[Chem. Pharm. Bull. 16(3) 430—436 (1968)]

UDC 547.963.3.07

Studies on Nucleosides and Nucleotides. X.[1] Nucleophilic Substitution of Secondary Sulfonyloxy Groups of Pyrimidine Nucleosides. III. Reaction of 2′,3′-Di-O-tosyluridine with Methanolic Ammonia[2]

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(Received April 25, 1967)

1-(2′,3′-Epoxy-β-D-lyxofuranosyl)isocytosine (IV) was obtained in the reaction of 2′,3′-di-O-tosyluridine (I) with methanolic ammonia through 2,2′-anhydro-1-(3′-O-tosyl-β-D-arabinofuranosyl)uracil (II) and 2-O-methyl-1-(2′,3′-epoxy-β-D-lyxofuranosyl)-uracil (III).

Ultraviolet absorption spectra of 2-substituted-uracils and their nucleosides were comparatively examined and their pK_a' values were determined.

From the nuclear magnetic resonance spectra it was found that amino group of isocytosine derivatives was existed as amino form and that H_1' of 2′,3′-epoxylyxosyl nucleosides did not couple with H_2' and its signal appeared as a sharp singlet.

This paper deals with the reaction of 2′,3′-di-O-tosyluridine (I) or 2,2′-anhydro-1-(3′-O-tosyl-β-D-arabinofuranosyl)uracil (II) with methanolic ammonia and also describes the physical properties of 2-substituted pyrimidine nucleosides obtained from above reactions.

Brown[4] and his coworkers demonstrated that reaction of 5′-O-acetyl-2′-O-tosyluridine with half saturated methanolic ammonia gives 2,2′-anhydro-1-β-D-arabinofuranosyluracil and 1-β-D-arabinofuranosylisocytosine, but 3′-tosyloxy derivative does not undergo such a reaction under the same conditions.

They[5] also reported that reaction of 2,5′-anhydro-1-(2′,3′-O-isopropylidene-β-D-ribofuranosyl)uracil with methanolic ammonia affords 1-(2′,3′-O-isopropylidene-β-D-ribofuranosyl)isocytosine *via* 2-O-methyluracil derivative.

It was suggested from Brown's result that treatment of I or II with methanolic ammonia or alkylamine would give isocytosine derivatives *via* 2-O-methyluracil derivative, but there were few reports concerned with isocytosine and 2-O-methyluracil derivatives.

As shown in Chart 1, compound (IV) or (V) was obtained in the reaction of I or II with methanolic ammonia or alkylamine, and treatment of II with dilute methanolic ammonia gave III, which in turn formed IV on treatment with concentrated methanolic ammonia. Compound (III) was also obtained in reaction of I or II with sodium methoxide in methanol. The above mentioned fact showed that II and III were the intermediates in this nucleophilic reactions. Ultraviolet absorption spectrum of III exhibited the two maxima at 228 and 251 mμ, and existence of one methoxy group was shown by elemental analysis. The spectra of IV and V were similar to that of 1-(2′,3′-O-isopropylidene-β-D-ribofuranosyl)isocytosine[5] and IV travelled toward an anode in electrophoresis. Furthermore, the reaction of III with 0.1 N hydrochloric acid or IV with nitrous acid gave the sole product which was identical with 1-(2′,3′-epoxy-β-D-lyxofuranosyl)uracil (VI), prepared from 2′,3′,5′-tri-O-mesyluridine by Fox.[6] Therefore, III, IV, and V were established as 2-O-methyl-1-(2′,3′-epoxy-β-D-

1) Part IX: *Chem. Pharm. Bull.* (Tokyo), **16**, 291 (1968).
2) Preliminary communication of this work appeared in *Chem. Pharm. Bull.* (Tokyo), **13**, 1258 (1965).
3) Location: *Minamifunabori-cho, Edogawa-ku, Tokyo.*
4) D.M. Brown, D.B. Parihar, Sir A. Todd, and S. Varadarajan, *J. Chem. Soc.*, **1958**, 3028.
5) D.M. Brown, Sir A. Todd, and S. Varadarajan, *J. Chem. Soc.*, **1957**, 868.
6) J.F. Codington, R. Fecher, and J.J. Fox, *J. Org. Chem.*, **27**, 163 (1962).

