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Studies on the Structure of a New Type of Cyclonucleoside, 6,2′-Triazacyclocytidine[1]

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Treatment of O^2,2′-cyclocytidine hydrochloride (I) with lithium azide in dimethylformamide at an elevated temperature gave 6,2′-triazacyclocytidine (II) (47.5%) and 2′-azido-2′-deoxycytidine (III) (10.8%). The structure of II was confirmed by physicochemical experiments.

Keywords——pyrimidine nucleoside; cyclonucleoside; azidosugar; lithium azide; nucleophilic substitution; catalytic hydrogenation; NMR; mass spectroscopy; UV spectroscopy

Cyclopyrimidine nucleosides are converted into a variety of nucleoside derivatives.[3–5] The cyclo-bonds in O^2-cyclouridines, such as O^2,5′-, O^2,3′-, and O^2,2′-cyclouridines, can be splitted[3–6] by a hydroxyl, sulfhydryl,[7] amino and dimethyloxosulfonium methylide[8] group, furnishing the derivatives substituted at the uracil moiety, and by a halogen, alkylthio,[9] thioacetate,[10] phosphate and azide[11–13] group, furnishing the derivatives substituted at the sugar moiety. Cleavage of the cyclo-bonds of O^2,5′-, O^2,3′-, and O^2,2′-cyclouridines by an azide ion has afforded azido-sugar nucleosides, such as 5′-azido-5′-deoxy-2′,3′-O-isopropylideneuridine (**1**),[11] 5′-azido-5′-deoxy-1-β-D-xylofuranosyluracil (**2**)[12] and 2′-azido-2′-deoxyuridine (**3**),[12,13] respectively, which have been subsequently converted into the corresponding amino-sugar nucleosides.

A few instances on the cleavage of cyclo-bonds of O^2,5′-, O^2,3′-, and O^2,2′-cyclocytidines by a sulfhydryl,[14] phosphate,[15] amino[16] and halide[17] are known and are similar to those reported on cyclouridines.

This paper relates to the treatment of O^2,2′-cyclocytidine (O^2,2′-anhydro-1-β-D-arabino-

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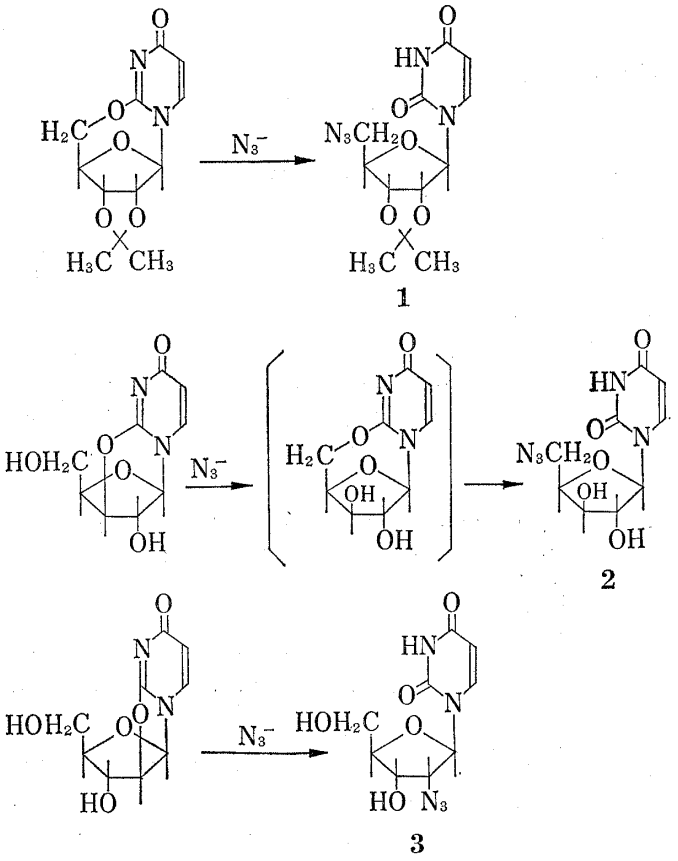

