

## Synthesis of 6-Cyano- and 5-Cyano-uridines and Their Derivatives (Nucleosides and Nucleotides. XXI<sup>1)</sup>)

HIDEO INOUE and TOHRU UEDA

*Faculty of Pharmaceutical Sciences, Hokkaido University<sup>2)</sup>*

(Received January 20, 1978)

5'-O-Acetyl-2',3'-O-isopropylidene-5-bromouridine (1) gave the 6-cyanouridine (2) by treatment with sodium cyanide at room temperature. Heating of 2 with sodium cyanide afforded the 5-cyanouridine derivative (3) by the addition-elimination mechanism. This method provides a convenient route of the preparation of 5-cyanouridine and 5-cyano-2'-deoxyuridine. The cyano function of 2 was converted to the amide, thioamide, carboxyl, hydroxymethyl, chloromethyl, methyl, cyanomethyl, and carboxymethyl group, respectively. The circular dichroism (CD) spectra of 6-substituted uridines prepared in this work showed all positive CD bands, though existed mainly as the *syn*-form as determined by the nuclear magnetic resonance measurements.

**Keywords**—pyrimidine nucleosides; orotidine; nucleophilic addition-elimination; sodium cyanide; nucleoside conformations; NMR; CD spectra

In the continuing studies of nucleoside conversions we have encountered an unique reaction of a 5-bromouridine derivative with sodium cyanide. As reported briefly<sup>3)</sup> 2',3'-O-isopropylidene-5-bromouridine gave three products by treatment with 4 molar equivalents of potassium cyanide in dimethylformamide at 80° for one hour, *i.e.* the 5-cyano, 6-cyano-, and a 0<sup>6</sup>,5'-cyclouridine derivatives. Later, the mechanism leading to give the cyano derivatives was elucidated.<sup>4)</sup> The formation of the 0<sup>6</sup>,5'-cyclouridine in this reaction was also explained<sup>1)</sup> by the attack of the 5'-hydroxyl group to the position 6 of the 6-cyanouridine derivative in which the cyano group behaved as a leaving group. The present report describes the full details of the substitution of 5-bromouridine derivative with cyanide ion, and the derivatization of the 6-cyano group to other functions. Some considerations on the conformations of 6-substituted pyrimidine nucleosides will also be presented.

In order to preclude the cyclonucleoside formation as encountered<sup>3)</sup> 5'-O-acetyl-2',3'-O-isopropylidene-5-bromouridine (1) was selected as the starting material. Treatment of 1 with 1.2 equivalents of sodium cyanide in dimethylformamide at room temperature for 24 hours afforded 5'-O-acetyl-2',3'-O-isopropylidene-6-cyanouridine (2) exclusively. Heating of 2 in the same solvent at 80° for 6 hours under the presence of an equivalent of sodium cyanide resulted in a formation of 5'-O-acetyl-2',3'-O-isopropylidene-5-cyanouridine (3), which was isolated in 70% yield. The most characteristic difference between 2 and 3 was found in the nuclear magnetic resonance (NMR) signals of their aromatic protons. The proton at position 5 of 2 at 6.33 ppm shifted by the reaction to 8.61 ppm which corresponded to the proton at position 6 of 3. This apparent migration of the cyano group from position 6 to 5 can be rationalized by the addition-elimination mechanism through the 5,6-dicyano-5,6-dihydro intermediate (B). It is reasonable to expect that the proton at 5 would be more acidic than that at 6 in B so that the elimination of hydrogen cyanide occurs to give 3 exclusively. It should be reminded that the treatment of 1 with toluenethiol afforded<sup>5)</sup> both 5-

1) Part XX: A. Matsuda, H. Inoue, and T. Ueda, *Chem. Pharm. Bull.* (Tokyo), **26**, 2340 (1978).

2) Location: *Kita-12, Nishi-6, Kita-ku, Sapporo, 060, Japan.*

3) H. Inoue and T. Ueda, *Chem. Pharm. Bull.* (Tokyo), **19**, 1743 (1971).

4) T. Ueda, H. Inoue, and A. Matsuda, *Ann. N.Y. Acad. Sci.*, **255**, 121 (1975).

5) H. Inoue, S. Tomita, and T. Ueda, *Chem. Pharm. Bull.* (Tokyo), **23**, 2614 (1975).

and 6-benzylthiouridine derivatives. In that case, however, no conversion of the 6-benzylthio derivative to the 5-benzylthio compound was observed by further treatment. Therefore the mechanisms producing 5-substituted derivatives are different in the two cases. Thus, in the formation of 5-benzylthiouridine from **1**, the reaction sequence should be addition-substitution-elimination. In the case of the cyanide attack the sequence should be addition (to give very labile intermediate **A**)-elimination-addition(Michael addition)-elimination.

