Chem. Pharm. Bull.
23(3) 604—610 (1975)

UDC 547.963.32.04 : 547.379.1.057

Nucleosides and Nucleotides. XII.[1] Synthesis and Properties of 2'-Deoxy-2'-mercaptouridine and Its Derivatives[2]

Masaoki Imazawa, Tohru Ueda,[3a] and Tyunosin Ukita[3b] (the late)

Faculty of Pharmaceutical Sciences, Hokkaido University[3a] and Faculty of Pharmaceutical Sciences, the University of Tokyo[3b]

(Received September 2, 1974)

New thiosugar nucleosides, 2'-deoxy-2'-mercaptouridine (III), its disulfide (IV), 2'-deoxy-2'-mercapto-3',5'-di-O-acetyluridine (V), and 2'-deoxy-2'-methyl-thiouridine (VI) have been synthesized. The present synthetic method involves the use of 2'-deoxy-2'-acetylthio-3',5'-di-O-acetyluridine (II) as the intermediate which was obtained by the reaction of 2,2'-cyclo-3',5'-di-O-acetyluridine (I) with thioacetic acid. The proton magnetic resonance (PMR) data of these compounds suggested that the introduction of sulfur atom at 2'-position resulted in the furanose ring puckering that is extremely biased to $C_{2'}$ endo-mode. 2'-Deoxy-2',6-epithio-5,6-dihydro-arabinofuranosyluracil (VIIIb), the 2'-epimer of III in an 2',6-epithio form, was also synthesized.

Subtle change in the sugar portion of the nucleosides that occur in the nucleic acids have led to analogs that possess interesting biological properties such as arabinosylcytosine and cordycepin.[4] A number of pyrimidine and purine nucleoside derivatives, which have a thiol function in the ribosyl moiety, have been synthesized in recent years.[5] Of the positional isomers, 2'-deoxy-2'-mercaptonucleoside has not been synthesized previously, though unique role of the substituent (hydrogen or hydroxyl) on the 2'-carbon atom of deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) is actively studied using many 2'-modified nucleosides, nucleotides, and polynucleotides.[6] The first attempt for substitution of 2'-hydroxyl group of nucleosides with thiol by Sekiya and Ukita was unsuccessfull. The reaction of 2,2'-cyclouridine derivatives with thiobenzoate or xanthogenate gave no sulfur-containing nucleoside but resulted in a partial liberation of uracil.[7] While a complex neighboring approach provided a synthesis of 2',3'-dideoxy-2'-mercapto-3'-aminouridine,[8] its conversion to 2'-deoxy-2'-mercaptouridine (III) by deamination reaction failed owing to glycosidic bond cleavage.[9] We wish to report the first synthesis of 2'-mercaptonucleoside, 2'-deoxy-2'-mercaptouridine. The synthesis of some of its derivatives and their properties are also described. After our preliminary report[2] had been published, a 2'-deoxy-2'-benzoylthioadenosine derivative was synthesized by Ryan *et al.*, but attempts to prepare 2'-deoxy-2'-mercaptoadenosine by its debenzoylation afforded adenine as only detectable purine derivative.[10]

