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One-Step Halogenation at the 2'-Position of Uridine, and Related Reactions of Cytidine and N^4-Acetylcytidine[1]

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The reaction of uridine with acyl bromide in acetonitrile (or ethyl acetate) afforded 3',5'-di-O-acyl-2'-bromo-2'-deoxyuridine (II, V) in good yield, which was converted to 2'-deoxyuridine by hydrogenation and subsequent deacylation. A silmiar reaction of N^4-acetylcytidine with acetyl bromide yielded 1-(2,5-di-O-acetyl-3-bromo-3-deoxy-β-D-xylofuranosyl)-N^4-acetylcytosine (VIII), which was converted to 3'-deoxycytidine (X) by hydrogenation and subsequent deacylation with a concomitant formation of 2',3'-dideoxycytidine (XII) and to 1-β-D-arabinofuranosyl cytosine (XI) by treatment with potassium hydroxide in ethanol. A similar reaction of cytidine with acyl bromide gave 2,2'-anhydro-1-(3,5-di-O-acyl-β-D-arabinofuranosyl)cytosine hydrobromide (XIV, XVI). These reaction mechanisms were also presented.

Fission of the ether linkage of 2,2'-anhydro-1-β-D-arabinofuranosyluracil (I) with a concomitant introduction of bromine atom at C2'[3] seems to be an attractive process for the synthesis of 2'-deoxyuridine because of easy accessibility of I from uridine.[4] In our efforts to look for an improved synthesis of 2'-bromo-2'-deoxyuridine, we tried to use acetyl bromide[5] for the fission in place of hydrogen bromide.[3] This paper deals with reactions of acyl halides with pyrimidine ribonucleosides as well as I.

Compound (I) was refluxed with acetyl bromide in ethyl acetate for 3—4 hr. The thin-layer chromatography (TLC) (silica gel, *n*-butanol saturated with water) of the reaction mixture revealed the presence of a single ultraviolet (UV)-absorbing spot. Purification of the reaction product afforded a white amorphous powder, mp 67—76°, in 94% yield, which was identified as 3',5'-di-O-acetyl-2'-bromo-2'-deoxyuridine (II)[6] on the basis of elemental analysis, nuclear magnetic resonance (NMR) and UV spectra. Butyl acetate, acetic acid, acetonitrile or dioxane was also useful as the solvent, and this indicates that the acetyl group in II originates from acetyl bromide, and not from ethyl acetate. The use of dimethyl formamide or nitromethane as the solvent resulted in a poor yield of II and a deep coloration of the reaction mixture. About three moles of acetyl bromide to one mole of I were satisfactory for the reaction. Catalytic hydrogenation of II in the presence of palladised barium sulfate followed by deacetylation with methanolic ammonia afforded 2'-deoxyuridine in an overall yield of 53% based on I.

1) This forms Part II of "Studies on the Synthesis of Pyrimidine Deoxynucleosides," Part I: Y. Furukawa, Y. Yoshioka, K. Imai and M. Honjo, *Chem. Pharm. Bull.* (Tokyo), **18**, 554 (1970). A part of this paper was presented at the 91st Annual Meeting of the Pharmaceutical Society of Japan, Fukuoka, April, 1971.

2) Location: *Juso-Nishinocho, Higashiyodogawa-ku, Osaka.*

3) J.F. Codington, I.L. Doerr and J.J. Fox, *J. Org. Chem.*, **29**, 558 (1964).

4) *a*) J.J. Fox, N. Miller and I. Wempen, *J. Med. Chem.*, **9**, 101 (1966); *b*) A. Hampton and A.W. Nichol, *Biochem.*, **5**, 2076 (1966); *c*) R. Marumoto and M. Honjo, *Takeda Kenkyusho Nempo*, **26**, 21 (1967); *d*) Y. Furukawa and M. Honjo, *Chem. Pharm. Bull.* (Tokyo), **16**, 2286 (1968).

5) The cleavage of aliphatic ethers by acyl halides is well documented. E. Müller (ed.), "Houben-Weyl Methoden der Organischen Chemie," Vol. 6/3, Georg Thieme Verlag, Stuttgart, 1965, p. 155.

6) R.J. Cushley, J.F. Codington and J.J. Fox, *Can. J. Chem.* **46**, 1131 (1968).

A similar reaction of I with acetyl chloride required a longer reaction period to afford 3′,5′-di-O-acetyl-2′-chloro-2′-deoxyuridine (III) in 91% yield.

Similarly, the reaction of 2,2′-anhydro-1-(3,5-di-O-acetyl-β-D-arabinofuranosyl)uracil (IV)[4d] with acetyl bromide afforded a mixture of II and a labile compound, which was detectable by thin-layer chromatography (silica gel, chloroform: tetrahydrofuran=5: 1 v/v) to be produced in *ca.* 50% yield and presumed to be N^3,3′,5′-(or O^2,3′,5′-)triacetyl-2′-bromo-2′-deoxyuridine. This labile compound, however, was not formed, when the reaction was carried out in the presence of two moles of methanol relative to IV.