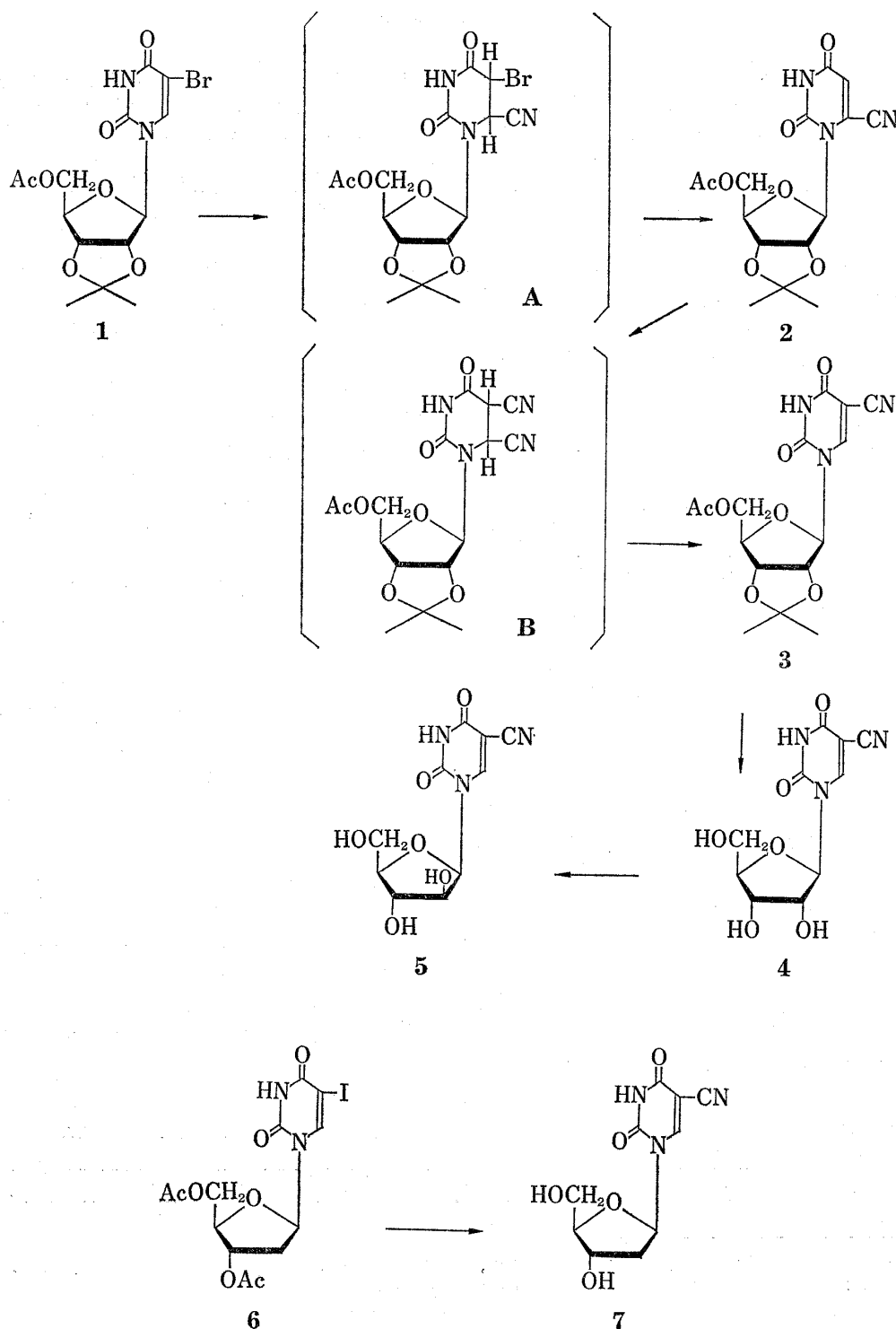


Chart 1

Deacetylation of **3** followed by deacetonation afforded 5-cyanouridine (**4**),<sup>6)</sup> thus established a facile route for the preparation of **4** as compared with the current condensation procedure.<sup>6)</sup> Compound **4** was converted to arabinofuranosyl-5-cyanouracil (**5**) by the conventional procedure. 3',5'-Di-*O*-acetyl-5-iodo-2'-deoxyuridine (**6**) was similarly treated with sodium cyanide to give the 6-cyano derivative, which was further converted to 5-cyano-2'-deoxy-