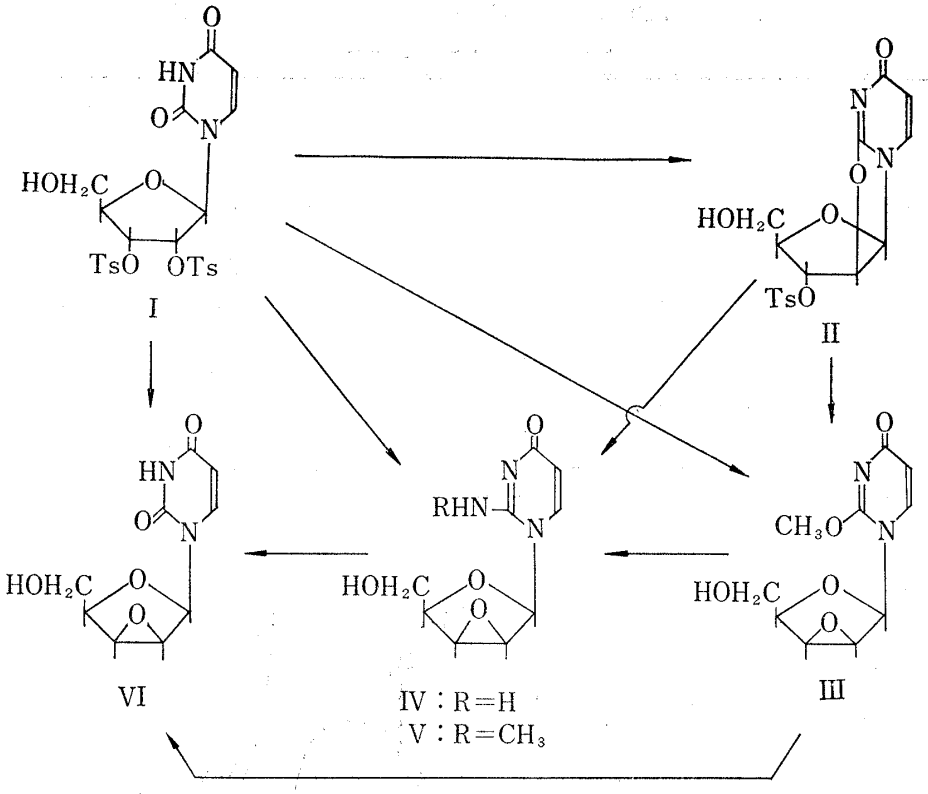

Chart 1

lyxofuranosyl)uracil, 1-(2′,3′-epoxy-β-D-lyxofuranosyl)isocytosine, and 2-N-methyl-1-(2′,3′-epoxy-β-D-lyxofuranosyl)isocytosine, respectively.

It was experimentally established that the reaction of I with base proceeded through II, which formed 2-O-methylpyrimidine derivative (III) by aryl-oxygen fission and epoxidation with methanolic base. Subsequent amination of methoxy group of III resulted in the formation of IV or V.

The spectroscopic properties of N_1-substituted isocytosine and 2-O-alkyluracil derivatives such as IV and III have been less investigated than cytosine derivatives. Therefore, ultraviolet absorption and nuclear magnetic resonance spectra of these nucleosides were examined and some new observations were gained, which are reported herein.

Ultraviolet Absorption Spectra——Recently, Ueda and Fox[7] reported on a difference in pK_{a1} values between 1-methyl- and 3-methyl-cytosine, whose pK_a values were 4.55 and 7.38, respectively, and demonstrated that this difference is explained in basicity of their site of protonation. Ultraviolet absorption spectra of IV and III were compared in different pH with those for 1-methylisocytosine (X), 3-methylisocytosine (XI), 2-O-ethyl-1-methyluracil (VIII), and 2-O-ethyl-3-methyluracil (IX) as model compounds, then apparent pK_a values of these six compounds were spectrophotometrically determined. These results are listed in Table I.

pK_a values of IV are similar to those of X and XI, and no difference in pK_a values is found between X and XI as in the case of cytosine, but ultraviolet absorption spectra exhibit an apparent difference between these two compounds.

