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Reaction of Pyrimidine Nucleosides with Sulfuryl Chloride

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Treatment of cytidine (I) with sulfuryl chloride in acetonitrile and pyridine gave 2,2′-anhydro-5,5′-dichloro-5′-deoxy-1-β-D-arabinofuranosylcytosine (II) as a main product, which was subsequently converted into 5,5′-dichloro-5′-deoxy-1-β-D-arabinofuranosylcytosine (III). The reaction of 1-β-D-arabinofuranosylcytosine (IX) with the reagent afforded 2,2′-anhydro-5,5′-dichloro-5′-deoxy-1-β-D-lyxofuranosylcytosine (XII). Uridine (XIV) was converted into 2,2′-anhydro-5,5′-dichloro-5′-deoxy-1-β-D-arabinofuranosyluracil 3′-O-chlorosulfate (XVI), which upon heating in water was transformed into 5,5′-dichloro-5′-deoxy-1-β-D-lyxofuranosyluracil (XVII). Treatment of I with the reagent in hexamethylphosphoroamide gave poly-sulfated derivatives of I.

Reactions of nucleosides with acid halids (RSO_2X, SOX_2, POX_3, and RCOX) have afforded useful derivatives of nucleoside or varsatile intermediates for nucleoside synthesis. Sulfonyl chloride (RSO_2X) may afford the corresponding O-sulfonate.[2] Thionyl chloride ($SOCl_2$) has exhibited various types of reactions depending on the structure of the substrate and the reaction conditions.[3–8] Thus, this reagent is capable of chlorinating the 5′-hydroxyl group of nucleoside[3,4] and in addition, with uridine and inosine, the hydroxyl group in the lactim form may also be chlorinated.[5] It may afford 2,2′-cyclized compounds with cytidine,[6] and 2′,3′-cyclic sulfinyl ribonucleosides[7] or a bis(ribonucleoside)-5′-sulfite.[8] Phosphorus oxychloride ($POCl_3$) has also shown various types of reactions with nucleosides.[9–12] It may phosphorylate a nucleoside to afford the corresponding nucleotide,[9] chlorinate the 6-hydroxyl group of purine nucleosides,[10] and convert uridine and cytidine into the 2,2′-cyclized products.[11,12] Reaction of acyl halides (RCOX), such as 2-acetoxyisobutyryl halide and acetyl bromide, with uridine and cytidine has afforded 3′-O-acetyl-2,2′-cyclized or 2′-halogeno-2′-deoxy-3′-O-acetyl derivatives,[13] and that with adenosine or its analog has afforded 3′-O-acetyl-2′-halogeno-2′-deoxy-1-β-D-arabinosyl and 2′-O-acetyl-3′-halogeno-3′-deoxy-1-β-D-xylosyl derivatives.[14]

1) Location: *51-Komiya-cho, Hachiouji, Tokyo.*
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The present paper describes the hitherto unknown reaction of sulfuryl chloride (SO_2Cl_2) with the pyrimidine nucleosides such as cytidine, 1-β-D-arabinofuranosylcytosine and uridine. It was found that the reaction gave several types of products depending on the structure of a substrate and the reaction conditions.

Cytidine (I) was treated with a 9 molar excess of sulfuryl chloride in acetonitrile in the presence of pyridine at room temperature. Two products were isolated by means of a cellulose column chromatography. The main product (II) detected on a paper chromatogram could not be isolated efficiently because it was quite unstable. It was isolated in a semi-sulfate form and exhibited ultraviolet (UV)-absorption maxima at 235, 278, and 298 (shoulder) nm (pH 1—7) similar to those of 2,2′-anhydro-5-chloro-1-β-D-arabinofuranosylcytosine.[15] Nuclear magnetic resonance (NMR) spectrum showed that a signal due to H_5 of I was absent and a signal due to H_2' shifted in a lower field, indicating that it had a 2,2′-anhydro-5-chloro structure. It was gradually transformed into III in an aqueous solution, which could be directly obtained in 49.8% yield from the reaction of I with SO_2Cl_2. The structure of III was demonstrated to be 5,5′-dichloro-5′-deoxy-1-β-D-arabinofuranosylcytosine by the following four lines of evidence: elemental analysis, UV-absorption spectrum similar to those of 5-chlorocytosine nucleosides,[15] metaperiodate titration studies, paper electrophoresis in borate buffer, and conversion into 5-chloro-2′,5′-anhydro-1-β-D-arabinofuranosylcytosine (IV) by heating with eth-