Chart 1

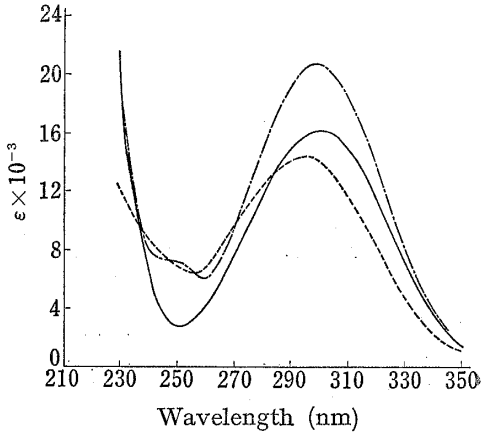

Fig. 1. UV Absorption Spectrum of Compound (II)

-----: pH 1, ——: pH 4, -----: pH 13

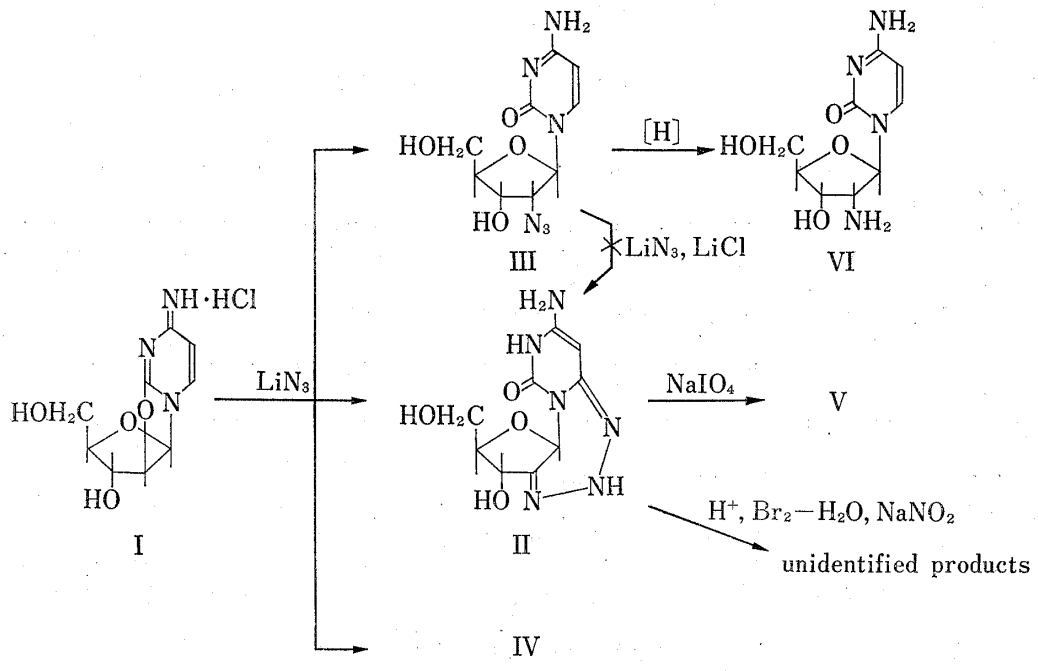

Chart 2

furanosylcytosine) (I)[17,18] with an azide ion and an unexpected formation of a new type of cyclonucleoside (II).

O^2,2′-Cyclocytidine hydrochloride (I) was treated with lithium azide in dimethylformamide at 80—90° for 72 hr. Paper chromatography of the reaction mixture revealed a major spot, the aqueous extract of which showed ultraviolet (UV) absorption maxima at 225 and 300 nm, corresponding to the product (II), and two minor spots corresponding to the products (III and IV). The reaction mixture was passed through a column of an anion exchange resin (bicarbonate form). A subsequent elution of the column with a mixture of methanol and triethylamine bicarbonate afforded the major product (II) in a yield of 47.5%. The non-absorbing fraction of the column was subjected to a successive cellulose column chromatography affording the two minor products, III (yield, 10.8%) and IV.

The major product (II) obtained as granules had a molecular formula of $C_9H_{12}O_4N_6$ and a molecular weight of 268 when estimated by an elemental analysis and a high resolution mass spectroscopy. It had the same empirical formula as the product (III) and three nitrogen atoms were introduced into the molecule of the starting material (I). An examination of its infrared (IR) absorption spectrum revealed the absence of absorption in the region of 2160—2120 cm^{-1}, whereas the absorption in this region is characteristic of an azide group.[19] Furthermore attempts to convert the compound into an amino nucleoside with catalytic hydrogenation or with sodium borohydride failed. Hence the three nitrogens introduced were not present as an azido function in the structure of II. A characteristic bathochromic shift in UV absorption spectrum ($\lambda_{max}^{pH\,4}$ 300 nm) (Fig. 1) suggested that a substitution occurred on the base moiety of I. It moved little towards an anode in paper electrophoresis (borate system),[20] indicating the absence of *cis*-glycol function in the sugar moiety.