We, then, subjected uridine to a similar reaction by the use of acetyl bromide. The thin-layer chromatography (silica gel, *n*-butanol saturated with water) revealed the presence of two UV absorbing compounds (A+B) having the same *Rf* values as those of II and 2′,3′,5′-tri-O-acetyluridine, respectively. After treatment of the mixture with methanolic ammonia, two UV absorbing compounds were detected by paper electrophoresis (0.05M borate buffer, pH 9.2), which had the same moving distances and UV spectra as those of I and uridine, respectively. Any attempts to isolate A and B were unsuccessful, owing to their close *Rf* values. The mixture (A+B) was thus subjected to catalytic hydrogenation followed by deacetylation, and was separated by Dowex-1 (borate form) column chromatography to isolate 2′-deoxyuridine. These results imply that the reaction of uridine with acetyl bromide provides a selective and one-step bromination of uridine at C2′[7] to afford II. As for the solvent, butyl acetate, ethyl cyanoacetate or acetonitrile was also useful. Acetonitrile was especially useful not only for reducing the reaction time (1—2 hr), but also for improving the yield of II up to 80%.

The reaction of uridine with propionyl bromide in place of acetyl bromide in acetonitrile afforded crystalline 3′,5′-di-O-propionyl-2′-bromo-2′-deoxyuridine (V), mp 134—135°,[8] in 63% yield, which was easily separated by filtration from the accompanying 2′,3′,5′-tri-O-propionyluridine in the reaction mixture and converted to 2′-deoxyuridine by dehalogenation and subsequent deacylation. Thus, this method opens a very useful route for the synthesis of 2′-deoxyuridine starting from uridine in high yield.

An intermediate in this selective bromination of uridine was confirmed to be 2,2′-anhydro-1-(3,5-di-O-acyl-β-D-arabinofuranosyl)uracil (VI) by thin-layer chromatography (silica gel, *n*-butanol saturated with water). The reaction mechanism, therefore, would involve formation of an oxonium cation (VII)[9] (*e.g.*, 2′,3′-acetoxonium ion) from uridine followed by nucleophilic "downward" attack of the 2-carbonyl oxygen on C2′[10] to form VI. Subsequent nucleophilic "upward" attack of bromide anion[3] on C2′ of VI would afford II or V (Chart 1). This reaction sequence receives support from the fact that both 5′- and 3′-O-acetyluridines react with acetyl bromide to afford II, though tri-O-acetyluridine is recovered unchanged and 2′-deoxyuridine is only acetylated to 3′,5′-di-O-acetyl-2′-deoxyuridine.

This selective bromination was applied to cytidine derivatives. The reaction of N^4-acetylcytidine with acetyl bromide afforded colorless platelets, mp 179—180° (decomp.), $C_{15}H_{18}O_7N_3Br$, in 30—40% yield. The structural determination was made by NMR analysis. The dihedral angle formed by the two planes ($H_{2'}$-$C_{2'}$-$C_{3'}$, $H_{3'}$-$C_{3'}$-$C_{2'}$) is found to be *ca.* 70—100°, calculated based on the coupling constant[11] ($J_{H1',H2'}<1$ Hz, $J_{H2',H3'}<1$ Hz),

7) The reaction of an α-acetoxyisobutyryl halide with uridine was reported by S. Greenberg and J.G. Moffatt (Abst. Papers, *Am. Chem. Soc.*, No. 155, 54C (1968)), which afforded, however, a mixture of 3′-O-acetyl-2′-halogeno-2′-deoxyuridines substituted at the 5′ by α-acetoxyisobutyrate and dioxolane groupings.

8) An authentic sample was prepared by the reaction of 2′-bromo-2′-deoxyuridine with propionic anhydride in pyridine.

9) The formation of VII would be explained as follows: the 3′-(or 2′-)hydroxyl reacts with acyl cation derived from acyl bromide to afford the protonated acyloxy group. The group is then attacked by the neighboring 2′-(or 3′-)hydroxyl to yield VII.

10) J.J. Fox and K.A. Watanabe, *Chem. Pharm. Bull.* (Tokyo), **17**, 211 (1969).

11) M. Karplus, *J. Chem. Phys.*, **30**, 11 (1959).