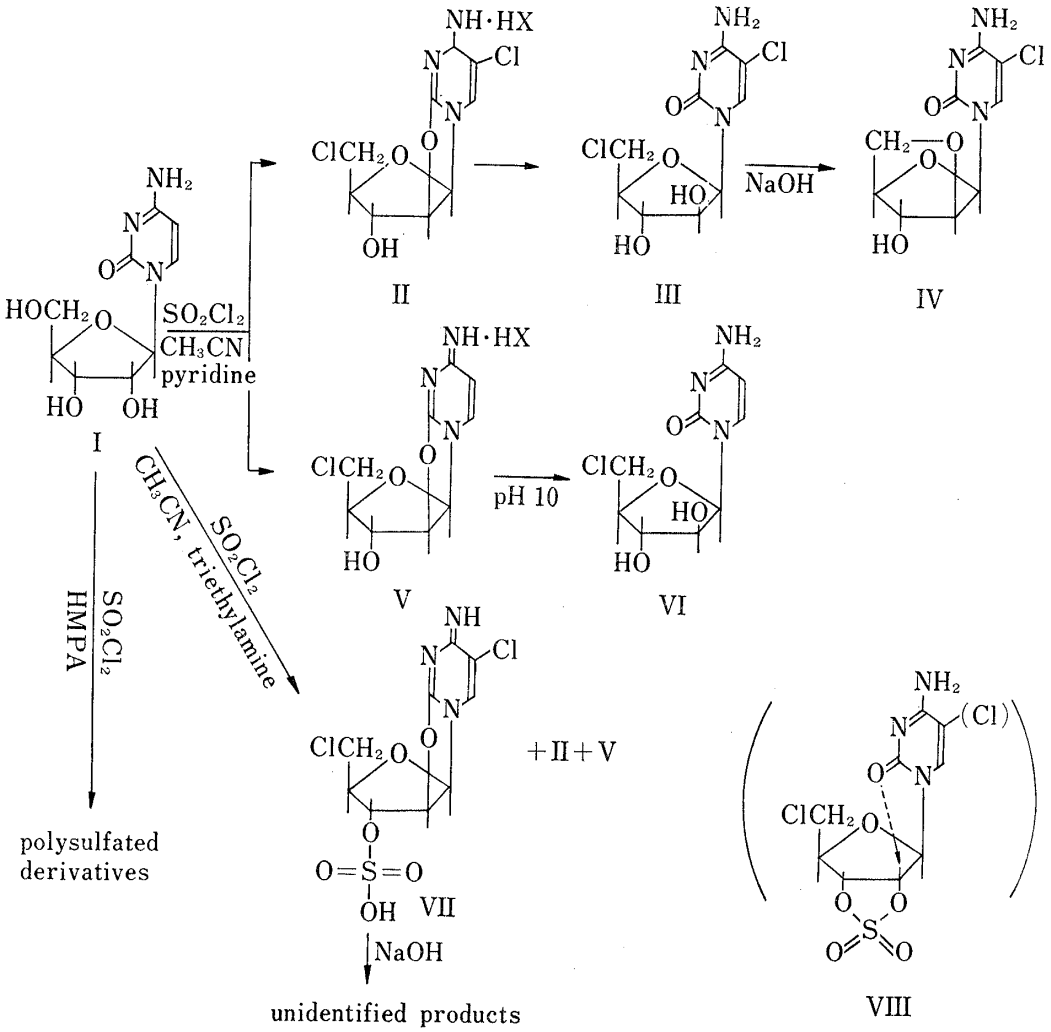

Chart 1

15) K. Kikugawa, I. Kawada, and M. Ichino, *Chem. Pharm. Bull.* (Tokyo), **23**, 35 (1975).

anolic NaOH. Thus, the structure of II was found to be 2,2′-anhydro-5,5′-dichloro-5′-deoxy-1-β-D-arabinofuranosylcytosine. The side product (V) obtained from the reaction of I with SO_2Cl_2 (2.8% yield) was identical with an authentic 2,2′-anhydro-5′-chloro-5′-deoxy-1-β-D-arabinofuranosylcytosine,[12] and it was converted into 5′-chloro-5′-deoxy-1-β-D-arabinofuranosylcytosine (VI)[12] on treatment at pH 10.

Cytidine (I) was treated with a 3 molar excess of SO_2Cl_2 in acetonitrile in the presence of triethylamine. Besides water-soluble products (II and V), an insoluble product (VII) was isolated in 11.4% yield. The structure of VII appeared to be 3′-O-sulfate of II on the basis of elemental analysis. In NMR spectrum, a signal due to H_5 was absent and those due to H_2' and H_3' were shifted in a lower field. UV-absorption spectrum was characteristic of the 5-chloro-2,2′-anhydro structure: the absorption maxima appeared at 243, 274.5, and 295 (shoulder) nm (pH 1—7), and a remarkable decrease in absorbance occurred in 0.1 N NaOH (Fig. 1). The absorbance of VII in 0.1 N NaOH at 22° gradually decreased to reach 4800 at $\lambda_{max}^{pH\,1}$ 285 nm, which was much lower than that of 5-chlorocytosine nucleosides: 11000 at $\lambda_{max}^{pH\,1}$ 289 nm.[15] Paper chromatography of VII treated in NaOH solution revealed the presence of several unidentified UV-absorbing spots. Thus, treatment of VII with NaOH gave products other than that derived by splitting the 2,2′-anhydro bond.

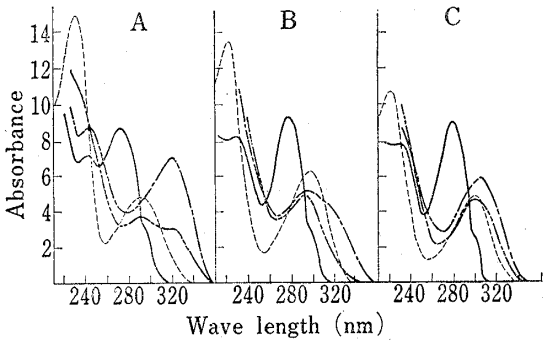

Fig. 1. UV-Absorption Spectra of 2,2′-Anhydro-5-chloro-1-β-D-arabinofuranosylcytosine Derivatives

A: 2,2′-anhydro-5,5′-dichloro-5′-deoxy-1-β-D-arabinofuranosylcytosine 3′-sulfate (VII)
B: 2,2′-anhydro-5,5′-dichloro-5′-deoxy-1-β-D-lyxofuranosylcytosine (XII)·1/2H_2SO_4
C: 2,2′-anhydro-5-chloro-1-β-D-arabinofuranosylcytosine·HCl (Ref. 15)
Spectra were taken in pH 1 HCl (———), in 0.1N NaOH for 5—10 min at 22° (— — —), in 0.1N NaOH for 30—100 min at 22° (———), and in pH 1 HCl after treatment of 0.1N NaOH (30—100 min) (--------).

Reaction of I with SO_2Cl_2 in acetonitrile included chlorination of 5- and 5′-position and 2,2′-cyclization affording the products, II, V, and VII. In these reactions the 2,2′-cyclization presumably proceeded *via* the 2′,3′-cyclic-O-sulfate (VIII).

When I was treated with SO_2Cl_2 in hexamethylphosphoroamide (HMPA), four negatively charged products were formed. These might be polysulfates of I or the epimerized compound (s) of I. These could not be isolated because their free forms were unstable. Reaction of I with SO_2Cl_2 in dimethylformamide (DMF) afforded 5′-chloro-5′-deoxycytidine and 2′,5′-anhydro-1-β-D-arabinofuranosylcytosine on subsequent treatment of the products with alkali, indicating that the reaction was 5′-chlorination and 2,2′-cyclization as was observed in the case of the reaction of I with $SOCl_2$–DMF.[12] In the reactions of I with SO_2Cl_2 in HMPA or DMF no 5-chloro-derivatives were found.