---

1) Part XI: K. Miura, T. Kasai, and T. Ueda, *Chem. Pharm. Bull.* (Tokyo), 23, 464 (1975).
2) A preliminary report of this work has appeared. M. Imazawa, T. Ueda, and T. Ukita, *Tetrahedron Letters*, **1970**, 4807.
3) Location: *a) Kita-12, Nishi-6, Kita-ku, Sapporo; b) 3-1, Hongo-7-chome, Bunkyo-ku, Tokyo.*
4) P. Roy-Burman, "Recent Results in Cancer Research," Vol. 25 ed. by P. Rentchnick, Springer-Verlag, Berlin, 1970, p. 66 and p. 77.
5) *a)* B. Bannister and F. Kagan, *J. Am. Chem. Soc.*, **82**, 3363 (1960); *b)* R.W. Chambers and V. Kurkov, *J. Am. Chem. Soc.*, **85**, 2160 (1963); *c)* E.J. Reist, A. Benitz, and L. Goodman, *J. Org. Chem.*, **29**, 554 (1964); *d)* N. Miller and J.J. Fox, *J. Org. Chem.*, **29**, 1772 (1964); *e)* K.J. Ryan, E.M. Acton, and L. Goodman, *J. Org. Chem.*, **33**, 1783 (1968); *f)* I. Wempen and J.J. Fox, *J. Org. Chem.*, **34**, 1020 (1969).
6) J.J. Fox, *Pure and Applied Chemistry*, **18**, 223 (1969); D. Wagner, J.P.H. Verheyden, and J.G. Moffatt, *J. Org. Chem.*, **37**, 1876 (1972); J. Hobbs, H. Sternbach, M. Sprinzl, and F. Eckstein, *Biochemistry*, **12**, 5138 (1973) and references cited therein.
7) T. Sekiya and T. Ukita, *Chem. Pharm. Bull.* (Tokyo), **15**, 1498 (1967).
8) T. Sekiya and T. Ukita, *Chem. Pharm. Bull.* (Tokyo), **15**, 1503 (1967).
9) T. Sekiya, unpublished.
10) K.J. Ryan, E.M. Acton, and L. Goodman, *J. Org. Chem.*, **36**, 2646 (1971).