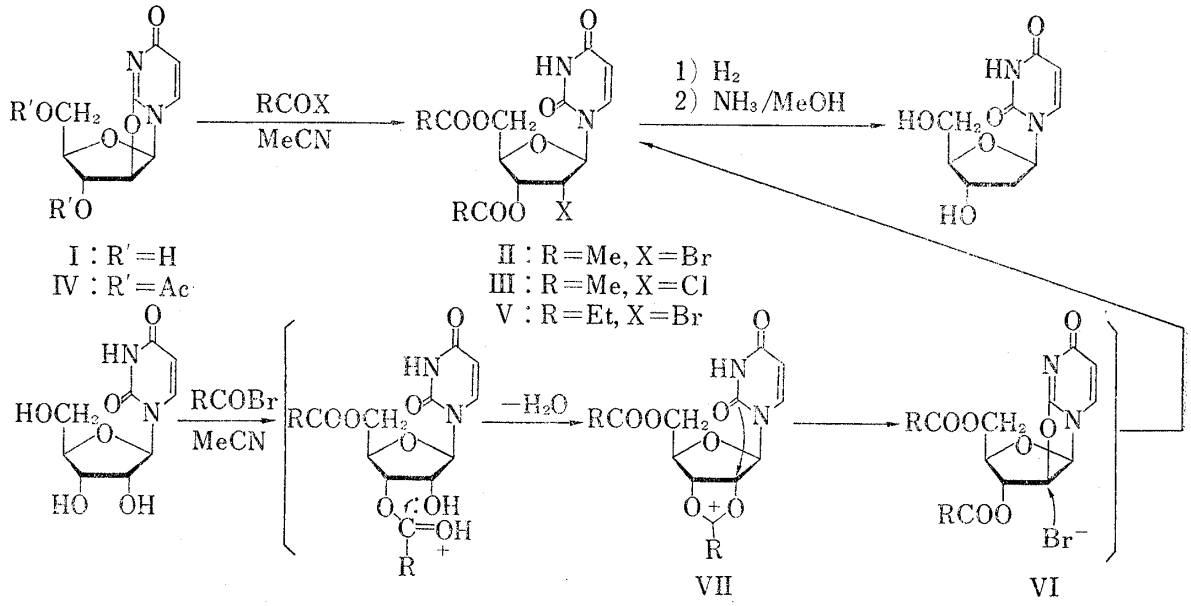

Chart 1

which reveals that the three protons ($H_{1'}$, $H_{2'}$, $H_{3'}$) are all in *trans* configuration. This compound was thus assigned the 1-(2,5-di-O-acetyl-3-bromo-3-deoxy-β-D-xylofuranosyl)-N^4-acetylcytosine structure (VIII)[12] and not the 1-(3,5-di-O-acetyl-2-bromo-2-deoxy-β-D-ribofuranosyl)-N^4-acetylcytosine structure. The assignment was further supported by the following experiments. 1) On catalytic hydrogenation with palladised barium sulfate, VIII absorbed one mole equivalent of hydrogen[13] to afford colorless needles, N^4,2',5'-triacetyl-3'-deoxycytidine (IX), mp 174—177°, $C_{15}H_{19}O_7N_3 \cdot 1/2H_2O$. The coupling constant ($J_{H1',H2'}<1$ Hz) of IX precludes the 2'-deoxy (*viz.* -CH_2-) structure. Treatment of this compound (IX) with methanolic ammonia afforded, after addition of sulfuric acid, 3'-deoxycytidine sulfate (X), mp 202—204° (decomp.) in good yield. 2) VIII was treated with 2.5% potassium hydroxide in refluxing ethanol to give 1-β-D-arabinofuranosylcytosine (XI) (hydrochloride, mp 188—189° (decomp.)) in 77% yield, presumably *via* the ribo-epoxide.[14]

The formation of VIII would be explained by formation of 2',3'-acetoxonium ion followed by nucleophilic "downward" attack of bromide anion on C3'. The preferential attack of bromide anion on C3' (rather than attack of the 2-carbonyl oxygen on C2') may be due to the diminished nucleophilicity of the 2-carbonyl as a result of the inductive effect of the N^4-acetyl substituent.

From the mother liquor of IX, after deacetylation with methanolic ammonia, a small amount of colorless rhombic crystals, mp 209—211°, $C_9H_{13}O_3N_3$ were obtained by cellulose column chromatography, which was identified as 2',3'-dideoxycytidine (XII)[15] on the basis of the melting point, elemental analysis, NMR spectrum and specific rotation. The reaction, therefore, implies that the hydrogenation of VIII led to a concomitant formation of N^4,5'-diacetyl-2',3'-dideoxycytidine (XIII). This appears to be a unique reductive elimination

12) The sugar conformation of VIII may be C_3'-endo type on the bases of the coupling constants ($J_{H1',H2'}$ and $J_{H2',H3'}$). C.D. Jardetzky, *J. Am. Chem. Soc.*, **82**, 229 (1960).

13) Complete hydrogenation of VIII, which absorbed two mole equivalents of hydrogen, afforded no UV-absorbing compound. The compound was assigned the N^4,2',5'-triacetyl-3'-deoxy-5,6-dihydrocytidine structure, on the basis of the NMR ($CDCl_3$) spectrum (δ: 2.1 (9H, s, 2-$OCOCH_3$, -$NCOCH_3$) 2.7 (2H, t, J=6 Hz, $2H_{5'}$), 3.5 (2H, m, $2H_6$), 4.25 (3H, broad s, $H_{4'}$, $2H_{5'}$), 5.3 (1H, m, $H_{2'}$), 5.8 (1H, d, J=4 Hz, $H_{1'}$), 8.8 (1H, broad s, H_{N^4}).

