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Studies of Nucleosides and Nucleotides. LXXIII.[1] Chlorination of Adenosine and Its N^6-Methyl Derivatives with *t*-Butyl Hypochlorite

Morio Ikehara, Yuko Ogiso, and Tokumi Maruyama

Faculty of Pharmaceutical Sciences, Osaka University[2]

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Adenosine was allowed to react with *t*-butyl hypochlorite in a variety of conditions. Using limited amount of the chlorinating agent at low temperature (−70°) a monochloro compound (IIa) was obtained. The structure of IIa was tentatively assigned as N^6-chloroadenosine. When the chlorination was conducted at higher temperature using excess reagent in dimethylformamide (DMF), 8-chloroadenosine (IIIa) was obtained as the main product. The structure of IIIa was elucidated by ultraviolet, mass and nuclear magnetic resonance spectra together with their chemical reactivities. The chlorination of 2′,3′,5′-tri-O-acetyladenosine also afforded 8-chloro derivative (IIIb) which gave 8-chloroadenosine by the treatment with ammonia.

When N^6-methyl or N^6-dimethyladenosine were chlorinated using *t*-butyl hypochlorite, only in the former case N^6-chloro compound (IIc) was obtained. 8-Chloro-N^6-methyl and -N^6-dimethyladenosine (IIIc,d) were obtained when the chlorination was conducted in DMF as the solvent using excess *t*-butyl hypochlorite.

Keywords——N^6-chloroadenosine; 8-chloroadenosine; UV; NMR; TLC; N^6-methyl-N^6-chloroadenosine; N^6-dimethyl-8-chloroadenosine; paper chromatography

The bromination of purine nucleosides has been extensively studied in recent years by a number of investigators.[3,4] It has been shown that 8-bromo adenosine adopted a *syn* conformation either in crystals[5] or in solution.[6], However, reports dealing with the chlorination of adenosine are rather limited in number.[7,8] This paper deals with the chlorination of adenosine, N^6-methyl- and N^6-dimethyladenosine using *t*-butyl hypochlorite as the chlorinating agent. We found that labile monochlorinated compounds (IIa,c) were obtained in the adenosine and N^6-methyladenosine cases when the reaction was conducted in rather weak conditions and that 8-chloro compounds (IIIa—d) were obtained under stronger reaction conditions.

Adenosine (Ia) was allowed to react with variable amounts of *t*-butyl hypochlorite in several solvents, such as methanol, dimethylformamide (DMF), dioxane and hexamethylphosphoroamide (HMPA) in conditions as summarized in Table I. A compound (IIa) having a ultraviolet (UV) absorption maximum in H_2O at 266 nm and in alkali at 274 nm was isolated as crystals in yield as shown in Table I. Elemental analysis of this sample gave values corresponding to monochloroadenosine. Behavior in thin-layer chromatography (TLC) with *Rf*'s higher than adenosine also suggested introduction of a lipophilic group. The ribose appeared to be intact because spots on paper chromatography were positive for the benzidine-periodate spray test.

1) Part LXXII of this series: M. Ikehara and T. Maruyama, "in preparation."
2) Location: *133-1 Yamadakami, Suita, Osaka, 565, Japan.*
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Table I

Exp. No.	Adenosine (mg)	*t*-Butyl hypochlorite (ml)	Solvent	Temperature	Time (hr)	Yield (%) IIa	Yield (%) IIIa
1	265	0.1	MeOH	−70°	3	72	
2	134	0.2	MeOH	−70°	5	55[a]	
3	160	0.18	DMF	−70°	3	56[a]	44[a]
4	265	0.1	MeOH	−20°	48	66	
5	1000	1.3	DMF	−70°	2	47[a]	63[a]
6	1000	0.45	HMPA	−20°	15(min)	31	
7	1000	0.5	DMF	−70°	1	17	16[b]

a) Converion estimated by the absorbance of spots in TLC.
b) isolated as triacetate

Compound (IIa) gave nomolecular ion peak in its mass spectrum showing that IIa is not a cyclonucleoside as shown previously.[9] Fragment ions *m/e* 169 (25%) and 171 (8%) in the ratio of chlorine isotopes corresponding to ions of chloroadenine were also observed. IIa showed signals in it's nuclear magnetic resonance (NMR) spectrum at δ 8.58 and 8.38, which are assignable to C_8- and C_2-protons. Signals of the sugar moiety appeared in similar positions to those of adenosine. When the compound (IIa) was treated with potassium mercaptide in a water-DMF mixture[10] or with triethylamine in DMF at room temperature only adenosine was recovered. From this evidence the chlorine atom introduced in the adenine moiety seems to be fairly unstable. Although its location could not be firmly established, the N^6-position is strongly implicated by these facts together with experiments described later.