1-β-D-Arabinofuranosylcytosine (IX) was treated with a 9 molar excess of SO_2Cl_2 in acetonitrile and pyridine at room temperature. The water-soluble main-product (XII) was isolated in a semi-sulfate form in 55% yield. Its UV-absorption maxima at pH 1—7 appeared at 234, 277, and 298 (shoulder) nm, characteristic of the 2,2′-anhydro-5-chloro structure, and the spectra in 0.1 N NaOH was similar to those of an authentic 2,2′-anhydro-5-chloro derivative (Fig. 1). The absence of a signal due to H_5 and downfield shift of a signal due to H_2' supported the 2,2′-anhydro-5-chloro structure. Although XII gave unidentified products on treatment with 0.1 N NaOH, it was transformed into XIII in 70% yield when treated in a buffer (pH 9—10). Its structure was elucidated to be 5,5′-dichloro-5′-deoxy-1-β-D-lyxofuranosylcytosine by the following four lines of evidence: elemental analysis, UV-absorption spectrum characteristic of 5-chlorocytosine nucleosides,[15] metaperiodate titration studies, and paper electrophoresis in borate buffer. Thus, the structure of XII was established to be 2,2′-anhydro-5,5′-dichloro-5′-deoxy-1-β-D-lyxofuranosylcytosine. Elemental analysis of XII also supported the structure.

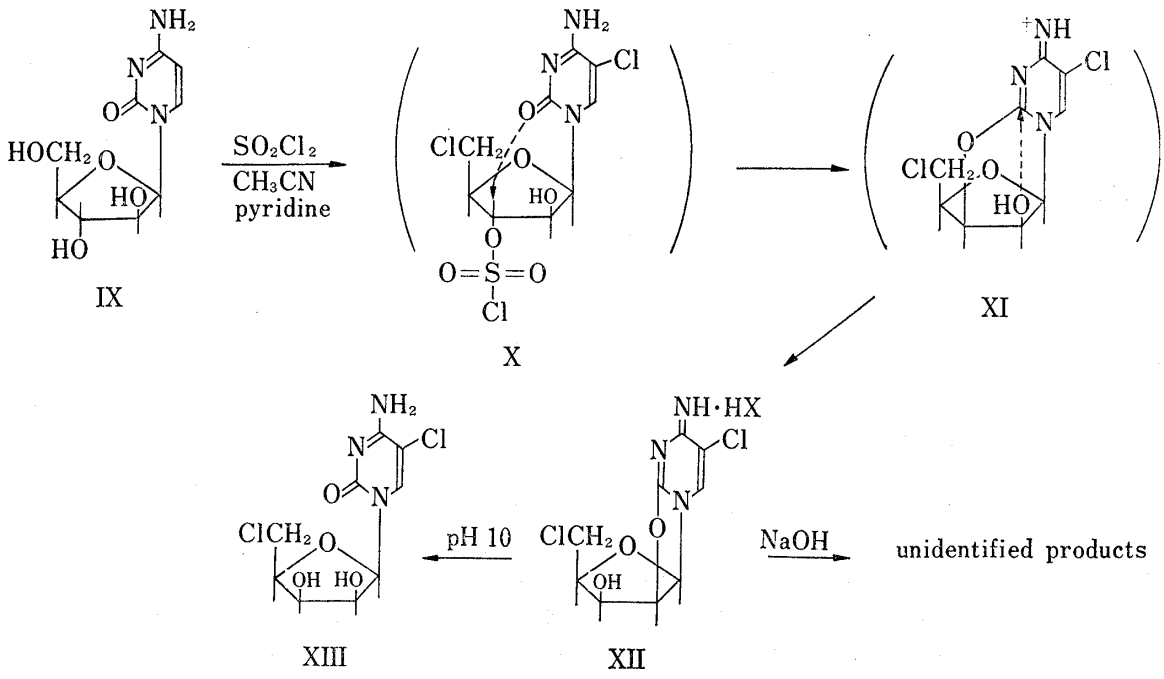

Chart 2

The mechanisms of the reaction of IX with SO_2Cl_2 might be as follows. Initially the 5,5′-dichloro-5′-deoxy-3′-O-chlorosulfate (X) was formed and in this intermediate the nucleophilic attack of the C_2 carbonyl group at the C_3' position occurred to form the 2,3′-anhydro-intermediate (XI). The nucleophilic attack of the 2′-hydroxyl function at the C_2 position occurred in XI to produce the final product (XII).

Uridine (XIV) was treated with SO_2Cl_2 in acetonitrile and pyridine. A water-insoluble product (XVI) was obtained in 19.4% yield. The UV-absorption maxima: 232, 260, and 280 (shoulder) nm suggested that it had a 2,2′-anhydro-5-chloro structure, since the spectrum was characteristic of 2,2′-anhydro derivatives and the maxima bathochromically shifted when compared to those of 2,2′-anhydro-1-β-D-arabinofuranosyluracil and 2,2′-anhydro-5-fluoro-1-β-D-arabinofuranosyluracil.[16] The absence of a signal due to H_5 and downfield shifts of signals due to H_2' and H_3' were observed in NMR spectrum. Treatment of XVI in

