ribofuranosyl-4-aminoimidazole 5-Carboxamidine (XII)——A solution of N_3,5′-cycloadenosine chloride (VIII) (300 mg, 1.05 mmol) in 5 ml of H_2O was passed through a column of Dowex 1×2 (OH^-) (10 ml). The column was washed with about 300 ml of H_2O until free of UV-absorbing material. The effluent and washings were combined and evaporated below 30° *in vacuo* to dryness. The residue was triturated with ethanol to afford plates of XII, 200 mg (yield 79.6%). Recrystallization from ethanol–H_2O gave a pure material, mp 218—222° (dec.). $[\alpha]_D^{24°}$ +117.9° (*c*: 0.38, H_2O). UV: $\lambda_{max}^{pH\,1}$ (ε) nm, 287 (9600), $\lambda_{max}^{pH\,3,7,9}$ 289 (10800), $\lambda_{max}^{pH\,13}$ 273 (9200). NMR (d_6-DMSO), ppm: 7.37 (1H, s, C_2H), 5.80 (1H, s, $C_{1'}H$), 4.40 (1H, dd, $C_{4'}H$, $J_{4',5'a}$=3 Hz, $J_{4',5'b}$=2 Hz), 4.27 (1H, d, $C_{2'}H$ or $C_{3'}H$, $J_{2',3'}$=7 Hz), 3.98 (1H, d, $C_{2'}H$ or $C_{3'}H$, $J_{2',3'}$=7 Hz), 3.40 (1H, dd, $C_{5'a}H$, $J_{4',5'a}$=3 Hz, J_{gem}=13 Hz), 2.90 (1H, dd, $C_{5'b}H$, $J_{4',5'b}$=2 Hz, J_{gem}=13 Hz). *Rf*: 0.03 (solvent 1) and 0.45 (solvent 2). *Anal.* Calcd. for $C_9H_{13}N_5O_3$; C, 45.18; H, 5.48; N, 29.28. Found: C, 45.10; H, 5.46; N, 28.97.

N_4,5′-Cyclo-3-β-D-ribofuranosyl-4-aminoimidazole 5-Carboxamide (XIII)——A solution of 200 mg (0.84 mmol) of XII in 5 ml of 0.1 N NaOH was heated at 80° for 2.5 hr. Crystals separated on cooling were collected by filtration. Recrystallization from H_2O gave pure columns of XIII, 100 mg (yield, 50.0%), mp 257—259° (dec.). $[\alpha]_D^{27°}$ +71.2° (*c*: 0.59, H_2O). UV: $\lambda_{max}^{pH\,1}$ (ε) nm, 266 (10500), $\lambda_{max}^{pH\,7}$ 273 (13100), $\lambda_{max}^{pH\,13}$ 274 (13600). NMR (d_6-DMSO), ppm: 7.50 (1H, s, C_2H), 5.90 (1H, s, $C_{1'}H$), 4.45 (1H, unresolved m, $C_{4'}H$), 4.26 (1H, d, $C_{2'}H$ or $C_{3'}H$, $J_{2',4'}$=6 Hz), 4.00 (1H, d, $C_{2'}H$ or $C_{3'}H$, $J_{2',3'}$=6 Hz), 3.57 (1H, dd, $C_{5'a}H$, $J_{4',5'a}$=3 Hz, J_{gem}=13 Hz), 2.97 (1H, dd, $C_{5'b}H$, $J_{4',5'b}$=2 Hz, J_{gem}=13 Hz). IR: ν_{max}^{KBr} cm^{-1}, 1635 (C=O). *Rf*: 0.21 (solvent 1) and 0.53 (solvent 2). *Anal.* Calcd. for $C_9H_{12}N_4O_4$; C, 45.00; H, 5.04; N, 23.32. Found: C, 45.00; H, 5.21; N, 23.19.

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