As shown in Table I, in some reactions we could obtain a monochloro compound (IIIa) other than IIa. In the case of Experiments No. 3, a large excess of *t*-butyl hypochlorite was used in the solvent DMF. In cases No. 5 and 7 DMF was also used as the solvent. Thus it seems likely that the use of a highly polar aprotic solvent DMF and excess of the chlorinating reagent lead to compound (IIIa) in addition to IIa. Compound (IIIa) showed a UV absorption maximum in water at 263.5 nm, which suggested C-8 as the position of chlorination, as in the case of 8-bromoadenosine.[11] The compound (IIIa) did not show any C-8 signal in its NMR spectra. Its mp 188—190°, UV absorption, mass spectrum and behavior in paper chromatography correspond with those reported in the literature,[7,12] showing that the IIIa was 8-chloroadenosine. This sample was further identical to 8-chloroadenosine obtained by the chlorination of 8-mercaptoadenosine[13] with N-chlorosuccinimide.

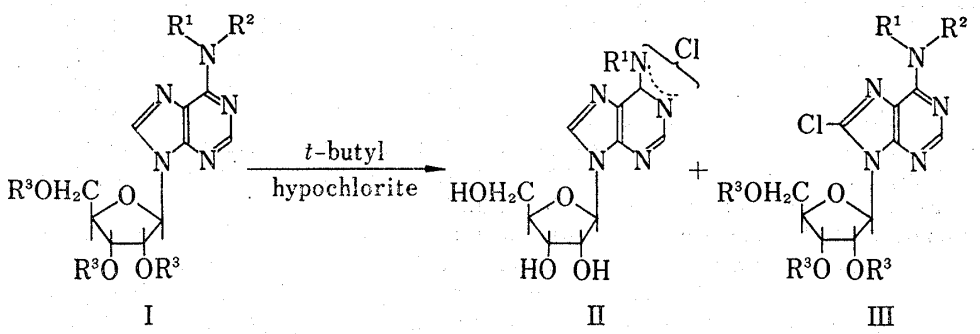
To confirm that the halogenation occurs at the C-8 position when using DMF and in rather strong conditions, 2′,3′,5′-tri-O-acetyladenosine (Ib) was chlorinated using excess *t*-butyl hypochlorite at 0°. In this case the 8-chloro compound IIIb was obtained in a yield of 24% by the silica gel column chromatography. Deprotection of (IIIb) with conc. ammonia gave 8-chloroadenosine in a yield of 73%. This sample was completely identical to that obtained above. The structure of 8-chloroadenosine was further supported by reaction with sodium methylmercaptide to give 8-methylthioadenosine.[12]

To obtain more information on the mechanism of this chlorination reaction we next chlorinated N^6-methyl- (Ic) and N^6-dimethyladenosine (Id) under the similar conditions. When N^6-methyladenosine was allowed to react with slight excess *t*-butyl hypochlorite at −20° for 18 hrs in methanol, a monochlorinated compound (IIc), mp 92—94° was obtained in a yield of 68%. The elemental analysis and mass spectrum suggested that a chloro atom was intro-

9) M. Ikeda, Y. Tamura, and M. Ikehara, *J. Heterocyclic. Chem.*, **7**, 1377 (1970).
10) M. Ikehara, E. Ohtsuka and S. Uesugi, *Chem. Pharm. Bull.* (Tokyo), **21**, 444 (1973).
11) M. Ikehara and H. Kaneko, *Tetrahedron*, **26**, 4251 (1970).
12) C.L. Schmidt and L.B. Townsend, *J. Org. Chem.*, **37**, 2300 (1972).
13) M. Ikehara and S. Yamada, *Chem. Pharm. Bull.* (Tokyo), **19**, 104 (1971).