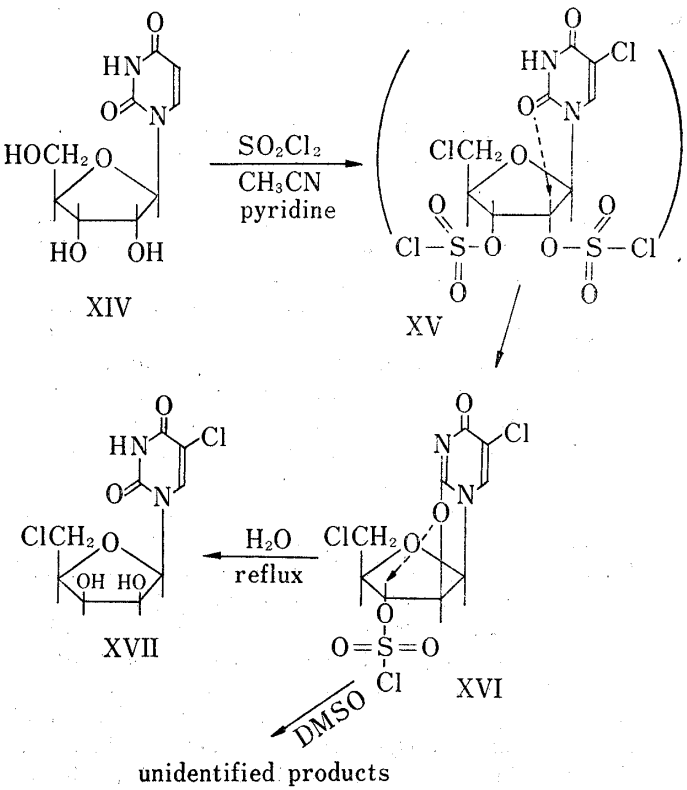

Chart 3

16) N.C. Yung, J.H. Burchenal, R. Fecher, R. Duschinsky, and J.J. Fox, *J. Amer. Chem. Soc.*, **83**, 4060 (1961).

water at refluxing temperature gave XVII quantitatively, which was elucidated to be 5,5′-dichloro-5′-deoxy-1-β-D-lyxofuranosyluracil by the following lines of evidence: elemental analysis, UV-absorption spectrum characteristic of 5-chlorouracil nucleosides,[15] NMR spectrum, and paper electrophoresis in borate buffer. Yung, *et al.*[16] have reported that treatment of 2,2′-anhydro-3′-O-methanesulfonyl-1-β-D-arabinofuranosyluracil in water at refluxing temperature gave 1-β-D-lyxofuranosyluracil. Analogous transformation of XVI into XVII indicated the structure of XVI to be 2,2′-anhydro-5,5′-dichloro-5′-deoxy-1-β-D-arabinofuranosyluracil-3′-O-chlorosulfate. Elemental analysis of XVI confirmed the structure, and the presence of the chlorosulfate group was substantiated by the reaction with aniline and pyridine to give a characteristic red dye.[17] The compound (XVI) was unstable in dimethylsulfoxide to form two unidentified 2,2′-anhydro-5-chloro compounds, probably due to the 3′-O-chlorosulfate function. In the reaction of XIV and SO_2Cl_2 the initial intermediate must be 2′,3′-di-O-chlorosulfate (XV).

Sulfuryl chloride has generally been known as a chlorination reagent of hydrocarbons and alcohols.[18] The reaction of the reagent with carbohydrates has become a well-established method for the preparation of chlorodeoxy sugars involving the initial formation of chlorosulfate.[17,19] In the case of the reaction of sucurose with the reagent, cyclic sulfate has been also formed.[20] In the present paper pyrimidine nucleosides, such as I, IX, and XIV, were treated with the reagent. In acetonitrile, 5,5′-chlorination and 2,2′-cyclization occurred in every compound *via* 2′,3′-cyclic-O-sulfate (VIII) or chlorosulfate (X and XV). In HMPA, poly-sulfated derivatives of I were formed.

Experimental[21]

Reaction of Cytidine (I) with Sulfuryl Chloride——(a) in Acetonitrile and Pyridine: Cytidine (I) (1.0 g, 4.12 mmoles) was suspended in 10 ml of acetonitrile and cooled in an ice-water bath. To the stirred suspension were added 3.0 ml (37.4 mmoles) of freshly distilled sulfuryl chloride and 0.4 ml (4.92 mmoles) of anhydrous pyridine. The mixture was stirred at room temperature overnight under anhydrous conditions. The clear

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21) Melting points were determined with a Buch melting point apparatus and uncorrected. UV-spectra were taken with a Hitachi recording spectrophotometer, EPS-3T. NMR spectra were taken in d_6-dimethylsulfoxide with a Varian T-60 spectrometer with tetramethylsilane as an internal standard. Optical rotation and infrared (IR) spectra were measured with a JASCO automatic polarimeter DIP-SL and a Hitachi 285 grating IR spectrophotometer, respectively. Elemental analyses (C,H,N) were carried out in our laboratory and those (Cl,S) at Shonan Analysis Center, Ltd. Paper chromatography was performed with the following solvent systems: 1, *n*-BuOH–H_2O (84: 16); 2, *n*-BuOH–AcOH–H_2O (2: 1: 1); 3, saturated $(NH_4)_2SO_4$–H_2O-iso-PrOH (79: 19: 2); 4, 5M NH_4OAc–0.5M ethylenediamine tetraacetic acid-Na_2–saturated borate–EtOH (60: 1.5: 240: 660). Rf_N refers to the *Rf* value obtained with the solvent N. Paper electrophoresis was done with the following buffer systems at 1000 V/25 cm for 1 hr: A, 0.02M NH_4OAc (pH 4.5); B, 0.02M phosphate (pH 7.5); C, borate (pH 6.0) (ref. 22). A qualitative metaperiodate consumption test on a paper sheet 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.*[23]: I consumed rapidly 1 mole of the reagent during 30 min and IX consumed slowly 0.94 mole of the reagent during 48 hr. Chlorosulfate group was detected with a spray reagent made of a *n*-BuOH solution of aniline and pyridine (ref. 17). OD and TOD refer to optical density and total optical density, respectively. The authors greatly acknowledge Mr. T. Kawashima and his co-workers of the laboratory for some physicochemical measurements.
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solution was evaporated *in vacuo* and dried over NaOH *in vacuo* at 50° for 2 hr. The gummy residue was dissolved in a mixture of 20 ml of ethyl acetate and 20 ml of water. The organic and the aqueous layers were separated. Paper chromatography of the organic layer revealed the presence of a weakly UV-absorbing spot (Rf_1 0.96 and Rf_2 0.95). The aqueous layer revealed the presence of a main tailing spot (Rf_1 0.5 and Rf_2 0.8) besides a spot arising from pyridine. The aqueous extract of the spot (Rf_1 0.5) had UV-absorption maxima at 234 and 274 nm (pH 1—7).