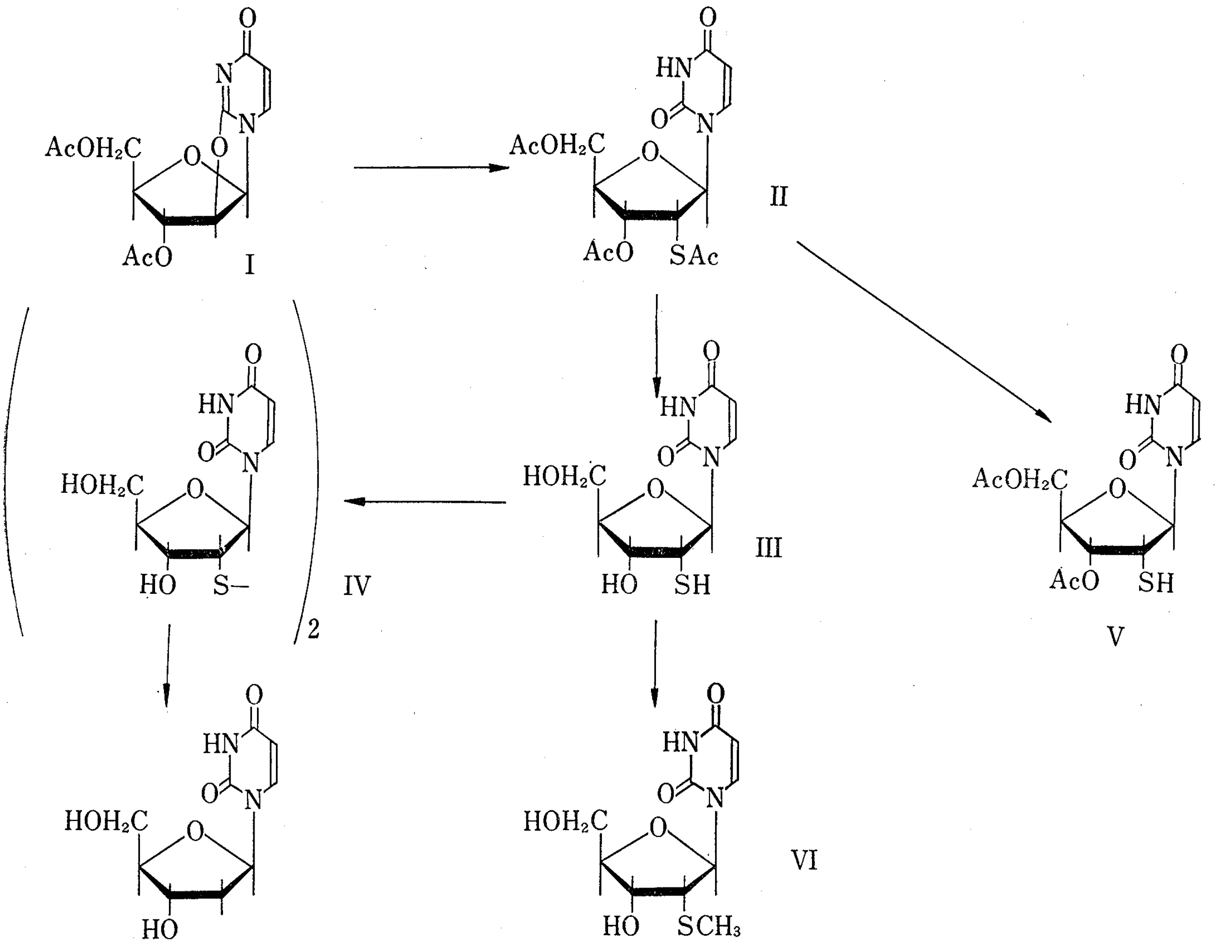

Chart 1

The reaction of 2,2′-cyclo-3′,5′-di-O-acetyluridine (I) with thioacetate in the presence of thioacetic acid gave uracil as a main product analogously to the reaction with thiobenzoate.[7] In the next experiment, I was brought in the reaction with free thioacetic acid which is also a good nucleophilic reagent.[11] When a solution of the reagent and I in dimethylformamide (DMF) was heated at 110° in a steel container, 2′-deoxy-2′-acetylthio-3′,5′-di-O-acetyluridine (II) was obtained in 30% yield. The characteristic ultraviolet (UV) absorbing spectrum of II ($\lambda_{\text{min}}^{\text{H}_2\text{O}}$ nm (ε): 229 (6400)) suggested that one acetylthio residue (λ_{max} nm (ε): 231 (4500))[12] was introduced to the ribose moiety of I with concomitant cleavage of 2,2′-cyclo bond. The low yield was mainly due to some reactivity of DMF with thioacetic acid.[13] So the solvent was replaced with dioxane and the yield was raised up to 65%. In these reaction mixtures, no uracil was detected. Treatment of II with potassium acetate-acetic acid (1: 1) in DMF at 80° for 3 hr afforded 40% of uracil, while II was stable in acetic acid-DMF (1: 1) at 110° for 6 hr. Presumably, acetate or thioacetate anion accelerates the neighboring attack[14] of the 2′-sulfur function to 1′-carbon in II with elimination of uracil. While deacetylation reaction of II with ammonia or metallic alkali at high or moderate temperature gave uracil as a main product, hydrolysis of II with KOH at a low temperature afforded crystalline 2′-deoxy-2′-mercaptouridine (III) almost quantitatively. Isolated III was fairly stable in neutral or

---

11) N. Nylen and A. Olsen, *Svensk Kem. Tid.*, **53**, 274 (1941).
12) L.H. Noda, S.A. Kuby, and H.A. Lardy, *J. Am. Chem. Soc.*, **75**, 913 (1953).
13) The formation of dimethylthioformamide was observed.
14) L. Goodman, "Advances in Carbohydrate Chemistry," Vol. 22 ed. by M.L. Wolfrom, Academic Press, New York and London, 1967, pp. 109—175.

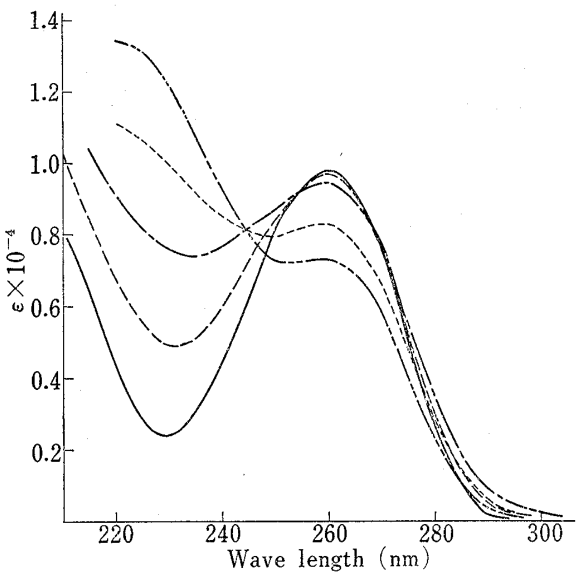

Fig. 1. UV Absorption Spectra of 2′-Deoxy-2′-mercaptouridine (III)