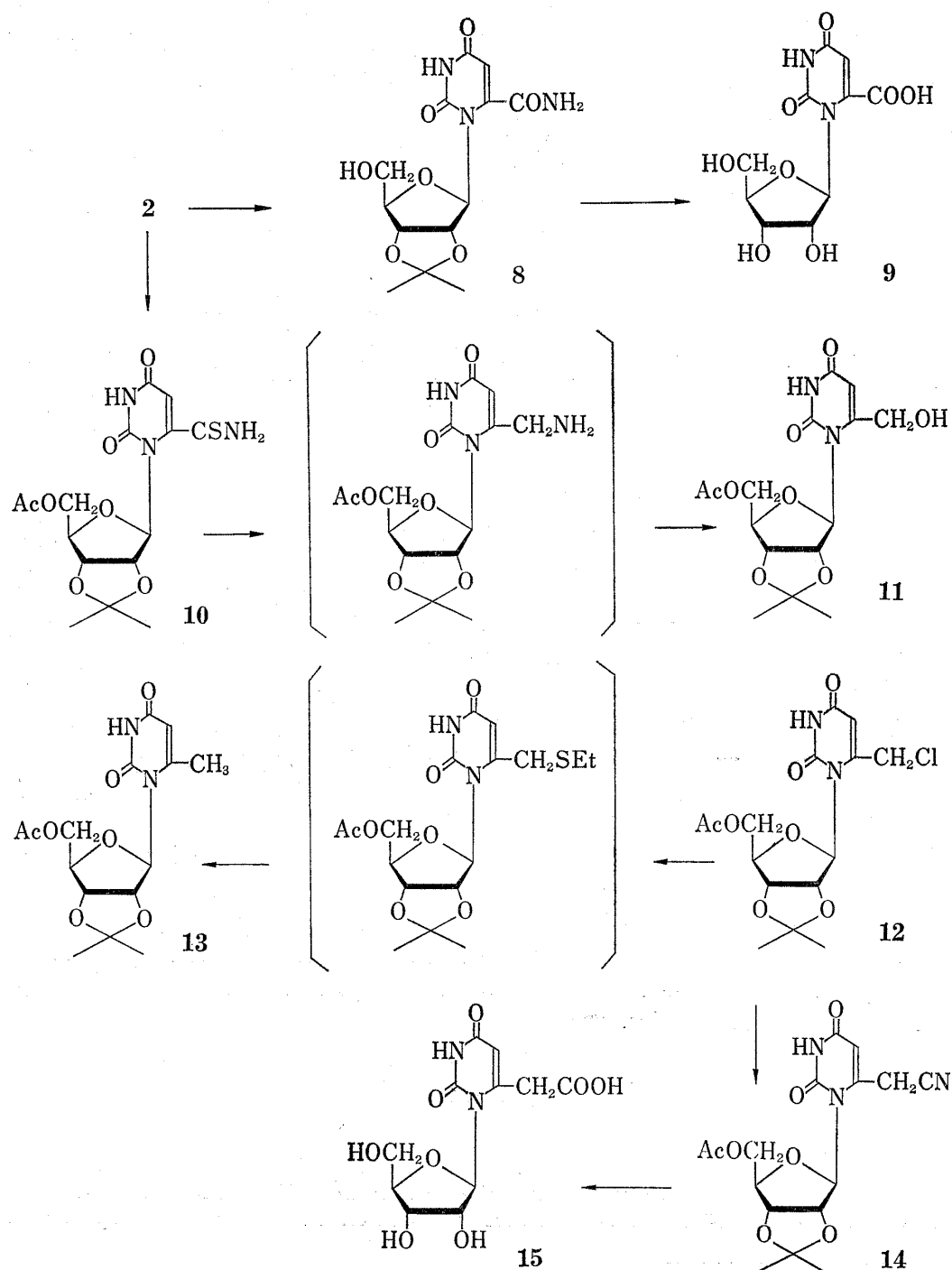


Chart 2

6) a) G. Shaw, R.N. Warrener, M.H. Maguire, and R.K. Ralph, *J. Chem. Soc.*, 1958 2294; b) N.J. Cusack, B.J. Hildick, D.H. Robinson, P.W. Rugg, and G. Shaw, *J. Chem. Soc. Perkin I*, 1973 1720; c) M. Prystas and F. Sorm, *Coll. Czech. Chem. Commun.*, 31, 3990 (1966); d) K.A. Watanabe and J.J. Fox, *J. Heterocycl. Chem.*, 6, 109 (1969).

uridine (7). After our previous report<sup>3,4)</sup> 6-cyano-2'-deoxyuridine<sup>7)</sup> and 7<sup>8,9)</sup> were prepared by essentially similar procedures. In addition, reaction of 5-bromocytidine with sodium cyanide was found to proceed rapidly to give 6-cyanocytidine.<sup>1)</sup>

Compound 2 was utilized for further derivatization. Treatment of 2 with 0.5 N sodium hydroxide solution at room temperature afforded 2',3'-*O*-isopropylideneuridine-6-carboxamide (8). Heating of 8 in the same solvent at 90—100° followed by mild acid treatment afforded orotidine (9) which was identified by the comparisons with the authentic material. This method provides a new route of preparation of orotidine<sup>10)</sup> from readily available uridine.

Treatment of 2 with hydrogen sulfide saturated in pyridine gave the 6-thiocarboxamide (10). Reductive desulfurization with Raney Ni and successive treatment with nitrous acid afforded a 6-hydroxymethyluridine (11). The hydroxymethyl group of 11 was converted to give the chloromethyluridine (12) by the treatment with *p*-toluenesulfonyl chloride and 2,6-lutidine in dimethylformamide at room temperature. Compound 12 was treated with sodium ethyl sulfide to give the 6-ethylthiomethyl derivative which was desulfurized to furnish 5'-*O*-acetyl-2',3'-*O*-isopropylidene-6-methyluridine (13).

Treatment of 12 with sodium cyanide in dimethylformamide at room temperature gave the 6-cyanomethyluridine (14). The large red-shift of the ultraviolet (UV) absorption maximum of 14 in an alkaline solution is indicative of the dissociation of the methylene group at position 6 of 14. Hydrolysis of 14 with sodium hydroxide solution followed by acid treatment afforded uridine-6-acetic acid (15), a homolog of orotidine.