The acidic aqueous layer was evaporated to dryness, redissolved in 5 ml of water, and stored at room temperature for several days. A crystalline solid of 5,5′-dichloro-5′-deoxy-1-β-D-arabinofuranosylcytosine (III), 606.8 mg (49.8% yield), was collected by filtration, mp 217—219° (decomp.). Recrystallization from H_2O afforded pure needles of III, mp 221—222.5° (decomp.). $[\alpha]_D^{28}$ +126.4° (c=0.37, H_2O). UV: $\lambda_{max}^{pH\,1}$ nm ($\varepsilon\times10^{-3}$), 218 (10.9), 297.5 (10.1), $\lambda_{min}^{pH\,1}$ 251 (1.5), $\lambda_{max}^{H_2O}$ 217.5 (11.3), 286 (7.6), $\lambda_{min}^{H_2O}$ 260 (4.1), $\lambda_{max}^{pH\,13}$ 289 (7.8), $\lambda_{min}^{pH\,13}$ 260 (3.7). NMR: δ, ppm, 7.70 (1H, s, H_6), 6.13 (1H, d, $H_{1'}$). Rf_1 0.69 and Rf_2 0.75. Paper electrophoresis (buffer C) showed that III did not migrate as did a reference standard, 5-chloro-1-β-D-arabinofuranosylcytosine. It consumed 0.7 mole of metaperiodate during the period of 48 hr. *Anal.* Calcd. for $C_9H_{11}O_4N_3Cl_2$: C, 36.50; H, 3.74; N, 14.19; Cl, 23.95. Found: C, 36.45; H, 3.72; N, 14.37; Cl, 23.51.

The above aqueous layer was evaporated to dryness, and the residue was purified through a cellulose column as follows. It was applied onto a column (1.8×50 cm) of cellulose and the column was eluted with *n*-BuOH–H_2O (84: 16) to afford two fractions, I (100—500 ml) and II (500—700 ml). Fraction I was evaporated *in vacuo* to dryness, and the residue was dissolved in 50 ml of water and adjusted to pH 5.5 by Diaion SA-11B (HCO_3^-) with stirring. The resin was filtered off and the filtrate was passed through a column of Dowex 1×2 (sulfate) (30 ml). The combined effluent and washing were evaporated *in vacuo* to dryness. The residue was treated with H_2O–EtOH to give 65 mg of II·1/2H_2SO_4, mp 150° (decomp.). The unstable product could not be purified further. Its structure should be 2,2′-anhydro-5,5′-dichloro-5′-deoxy-1-β-D-arabinofuranosylcytosine·1/2H_2SO_4 as judged from the following evidence. UV-absorption spectra, measured in H_2O or 0.1N HCl, showed maxima at 235, 278 and 298 (shoulder) nm, and those measured in 0.1N NaOH showed a characteristic one of 2,2′-anhydro-5-chloro-1-β-D-arabinofuranosylcytosine structure.[15] NMR spectrum revealed the presence of signals at δ, ppm, 8.53 (1H, s, H_6), 6.26 (1H, d, $H_{1'}$, $J_{1',2'}$=6 Hz) and 5.63 (1H, t, $H_{2'}$, $J_{1',2'}$=6 Hz), indicating the downfield shift of a signal due to $H_{2'}$ characteristic of 2,2′-anhydro structure. The product traveled as a single spot (Rf_1 0.11) on a paper chromatogram and migrated towards the cathod (−0.9 cm) in paper electrophoresis (buffer A) as did 2,2′-anhydro-1-β-D-arabinofuranosylcytosine·HCl. This sample showed the positive test for analysis of chlorine atom and sulfate ion. It gradually decomposed to III in water.

Fraction II was then evaporated *in vacuo* to dryness. The residue was dissolved in 15 ml of water and adjusted to pH 4 by Diaion SA-11B (HCO_3^-) with stirring. The resin was filtered off and the filtrate was passed through a column of Dowex 1×2 (sulfate) (10 ml). The combined effluent and washings were evaporated *in vacuo* to dryness. The residue was crystallized from EtOH to afford 2,2′-anhydro-5′-chloro-5′-deoxy-1-β-D-arabinofuranosylcytosine (V)·1/2H_2SO_4, 33 mg (2.8% yield), mp 204—208° (decomp.). Recrystallization from EtOH gave a pure sample melting at 229—232° (decomp.). UV: $\lambda_{max}^{pH1\text{-}7}$ nm ($\varepsilon\times10^{-3}$), 233 (9.1), 262.5 (10.2), 283 (shoulder) (2.9), $\lambda_{min}^{pH1\text{-}7}$ 244 (7.1). Rf_1 0.05 and Rf_2 0.64. *Anal.* Calcd. for $C_9H_{10}O_3N_3Cl$·1/2H_2SO_4: C, 36.93; H, 3.79; N, 14.36. Found: C, 36.45; H, 3.79; N, 14.27.

The compound (V·1/2H_2SO_4) was converted into the HCl salt, which was found identical with an authentic 2,2′-anhydro-5′-chloro-5′-deoxy-1-β-D-arabinofuranosylcytosine·HCl.[12] Thus, the comparisons of Rf_1 value and mixed fusion test showed that both compounds were identical. The compound (V·1/2H_2SO_4) (101 mg) was dissolved in 5 ml of water and adjusted to pH 9 with NH_4OH. After standing at room temperature for 15 min, the mixture was evaporated *in vacuo* to dryness. The residue was crystallized from H_2O–EtOH to afford VI, 65 mg, mp 202—204° (decomp.). UV: λ_{max}^{pH1} nm, 281.5, $\lambda_{max}^{H_2O.pH13}$ 273. Rf_1 0.51 and Rf_2 0.65. Comparison of IR spectra (KBr) and mixed fusion test of VI with an authentic sample[12] showed that the structure of VI was 5′-chloro-5′-deoxy-1-β-D-arabinofuranosylcytosine.