14) The analogous compound, 1-(3'-deoxy-3'-iodo-β-D-xylofuranosyl)uracil was converted to I on treatment with ethanolic potassium hydroxide. G.A.R. Johnston, *Aust. J. Chem.*, **21**, 515 (1968).

15) J.P. Horwitz, J. Chua, M. Noel and J.T. Danatti, *J. Org. Chem.*, **32**, 817 (1967).

of a *trans*-bromohydrin acetate in the nucleoside series.[16] A plausible interpretation of the elimination is that palladium would withdraw bromine to furnish the C3′ cation, which accepts two electrons from the catalyst to form the anion, and the negative charge on C3′ would then give rise to an electronic displacement resulting in the ejection of an acetate ion from C2′ to form the double bond and the hydrogenation of the double bond would finally lead to XIII.

Reaction of cytidine with acetyl bromide in acetonitrile afforded two products, which could be separated by silica gel column chromatography. One product, colorless needles, mp 228—229° (decomp.), $C_{13}H_{16}O_6N_3Br$, was obtained in 40% yield and assigned the 2,2′-anhydro-1-(3,5-di-O-acetyl-β-D-arabinofuranosyl)cytosine hydrobromide structure (XIV), on the basis of the elemental analysis, UV and NMR spectra. The other product, a white amorphous powder, turned out to be a mixture of two compounds from the NMR spectrum. One of them was labile at room temperature and was gradually converted to XIV, which was detected by thin-layer chromatography (silica gel, chloroform: methanol=2: 1 v/v). The mixture was converted to 1-β-D-arabinofuranosylcytosine and cytidine when treated with methanolic ammonia. These observations indicate that the reaction mixture from cytidine and acetyl bromide contained 3′,5′-di-O-acetyl-2′-bromo-2′-deoxycytidine (XV) and 2′,3′,5′-tri-O-acetylcytidine in addition to XIV. The difference of the reactivity of cytidine from that of uridine with acyl bromide might be due to the ease[17] of XV to return to XIV. A similar reaction of cytidine with isobutyryl bromide afforded the isobutyryl analog (XVI) (Chart 2).