As shown in Fig. 2 and 3, the spectra of X show almost no shift of the absorption maxima at about 260 mμ in all states, while the spectra of XI show a shift of absorption maxima at about 260 mμ in acidic state to a longer wave-length region (285—287 mμ) in neutral

7) T. Ueda and J.J. Fox, *J. Am. Chem. Soc.*, **85**, 4024 (1963).

Table I. pK_a Values of 2-O-Alkyluracil, Isocytosine Derivatives, and Related Compounds

Compound	pK_{a1}	pK_{a2}
cytosine	4.61[8]	12.2[9]
1-methylcytosine	4.55[10]	
3-methylcytosine	7.38[7]	13—14[7]
isocytosine	3.9[11]	10.8[11]
1-methylisocytosine (X)	4.0	12.4
3-methylisocytosine (XI)	4.2	12.5
1-(2′,3′-epoxy-β-D-lyxofuranosyl)isocytosine (IV)	4.2	12.9
2-O-ethyl-1-methyluracil (VIII)	1.2	
2-O-ethyl-3-methyluracil (IX)	1.0	
2-O-methyl-1-(2′,3′-epoxy-β-D-lyxofuranosyl)uracil (III)	1.0	

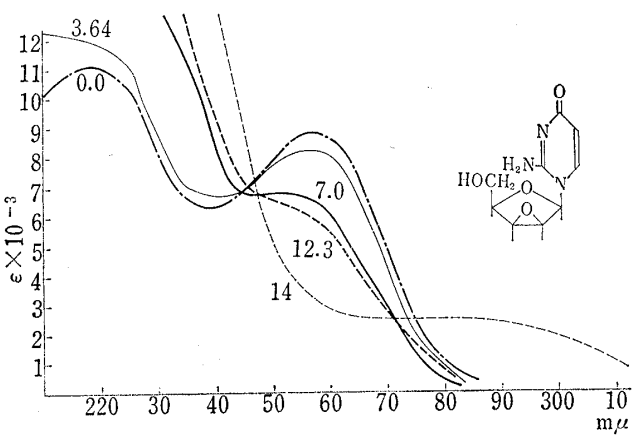

Fig. 1. Ultraviolet Spectra of 1-(2′,3′-Epoxy-β-D-lyxofuranosyl)isocytosine (IV) at pH Values indicated