duced to the methyladenine moiety. The compound (IIc) has a UV absorption maximum at 275 nm in neutral pH and at 273 nm in alkaline conditions. In the mass spectrum fragment ion peaks of the base (*m/e* 183 and 185) appeared in a ratio of 3: 1. If the same reaction was conducted in DMF at −70° for 2 hr, the compound (IIc) was obtained in a yield of 78%. Raising the reaction temperature to −20° for 15 hr and then to room temperature for 24 hr, a compound having λ_{max} 270 nm at neutrality was observed on TLC. Although this compound (IIIc) was not isolated, the UV absorption properties closely resembled those of 8-bromo-N^6-methyladenosine,[14] suggesting that the compound (IIIc) is probably 8-chloro-N^6-methyladenosine. Thus in the chlorination of N^6-methyladenosine the monochlorinated compound (IIc) seems to be rather stable and easily isolable. This may suggest that the pK of N^6 (or N^1) was increased by virtue of the introduction of the methyl group which may stabilize the N-chloro compound (IIc.)

N^6-Dimethyladenosine (Id) was then chlorinated using *t*-butyl hypochlorite in DMF at 0° for 30 min. A chlorinated compound (IIId) having mp 135—137° was isolated in a yield of 41%. This compound had UV $\lambda_{max}^{H_2O}$ at 277 nm which was similar to 8-bromo-dimethyladenosine.[15] Elemental analyses suggested the introduction of a chlorine atom. The mass spectrum showed fragment ion peaks *m/e* 455—458 corresponding to the chlorinated base moiety. Although position 2 is also available for chlorination, the results suggested the formation of 8-chloro-N^6-dimethyladenosine. From this experiment, it was confirmed that if the N^6-position of adenosine was fully substituted, the chlorine atom attacked the 8-position in similar reaction conditions to the case of adenosine. Thus it was shown that the use of *t*-butyl hypochlorite for the chlorination of adenosine and it's N^6-methyl derivatives gave 8-chloro compounds as the final products. The present method may be a convenient way to prepare 8-chloroadenosine, as well as it's N^6-methyl derivatives. If an N^6-H atom of adenosine is available, the reaction goes first to the N^6-position (or N^1-position) and a labile monochlorinated compound is obtained. The exact molecular structure of this compound awaits elucidation by X-ray crystallography or by other methods.



a: R^1, R^2, R^3=H
b: R^1 R^2=H, R^3=$COCH_3$
c: R^1=CH_3, R^2, R^3=H
d: R^1,R^2=CH_3, R^3=H

Chart 1

Experimental

UV absorption spectra were measured with a Hitachi EPS-3 spectrophotometer, and NMR spectra with a Hitachi R22 (90 MHz) spectrometer in dimethyl sulfoxide (DMSO-d_6) with tetramethylsilane (TMS) as external standard. Paper chromatography was performed on Toyo filter paper No. 51A in solvent system: A, *n*-butanol–water (86: 14); B, *n*-butanol–acetic acid–water (5: 2: 3). TLC was performed on Kieselgel HF 254.

14) H. Morisawa, unpublished experiments.
15) M. Ikehara and H. Morisawa, *Chem. Pharm. Bull.* (Tokyo), **19**, 2593 (1971).

Chlorination of Adenosine——i) In Methanol: Adenosine (265 mg, 1 mmole) was dissolved in anhydrous methanol (100 ml). The solution was cooled to −70° with a dry ice–acetone bath and *t*-butyl hypochlorite (0.1 ml) was added. The solution was kept at −20° (in a deep freezer) overnight. Compound (IIa) obtained as tiny needles. Yield was 216 mg (72%). *Anal.* Calcd. for $C_{10}H_{12}O_4N_5Cl$: C, 39.81; H, 4.01; N, 23.21; Cl; 11.75. Found: C, 39.60; H, 3.90; N, 23.08; Cl, 11.80. UV: $\lambda_{max}^{H_2O}$ 266 nm (ε 13500); $\lambda_{max}^{0.1N\ HCl}$ 261 (12100); $\lambda_{max}^{0.1N\ NaOH}$ 274 (11100), 300 (8900, sh). NMR (d_6-DMSO): δ 8.58 (s, 1H, H-8), 8.38 (s, 1H, H-2), 5.91 (d, 1H, H-1′, $J_{1'-2'}$=5.5 Hz), 4.53 (t, 1H, H-2′), 4.18 (t, 1H, H-3′), 3.98 (m, 1H, H-4′), 3.62 (m, 2H, H-5′). Massum Spectr *m/e*: 169, 171 (ratio3: 1, chloroadenin); 142, 144 (chloroadenine-HCN); 135, 136 (adenine). TLC ($CHCl_3$–EtOH, 3: 2) *Rf* 0.40 (adenosine, 0.27).