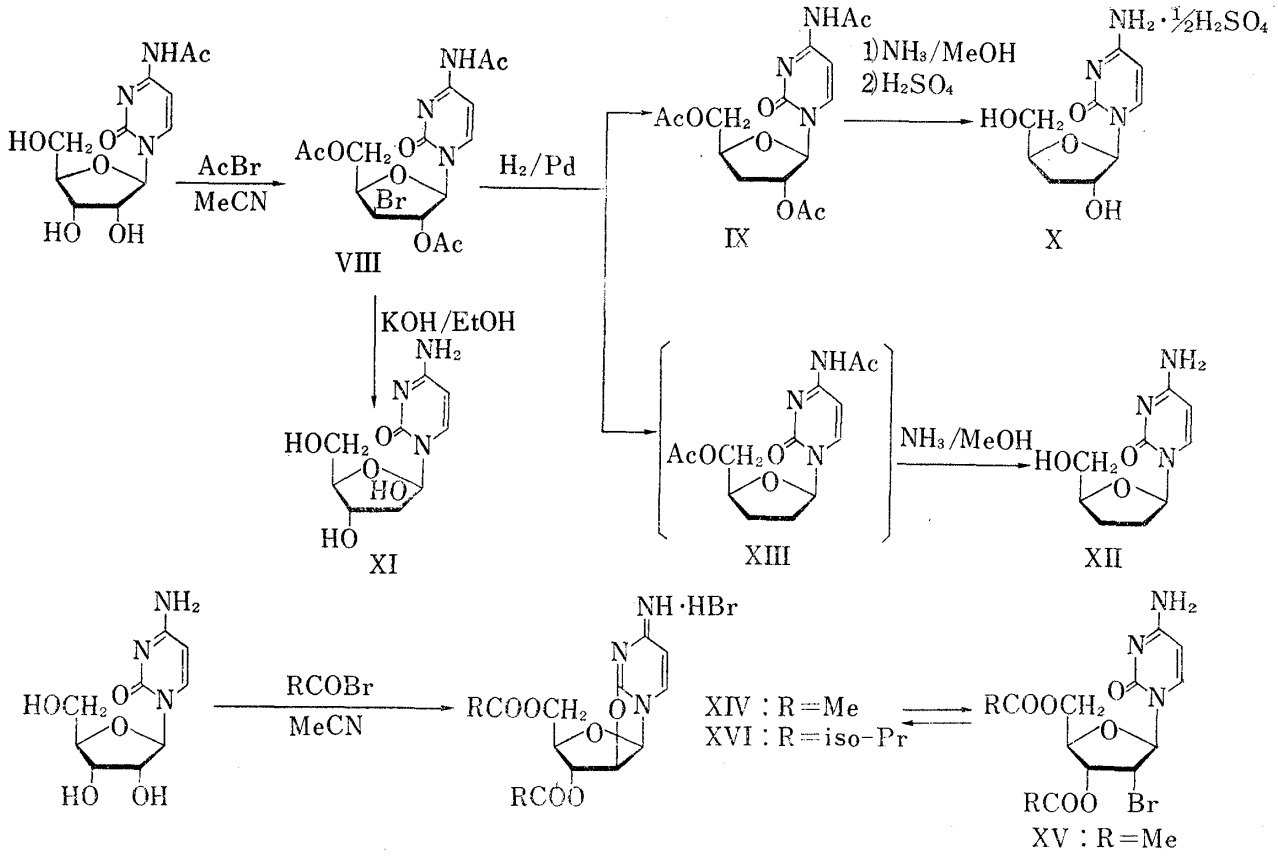

Chart 2

16) It has been reported from our laboratories that another reductive elimination takes place in a *cis*-bromohydrin mesylate to give 2′,3′-dideoxyuridine in a better yield. Y. Furukawa, Y. Yoshioka, K. Imai and M. Honjo, *Chem. Pharm. Bull.* (Tokyo), **18**, 554 (1970).

17) I.L. Doerr and J.J. Fox, *J. Org. Chem.*, **32**, 1467 (1967).

Experimental[18]

3′,5′-Di-O-acetyl-2′-bromo-2′-deoxyuridine (II)——a) To a stirred suspension of I[4b] (25 g, 0.11 mole) in a mixture of DMF (50 ml) and AcOEt (450 ml) was added AcBr (25 ml, 0.34 mole). The mixture was refluxed for 1.5 hr and evaporated to dryness *in vacuo*. The residue was dissolved in AcOEt (1 liter), the solution washed with water (0.5 liter×2) and the AcOEt layer was evaporated to dryness leaving a colorless powder, mp 67—76° (40.5 g, 94%). *Anal.* Calcd. for $C_{13}H_{15}O_7N_2Br$: C, 39.9; H, 3.84; N, 7.17; Br, 20.4. Found: C, 39.7; H, 3.95; N, 7.36; Br, 20.5. UV λ_{max}^{EtOH} nm (ε): 256 (10.0×10^3). IR ν_{max}^{KBr} cm^{-1}: 1750, 1690, 1450, 1380, 1270, 1230. NMR ($CDCl_3$) δ: 2.13 (3H, s, -OCOCH$_3$), 2.18 (3H, s, -OCOCH$_3$), 4.4—4.7 (4H, m, $H_{2'}$, $H_{4'}$, $2H_{5'}$), 5.15 (1H, m, $H_{3'}$), 5.80 (1H, d, J=8 Hz, H_5), 6.22 (1H, d, J=5 Hz, $H_{1'}$), 7.46 (1H, d, J=8 Hz, H_6), 9.65 (1H, broad s, H_{N^3}).

b) To a boiling and stirred mixture of IV[4d] (3.1 g, 0.01 mole), AcOEt (100 ml), and MeOH (8 ml, 0.02 mole) was added AcBr (3 ml, 0.04 mole). After the reaction for 2 hr, the solvent was evaporated *in vacuo*. The residue was dissolved in $CHCl_3$ (50 ml), the solution was washed with water (50 ml), and the $CHCl_3$ layer was evaporated to dryness to furnish a colorless powder (3.8 g, 98%). Its NMR ($CDCl_3$) spectrum is in good accord with that of an authentic sample (II).

(c) To a stirred suspension of uridine (40 g, 0.164 mole) in MeCN (3 liters) was added dropwise AcBr (80 ml, 1.07 moles) and the mixture was refluxed with stirring for 1 hr. The solvent was evaporated to dryness *in vacuo*, the residue was dissolved in $CHCl_3$ (600 ml) and the resulting solution was washed with water (400 ml). The $CHCl_3$ layer was evaporated to dryness *in vacuo* to give a pale yellow powder (61 g). A portion of the material was dissolved in 20% methanolic ammonia, kept overnight, and gave two UV absorbing spots in PE, which had the same mobilities as those of I (75%) and uridine (25%), respectively. This fact indicates that the pale yellow powder was a mixture of II and 2′,3′,5′-tri-O-acetyluridine.

3′,5′-Di-O-acetyl-2′-chloro-2′-deoxyuridine (III)——To I (1 g, 4.4 mmoles) suspended in MeCN (100 ml) was added AcCl (8 ml, 0.112 mole). The mixture was refluxed at 120° for 7 hr and evaporated to dryness *in vacuo*. The residue was dissolved in $CHCl_3$ (100 ml) and washed with water (100 ml). The $CHCl_3$ layer was evaporated to dryness *in vacuo*. The residue was recrystallized from EtOH to give colorless needles (1.4 g, 91%). mp 137° (lit.[17] mp 127—130°). *Anal.* Calcd. for $C_{13}H_{15}O_7N_2Cl$: C, 45.0; H, 4.33; N, 8.08; Cl, 10.2. Found: C, 44.7; H, 4.59; N, 8.09; Cl, 10.5. NMR ($CDCl_3$) δ: 2.2 (6H, 2s, -OCOCH$_3$), 4.4—4.8 (4H, m, $H_{2'}$, $H_{4'}$, $2H_{5'}$), 5.3 (1H, m, $H_{3'}$), 5.87 (1H, d, J=9 Hz, H_5), 6.14 (1H, d, J=5 Hz, $H_{1'}$), 7.55 (1H, d, J=9 Hz, H_6), 9.95 (1H, s, H_{N^3}).