### Conformations of 6-Substituted Uridines

In consistent with the previous results<sup>5)</sup> the 6-substituted uridine derivatives, 2, 10—14, prepared in the present work are in *syn*-conformations as determined by the down-field shifts of the 2' (and 3')-protons in NMR measurements as shown in Table I. The circular dichroism (CD) spectra of these 6-substituted uridines (2, 9, 12—14) exhibited positive Cotton bands in their main absorption regions regardless of the electro-negative or -positive nature of the substituents at the 6-position. It appears that 6-methyluridine and 6-methylcytidine are the exceptions which exhibit negative CD bands.<sup>11)</sup> Since the reversal of the sign of CD band of 6-methyluridine was observed by the protection of the sugar moiety (as in 13), it should be careful to draw a conclusion simply from a sign of the CD bands as to the *syn-anti* conformations. Assignment of the *anti*-conformation for orotidine by the CD measurement<sup>12)</sup> alone should be revised at the present stage. The 6-substituents so far introduced in the pyrimidine nucleosides, however, may not be bulky enough to preclude the possible existence of the these nucleosides in the *anti*-conformations. The introduction of the bulkier substituents into the 6-position should be desirable to solve the relation of the sign of CD bands in terms of *syn-anti* conformations. Studies on the synthesis of such model compounds are in progress in our laboratories.

### Experimental

Melting points were determined by a Yamato MP-1 melting point apparatus and were uncorrected. Thin-layer chromatography was performed with Wako gel B-5F and silica gel column chromatography was performed with Wako gel C-200. UV spectra were measured on a Shimadzu D-40R spectrophotometer.

- 7) A. Holy, *Coll. Czech. Chem. Commun.*, **40**, 738 (1975).
- 8) R.C. Bleackley, A.S. Jones, and R.T. Walker, *Nucleic Acids Res.*, **2**, 683 (1975).
- 9) P.F. Torrence, B. Bhooshan, J. Descamps, and E. De Clercq, *J. Med. Chem.*, **20**, 974 (1977).
- 10) W.V. Curran and R.B. Angier, *J. Org. Chem.*, **31**, 201 (1966).
- 11) D.W. Miles, M.J. Robins, R.K. Robins, M.W. Winkley, and H. Eyring, *J. Am. Chem. Soc.*, **91**, 824, 831 (1969).
- 12) A. Holy, *Coll. Czech. Chem. Commun.*, **37**, 1555 (1972).

Infrared (IR) spectra were recorded on a Hitachi 215 spectrophotometer. NMR spectra were recorded on a Hitachi R-24 spectrometer with tetramethylsilane as an internal standard. Mass spectra (MS) were recorded on a Hitachi RMU-7 spectrometer. CD spectra were measured by a JASCO J-40 spectropolarimeter.

**5'-O-Acetyl-2',3'-O-isopropylidene-5-bromouridine (1)**—2',3'-O-Isopropylidene-5-bromouridine (9 g) and acetic anhydride (10 ml) in 50 ml of pyridine were kept for 4 hr at room temperature. The mixture was evaporated and the residue was dissolved in EtOH and concentrated to leave a sirup. This was repeated several times and the final sirup was dissolved in hot EtOH. On cooling colorless crystals (1, 9.1 g, 88%) were separated, mp 166—167°. *Anal.* Calcd. for  $C_{14}H_{17}BrN_2O_7$ : C, 41.50; H, 4.23; Br, 19.72; N, 6.92. Found: C, 41.46; H, 4.24; Br, 19.61; N, 7.06. UV  $\lambda_{max}^{EtOH}$ , 276 nm ( $\epsilon$ , 9600). NMR ( $CDCl_3$ )  $\delta$ : 9.68 (bs,  $N^3-H$ ), 2.12 (s, 3, Ac), 1.57, 1.36 (s, 3+3,  $Me_2C$ ). Other signals were recorded in Table I.

TABLE I. NMR Chemical Shifts ( $\delta$ ) of 6 (5)-Substituted 5'-O-Acetyl-2',3'-O-isopropylideneuridines

Substituent	5-H or 6-H	1'-H	2'-H	3'-H	4'- and 5'-H	Solvent
5-Br (1)	7.69	5.76	4.96	4.79	4.6—4.2	$CDCl_3$
5-CN (3)	8.61	5.83	4.96	4.78	4.6—4.2	$CDCl_3$
6-CN (2)	6.33	6.03	5.22	4.88	4.31	$CDCl_3$
6- $CSNH_2$ (10)	5.50	5.73	5.15	4.79	4.4—3.9	DMSO- $d_6$
6- $CH_2OH$ (11)	5.70	5.79	5.20	4.80	4.5—3.9	DMSO- $d_6$
6- $CH_2Cl$ (12)	5.83	5.83	5.28	4.93	4.29	$CDCl_3$
6- $CH_3$ (13)	5.58	5.70	5.24	4.92	4.28	$CDCl_3$
6- $CH_2CN$ (14)	6.02	5.53	5.28	4.90	4.26	$CDCl_3$

**5'-O-Acetyl-2',3'-O-isopropylidene-6-cyanouridine (2)**—Compound 1 (2.03 g) was dissolved in 10 ml of DMF and to which was added 370 mg (1.5 eq.) of NaCN and stirred for 24 hr at room temperature. The mixture was poured in 100 ml of EtOAc and extracted with 50 ml of  $H_2O$ , while adjusting the pH of the aqueous layer to 6—7 by adding 1N HCl. The organic layer was washed twice with  $H_2O$  (20 ml), dried over  $Na_2SO_4$ , and evaporated to a sirup. This was dissolved in EtOH and evaporated again to leave crude 2 (1.8 g). Purification of 2 was performed by a silica gel chromatography (silica gel 60 g,  $2.7 \times 19.5$  cm) with 2% EtOH- $CHCl_3$  as an eluent. Evaporation of the solvent afforded colorless solid of 2 (1.69 g, 96%). *Anal.* Calcd. for  $C_{15}H_{17}N_3O_7$ : C, 51.28; H, 4.88; N, 11.96. Found: C, 51.42; H, 4.90; N, 11.96. UV  $\lambda_{max}^{EtOH}$ , 281 nm ( $\epsilon$ , 8600),  $\lambda_{max}^{EtOH}$ , 229 nm ( $\epsilon$ , 2000); IR (Nujol)  $2250\text{ cm}^{-1}$  (CN); MS  $m/e$ : 336 ( $M-Me$ )<sup>+</sup>; NMR ( $CDCl_3$ )  $\delta$ : 9.97 (bs, 1,  $N^3-H$ ), 2.07 (s, 3, Ac), 1.57, 1.36 (s, 3+3,  $Me_2C$ ), other protons, see Table I. CD (MeOH) nm ( $\theta$ ): peak 278 (+5500), 243 (0), sh 234 (−1100).