ii) In DMF: Adenosine (1.0 g, 3.75 mmoles) was dissolved in DMF (20 ml) and cooled to −70° with a dry ice–acetone bath. *t*-Butyl hypochlorite (0.5 ml) was added and the mixture was kept at this temperature for 1 hr. The solvent was concentrated *in vacuo* and pink crystals of compound (IIa) (196 mg, 17%) were obtained as precipitates. The solvent was completely evaporated *in vacuo* and the residue was stirred with acetic anhydride (3 ml) and pyridine (6 ml) at 0° for 2 hr. The solvent was removed *in vacuo*, ethanol was added and removed, and the residue was dissolved in $CHCl_3$. The solution was applied to a column (1.5×36 cm) of silica gel and eluted with $CHCl_3$ containing 2% EtOH. 2′,3′,4′-Tri-O-acetyl-8-chloroadenosine, mp 180—182°, was obtained in a yield of 251 mg (0.59 mmole, 16%). This sample was identical with that obtained below. The triacetyl-8-chloroadenosine (65 mg, 0.15 mmole) was dissolved in EtOH (2 ml) and 28% conc. aqueous ammonia (1 ml) was added. After 24 hr at 5°, the mixture was evaporated and the residue was recrystallized from EtOH–H_2O. 8-Chloroadenosine, mp 188—190° (33 mg, 73%) was obtained. *Anal.* Calcd. for $C_{10}H_{12}O_4N_5$ Cl: C, 38.81; H, 4.01; N, 23.21; Cl, 11.75. Found: C, 38.65; H, 3.98; N, 23.24; Cl, 11.88. UV: $\lambda_{max}^{50\%\ EtOH}$ 263.5 nm (ε, 13500), $\lambda_{max}^{0.1N\ HCl}$ 261.5 (13300), $\lambda_{max}^{0.1N\ NaOH}$ 263.5 (11400). Paper chromatography: *Rf* (A) 0.48, *Rf* (B) 0.74. TLC ($CHCl_3$–EtOH, 3: 2): *Rf* 0.70. This sample was completely identical with an authentic sample.[7]

iii) In HMPA: Adenosine (1.0 g, 3.75 mmole) was dissolved in HMPA (10 ml) and cooled to −20°. To the solution *t*-butyl hypochlorite (0.45 ml, 4 mmole) was added. After 15 min water (100 ml) was added and the solution was extracted with $CHCl_3$ (50 ml×7) to remove HMPA. After 5 extractions, brownish crystalline platelets precipitated. Recrystallization from methanol gave chloroadenosine (IIa) in a yield of 352 mg, (1.17 mmole, 31%). This sample was identical with that obtained above.

8-Chloroadenosine from 8-Mercaptoadenosine——8-Mercaptoadenosine (140 mg) was dissolved in 80% MeOH (25 ml) and N-chlorosuccinimide (267 mg, 4 equiv.) was added. The solution was kept at room temperature for 3 hr. The solvent was evaporated *in vaeuo* and the residue was recrystallized from EtOH–H_2O. Crystalline 8-chloroadenosine, mp 187—190, was obtained in a yield of 47%. This sample was identical with the sample obtained above.