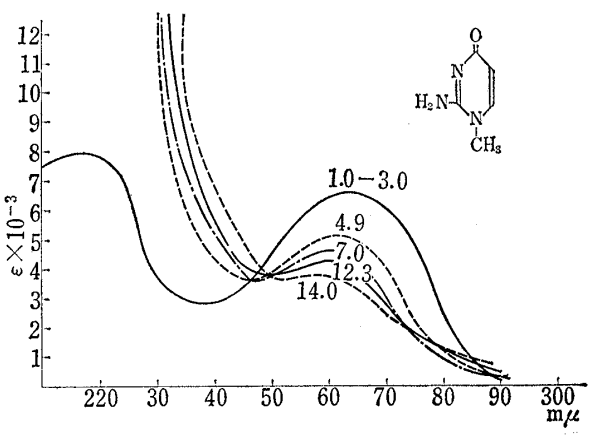

Fig. 2. Ultraviolet Spectra of 1-Methylisocytosine (X) at pH Values indicated

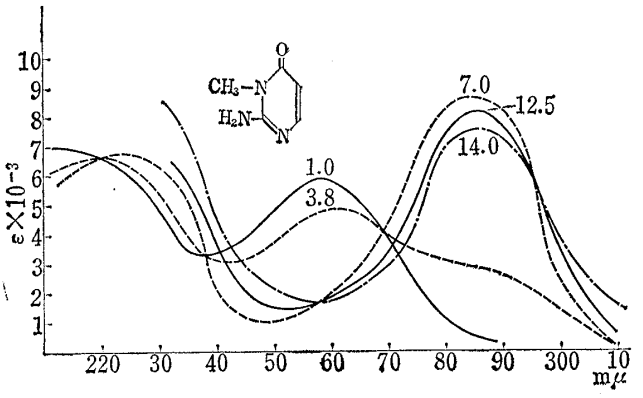

Fig. 3. Ultraviolet Spectra of 3-Methylisocytosine (XI) at pH Values indicated

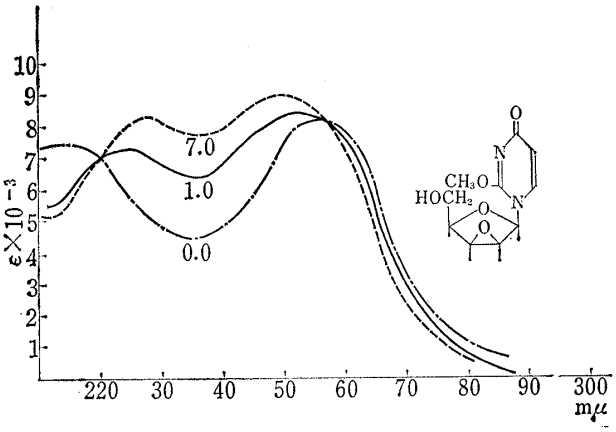

Fig. 4. Ultraviolet Spectra of 2-O-Methyl-(2′,3′-epoxy-β-D-lyxofuranosyl)uracil (III) at pH Values indicated

8) I. Wempen, R. Duschinsky, L. Kaplan, and J.J. Fox, *J. Am. Chem. Soc.*, **83**, 4755 (1961).
9) D. Shugar and J.J. Fox, *Biochim. Biophys. Acta*, **9**, 199 (1952).
10) J.J. Fox and D. Shugar, *Biochim. Biophys. Acta*, **9**, 369 (1952).
11) C.W. Whitehead and J.J. Traverso, *J. Am. Chem. Soc.*, **82**, 3971 (1960).

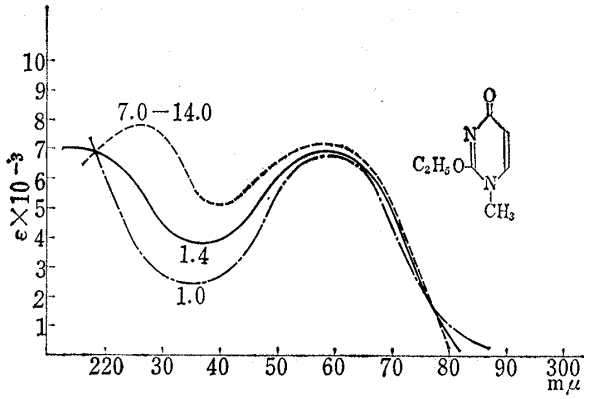

Fig. 5. Ultraviolet Spectra of 2–O–Ethyl–1–methyluracil (VIII) at pH Values Indicated

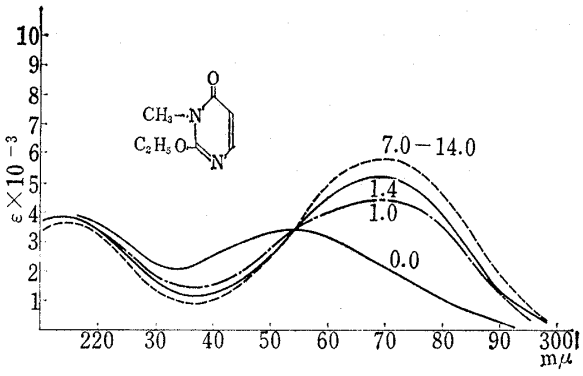

Fig. 6. Ultraviolet Spectra of 2–O–Ethyl–3–methyluracil (IX) at pH Values Indicated

and basic states. On the other hand, ultraviolet absorption spectra of IV (Fig. 1) have maxima at 256 mμ in acidic state and show no shift of absorption maxima both in neutral and basic states and then a significant difference is not observed between IV and X. These spectral evidences suggest IV as N_1–substituted isocytosine.

The same comparison as above was carried out among III, VIII, and IX (Fig. 4, 5, 6). pK_a values of III, VIII, and IX were similar and no significant difference between VIII and IX was observed as in the case of cytosine, but ultraviolet absorption spectra exhibited an apparent difference between these two compounds. As shown in Fig. 5 and 6, VIII and IX have quite different absorption spectra in all states. Especially large differences are observed in their maxima in neutral and basic states, VIII having its two maxima at 226 (ε 7800) and 258 mμ (ε 7200) (twin maxima), while IX having its two maxima at 216 (ε 3700) and 271 mμ (ε 5900). The absorption spectra of III (Fig. 4) are very similar to that of VIII and then a significant difference is not observed. These spectral features indicate III to be N_1–substituted 2–O–alkyluracil derivative.