(b) In Acetonitrile and Triethylamine: Cytidine (I) (1.0 g, 4.12 mmoles) was suspended in 4 ml of acetonitrile and was cooled in an ice-water bath. To the stirred suspension were added 1.0 ml (12.5 mmoles) of sulfuryl chloride and 0.2 ml (1.44 mmoles) of triethylamine. The mixture was stirred at room temperature overnight. The solution was evaporated *in vacuo* to dryness and dried. The gummy residue was shaken with a mixture of 80 ml of ethyl acetate and 80 ml of water. The organic and the aqueous layers were separated. Paper chromatography of the aqueous layer revealed the presence of a main tailing spot as observed in the reaction (a) described above. The organic layer was evaporated *in vacuo* to dryness, and the residue was triturated with EtOH to give a white crystalline solid. It was collected by filtration, dried and weighed, 549 mg. It was suspended in 85 ml of water, and heated at 80° for 25 min. The mixture was stored in a refrigerator overnight to afford white needles of 2,2′-anhydro-5,5′-dichloro-5′-deoxy-1-β-D-arabinofuranosylcytosine 3′-O-sulfate (VII), 169 mg (11.4% yield), mp 236—250° (decomp.). Recrystallization from a large amount of water afforded pure needles, mp 243—247° (decomp.). $[\alpha]_D^{26}$ −70.8° (c=0.50, H_2O). UV: $\lambda_{max}^{pH1\text{-}6}$ nm ($\varepsilon\times10^{-3}$), 243 (7.4), 274.5 (9.2), 295 (shoulder) (3.3), $\lambda_{min}^{pH1\text{-}7}$ 252 (6.4). Full spectra are shown in Fig. 1. NMR: δ, ppm, 8.73 (1H, s, H_6), 6.24 (1H, s, $H_{1'}$), 5.6 (2H, unresolved m, $H_{2'\text{ and }3'}$), 4.70 (1H, t, $H_{4'}$), 4.02 (1H,

s, $H_{5'a}$), 3.93 (1H, s, $H_{5'b}$). Rf_1 0.38 and Rf_2 0.62. Chlorine and sulfur atoms were present in the molecule, but chlorosulfate group was absent. *Anal.* Calcd. for $C_9H_9O_6N_3SCl_2$: C, 30.18; H, 2.53; N, 11.73. Found: C, 29.82; H, 2.54; N, 11.74.

(c) In HMPA: A mixture of 25 ml of HMPA and 2.5 ml (31.2 mmoles) of sulfuryl chloride was cooled in an ice-water bath, and to this was added 1.0 g (4.12 mmoles) of I under cooling. The mixture was stirred at room temperature for 5 hr, and it was poured into 300 ml of stirred ice-water. Paper chromatography of the aqueous mixture (solvent 1) revealed the presence of a spot at the origin, aqueous extract of which showed UV-absorption maxima at 280 nm (pH 1) and 272 (pH 7) nm. The solution was adsorbed to a column of activated charcoal (40 g). The column was washed with water until the washings became neutral and eluted with 1.0 liter of 10% NH_4OH–EtOH (1: 1). The eluate was evaporated *in vacuo* to dryness and redissolved in 100 ml of water. The solution was filtered through cotton fibers and passed through a column of Dowex 50×8 (H^+) (50 ml). The effluent and washings (100 ml) were combined. TOD (at 280 nm and pH 1) of the solution was 57600, and the ratio of OD (at 280 nm and pH 1) to that (at 260 nm and pH 1) was 2.15. Paper electrophoresis (buffer B) of the solution showed three spots: all migrating to the anode; +6.2 cm, +8.5 cm and +10.5 cm (reference standards: I, +1.0 cm; 5′-CMP, +7.5 cm). One fourth (50 ml) of the solution, after adjusted to pH 9 with NH_4OH, was applied to a column of diethylaminoethyl (DEAE) cellulose. The column was eluted with triethylamine bicarbonate buffer (Fig. 2). Three fractions were obtained: I [recovery based on TOD (at 280 nm and pH 1): 18.9%], II (46.5%) and III (32.2%). Each of the fractions was evaporated *in vacuo* to dryness. Paper electrophoresis (buffer B) and paper chromatography of these fractions showed the following mobilities and *Rf* values: I, +6.2 cm and Rf_3 0.6; II, +8.5 cm and Rf_3 0.5 and 0.6 (two spots); and III, +10.5 cm and Rf_3 0.6. These data indicated that the products were negatively charged and must be sulfate esters of I or the epimerized compound(s) derived from I. Isolation of these products could not be performed because they were unstable.

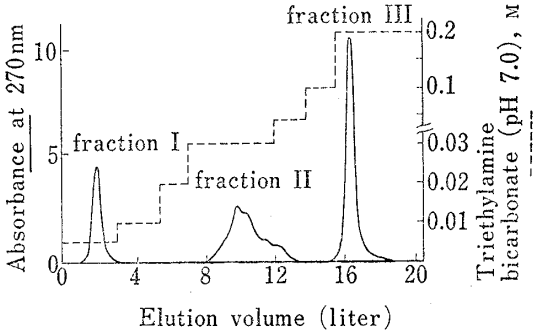

Fig. 2. DEAE Cellulose Column Chromatography of the Reaction Products of Cytidine (I) with Sulfuryl Chloride in HMPA

column: DEAE cellulose (HCO_3^-) (2.3×31 cm)

(d) In DMF: To a mixture of DMF (12.5 ml) and 1.3 ml (16.2 mmoles) of sulfuryl chloride was added 500 mg (2.06 mmoles) of I. The mixture was stirred at room temperature for 3 days. The mixture was then poured into 100 ml of stirred ice-water, and the aqueous mixture was adsorbed to a column of Diaion SK-1B (H^+) (150 ml). The column, after being washed with water, was eluted with 500 ml of 3N NH_4OH. The combined effluent and washings were evaporated to a small volume and submitted to paper chromatography. Two UV-absorbing spots (solvent 1 and 4) were detected: one corresponded to 5′-chloro-5′-deoxycytidine[12] and another to 2′,5′-anhydro-1-β-D-arabinofuranosylcytosine.[12] UV-absorption spectra and metaperiodate consumption test confirmed the structures.