2′,3′,5′-Tri-O-acetyl-8-chloroadenosine——2′,3′,5′-Tri-O-acetyladenosine (1.11 g, 2.82 mmole) was dissolved in DMF (9 ml). To the solution *t*-butyl hypochlorite (0.38 ml) was added. The mixture was kept at 25° for 30 min. DMF was evaporated *in vacuo*, the residue was dissolved in $CHCl_3$ and applied to a column (1.8×35 cm) of silica gel. Elution with $CHCl_3$ containing 2% EtOH gave a fraction containing the product. Evaporation of the solvent gave 2′,3′,5′-tri-O-acetyl-8-chloroadenosine mp 180—182° in a yield of 295 mg (0.69 mmole, 24%). *Anal.* Calcd. for $C_{16}H_{18}O_7N_5Cl$: C, 44.92; H, 4.24; N, 16.39; Cl, 8.29. Found: C, 44.89; H, 4.29; N, 16.22; Cl, 8.26. UV: $\lambda_{max}^{50\%\ EtOH}$ 261.5 nm (ε, 16200), $\lambda_{max}^{0.1N\ HCl}$ 260 (17500), $\lambda_{max}^{0.1N\ NaOH}$ 263 (16400). Paper chromatography: *Rf* (A) 0.79, *Rf* (B) 0.91.

Chlorination of N^6-Methyladenosine——i) In MeOH: N^6-Methyladenosine (56 mg, 0.20 mmole) was dissolved in MeOH (3 ml) and *t*-butyl hypochlorite (0.03 ml, 0.26 mmole) was added. The mixture was kept at −20° for 18 hr. Upon evaporation of the solvent chloro-N^6-methyladenosine was obtained as white needles mp 92—94° in a yield of 43 mg (0.137 mmole, 68%). *Anal.* Calcd. for $C_{11}H_{14}O_4N_5Cl$: C, 40.69; H, 4.66; N, 21.57; Cl, 10.92. Found: C, 40.64; H, 4.41; N, 21.24, Cl, 10.58. UV: $\lambda_{max}^{50\%\ EtOH}$ 275 nm, $\lambda_{max}^{0.1N\ NaOH}$ 273 nm. In 0.1 N HCl it showed λ_{max} 263 nm which should be λ_{max} of N^6-methyladenosine because re-alkalination showed λ_{max} 267 nm. Mass Spectrum *m/e*: 183 (71.5%), 185 (23.5%) corresponding to monochloro-N^6-methyladenine.

ii) In DMF: The same reaction was conducted at −70° for 2 hr. Chloro-N^6-methyladenosine was obtained in a yield of 49 mg (78%). When the above reaction was performed at −20° for 15 hr and then at room temperature for 24 hr, a spot corresponding to 8-chloro-N^6-methyladenosine was observed at *Rf* 0.65 in TLC ($CHCl_3$–EtOH, 5: 1). The conversion ratio from N^6-monomethyladenosine was *ca.* 50%. UV: $\lambda_{max}^{50\%\ EtOH}$ 270 nm, $\lambda_{max}^{0.1N\ HCl}$ 267, $\lambda_{max}^{0.1N\ NaOH}$ 270.

8-Chloro-N^6-dimethyladenosine——N^6-Dimethyladenosine (1.23 g, 4.18 mmole) was dissolved in DMF (30 ml) and *t*-butyl hypochlorite (0.8 ml) was added. The mixture was kept at 0° for 30 min. DMF was evaporated, and the residue was dissolved in H_2O and extracted with *n*-butanol. The butanol layer was washed with water and evaporated *in vacuo*. Recrystallization of the residue from EtOH gave 577 mg (41%) of 8-chloro-N^6-dimethyladenosine, mp 135.5—137°. *Anal.* Calcd. for $C_{12}H_{16}O_4N_5Cl$–1/2H_2O: C, 42.55; H, 5.06; N, 20.67; Cl, 10.47. Found: C, 42.49; H, 5.02; N, 20.72; Cl, 10.41. UV $\lambda_{max}^{H_2O}$ 277 nm (ε 18600), $\lambda_{max}^{0.1N\ HCl}$ 271 (19700), $\lambda_{max}^{0.1N\ NaOH}$ 277.5 (19300). The mass spectrum of the triacetate *m/e*: 455, 457 (3: 1) (M^++H); 157, 160 (3: 1) (Cl-dimethylA+H). Paper chromatography: *Rf* (A) 0.80, *Rf* (B) 0.82 (N^6-Dimethyladenosine, *Rf* (A) 0.62, *Rf* (B) 0.72; 8-bromo-N^6-dimethyladenosine, *Rf* (A) 0.81.