**5'-O-Acetyl-2',3'-O-isopropylidene-5-cyanouridine (3)**—Compound 2 (1.3 g) and NaCN (184 mg) were dissolved in 37 ml of dimethylformamide (DMF) and stirred for 6 hr at 80°. The mixture was poured into 250 ml of EtOAc and washed with 100 ml of  $H_2O$ , adjusting the pH of the aqueous layer at 6—7 by 1N HCl, and the organic layer was separated. After washing with 50 ml of  $H_2O$  twice and drying over  $Na_2SO_4$ , the organic layer was concentrated and the residue was crystallized from EtOH to give 910 mg, 70%, of 3, mp 174—176°. *Anal.* Calcd. for  $C_{15}H_{17}N_3O_7$ : C, 51.28; H, 4.88; N, 11.96. Found: C, 51.10; H, 4.95; N, 12.02. UV  $\lambda_{max}^{EtOH}$  275 nm ( $\epsilon$ , 12300),  $\lambda_{max}^{EtOH}$  236 nm ( $\epsilon$ , 1850); IR (Nujol)  $2245\text{ cm}^{-1}$  (CN); NMR ( $CDCl_3$ )  $\delta$ : 9.85 (bs, 1,  $N^3-H$ ), 2.12 (s, 3, Ac), 1.57, 1.36 (s, 3+3,  $Me_2C$ ), other protons, see Table I.

**5-Cyanouridine<sup>8a,9</sup>**—Compound 3 (5 g) was dissolved in 100 ml of MeOH saturated with  $NH_3$  and kept overnight at room temperature. On evaporation of the solvent the crystalline material separated (2',3'-O-isopropylidene-5-cyanouridine), which was washed with EtOH and dissolved in 100 ml of 50%  $HCO_2H$ , and kept at room temperature overnight. The solvent was evaporated and the residue was taken in 50 ml of 10%  $NH_4OH$  and set aside at room temperature for 1 hr. After removal of the solvent the residue was crystallized from  $H_2O$ -EtOH to give 3.3 g (87%) of 4. The analytical sample was obtained by recrystallization, mp 191—192°. *Anal.* Calcd. for  $C_{10}H_{11}N_3O_6$ : C, 44.61; H, 4.12; N, 15.61. Found: C, 44.73; H, 4.12; N, 15.42.

**1- $\beta$ -D-Arabinofuranosyl-5-cyanouracil (5)**—Compound 4 (1.35 g) was treated in 2.5 ml of DMF with 1.35 g of diphenyl carbonate and 28 mg of  $NaHCO_3$  at 150° for 30 min under stirring. MeOH (2.5 ml) was added to the mixture and poured into 100 ml of ether, and triturated. The collected precipitate was taken in  $H_2O$ -MeOH, decolorized with active carbon, and evaporated to leave a residue. The solid was dissolved in 5 ml of 0.5N NaOH and kept overnight at room temperature. After neutralization of the solution with Dowex 50 ( $H^+$ ) resin the filtrate was concentrated, and the separated crystals (5, 300 mg) were recrystallized from EtOH- $H_2O$ , mp 231.5—232.5°. *Anal.* Calcd. for  $C_{10}H_{11}N_3O_6$ : C, 44.61; H, 4.12; N, 15.61. Found: C, 44.70; H, 4.03; N, 15.72. UV  $\lambda_{max}^{EtOH}$  278 nm ( $\epsilon$ , 12900), 218 ( $\epsilon$ , 10400),  $\lambda_{max}^{EtOH}$  277 nm ( $\epsilon$ , 9600); IR (Nujol)  $2250\text{ cm}^{-1}$  (CN); NMR ( $D_2O$ )  $\delta$ : 8.62 (6-H), 6.02 (1'-H). *Rf* (EtOH-0.5N  $NH_4OAc$ , 5:2) 0.55 (compound 4, 0.49).

**3',5'-Di-O-acetyl-5-iodo-2'-deoxyuridine (6)**—5-Iodo-2'-deoxyuridine (purchased from Yamasa Shoyu Co., 5 g) was suspended in 10 ml of pyridine and 10 ml of  $\text{Ac}_2\text{O}$  and stirred overnight at room temperature. After the removal of the solvent the residue was taken in  $\text{CHCl}_3$ , washed with  $\text{H}_2\text{O}$ , dried over  $\text{Na}_2\text{SO}_4$ , and evaporated to give a crystalline solid, which was recrystallized from  $\text{H}_2\text{O}$ -EtOH to afford 6.1 g (quant.) of 6, UV  $\lambda_{\text{max}}^{\text{EtOH}}$  281 nm.