Reaction of 1-β-D-Arabinofuranosylcytosine (IX) with Sulfuryl Chloride in Acetonitrile and Pyridine——1-β-D-Arabinofuranosylcytosine (IX)[12] (1.0 g, 4.12 mmoles) was suspended in 10 ml of acetonitrile and the mixture was cooled in an ice-water bath. To the stirred suspension were added 3.0 ml (37.4 mmoles) of sulfuryl chloride and 0.4 ml (4.92 mmoles) of anhydrous pyridine. The mixture was stirred at room temperature overnight. The solution was evaporated *in vacuo* below 30° and then dried. The white gummy residue was shaken with a mixture of 20 ml of ethyl acetate and 20 ml of water. The organic layer was extracted with 20 ml of water again, and the combined aqueous layers were evaporated *in vacuo* to dryness. The residue was dissolved in 50 ml of water and a small amount of an insoluble material was filtered off. Paper chromatography of the solution revealed the presence of a main tailing spot (Rf_1 0.5), the aqueous extract of which showed UV-absorption maxima at 234 and 275 nm (pH 1—7). The solution was passed through a column of Diaion SA-11B ($HCOO^-$) (90 ml) and the column was washed with 70 ml of water. The combined effluent and washings were adjusted to pH 2.4 with formic acid and lyophillized. The residue was dissolved in 30 ml of water and evaporated *in vacuo* to remove remaining pyridine. The residue was redissolved in 30 ml of water and passed through a column of Dowex 1×2 (sulfate) (15 ml). The column was washed with 35 ml of water. The combined effluent and washings were adjusted to pH 4 by Diaion SA-11B (HCO_3^-) with stirring. The resin was filtered off and the filtrate was evaporated *in vacuo* to dryness. The residue was triturated with 3 ml of EtOH to afford crystals. 2,2′-Anhydro-5,5′-dichloro-5′-deoxy-1-β-D-lyxofuranosylcytosine (XII)·1/2H_2SO_4 was collected by filtration, dried and weighed 780.8 mg (55.0% yield), mp 155.5—164° (decomp.). Recrystallization from EtOH–H_2O gave white columns of XII·1/2H_2SO_4, mp 155—158° (decomp.). $[\alpha]_D^{24}$ −69.0° (c=0.44, H_2O). UV: λ_{max}^{pH1} nm ($\varepsilon\times10^{-3}$), 234 (7.9), 277 (9.3), 298 (shoulder) (3.0), λ_{min}^{pH1} 251 (4.3), $\lambda_{max}^{H_2O}$ 234 (8.1), 277 (9.5), 298 (shoulder) (3.0), $\lambda_{min}^{H_2O}$ 251 (4.3). Full spectra are

shown in Fig. 1. NMR: δ, ppm, 8.66 (1H, s, H_6), 6.40 (1H, d, $H_{1'}$, $J_{1',2'}=5.5$ Hz), 5.75 (1H, t, $H_{2'}$, $J_{1',2'}=5.5$ Hz). Rf_1 0.07 and Rf_2 0.66. Paper electrophoresis (buffer A) showed that XII migrated towards the cathod (−9.0 cm) as did 2,2′-anhydro-1-β-D-arabinofuranosylcytosine·HCl. *Anal.* Calcd. for $C_9H_9O_3N_3Cl_2\cdot 1/2H_2SO_4\cdot H_2O$: C, 31.32; H, 3.50; N, 12.18; Cl, 20.54; S, 4.65. Found: C, 31.16; H, 3.39; N, 12.55; Cl, 20.86; S, 4.82.

Reaction of Uridine (XIV) with Sulfuryl Chloride in Acetonitrile and Pyridine——Uridine (XIV) (1.0 g, 4.10 mmoles) was suspended in 10 ml of acetonitrile and was cooled in an ice-water bath. To the stirred suspension were added 3.0 ml of sulfuryl chloride and 0.4 ml of anhydrous pyridine. The mixture was stirred at room temperature overnight. The solution was evaporated *in vacuo* to dryness and dried. The gummy solid was treated with a mixture of 50 ml of ethyl acetate and 50 ml of water. Crystals separated in the mixture were collected by filtration. Paper chromatography of the aqueous layer of the filtrate showed no UV-absorbing spots other than those arising from pyridine. The organic layer was washed with water and evaporated to dryness. The residue was triturated with EtOH to give additional crystals. The crystals were combined and recrystallized from acetonitrile to give granules of 2,2′-anhydro-5,5′-dichloro-5′-deoxy-1-β-D-arabinofuranosyluracil 3′-O-chlorosulfate (XVI), 300 mg (19.4% yield), mp 216—218° (decomp.). $[\alpha]_D^{25}$ −122.6° ($c=0.42$, CH_3CN). UV: $\lambda_{max}^{CH_3CN}$ nm ($\varepsilon\times10^{-3}$), 232 (6.8), 260 (7.4), 283 (shoulder) (2.0), $\lambda_{min}^{CH_3CN}$ 213 (4.6), 243 (6.0). NMR: δ, ppm, 8.50 (1H, s, H_6), 6.50 (1H, d, $H_{1'}$), 5.6—6.2 (2H, unresolved m, $H_{2'\text{ and }3'}$), 4.85 (1H, d, $H_{4'}$), 3.92 (1H, s, $H_{5'a}$), 3.83 (1H, s, $H_{5'b}$). Rf_2 0.88. It produced red dye by spraying aniline and pyridine. *Anal.* Calcd. for $C_9H_7O_6N_2SCl_3$: C, 28.63; H, 1.87; N, 7.42; Cl, 28.17; S, 8.49. Found: C, 28.89; H, 1.92; N, 7.52; Cl, 28.51; S, 8.98.