Examination of nuclear magnetic resonance (NMR) spectrum of II taken in deuterated dimethylsulfoxide (Fig. 2A) revealed six proton signals at 3.52 (broad singlet, 2H), 3.64 (multiplet, 1H), 4.40 (doublet, 1H), 6.95 (singlet, 1H) and 8.43 (singlet, 1H) ppm and other six proton signals substituted in the presence of deuteroxide. The results indicated that the coupled C_5–C_6 vinylic protons of the cytosine moiety of the compound (I) had disappeared, suggesting the substitution occurred at C_5 or C_6-position of I. The singlet at 6.95 ppm must correspond to $C_{1'}$-proton of the sugar moiety, whereas the $C_{1'}$-proton signal of I appeared at 6.7—6.8 ppm as a doublet and those of cytidine and 1-β-D-arabinofuranosylcytosine at 5.9—6.2 ppm as dou-

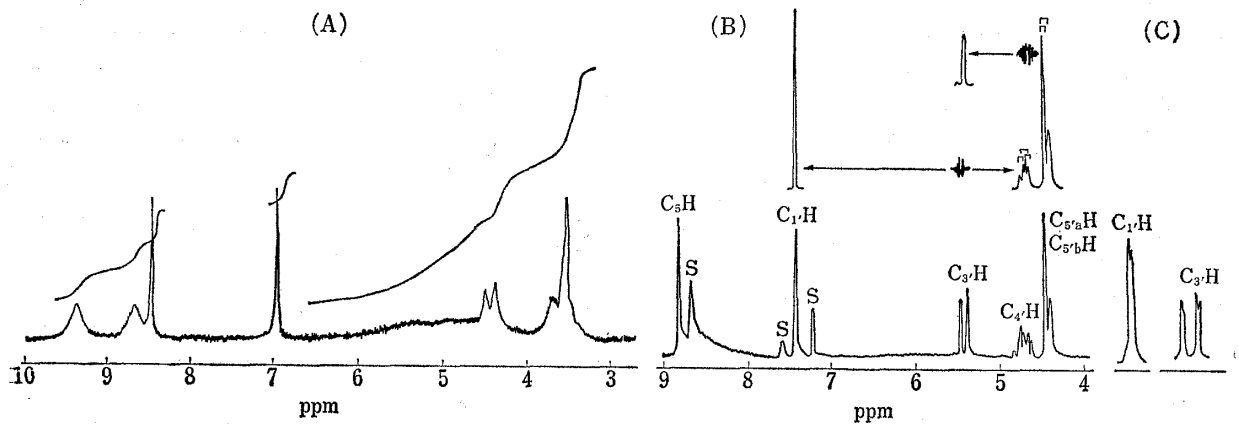

Fig. 2. NMR Spectra of Compound (II)

(A): NMR spectrum (60 MHz) taken in d_6-dimethylsulfoxide
(B): NMR spectrum (100 MHz) taken in d_5-pyridine–deuteroxide mixture with tetramethylsilane as an internal standard
Signals derived from the solvent are indicated by the symbol, S.
(C): Enlargement of the $C_{1'}H$ and $C_{3'}H$ signals in (B).

18) K. Kikugawa, *J. Pharm. Sci.*, **64**, 424 (1975).
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20) J.F. Codington, R. Fecher, and J.J. Fox, *J. Am. Chem. Soc.*, **82**, 2794 (1960).

blets. These results showed as well as C_5 or C_6-position of the base moiety $C_{2'}$-position of the sugar moiety was modified in the compound (II).