**5-Cyano-2'-deoxyuridine (7)<sup>8,9)</sup>**—Compound 6 (2.19 g) was treated with 0.4 g of NaCN in 10 ml of DMF under stirring for 6 hr at room temperature. After the work-up as performed in the preparation of 2, the sirupy product, 3',5'-di-O-acetyl-6-cyano-2'-deoxyuridine (1.7 g), UV  $\lambda_{\text{max}}$  280 nm, was obtained. This sirup was again treated with 0.25 g of NaCN in 20 ml of DMF under stirring at 80° for 5 hr. A similar work-up of the mixture afforded a sirup (1.7 g) of the 5-cyano derivative, UV  $\lambda_{\text{max}}^{\text{EtOH}}$  275 nm. This sirup was taken in 100 ml of MeOH saturated with  $\text{NH}_3$  and kept overnight at room temperature. After evaporation of the solvent the residue was crystallized from EtOH- $\text{H}_2\text{O}$  to give 0.8 g (64%) of 7, mp 172–173°. *Anal.* Calcd. for  $\text{C}_{10}\text{H}_{11}\text{N}_3\text{O}_5$ : C, 47.43; H, 4.38; N, 16.60. Found: C, 47.56; H, 4.39; N, 16.41. UV  $\lambda_{\text{max}}^{\text{EtOH}}$  278 nm ( $\epsilon$ , 12200), 217 ( $\epsilon$ , 10400),  $\lambda_{\text{max}}^{\text{OH}^-}$  277 nm ( $\epsilon$ , 8800); IR (Nujol) 2240  $\text{cm}^{-1}$  (CN).

**2',3'-O-Isopropylideneuridine-6-carboxamide (8)**—Compound 2 (3.5 g) was dissolved in 80 ml of 0.5N NaOH, kept for 110 min at room temperature, and neutralized by addition of Dowex 50 ( $\text{H}^+$ ) resin to pH 6–7. The filtrate was concentrated and the residue was dissolved in MeOH, mixed with silica gel (14 g), and dried. This powder was applied on the top of a column (silica gel, 70 g,  $2.8 \times 18$  cm) and developed with EtOH-EtOAc. The eluate of 10–20% EtOH-EtOAc solution was collected, concentrated, and the residue was again concentrated from MeOH to leave a foam of 8 (2.97 g, 91%). *Anal.* Calcd. for  $\text{C}_{13}\text{H}_{17}\text{N}_3\text{O}_7$ : C, 47.71; H, 5.23; N, 12.84. Found: C, 47.72; H, 5.29; N, 12.71. UV  $\lambda_{\text{max}}^{\text{EtOH}}$  267 nm ( $\epsilon$ , 8800),  $\lambda_{\text{max}}^{\text{EtOH}}$  265 nm ( $\epsilon$ , 6600); MS  $m/e$ : 312 ( $\text{M}-\text{Me}$ )<sup>+</sup>; NMR (DMSO- $d_6$ )  $\delta$ : 8.40, 8.06 (s, 1+1, CONH<sub>2</sub>), 5.67 (s, 2, 5-H and 1'-H), 5.15 (bs, 1, 2'-H), 4.7 (m, 2, 3'-H and 5'-OH), 3.9 (s, 1, 4'-H), 3.7–3.1 (m, 2, 5'-H overlapped with  $\text{H}_2\text{O}$ ), 1.45, 1.26 (s, 3+3,  $\text{Me}_2\text{C}$ ).

**Orotidine (9)**—Compound 8 (646 mg) was dissolved in 12 ml of 0.5N NaOH and kept at 90–100° for 5 hr. The solution was added Dowex 50 ( $\text{H}^+$ ) resin to adjust pH 2–3. After filtration of the resin the filtrate was concentrated to 20 ml and kept for 22 hr at 36°. Triethylamine- $\text{H}_2\text{O}$  was added to adjust the pH at 6.1 and the solution was applied to a column of DEAE-cellulose ( $2.7 \times 34$  cm,  $\text{HCO}_3^-$ ). A linear gradient elution with  $\text{H}_2\text{O}$  (2000 ml) and 0.1M triethylammonium bicarbonate (2000 ml) gave the fractions (No. 61–69, one fraction, 20 ml) containing 9 (11000 OD Unit at 267 nm) which was concentrated to a small volume. Addition of  $\text{H}_2\text{O}$  and evaporation was repeated several times to remove the salts and the final solution was passed through a column of Dowex 50 ( $\text{H}^+$ ,  $1.6 \times 8$  cm) resin. Evaporation of the eluent and co-evaporation with EtOH afforded a solid of 9 (305 mg, 54%); UV  $\lambda_{\text{max}}^{\text{EtOH}}$  267 nm,  $\lambda_{\text{min}}^{\text{EtOH}}$  235 nm,  $\lambda_{\text{max}}^{\text{EtOH}}$  267 nm,  $\lambda_{\text{min}}^{\text{EtOH}}$  247 nm. *Rf* (solvent-iso-PrOH-conc.  $\text{NH}_4\text{OH}$ ,  $\text{H}_2\text{O}=7:1:2$ ) 0.40. *Rf* (EtOH-1M  $\text{NH}_4\text{OAc}=5:2$ , pH 7) 0.43. Paper electrophoretic migration (0.05M NaOAc, pH 5.0, 700 V, 70 min), +9.3 cm. These values are identical with those of the commercially available authentic sample. NMR (DMSO- $d_6$ - $\text{D}_2\text{O}$ )  $\delta$ : 5.77 (s, 1, 5-H), 5.45 (d, 1, 1'-H), 4.51 (1, 2'-H), 4.03 (1, 3'-H), 3.3–3.8 (3, 4',5'-H).