3′,5′-Di-O-propionyl-2′-bromo-2′-deoxyuridine (V)——a) To a boiling and stirred suspension of uridine (40 g, 0.164 mole) in MeCN (2 liters) was added EtCOBr[19] (68 ml, 0.73 mole) dropwise over a period of 1 hr (bath temp. 120°). After the reaction for 2 hr, the solvent was evaporated *in vacuo*, and the residue was recrystallized from EtOH to give colorless imbricate plates (43 g, 63%). mp 133—134°. *Anal.* Calcd. for $C_{15}H_{19}O_7N_2Br$: C, 42.97; H, 4.54; N, 6.69; Br, 19.07. Found: C, 42.94; H, 4.67; N, 6.69; Br, 18.88. NMR ($CDCl_3$) δ: 1.2 (6H, 2t, 2-$CH_2\underline{CH_3}$), 2.4 (4H, m, 2-$C\underline{H_2}$-), 4.4—4.6 (4H, m, $H_{2'}$, $H_{4'}$, $2H_{5'}$), 5.2 (1H, m, $H_{3'}$), 5.8 (1H, d, J=8.5 Hz, H_5), 6.22 (1H, d, J=5.5 Hz, $H_{1'}$), 7.46 (1H, d, J=8.5 Hz, H_6), 9.70 (1H, s, H_{N^3}).

b) $(EtCO)_2O$ (1 ml) was added to a solution of 2′-bromo-2′-deoxyuridine[3] (0.3 g) in pyridine (3 ml). The mixture was left at room temperature for 2 hr, and poured into a stirred ice-water. The solid was collected by filtration (0.4 g), and recrystallized from EtOH to give colorless imbricate plates (mp 133—134°), which were identical with the sample obtained in a) by comparison of NMR ($CDCl_3$) spectrum and by mixed melting point.

2′-Deoxyuridine——a) To a suspension of Pd-$BaSO_4$ catalyst[20] (10 g) in 50% MeOH (60 ml) was added a solution of II (40 g, 0.102 mole) and NaOAc (24 g, 0.292 mole) in 50% MeOH (400 ml). The mixture was shaken in H_2 atmosphere at 760 mmHg and 25° for 3 hr (H_2 absorbed, 2.4 liters). The catalyst was filtered off, the filtrate concentrated, and the resulting emulsion (50 ml) was diluted with water (200 ml) and extracted with $CHCl_3$ (400 ml×2). The combined extracts were evaporated to dryness *in vacuo*. The residue was dissolved in 20% methanolic ammonia (600 ml) and kept overnight at 5°. The reaction mixture was concentrated to give the oily residue which was in turn recrystallized from MeOH to give colorless crystals (13 g, 56%). mp 160—161°. The sample gave only one UV absorbing spot on PE and its NMR spectrum was in good accord with that of an authentic 2′-deoxyuridine.

b) A mixture (61 g) of II and 2′,3′,5′-tri-O-acetyluridine, which was prepared by the reaction of uridine with AcBr, was similarly treated as in a). The oily residue was rinsed with AcOEt (100 ml) and recrystallized from MeOH (100 ml) to give 2′-deoxyuridine (9.87 g). The mother liquor was evaporated,

18) All melting points were uncorrected. Paper electrophoresis (PE) was carried out on Whatman No. 1 filter paper at 22 v/cm for 60 min using 0.05M borate buffer (pH 9.2). Thin-layer chromatography (TLC) was performed on 0.25 mm layer of E. Merck DC-Alufolien Kieselgel F254.

19) EtCOBr (bp 104—106°) was prepared in 70% yield according to the modified A. Kirrmann's procedure (*Ann. Chim.* X. **11**, 280 (1929)).

20) R. Kuhn and H.J. Haas, *Angew. Chem.*, **77**, 785 (1965).

the residue dissolved in water (400 ml), and the solution was passed through a column of Dowex 1×8 (100—200 mesh, borate form, 70 ml). The effluent was evaporated to dryness *in vacuo* and the residue was recrystallized from MeOH to give additional 2′-deoxyuridine (2.3 g). Total yield was 12.2 g (33%). Its mobility in PE and NMR spectrum (D_2O) were similar to those of the authentic 2′-deoxyuridine.

c) V (20 g, 47.7 mmoles) was similarly treated as in a) to give crystals (6.18 g, 57%). mp 155—157°. The NMR spectrum (D_2O) and mobility in PE of the material were in accord with those of the authentic 2′-deoxyuridine.