Model compounds (VIII), (IX), (X), and (XI) used in this spectroscopic study were prepared as shown in Chart 2.

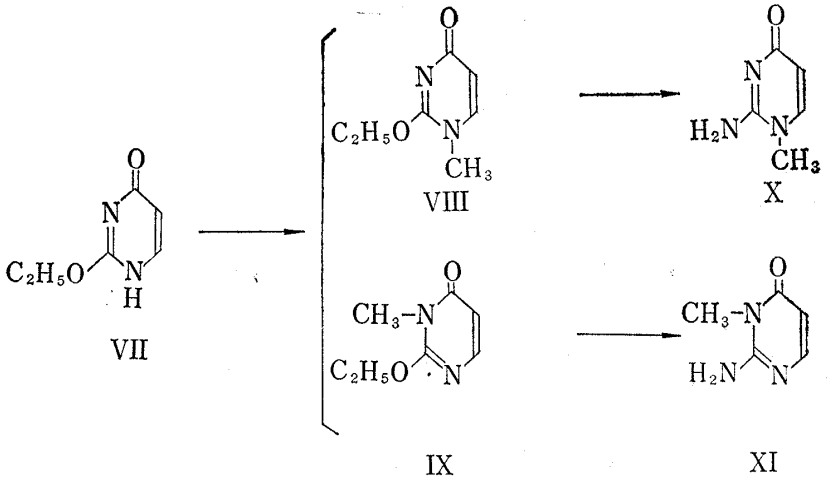

Chart 2

The products obtained by treatment of 2–O–ethyluracil (VII)[12,13] with dimethylsulfate in sodium hydroxide solution were separated into VIII and IX by chromatography. In

12) G.E. Hilbert and T.B. Johnson, *J. Am. Chem. Soc.*, **52**, 1152 (1930).
13) G.E. Hilbert and E.F. Johnson, *J. Am. Chem. Soc.*, **57**, 552 (1935).

order to establish the structure of VIII and IX, these compounds were converted by hydrochloric acid treatment to 1–methyl– and 3–methyl–uracil, respectively. Compounds (X) and (XI) were obtained from VIII and IX, respectively, by treatment with alcoholic ammonia at 100° for four hours or three and a half days.

Nuclear Magnetic Resonance Spectra——The structures of cytosine and its nucleoside have been already established to be of 4–amino–2–oxo form on the basis of ultraviolet, infrared, and nuclear magnetic resonance spectral studies.[7,10,14-18] 2–Amino group of 1–substituted– or 3–substituted–isocytosine derivatives were expected to be in either amino or imino form, but their tautomeric form have not been established.

Table II. Chemical Shifts and Coupling Constants of the Pyrimidine Derivatives

Compound	Solvent	Chemical Shift (τ Value)			
		H6	H5	H_1'	2–NH_2
1–(2′,3′–epoxy–β–D–lyxofuranosyl)isocytosine (IV)	DMSO	2.55	4.37	4.22 ($J=0$)	2.85 (2H)
3–methylisocytosine (XI)	DMSO	3.95	5.85		4.34 (2H)
2–O–methyl–1–(2′,3′–epoxy–β–D–lyxofuranosyl)uracil (III)	D_2O	2.14	3.97	3.84 ($J=0$)	
1–(2′,3′–epoxy–β–D–lyxofuranosyl)uracil (VI)	D_2O	2.70	4.74	4.41 ($J=0$)	

Nuclear magnetic resonance spectrum was determined on a J.N.H. 3H–60 spectrophotometer and tetramethylsilane was used as an internal reference.

Nuclear magnetic resonance spectra of IV and XI in DMSO showed 2H signals corresponding to amino group at 2.85 (τ) and 4.34 (τ), respectively (see Table II), showing that 2–amino group of 1–substituted– and 3–substituted–isocytosine derivatives is represented by amino form as in the case of cytosine derivatives.