**5'-O-Acetyl-2',3'-O-isopropylideneuridine-6-thiocarboxamide (10)**—Compound 2 (7.1 g) was dissolved in 100 ml of pyridine and to which was bubbled  $\text{H}_2\text{S}$  for 20 min at room temperature, sealed and kept for 1 hr at room temperature. After vaporization of  $\text{H}_2\text{S}$  by passing  $\text{N}_2$  stream the solution was evaporated to leave a residue. Dissolution of the residue in EtOH and standing for several hours afforded crystals of 10 (6.2 g, 81%), which was recrystallized from MeOH, mp 240–242° (dec.). *Anal.* Calcd. for  $\text{C}_{15}\text{H}_{19}\text{N}_3\text{O}_7\text{S}$ : C, 46.75; H, 4.97; N, 10.90; S, 8.32. Found: C, 46.63; H, 4.90; N, 10.84; S, 8.41. UR  $\lambda_{\text{max}}^{\text{EtOH}}$  276 nm ( $\epsilon$ , 12600); NMR (DMSO- $d_6$ )  $\delta$ : 11.57 (bs, 1,  $\text{N}^3\text{-H}$ ), 10.54, 10.21 (bs, 1+1,  $\text{CSNH}_2$ ), 2.01 (s, 3, Ac), 1.44, 1.28 (s, 3+3,  $\text{Me}_2\text{C}$ ), other signals, see Table I. MS  $m/e$ : 385 ( $\text{M}^+$ ).

**5'-O-Acetyl-2',3'-O-isopropylidene-6-hydroxymethyluridine (11)**—Compound 10 (3.86 g) was dissolved in hot 90% EtOH (220 ml) and kept under stirring with 30 g of Raney Ni (W-2) for 2 hr. The Ni was removed by filtration, washed with 200 ml of EtOH, and the filtrate and washings were combined and evaporated to a residue (2.5 g). This was dissolved in 5 ml of acetic acid and partitioned with 50 ml of  $\text{H}_2\text{O}$  and 20 ml of  $\text{CHCl}_3$ . The aqueous layer was separated and added 1 ml of AcOH and 5 ml of 2N  $\text{NaNO}_2$  under ice-cooling. After 30 min the mixture was extracted with EtOAc (60 ml, then 20 ml) and the organic layer was concentrated. The residue was taken in EtOH from which colorless crystals of 11 (770 mg, 22%) were separated, mp 238–240°. Purification by a column chromatography with silica gel gave the analytically pure sample, mp 249.5–250.5°. *Anal.* Calcd. for  $\text{C}_{15}\text{H}_{20}\text{N}_2\text{O}_8$ : C, 50.56; H, 5.66; N, 7.86. Found: C, 50.33; H, 5.61; N, 7.76. UV  $\lambda_{\text{max}}^{\text{EtOH}}$  260 nm ( $\epsilon$ , 10000); MS  $m/e$ : 341 ( $\text{M}-\text{Me}$ )<sup>+</sup>; NMR (DMSO- $d_6$ )  $\delta$ : 11.35 (bs, 1,  $\text{N}^3\text{-H}$ ), 5.79 (1, OH), 4.31 (s, 2, 6- $\text{CH}_2$ ), 2.00 (s, 3, Ac), 1.47, 1.28 (s, 3+3,  $\text{Me}_2\text{C}$ ), other protons, see Table I.

**5'-O-Acetyl-2',3'-O-isopropylidene-6-chloromethyluridine (12)**—Compound 11 (1.78 g) and TsCl (1.14 g, 1.2 eq.) were dissolved in 15 ml of DMF and 0.81 ml of 2,6-lutidine was added and kept at room temperature overnight. After standing few minutes on addition of 2 ml of  $\text{H}_2\text{O}$  to the mixture it was diluted with 50 ml of  $\text{H}_2\text{O}$  and extracted with 100 ml of EtOAc. The organic layer was washed twice with 20 and 10 ml of  $\text{H}_2\text{O}$ , respectively, dried over  $\text{Na}_2\text{SO}_4$ , and evaporated to leave a solid. Crystallization from EtOH afforded 12 (1.4 g, 75%), mp 188–189°. *Anal.* Calcd. for  $\text{C}_{15}\text{H}_{19}\text{ClN}_2\text{O}_7$ : C, 48.07; H, 5.11; Cl, 9.46; N, 7.47. Found: C, 47.94; H, 5.06; Cl, 9.43; N, 7.29. UV  $\lambda_{\text{max}}^{\text{EtOH}}$  265 nm ( $\epsilon$ , 10100); MS  $m/e$ : 359 ( $\text{M}-\text{Me}$ )<sup>+</sup>; NMR ( $\text{CDCl}_3$ )  $\delta$ :

10.04 (bs, 1, N<sup>3</sup>-H), 4.38 (s, 2, 6-CH<sub>2</sub>), 2.06 (s, 3, Ac), 1.54, 1.35 (s, 3+3, Me<sub>2</sub>C), other signals, see Table I; CD (MeOH) nm ( $\theta$ ): peak 260 (+5800), 235 (0).