1-(2,5-Di-O-acetyl-3-bromo-3-deoxy-β-D-xylofuranosyl)-N⁴-acetylcytosine (VIII)——A suspension of N⁴-acetylcytidine[21] (4 g, 14 mmoles) in MeCN (200 ml) was refluxed, to which was added AcBr (10 ml, 0.137 mole) dropwise (30 min), and the mixture was further refluxed for 3 hr. The solvent was distilled off, the syrup dissolved in $CHCl_3$ (100 ml) and the solution was washed with water (100 ml). Evaporation of the $CHCl_3$ layer and recrystallization of the residue from EtOH (10 ml) gave colorless platelets (2 g, 33%). mp 179—180° (decomp.). $[\alpha]_D^{28}$: +63.8° (c=1.0, $CHCl_3$). *Anal.* Calcd. for $C_{15}H_{18}O_7N_3Br$: C, 41.69; H, 4.17; N, 9.72; Br, 18.50. Found: C, 41.50; H, 4.06; N, 9.75; Br, 19.13. NMR ($CDCl_3$) δ: 2.0—2.4 (9H, 3s, 2-$OCOCH_3$, N-$COCH_3$), 4.2—4.7 (4H, m, $H_{3'}$, $H_{4'}$, $2H_{5'}$), 5.5 (1H, d, J<1 Hz, $H_{2'}$), 6.0 (1H, d, J<1 Hz, $H_{1'}$), 7.5 (1H, d, J=7.5 Hz, H_5), 8.1 (1H, d, J=7.5 Hz, H_6), 10.2 (1H, broad s, H_{N^4}).

N⁴,2′,5′-Tri-acetyl-3′-deoxycytidine (IX)——A mixture of VIII (1 g, 2.3 mmoles), $CaCO_3$ (1 g), Pd-$BaSO_4$ catalyst (0.2 g) and 50% MeOH (50 ml) was shaken in H_2 atmosphere at 760 mmHg and 25° for 1 hr (H_2 absorbed, 60 ml). The catalyst was filtered off, the filtrate concentrated. To the concentrate were added water (100 ml) and $CHCl_3$ (100 ml) and the mixture was shaken. The $CHCl_3$ layer was evaporated to dryness *in vacuo*, and the residue was recrystallized from EtOH (10 ml) to give colorless needles (430 mg, 51%). mp 174—177° (sintered at 170°). *Anal.* Calcd. for $C_{15}H_{19}O_7N_3 \cdot 1/2H_2O$: C, 49.72; H, 5.52; N, 11.60. Found: C, 49.87; H, 5.25; N, 11.60. NMR ($CDCl_3$) δ: 2.10, 2.27 (9H, 2s, 2-$OCOCH_3$, N-$COCH_3$), 4.30—4.80 (3H, m, $H_{4'}$, $2H_{5'}$), 5.46 (1H, m, $H_{2'}$), 5.90 (1H, d, J=1 Hz, $H_{1'}$), 7.43 (1H, d, J=7.5 Hz, H_5), 8.05 (1H, d, J=7.5 Hz, H_6), 10.30 (1H, broad s, H_{N^4}).

2′,3′-Dideoxycytidine (XII)——The mother liquor of IX was evaporated to dryness. The residue was dissolved in 20% methanolic ammonia (10 ml) and kept overnight at 5°. The PC (Whatman No. 1, n-PrOH: H_2O=4: 1 v/v, ascending method) of the sample revealed the presence of two UV absorbing spots of Rf values 0.42 (50%) and 0.54 (50%) which were in good accord with those of X and XII, respectively. The column chromatography of the reaction mixture using cellulose powder (Toyo Roshi, 100—200 mesh, 2.5×55 cm, n-PrOH: H_2O=4: 1 v/v) and recrystallization of the crude product from EtOH gave colorless rhombic crystals (34 mg, 7%). mp 209—211° (lit.[15] 215—217°), $[\alpha]_D^{22.2}$: +75.9° (c=0.6, H_2O) (lit.[15] $[\alpha]_D^{25}$: +81° (c=0.635, H_2O)). *Anal.* Calcd. for $C_9H_{13}O_3N_3$: C, 51.18; H, 6.20; N, 19.89. Found: C, 51.04; H, 6.13; N, 19.68. NMR (D_2O) δ: 1.7—2.7 (4H, m, $2H_{2'}$, $2H_{3'}$), 3.85 (2H, m, $2H_{5'}$), 4.3 (1H, m, $H_{4'}$), 6.05 (1H, m, $H_{1'}$), 6.05 (1H, d, J=7.5 Hz, H_5), 7.9 (1H, d, J=7.5 Hz, H_6).