In the nuclear magnetic resonance spectra of 2′,3′–epoxylyxosyl nucleosides (III), (IV), and (VI), the coupling between H_1' and H_2' was very small and a peak due to H_1' appeared as a sharp singlet. Recently, similar results[19,20] were obtained in the case of anhydrofuranose derivatives whose anomeric hydrogen did not couple with C_2 hydrogen and its signal appeared as a sharp singlet.

Experimental[21]

Buffer Solution——According to the report fo Fox,[9] 0.01 N HCl was taken as pH 2.0, 0.1 N HCl as pH 1.0 and 1 N HCl as pH 0.0. The solution of nearby pH 4.0 was prepared by dilution of 0.01 N HCl with H_2O and immediately after dissolution of substance, pH of the solution was measured accurately by Hitachi–Horiba pH–meter type M–4. Phosphate buffer was taken as pH 7.0, borate buffer as pH 9.18. 0.01 N NaOH was taken as essential equal to pH 12.0, 0.1 N NaOH as pH 13.0 and 1.0 N NaOH was assumed to be pH 14.0. Between pH 12.0 and 9.18 dilute NaOH was used as follows. 0.01 N NaOH was diluted with H_2O and immediately after dissolution of substance, pH of the solution was measured by pH–meter described above.

2–O–Methyl–1–(2′,3′–epoxy–β–D–lyxofuranosyl)uracil (III)——A) To a suspension of I (2.8 g, 5 mmoles) in MeOH (30 ml), 1N NaOMe (110 ml, 11 mmoles) was added and the solution was allowed to stand at room temperature overnight. After neutralization by bubbling of CO_2, the reddish solution was concentrated

14) P. Brooks and P.D. Lawley, *J. Chem. Soc.*, **1962**, 1348.
15) A.R. Katritzky and A.J. Warning, *Chem. Ind.* (London), **1962**, 695.
16) D.J. Brown and J.M. Lyall, *Australian J. Chem.*, **15**, 851 (1962).
17) H.T. Miles, *J. Am. Chem. Soc.*, **85**, 1007 (1963).
18) O. Jardetzky, P. Pappas, and N.G. Wade, *J. Am. Chem. Soc.*, **85**, 1657 (1963).
19) L.D. Hall, *Chem. Ind.* (London), **1963**, 950.
20) T. Hiraoka, T. Iwashige, and I. Iwai, *Chem. Pharm. Bull.* (Tokyo), **13**, 285 (1965).
21) All melting points are uncorrected. Paper chromatography (ppc) was carried out on Toyo Roshi No. 51 filter paper. Ultraviolet absorption spectra were measured with Hitachi Model EPS–2U spectrophotometer.

to dryness under reduced pressure. The residual solid was extracted twice with acetone (30 ml) and concentrated to leave white prisms, mp 168—174°. Yield, 850 mg (80.5%). Recrystallization from MeOH three times afforded white needles, mp 179—183°. $[\alpha]_D^{21.5}$ +51.8° (c=0.66, MeOH). UV $\lambda_{max}^{H_2O}$ mμ (ε): 228 (8300), 251 (9000). $\lambda_{min}^{H_2O}$ mμ (ε): 236 (7700). *Anal.* Calcd. for $C_{10}H_{12}O_5N_2$: C, 50.10; H, 5.01; N, 11.63; CH_3O, 12.91. Found: C, 49.91; H, 5.26; N, 11.23; CH_3O, 13.13.

B) To a suspension of II (19 g, 50 mmoles) in MeOH (100 ml), 1N NaOMe (55 ml, 55 mmoles) was added and the solution was allowed to stand at room temperature overnight. There was obtained III in yield of 7.9 g (66%) by the procedure as described above.