The most probable structure of the compound (II) was 6,2′-triazacyclocytidine, when the molecular model was examined as it fitted to the empirical formula containing three added nitrogens and to the structure with modification at $C_{2'}$-, and C_5- or C_6-position of I. NMR spectra (100 MHz) of II in deuterated pyridine–deuteroxide were examined and the typical charts are shown in Fig. 2B and 2C. The assignments of sugar proton resonances were confirmed by spin-decoupling experiments. The $C_{3'}H$ (5.42 ppm, doublet, 1H, $J_{3',4'}=7$ Hz) could be readily shown to be spin-coupled to the $C_{4'}H$ (4.69 ppm, multiplet, 1H), and the $C_{4'}H$ ($J_{4',5'_a}=5.5$ Hz, $J_{4',5'_b}=4$ Hz) was shown to be spin-coupled to the $C_{5'_a}H$ (4.45 ppm, doublet, 1H, $J_{4',5'_a}=5.5$ Hz) and to the $C_{5'_b}H$ (4.46 ppm, doublet, 1H, $J_{4',5'_b}=4$ Hz) with an ABX system. The signals of the $C_{5'_a}H$ and the $C_{5'_b}H$ appeared as an apparent doublet together with each other (Fig. 2B). The difference of the chemical shifts between the $C_{5'_a}H$ and the $C_{5'_b}H$ must be small (0.01 ppm) and both the protons might be spin-coupled to the $C_{4'}H$ with slightly differing intensity, 4 and 5.5 Hz, respectively. The $C_{1'}H$ and the $C_{3'}H$ must be long range-coupled, since spin-decoupling studies of the $C_{1'}H$ and the $C_{3'}H$ showed that the height of the signal of the $C_{1'}H$ became larger (Fig. 2B), and the enlargement of the signals of the $C_{1'}H$ (7.41 ppm) and the $C_{3'}H$ (5.42 ppm) revealed that the small coupling existed between these two protons (Fig. 2C). These results from 100 MHz NMR spectra of II were consisted with the assumed structure of II, 6,2′-triazacyclocytidine.

The other by-product (III), having the same empirical formula as II, was found to be 2′-azido-2′-deoxycytidine on the basis of its IR, UV, and NMR spectra. The compound (III) was readily converted into the known 2′-amino-2′-deoxycytidine (VI)[13] with catalytic hydrogenation. Another by-product (IV), which contained relatively high amount of nitrogen and no azido group, and consumed metaperiodate, could not be identified.

Several reactions were performed on 6,2′-triazacyclocytidine (II). Metaperiodate titration studies, initially intended to clarify the absence of *cis* or *trans*-glycol function in the sugar moiety, showed that II consumed unexpectedly 2 moles of the reagent (Fig. 3). The reaction product (V) was readily isolated. However, the structure of V could not be confirmed, even though the physicochemical data of V were determined by means of mass, UV and NMR spectroscopies, and elemental analysis.

Treatment of II in 0.1 N HCl at 80° for 2 hr gave two unidentified UV-absorbing substances, while II was intact in a caustic alkali at an elevated temperature. While the compound (II) was treated with bromine–water or an aqueous solution of sodium nitrite in order to examine whether bromination on the pyrimidine ring and deamination of an amino group of II took place or not, neither of reaction products showed UV-absorption maximum in region over 240 nm and were expected ones.

The formation of 2′-azido-2′-deoxycytidine (III) from I may be readily understandable from the known reaction of azide ion with O^2,2′-cyclouridine.[12,13] The formation of the major product, 6,2′-triazacyclocytidine (II), from I was, however, quite unexpected. The processes of the formation of this complicated compound (II) could not involve III as one of intermediate compounds, since treatment of III with lithium azide or lithium chloride in dimethylformamide at 80—90° did not give II. When 2′,3′-O-isopropylidene-O^2,5′-cyclocytidine[21] was treated with lithium azide under the same conditions, only 5′-azido-5′-deoxy-like compound was detected on a chromatogram, as was in the case of O^2,5′-cyclouridine derivative.[11] Thus, the formation of this unique type of cyclonucleoside as II might be attributed to the property of O^2,2′-cyclocytidine.

The compound (II) did not show any pharmacological activities including antitumor activity, when tested at Panlabs, Inc. and at the laboratories of this company.

21) J. Zěmlička and J. Smrt, *Tetrahedron Letters*, **1964**, 2081.