3′-Deoxycytidine (X) Sulfate——A solution of IX (1.2 g, 3.3 mmoles) in 20% methanolic ammonia (30 ml) was kept at 5° for 20 hr. The solvent was distilled off and the residue was dissolved in EtOH (30 ml). The solution was adjusted to pH 3 with 2N sulfuric acid and a solid precipitated. The solid was dissolved in a small amount of water, to which was added EtOH to give colorless needles (1.3 g, 71%). mp 202—204° (decomp.) (lit.[22] mp 201—202°). $[\alpha]_D^{22}$: +51.5° (c=1.0, H_2O) (lit.[22] $[\alpha]_D$: +48° (c=0.72, H_2O)). UV λ_{max} nm: 280 (pH 1), 271 (pH 14); λ_{min} nm: 240 (pH 1), 250 (pH 14). *Anal.* Calcd. for $C_9H_{13}O_4N_3 \cdot 1/2H_2SO_4$: C, 39.13; H, 5.11; N, 15.21. Found: C, 38.92; H, 5.01; N, 14.99. NMR (D_2O) δ: 1.95—2.15 (2H, m, $2H_{3'}$), 3.9 (3H, m, $H_{4'}$, $2H_{5'}$), 4.6 (1H, m, $H_{2'}$), 5.84 (1H, d, J=1 Hz, $H_{1'}$), 6.27 (1H, d, J=8 Hz, H_5), 8.23 (1H, d, J=8 Hz, H_6).

1-β-D-Arabinofuranosylcytosine (XI) Hydrochloride——A solution of VIII (0.2 g) in 2.5% ethanolic potassium hydroxide (20 ml) was refluxed for 2 hr. The solvent was evaporated *in vacuo* and the residue was dissolved in water (5 ml). The solution was adjusted to pH 3 and desalted using a column of activated charcoal (2 g). The eluate was evaporated to dryness and the residue was dissolved in 5% methanolic hydrogen chloride (2 ml). After evaporation of the solvent *in vacuo*, EtOH (5 ml) was added to the residue, the solution was evaporated again and this procedure was repeated to remove a trace of hydrogen chloride giving crystallines (0.1 g, 77%). mp 188—189° (decomp.). This sample was found to be identical with an authentic XI (hydrochloride) by comparison of the NMR (D_2O) and IR (KBr) spectra, PE and PC (n-propanol: H_2O (4: 1 v/v)).

2,2′-Anhydro-1-(3,5-di-O-acetyl-β-D-arabinofuranosyl)cytosine Hydrobromide (XIV)——To a boiling suspension of cytidine (1 g, 4.1 mmoles) in MeCN (100 ml) was added AcBr (4 ml, 55 mmoles) dropwise (30 min), and the mixture was further refluxed for 2 hr. The reaction mixture gave two UV absorbing spots (Rf=0.61 (50%), 0.18 (50%)) in TLC (silica gel, $CHCl_3$: MeOH=2: 1, v/v). The solution was evaporated to dryness and the residue was chromatographed on silica gel[23] column (25 g). The column was

21) K.A. Watanabe and J.J. Fox, *Angew. Chem.*, 78, 589 (1966).
22) E. Walton, F.W. Holly, G.E. Boxer and R.F. Nutt, *J. Org. Chem.*, 31, 1163 (1966).
23) E. Merck Kieselgel 0.05—0.2 mm für die Säulen-Chromatographie.

eluted with $CHCl_3$: MeOH (9: 1 v/v, 200 ml) and the eluate ($Rf=0.61$) was evaporated to dryness giving white amorphous powder (900 mg). A portion of the sample was treated with 20% methanolic ammonia for 3 hr, and the solution gave two UV absorbing spots in PE corresponding to cytidine and XI. The column was further eluted with $CHCl_3$: MeOH (4: 1 v/v, 500 ml) and the eluate ($Rf=0.18$) was evaporated to dryness. The residue (300 mg) was recrystallized from EtOH giving colorless needles. mp 228—229° (decomp.). $[\alpha]_D^{25}$: −88.6° ($c=0.49$, DMF). UV λ_{max}^{EtOH} nm: 234.5, 263; λ_{min}^{EtOH} nm: 245. *Anal.* Calcd. for $C_{13}H_{15}O_6N_3 \cdot HBr$: C, 40.01; H, 4.13; N, 10.76. Found: C, 40.13; H, 4.07; N, 10.62. NMR (d_6-DMSO) δ: 1.86 and 2.11 (6H, 2s, 2-$OCOCH_3$), 4.08 (2H, m, $2H_{5'}$), 4.70 (1H, m, $H_{4'}$), 5.37 (1H, m, $H_{3'}$), 5.78 (1H, d, $J=6$ Hz, $H_{2'}$), 6.68 (1H, d, $J=6$ Hz, $H_{1'}$), 6.71 (1H, d, $J=7$ Hz, H_5), 8.42 (1H, d, $J=7$ Hz, H_6), 9.52 (2H, broad s, $=NH_2$).

2,2′-Anhydro-(3,5-di-O-isobutyryl-β-D-arabinofuranosyl)cytosine Hydrobromide (XVI)———The mixture of cytidine (1 g), MeCN (50 ml) and isobutyryl bromide (2.5 ml) was treated in a similar manner as for (XIV) to give a crystalline solid (200 mg). mp 260—261° (decomp.). UV λ_{max}^{EtOH} nm: 235, 264; λ_{min}^{EtOH} nm: 246. *Anal.* Calcd. for $C_{17}H_{24}O_6N_3Br$: C, 45.74; H, 5.42; N, 9.41. Found: C, 45.63; H, 5.07; N, 9.10.

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