1-(2′,3′-Epoxy-β-D-lyxofuranosyl)isocytosine (IV)——A) I (2.8 g, 5 mmoles) was added to NH_3-saturated MeOH (50 ml) and the suspension was kept at room temperature overnight with occasional shaking. After evaporation of the solvent, the residue was dissolved in MeOH (50 ml) and the solution was passed through a column of Amberite IRA-410 (OH-form, 30 ml). The eluate was concentrated to a syrup and it was dissolved in EtOH, whereupon solidification occurred. This solid was purified by column chromatography of celite (25 g; solvent: H_2O-saturated iso-BuOH). Fractions of each 10 ml were collected and fraction Nos. 16—25 were concentrated to syrup and it was, after addition of EtOH, evaporated repeatedly *in vacuo*. The residue was crystallized from MeOH-EtOH to give 160 mg (13.7%) of IV, colorless prisms, mp 132—135°. Another run, using 28 g of I, resulted in higher yield (57%). $[\alpha]_D^{21.5}$ −29.6° (c=0.64, H_2O). UV $\lambda_{max}^{H_2O}$ mμ (ε): 250 (shoulder) (6900). *Anal.* Calcd. for $C_9H_{11}O_4N_3 \cdot \frac{1}{2}H_2O$: C, 46.20; H, 5.55; N, 17.90. Found: C, 46.35; H, 5.34; N, 17.70.

B) II (3.8 g, 10 mmoles) was added to NH_3-saturated MeOH (50 ml) and the suspension was kept at room temperature overnight. There was obtained IV, mp 129—133°, in yield of 1.4 g (59.6%) by the procedure described above.

C) To a solution of II (380 mg) in MeOH (100 ml), 2 ml of 25% NH_3-MeOH was added and the solution was kept at room temperature overnight. Examination of the solution by ppc (solvent: H_2O-saturated MEK, detection: UV rays)showed that II was converted into III completely. The solvent was evaporated to dryness under reduced pressure and a resulting syrup was dissolved in 25% NH_3-MeOH (40 ml) and the solution was allowed to stand at room temperature for 2 days. It was concentrated *in vacuo* and the residue was crystallized from MeOH. IV, White needles, mp 132—135°, was obtained in yield of 160 mg (67%).

2-N-Methyl-1-(2′,3′-epoxy-β-D-lyxofuranosyl)isocytosine (V)——A) II (1.9 g, 5 mmoles) was dissolved in 20% CH_3NH_2-MeOH (60 ml) and after standing at room temperature for 4 hr, colorless prisms, mp 186°, were separated out from the solution. Yield, 550 mg (46%). $[\alpha]_D^{20}$ −47.2 (c=0.66, H_2O). UV $\lambda_{max}^{H_2O}$ mμ (ε): 215 (18500), 256 (shoulder) (4400). *Anal.* Calcd. for $C_{10}H_{13}O_4N_3$: C, 50.21; H, 5.48; N, 17.57. Found: C, 50.44; H, 6.01; N, 17.84.

B) III (500 mg) was dissolved in 20% CH_3NH_2-MeOH (40 ml) and after standing at room temperature for 4 hr, the solution was concentrated *in vacuo*. The residual syrup was dissolved in MeOH, and was allowed to stand at room temperature. The colorless needles, mp 183—184°, were obtained. Yield, 400 mg (80%). This compound was identical with the substance prepared by the procedure described above.

1-(2′,3′-Epoxy-β-D-lyxofuranosyl)uracil (VI)——A) I (11 g, 20 mmoles) was suspended in 1N NaOH (60 ml, 60 mmoles) and the suspension was stirred at room temperature for 3 hr. After dilution to 500 ml with H_2O, the solution was passed through Amberite IR-120 (H-form, 30 ml) and Amberite IRA-410 (OH-form, 40 ml) columns to remove Na tosylate. The eluate was concentrated to a syrup and it was chromatographed through a celite (225 g) column (solvent: H_2O-saturated MEK). The fractions containing VI were concentrated to a syrup which was crystallized from EtOH to prisms, mp 125—128°, 2.5 g (55%). Recrystallization from EtOH twice to give an analytical sample of VI, mp 137—139°. This compound was identical with an authentic specimen prepared by Fox.[6]

B) III (240 mg, 1 mmole) was dissolved in 0.1 N HCl (10 ml) and the solution was kept at room temperature overnight. After passing through a column of IRA-410 (OH-form, 1 ml), the eluate was concentrated to a syrup and it was crystallized from EtOH to prisms, mp 125—128°, 130 mg (57%). Recrystallization from EtOH afforded colorless prisms, mp 132—135°, undepressed on admixture with a sample described above.