NMR spectrum of XVI in d_6-dimethylsulfoxide stored at room temperature for a few days was different from that measured immediately. Thus, the spectrum revealed the presence of signals at δ, ppm, 8.50 (1H, broad s, H_6), 6.40 (1H, broad d, $H_{1'}$), 5.65 (1/2H, d, $H_{2'}$) and 5.37 (1/2H, d, $H_{2'}$). Paper chromatography of the solution revealed the presence of two spots (Rf_1 0.25 and 0.66, and Rf_2 0.51 and 0.77), neither of which corresponded to XVI. An aqueous extract of every spot showed the similar UV-absorption spectrum as XVI (λ_{max}^{pH1,H_2O} nm, 228 (shoulder), 264, 288 (shoulder)). The results indicated that XVI was converted into two compounds having 2,2′-anhydro-5-chloro-1-β-D-arabinofuranosyluracil structure.

5-Chloro-2′,5′-anhydro-1-β-D-arabinofuranosylcytosine (IV)——5,5′-Dichloro-5′-deoxy-1-β-D-arabinofuranosylcytosine (III) (500 mg, 1.68 mmoles) was dissolved in 62 ml of EtOH, and to this was added 16.8 ml of 0.1N NaOH. The mixture was refluxed for 2.5 hr. After the addition of further 2.0 ml of 0.1N NaOH, the mixture was refluxed for additional 30 min. The mixture was evaporated *in vacuo* to dryness and the residue was dissolved in 5 ml of water. Crystalline solid of IV separated was collected by filtration and dried, 279.2 mg (64% yield), mp 241.5—243° (decomp.). Recrystallization from EtOH gave pure specimen, mp 242—245° (decomp.). $[\alpha]_D^{22}$ +176.6° ($c=0.47$, H_2O). UV: λ_{max}^{pH1} nm ($\varepsilon\times10^{-3}$), 219 (10.2), 300 (10.7), λ_{min}^{pH1} 253 (0.95), $\lambda_{max}^{H_2O}$ 219 (11.6), 288 (7.59), $\lambda_{min}^{H_2O}$ 260 (3.5), λ_{max}^{pH13} 290 (7.6), λ_{min}^{pH13} 260 (3.1). NMR: δ, ppm, 7.96 (1H, s, H_6), 6.04 (1H, s, $H_{1'}$). Rf_1 0.42 and Rf_2 0.63. *Anal.* Calcd. for $C_9H_{10}O_4N_3Cl$: C, 41.63; H, 3.88; N, 16.18: Cl, 13.66. Found: C, 41.37; H, 3.91; N, 16.14; Cl, 13.69.

5,5′-Dichloro-5′-deoxy-1-β-D-lyxofuranosylcytosine (XIII)——2,2′-Anhydro-5,5′-dichloro-5′-deoxy-1-β-D-lyxofuranosylcytosine (XII)·$1/2H_2SO_4$ (300 mg, 0.87 mmoles) was dissolved in 50 ml of 0.1M triethylamine bicarbonate (pH 9.65), and the mixture was stored at room temperature overnight. The mixture was evaporated *in vacuo* to dryness, and the residue was crystallized from EtOH. 5,5′-Dichloro-5′-deoxy-1-β-D-lyxofuranosylcytosine (XIII) was obtained in 70.0% yield (180 mg), mp 192—194° (decomp.). Recrystallization from EtOH–H_2O gave pure sample of XIII, mp 202—206° (decomp.). $[\alpha]_D^{26}$ +105.7° ($c=0.40$, H_2O). UV: λ_{max}^{pH1} nm ($\varepsilon\times10^{-3}$), 218 (11.3), 298 (10.7), λ_{min}^{pH1} 251 (1.0), $\lambda_{max}^{H_2O}$ 218 (11.9), 287 (7.5), $\lambda_{min}^{H_2O}$ 261 (3.9), λ_{max}^{pH13} 289 (7.1), λ_{min}^{pH13} 260 (3.4). NMR: δ, ppm, 8.03 (1H, s, H_6), 6.13 (1H, d, $H_{1'}$). Rf_1 0.63 and Rf_2 0.73. Paper electrophoresis (buffer C) showed that the compound migrated towards the anode: +5.7 cm (reference standards: 5-chlorocytidine, +3.8 cm and 5-chloro-1-β-D-arabinofuranosylcytosine, 0 cm). It consumed rapidly 1 mole of metaperiodate within 30 min. *Anal.* Calcd. for $C_9H_{11}O_4N_3Cl_2$: C, 36.50; H, 3.74; N, 14.19; Cl, 23.95. Found: C, 36.64; H, 3.75; N, 14.24; Cl, 23.93.

5,5′-Dichloro-5′-deoxy-1-β-D-lyxofuranosyluracil (XVII)——2,2′-Anhydro-5,5′-dichloro-5′-deoxy-1-β-D-arabinofuranosyluracil 3′-O-chlorosulfate (XVI) (200 mg) was suspended in 50 ml of water and refluxed for 5 hr. The solution was evaporated to dryness and the residue was crystallized and recrystallized from a small amount of water, mp 191—193° (decomp.). $[\alpha]_D^{28}$ −0.84° ($c=0.36$, dimethylsulfoxide). UV: λ_{max}^{pH1} nm ($\varepsilon\times10^{-3}$), 277.5 (9.1), λ_{min}^{pH1} 240 (1.8), $\lambda_{max}^{H_2O}$ 277.5 (9.1), $\lambda_{min}^{H_2O}$ 240 (1.8), λ_{max}^{pH13} 277 (6.9), λ_{min}^{pH13} 250 (3.4). NMR: δ, ppm, 8.26 (1H, s, H_6), 6.13 (1H, d, $H_{1'}$). Rf_1 0.75, Rf_2 0.79 and Rf_4 0.80. A metaperiodate consumption test on a paper sheet was positive. Paper electrophoresis (buffer C) of XVII migrated to the anode: +6.1 cm (reference standards: 5-chlorouridine, +3.4 cm: 5-chloro-1-β-D-arabinofuranosyluracil, 0 cm). *Anal.* Calcd. for $C_9H_{10}O_5N_2Cl_2$: C, 36.38; H, 3.39; N, 9.43. Found: C, 36.41; H, 3.31; N, 9.54.