———: pH 4.2, ———: pH 7.2 ———: pH 8.6
--------: pH 10.0 ———: pH 12.0

slight acidic aqueous solution at room temperature. In a crystalline state III stayed unchanged after one year storage at −20°. Compound III was oxidized by iodine solution to give the disulfide (IV). 2′-Deoxy-2′-mercapto-3′,5′-di-O-acetyluridine (V) was also obtained from II by selective S-deacetylation with *n*-butylamine in ethyl acetate.

The 2′-deoxy-2′-thio structure of the sugar portion of III was confirmed as follows. Proton magnetic resonance (δ=2.38) and infrared absorption (ν=2560 cm^{-1}) of III clearly indicated the existence of a mercapto group. The remarkable highfield shift (0.6—0.7 ppm) of 2′-proton resonance relative to that of normal uridine derivatives indicated that the sulfur was substituted at 2′-position. In addition, desulfurization of IV with Raney nickel afforded 2′-deoxyuridine along with uracil (the molar ratio, 4: 6).

By ultraviolet spectroscopy, III showed the pK_{a1} value of 7.3 due to the ionization of the 2′-mercapto group (Fig. 1). Thiolate ion has a strong absorbancy (ε=5000) about 235 nm where absorption of the undissociated thiol group is negligible.[15] The similar spectral observation has been reported in the case of 1-(2,3-dideoxy-3-mercapto-β-D-*threo*-pentofuranosyl)-thymine by Wempen and Fox.[5b,16] The formation of the 2′-thiolate in III would suppress the second ionization of the N^3 hydrogen in the aglycon. Indeed, the pK_a of N^3 in III is 9.85, whereas that in uridine and in IV is 9.2. Treatment of III with methyl iodide at pH 7.3 afforded immediately 2′-deoxy-2′-methylthiouridine (VI) as a sole product. Compound VI showed a single spectral shift associated with the dissociation of N^3 proton, the pK_a being 9.2.

Table I. Proton Coupling Constants of the Furanose Ring of 2′-Deoxy-2′-thiouridine Derivatives

Compd.	$J_{1',2'}$, Hz	$J_{2',3'}$, Hz	$J_{3',4'}$, Hz	Solvent
II	9.2	5.4	1.2	$CDCl_3$
III	8.6	5.5	1.6	DMSO-d_6
	8.4	5.3	2.1	D_2O
IV	7.9	5.8	2.5	D_2O
V	8.3	5.8	1.5	$CDCl_3$
VI	8.3	5.4	2.2[e]	DMSO-d_6
Uridine[a]	4.4[b]	5.3[b]	5.5[b]	D_2O
80% S type[c]	8	5—6	2—3	
2′ Endo[d]	8.3	5.9	0	

a) B.J. Blackburn, A.A. Grey, I.C.P. Smth, and F.E. Hruska, *Can. J. Chem.*, **48**, 2866 (1970)
b) Values measured at 28°.
c) predicted values, lit. 19)
d) predicted values, lit. 17*b*)
e) detectable only after addition of 5% volume of D_2O

---

15) R.J. Irving, L. Nelander, and Y. Wadsö, *Acta Chem. Scand.*, 18, 769 (1964).

16) They interpreted the increase in absorption about 235 nm as a reflection of rupture of hydrogen bonding between the 2-carbonyl and the 3′-thiol of the sugar moiety by thiolate ion formation.