Experimental[22]

Reaction of O²,2′-Cyclocytidine Hydrochloride (I) with Lithium Azide——O²,2′-Cyclocytidine hydrochloride (I)[17,18] (800 mg, 3.05 mmoles) was added to a solution of 5.0 ml of dimethylformamide containing 800 mg of lithium azide prepared from sodium azide by use of a cation exchange resin, Diaion SK-1B (lithium form). The mixture was heated at 80°—90° for 72 hr with continuous shaking. Paper chromatography (solvent 1) of the amber-colored reaction mixture showed a major spot having *Rf* around 0.2 accompanying other two minor spots. Aqueous extract of the major spot showed UV absroption maximum at 300 nm, and those of the two minor spots showed maximum at 270 nm and 240 nm. The mixture was diluted with 150 ml of water (Total $OD^{pH\ 1}_{310}$: 17640) and applied to a column of Dowex 1×4 (HCO_3^-) (50 ml). The column which was washed with 1.0 liter of water was eluted with MeOH–0.1 M triethylamine bicarbonate (pH 7.5) (1: 1). Fractions containing the product (II) (Total $OD^{pH\ 1}_{310}$: 15435, recovery: 88%) were combined and evaporated *in vacuo* to dryness. The residue was co-evaporated with EtOH repeatedly to remove salt. Crystallization of the residue from EtOH gave 398 mg of the product (II) (yield, 47.5%). Recrystallization from water gave granules of 6,2′-triazacyclocytidine (II), mp 182—186° (decomp.). Paper chromatography showed single spot, *Rf* 0.21 (solvent 1) and *Rf* 0.52 (solvent 2), both consumed metaperiodate. $[\alpha]_D^{25}$ −13° (c=0.5, water).

Elemental analysis of II showed C, 39.42; H, 4.41; N, 30.96%; which was coincided with the calculated value for $C_9H_{12}O_4N_6\cdot1/3H_2O$ (Molecular weight: 268+6=274); C, 39.42; H, 4.66; N, 30.64%. High resolution mass spectroscopy of II revealed a molecular ion peak at *m/e* 268 and an elemental composition of $C_9H_{12}O_4N_6$. These results established the elemental composition of II to be $C_9H_{12}O_4N_6$. IR absorption spectrum of II (KBr) showed no azide absorption at 2160—2120 cm^{-1} region. UV $\lambda^{pH\ 1}_{max}$ nm (ε) 295 (14200), $\lambda^{pH\ 1}_{min}$ 257 (6300), $\lambda^{pH\ 4}_{max}$ 300 (16200), $\lambda^{pH\ 4}_{min}$ 250 (2600), $\lambda^{pH\ 13}_{shoulder}$ 250 (7200), $\lambda^{pH\ 13}_{max}$ 300 (20700), $\lambda^{pH\ 13}_{min}$ 260 (6000). Full UV absorption spectra are shown in Fig. 1. pK_a's determined spectrophotometrically were 2.0 and 7.0. NMR spectra (60 MHz and 100 MHz) are presented in Fig. 2. The assignments of sugar proton resonances were confirmed by spin-decoupling experiments. NMR (d_5-pyridine–deuteroxide, tetramethylsilane as an internal standard) δ, ppm: 8.81 (1H, singlet, C_5H), 7.41 (1H, singlet, $C_{1'}H$), 5.42 (1H, doublet, $C_{3'}H$, $J_{3',4'}$=7Hz), 4.69 (1H, multiplet, $C_{4'}H$), 4.45 (1H, doublet, $C_{5'a}H$, $J_{4',5'a}$=5.5 Hz), 4.46 (1H, doublet, $C_{5'b}H$ $J_{4',5'b}$=4Hz). Paper electrophoretic mobility of II was +3.0 cm, whereas those of cytidine and 1-β-D-arabinofuranosyl cytosine were +5.5 cm and +2.5 cm, respectively. A solution of II in EtOH which was vigorously shaken in a hydrogen atmosphere with 10% palladium-barium sulfate or 5% palladium-carbon catalyst for more than 10 hr contained no other compounds than II. The compound (II) was also recovered from a solution of II and $NaBH_4$ in water stored at room temperature for 6 hr.