**5'-O-Acetyl-2',3'-O-isopropylidene-6-methyluridine (13)**—Compound 12 (379 mg) was dissolved in 10 ml of EtOH previously added 1 ml of 2 N NaOEt and 0.3 ml of C<sub>2</sub>H<sub>5</sub>SH, and kept overnight at room temperature. After addition of a small amount of AcOH the solution was evaporated and the residue was treated with 1 ml of Ac<sub>2</sub>O for 12 hr. The solution was diluted with 5 ml of EtOH and evaporated to leave a residue. This was partitioned with EtOAc (3 ml) and H<sub>2</sub>O (10 ml) and the organic layer was concentrated and the residue was taken in 95% EtOH. Stirring of the solution with 1 g of Raney Ni (W-2) for 4 hr followed by filtration and evaporation gave a solid. This was applied to a column of silica gel (1.9 × 4 cm) and eluted with 2.5% EtOH in CHCl<sub>3</sub>. The eluates were concentrated and the residue was decolorized and crystallized from EtOH to give 190 mg (56%) of 13, mp 229.5–230.5°. *Anal.* Calcd. for C<sub>15</sub>H<sub>20</sub>N<sub>2</sub>O<sub>7</sub>: C, 52.94; H, 5.92; N, 8.23. Found: C, 52.74; H, 5.94; N, 8.18. UV  $\lambda_{\text{max}}^{\text{EtOH}}$  258 nm ( $\epsilon$ , 11200); MS *m/e*: 325 (M-Me)<sup>+</sup>; NMR (CDCl<sub>3</sub>)  $\delta$ : 10.12 (bs, 1, N<sup>3</sup>-H), 2.33 (s, 3, 6-Me), 2.06 (s, 3, Ac), 1.53, 1.34 (s, 3+3, Me<sub>2</sub>C), other signals, see Table I; CD (MeOH) nm ( $\theta$ ): peak 250 (+6300), 228 (0).

**5'-O-Acetyl-2',3'-O-isopropylidene-6-cyanomethyluridine (14)**—Compound 12 (749 mg) was dissolved in 5 ml of DMF and 197 mg of NaCN (dissolved in 15 ml of DMF) was added and stirred for 10 hr at room temperature. The mixture was poured in 100 ml of EtOAc and washed with 100 ml of H<sub>2</sub>O, while the pH of the aqueous layer was adjusted to 6–7. The separated organic layer was washed twice with H<sub>2</sub>O (20 ml, each), dried over Na<sub>2</sub>SO<sub>4</sub>, and evaporated to leave a sirup. The CHCl<sub>3</sub> solution of the residue was applied to a column of silica gel and developed with EtOH-CHCl<sub>3</sub>. The concentrate of the eluate, 14 (440 mg, 61%), was crystallized from EtOH, mp 199.5–202.5°. *Anal.* Calcd. for C<sub>16</sub>H<sub>19</sub>N<sub>3</sub>O<sub>7</sub>: C, 52.60; H, 5.24; N, 11.50. Found: C, 52.48; H, 5.24; N, 11.27. UV  $\lambda_{\text{max}}^{\text{MeOH}}$  257 nm ( $\epsilon$ , 10600),  $\lambda_{\text{max}}^{\text{pH} 14}$  332.5 nm. IR (Nujol) 2270 cm<sup>-1</sup>; MS *m/e*: 350 (M-Me)<sup>+</sup>; NMR (CDCl<sub>3</sub>)  $\delta$ : 9.91 (bs, 1, N<sup>3</sup>-H), 3.79 (s, 2, 6-CH<sub>2</sub>), 2.06 (s, 3, Ac), 1.53, 1.34 (s, 3+3, Me<sub>2</sub>C); CD (MeOH) nm ( $\theta$ ): peak 256 (+6500), 229 (0).

**Uridine-6-acetic Acid (15)**—Compound 14 (22 mg) was dissolved in 6 ml of 2 N NaOH and kept for 18 hr at room temperature. The solution was neutralized with Dowex 50 (H<sup>+</sup>) resin and filtered. The filtrate was concentrated and the residue was added 2 ml of 98% HCO<sub>2</sub>H and kept for 7 hr at room temperature. The solution was evaporated, added H<sub>2</sub>O, and evaporated. This was repeated several times to remove HCO<sub>2</sub>H. The final residual solid (15) was unable to crystallize due, probably, to its small quantity. *Rf* (EtOH-1 M NH<sub>4</sub>OAc=5:2, pH 7) 0.34, *Rf* (iso-PrOH-conc. NH<sub>4</sub>OH-H<sub>2</sub>O=7:1:2) 0.15. The *Rf* values of uridine in these solvents were 0.52 and 0.32, respectively. Paper electrophoretic migrations (0.05 M triethylammonium bicarbonate, pH 8, 700 V, 1 hr) +4 cm (UMP, +7 cm; uridine, 0 cm). UV  $\lambda_{\text{max}}^{\text{H}_2\text{O}}$  262 nm;  $\lambda_{\text{max}}^{\text{pH} 1}$  260 nm;  $\lambda_{\text{max}}^{\text{pH} 13}$  262.5 nm. Periodate-benzidine spray test: positive.

**Acknowledgement** This work was supported, in part, by the Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture, Japan.