It is of interest to investigate the effect of sulfur substitution at 2′-position on the sugar ring conformation. Table I shows three vicinal coupling constants of the furanose ring of 2′-deoxy-2′-thiouridine derivatives. It was found that in all derivatives (II—VI) $J_{1',2'}$ and $J_{3',4'}$ values (8—9 and 1—2 Hz, respectively) are quite different from those of uridine. These J values resembled with predicted values for $C_{2'}$ endo[17] or 80% S type[18] conformation in ribofuranosides. From this evidence, furanose ring puckering that is extremely biased to 2′ endo-mode is infered in compound II—VI, while normal uridine derivatives are close to a $C_{2'}$ endo-$C_{3'}$ endo equilibrium constant of unity.[19] Though the relative inductive effect of the 2′-substituents accounts for the different $J_{1',2'}$ values,[20] this effect is rather small, since examinations of the variation of the vicinal coupling constant in a series of substituted ethanes indicate that replacement of a sulfur for oxygen could increase the vicinal coupling by no more than 0.5 Hz.[21] This is also supported by the evidence that $J_{2',3'}$ values, which are practically independent of the $C_{2'}$ endo-$C_{3'}$ endo equilibrium,[19] are little affected by the sulfur substitution at $C_{2'}$ position. Therefore it is apparent that the large values of $J_{1',2'}$ in 2′-deoxy-2′-thiouridine derivatives are due primarily to the change of dihedral angle ($\phi_{1',2'}$).

It remained to be explained why $C_{2'}$ endo-mode is the preferred conformation in 2′-thiouridines. One of the possible factors is a steric effect. The bulky sulfur atom at $C_{2'}$ position of II—VI will prefer to take up the *quasi*-equatorial orientation in the $C_{2'}$ endo-mode, rather than the *quasi*-axial one in the $C_{3'}$ endo conformation. It is noteworthy that derivatives of 2′-deoxy-2′-iodo-[22] and 2′-deoxy-2′-acetamidouridine,[23] having a bulky substituent at $C_{2'}$, showed large $J_{1',2'}$ and small $J_{3',4'}$ coupling constants similar to those of the 2′-deoxy-2′-thiouridine derivatives. The preferency of $C_{2'}$ endo conformation is probably a general property of 2′-deoxy-2′-thioribonucleoside derivatives, as large $J_{1',2'}$ values are also reported for the 6-substituted 9-purinyl nucleosides of 2-thio-D-ribose.[10] It will be interesting to study the effect of this sugar ring conformational change on biochemical properties of the nucleotides both at monomer and polymer levels. The synthetic study of the 2′-deoxy-2′-thionucleotide derivatives is now in progress.

We also tried to synthesize 2′-deoxy-2′-mercapto-1-β-D-arabinofuranosyluracil (VIIIa), a 2′-epimer of III. Ueda and Shibuya had reported that (*S*)-2,2′-cyclo-2-thiouridine (VII) yielded in alkaline solution a uridine-like UV absorbing compound suggestive of aryl-S bond cleavage.[24] After treatment of VII with 1 N NaOH at 25° for 5 hr and subsequent neutraliza-