The non-absorbing eluate of the Dowex 1 column was adjusted to pH 1 with concentrated hydrochloric acid and was adsorbed to a column of a Dowex 50×4 (H^+) (70 ml). The column was washed with water until the washings became neutral, and it was eluted with 200 ml of 3 N NH_4OH. The eluate was evaporated *in vacuo* to dryness, and the residue was applied onto a cellulose column (1.7×40 cm), which was eluted with *n*-butanol–water (84: 16) to obtain two UV-absorbing fractions. The first fraction was evaporated *in vacuo* to dryness and the residue was triturated with EtOH to give 2′-azido-2′-deoxycytidine (III) in a yield of 10.8% (88 mg), mp 216—222° (decomp.). Recrystallization from MeOH gave pure leaflets of III, mp 222—225° (decomp.). UV $\lambda^{pH\ 1}_{max}$ nm (ε) 280 (10600), $\lambda^{pH\ 1}_{min}$ 242 (1400), $\lambda^{pH\ 7}_{max}$ 272 (7200), $\lambda^{pH\ 7}_{min}$ 252 (5300), $\lambda^{pH\ 13}_{max}$ 273 (7500), $\lambda^{pH\ 13}_{min}$ 252 (4800). $[\alpha]_D^{25}$ +281° (c=0.68, water). NMR (d_6-dimethylsulfoxide, tetramethylsilane as an internal standard) δ, ppm: 7.85 (1H, doublet, C_6H, $J_{5,6}$=7 Hz), 7.22 (2H, broad singlet, NH_2), 5.93 (1H, doublet, $C_{1'}H$), 5.80 (1H, doublet, C_5H, $J_{5,6}$=7 Hz), 4.30 (1H, triplet, $C_{3'}H$), 3.9 (2H, multiplet, $C_{2'}H$ and $C_{4'}H$), 3.7 (2H, multiplet, $C_{5'}H$). IR ν^{KBr}_{max} cm^{-1} 2120 ($-N_3$). Paper chromatography showed single spot having *Rf* 0.32

22) Melting points were determined with Buchi melting point apparatus and uncorrected. UV absorption spectra were taken with a Hitachi Recording Spectrophotometer, EPS-3T. NMR spectra were taken with a Varian T-60 and a Varian HA-100 Spectrometer, and the measurements were greatly acknowledged to Mr. T. Kawashima and Mr. K. Gohgi of this company, and to Mr. S. Satoh of Nippon Electronic Varian Company, Ltd. High resolution mass spectroscopy was measured with a CEC 110B double-focus mass spectrometer, and the measurements were done by Mr. N. Wasada of the National Chemistry Laboratory for Industry. Optical rotation and IR absorption spectra were measured with a JASCO automatic polarimeter, DIP-SL and a Hitachi 285 Grating Infrared Spectrophotometer, respectively. Paper chromatography was carried out on Toyo Roshi No. 51A with solvent 1: *n*-butanol–water (84: 16) and solvent 2: iso-propanol–NH_4OH–water (7: 1: 2). Paper electrophoresis was performed with 0.02 M boric acid (pH 6.0) (ref. 20). Spots were detected by UV-ray. Cellulose column was prepared with cellulose powder (100—200 mesh) (Toyo Roshi Kaisha, Ltd.). A qualitative metaperiodate consumption test was done by spraying a 0.5% $NaIO_4$ solution and subsequently a 5% KI-starch solution. Metaperiodate titration studies were performed according to the method of Fox, *et al.* (ref. 23)

23) J.J. Fox, N. Yung, J. Davoll, and G.B. Brown, *J. Am. Chem. Soc.*, **78**, 2117 (1956).

(solvent 1) and *Rf* 0.61 (solvent 2) which did not consume metaperiodate. *Anal.* Calcd. for $C_9H_{12}O_4N_6$; C, 40.29; H, 4.50; N, 31.33%. Found: C, 40.16; H, 4.40; N, 31.21%.

The second fraction from the cellulose column was evaporated *in vacuo* to dryness, and the residue was triturated with water to afford yellow leaflets of the compound (IV), 20 mg, which did not melt below 270°. UV $\lambda_{max}^{pH\ 1}$ nm 221, $\lambda_{max}^{pH\ 7}$ 213, 244, $\lambda_{shoulder}^{pH\ 13}$ 233. IR spectrum showed no absorption at an azide region. Paper chromatography showed single spot having *Rf* 0.11 (solvent 1) and *Rf* 0.49 (solvent 2) both consumed metaperiodate. *Anal.* C, 32.09; H, 4.53; N, 61.05%.