C) To a solution of IV (1.17 g, 5 mmoles) in 2 N AcOH (20 ml), $NaNO_2$ (1.75 g, 25 mmoles) was added in five portions for 2 hr at 0—5°. After standing at room temperature overnight, the pale yellow solution was passed through Amberite IR-120 (H-form, 20 ml) column. The eluate was concentrated to a syrup (1 g) *in vacuo* and it was chromatographed through a cellulose powder (100 g) column, and was developed with H_2O-saturated MEK. The fractions containing VI (examined by UV rays) were evaporated *in vacuo* to a syrup. It was crystallized from EtOH to colorless prisms, 370 mg (36.2%), mp 132—135°, undepressed on admixture with a sample described above.

2-O-Ethyl-1-methyluracil (VIII) and 2-O-Ethyl-3-methyluracil (IX)——To a solution of dimethylsulfate (0.75 g, 5.5 mmoles) in 20% NaOH (2.2 ml, 11 mmoles), VII (1.4 g, 10 mmoles) was added under chilling. The solution was warmed gradually to 80° and after keeping the same temperature for 30 min, the solution was allowed to stand at room temperature overnight. Examination of the solution by ppc (solvent: H_2O-

saturated MEK) showed two new UV absorbing spots at *Rf* 0.87 and 0.33. This mixture was chromatographed through a celite column (celite: 150 g; solvent: described above). Fractions showing a spot at *Rf* 0.87 were concentrated to leave IX. Recrystallization from ligroin gave 370 mg (24.3%) of IX, colorless needles, mp 55—60°. UV $\lambda_{max}^{H_2O}$ mμ (ε): 216 (3700), 271 (5900). *Anal.* Calcd. for $C_7H_{10}O_2N_2$: C, 54.59; H, 6.49; N, 18.18. Found: C, 54.64; H, 6.76; N, 17.78.

From fractions showing a spot at *Rf* 0.33, 270 mg (18%) of VIII, colorless needles, mp 112—115°. UV $\lambda_{max}^{H_2O}$ mμ (ε): 226 (7800), 258 (7200). $\lambda_{min}^{H_2O}$ mμ (ε): 240 (5100). *Anal.* Calcd. for $C_7H_{10}O_2N_2$: C, 54.59; H, 6.49; N, 18.18. Found: C, 54.15; H, 6.58; H, 18.35.

1-Methylisocytosine (X)——VIII (100 mg) was dissolved in NH_3–EtOH (4 ml) saturated at 0° and then the solution was heated in a sealed tube at 100° for 4 hr, then cooled. After evaporation of the solvent, the residue was recrystallized from MeOH to colorless prisms, mp 276—279° (decomp.). Yield, 44 mg (54%). *Rf* value: 0.01 (solvent: described above). UV $\lambda_{max}^{H_2O}$ mμ (ε): 261 (4650). $\lambda_{min}^{H_2O}$ mμ (ε): 246 (3500). *Anal.* Calcd. for $C_5H_7ON_3$: N, 33.60. Found: N, 34.01.

3-Methylisocytosine (XI)——IX (100 mg) was dissolved in NH_3–EtOH (4 ml) saturated at 0° and the solution was heated at 90° for 3.5 days in a sealed tube, then cooled. After evaporation of the solvent, the crystalline residue was recrystallized from MeOH to colorless plates, mp 257—260° (decomp.). Yield, 50 mg (62%). *Rf* value: 0.37. UV $\lambda_{max}^{H_2O}$ mμ (ε): 285 (8800). $\lambda_{min}^{H_2O}$ mμ (ε): 248 (1000). *Anal.* Calcd. for $C_5H_7ON_3$: N, 33.60. Found: N, 33.82.

Acknowledgement The author wishes to express his deep gratitude to Prof. T. Ukita of the Faculty of Pharmaceutical Science, University of Tokyo, for reviewing this manuscript, and to the member of the Laboratory of Hygiene and Forensic Chemistry of the University for valuable discussion. He is indebted to Dr. T. Ishiguro, President, and Dr. M. Shimizu, Director of this Laboratory, for kind encouragement throughout the course of this work and for permission to publish this work, and to Dr. Takeo Naito, Head of Organic Chemistry Division of this Laboratory, for kind guidance. Thanks are due to Mr. B. Kurihara and Miss E. Kosaka for elemental analyses and to Mr. I. Ito for measurement of nuclear magnetic resonance spectra.