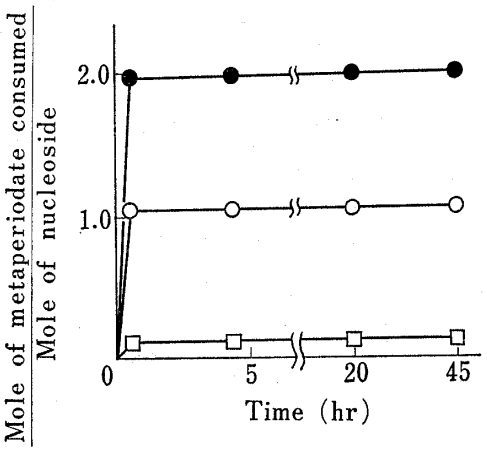

Fig. 3. Metaperiodate Titration Studies (at 20°) of Compound (II)

●: compound (II)
○: cytidine
□: O^2,2′-cyclocytidine hydrochloride

Reaction of 6,2′-Triazacyclocytidine (II)——(A) Metaperiodate Oxidation: Metaperiodate titration studies showed that II rapidly consumed 2 moles of the reagent, whereas the control compounds, cytidine and O^2,2′-cyclocytidine hydrochloride (I), consumed 1 mole and less than 0.2 mole of the reagent, respectively (Fig. 3). In order to obtain the reaction product (V), 40 mg (0.15 mmole) of II dissolved in 50 ml of water was treated with 150 ml of 0.008 M $NaIO_4$ (0.6 mmole). Precipitates thus formed were collected by filtration and recrystallized from dimethylformamide–water, 24 mg (yield 78%), mp above 270°. UV $\lambda_{max}^{pH\ 1}$ nm (ε) 315 (17100), $\lambda_{max}^{pH\ 7}$ 333 (27700), $\lambda_{max}^{pH\ 13}$ 342 (25700). NMR (d_6-dimethylsulfoxide, tetramethylsilane as an internal standard) δ, ppm: 9.67 (1H, singlet), 9.45 (2H, broad singlet), 8.64 (1H, singlet), 8.26 (1H, singlet). High resolution mass spectroscopy revealed a molecular ion peak at *m/e* 206. Paper chromatography showed *Rf* 0.51 (solvent 2). *Anal.* Calcd. for $C_7H_6O_2N_6$; C, 40.77; H, 2.93; N, 40.76%. Found: C, 40.65; H, 2.99; N, 40.80%. While V was intact in acid, treatment of V in 0.1 N NaOH at 80° for 10 min yielded a substance having UV absorption maxima at 278 (pH 1), 243, 312 (pH 7) and 241, 314 (pH 13) nm.

(B) Treatment with Acid and Alkali: While II was stable in 0.1 N NaOH at 80° for 2 hr, it was degraded into two compounds in 0.1 N HCl at 80° for 2 hr. Thus, paper chromatography (solvent 1) of the heated solution of II in 0.1 N HCl revealed two spots having *Rf* of 0.60 and 0.14. The aqueous extract of the higher spot had UV absorption maxima at 293 (pH 1), 299 (pH 7) and 301 (pH 13) nm, similar to those of II, while that of the lower had maxima at 269 (pH 1), 276 (pH 7) and 294 (pH 13) nm. Both spots consumed metaperiodate. Several attempts to isolate these two compounds were unsuccessful.

(C) Treatment with Bromine–Water: The compound (II) (50 mg) was treated with 10 ml of water containing 50 mg of bromine at room temperature overnight. The mixture was subjected to paper chromatography (solvent 1), which showed two spots having *Rf* of 0.35 and 0.17. Both spots showed UV absorption maximum at 240 nm (pH 13) after extraction with water.

(D) Treatment with Sodium Nitrite: The compound (II) gave non-UV-absorbing substance(s) by treatment with an aqueous solution of $NaNO_2$ at room temperature.

Reduction of 2′-Azido-2′-deoxycytidine (III)——2′-Azido-2′-deoxycytidine (III) (100 mg, 0.37 mmole) in 30 ml of MeOH was shaken in a hydrogen atmosphere with 100 mg of 5% palladium-carbon for 5 hr. The catalyst was removed by filtration and the filtrate was evaporated *in vacuo* to leave a colorless gum, which was crystallized from EtOH to afford 2′-amino-2′-deoxycytidine (VI), mp 195—196°. UV $\lambda_{max}^{pH\ 1}$ nm 279, $\lambda_{max}^{pH\ 7}$ 273. Paper chromatography (solvent 1) showed single spot having *Rf* 0.05, which was positive to ninhydrin reagent (lit.[13] mp 196—197°).