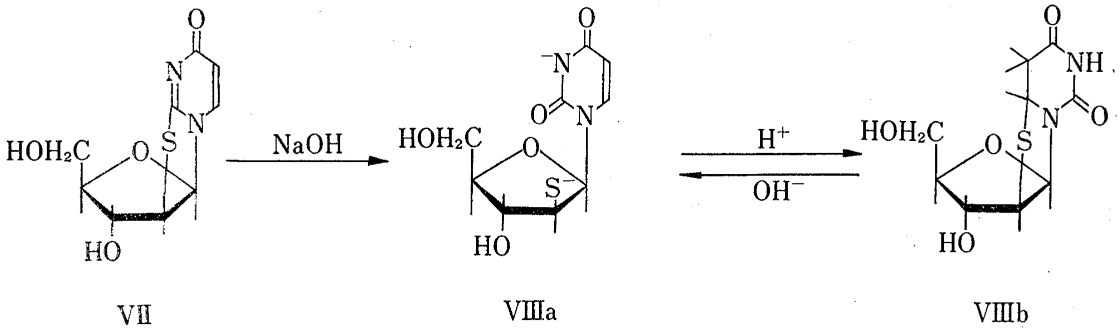

Chart 2

---

[17] *a*) C.D. Jardetzky, *J. Am. Chem. Soc.*, **82**, 229 (1960); *b*) T. Schleich, B.J. Blackburn, R.D. Lapper, and I.C.P. Smith, *Biochemistry*, **11**, 137 (1972).
[18] C. Altona and M. Sundaralingam, *J. Am. Chem. Soc.*, **94**, 8205 (1972).
[19] C. Altona and M. Sundaralingam, *J. Am. Chem. Soc.*, **95**, 2333 (1973).
[20] S. Sternhell, *Quart. Rev.*, **23**, 236 (1969).
[21] R.J. Abraham and K.G.R. Pachaler, *Mol., Phys.*, **7**, 165 (1964).
[22] J.P.H. Verheyden and J.G. Moffatt, *J. Org. Chem.*, **35**, 2868 (1970).
[23] J.P.H. Verheyden, D. Wagner, and J.G. Moffatt, *J. Org. Chem.*, **36**, 250 (1971).
[24] T. Ueda and S. Shibuya, *Chem. Pharm. Bull.* (Tokyo), **22**, 930 (1974).

tion, a crystalline product was obtained which was found to be 2′-deoxy-2′,6-epithio-5,6-dihydro-arabinofuranosyluracil (VIIIb), an 6,2′-epithio form of compound VIIIa. The proton magnetic resonance (PMR) spectrum of VIIIb gave a multiplet signal centered at 2.85 ppm which was assignable to two protons at C_5 and a doublet of doublet signal at 5.15 ppm due to a proton at C_6. Compound VIIIb had no UV absorption maximum in neutral solution but showed shoulder at about 260 nm at pH≥12 due to the episulfide bond opening. The similar behavior of 5′-deoxy-5′-thio-2′,3′-O-isopropylideneuridine had been reported.[5a,5b]

Experimental

All melting points were uncorrected. The PMR spectra were recorded at 35° on a Hitachi H-60 spectrometer using tetramethyl silane (TMS) as internal or external (when the solvent was D_2O) standard. The samples were dissolved in solvents at concentrations about 0.20 M. UV and infrared (IR) spectra were measured on a Hitachi recording spectrophotometer 3T and Hitachi 215 spectrophotometer, respectively. The mass spectra were determined on a Hitachi RMU-7 spectrometer. Silica gel column and thin-layer chromatography were performed using silicic acid (Mallinckrodt) (100 mesh), and Wakogel B-5F respectively. Cellulose paper and column chromatography was carried out on Toyo Roshi No. 51A paper and cellulose powder (200—300 mesh) (Jujo Seishi Ltd.) using the solvent system: n-BuOH–H_2O (86: 14) unless otherwise described.

O^2,2′-Cyclo-3′,5′-di-O-acetyluridine (I)——The title compound was prepared by the procedure of Furukawa and Honjo[25] with slight modification. Partially hydrolyzed $POCl_3$ prepared from 115 g (0.75 mol) of $POCl_3$ and 13.5 ml (0.75 mol) of H_2O was poured to a suspension of uridine (16 g, 65.5 mmol) in EtOAc (1.3 liter). The mixture was refluxed for 40 min, concentrated *in vacuo* to about a half volume, and poured into ice-water (1.5 liter) containing $NaHCO_3$ (160 g) under stirring. Aqueous layer was separated and pH was adjusted to 6 using additional $NaHCO_3$, if neccessary, and the volume was reduced to about one-third from which crystalline needles separated. The mixture was left standing in a cold room and separated crystals were collected. The yield was 13.6 g (66%), mp 179—182°. The product was recrystallized from water to yield 11.3 g (56%), mp 182—184° (lit.[25] 178—179°).

Reaction of I with Potassium Thioacetate in the Presence of Thioacetic Acid——To a solution of 310 mg (1 mmol) of I in 5 ml of DMF was added 800 mg (7 mmol) of potassium thioacetate and 0.5 ml (7 mmol) of thioacetic acid, and the mixture was heated at 110° for 6 hr in a steel container. The reaction mixture was then applied to paper chromatography. The chromatogram revealed two main spots of uracil ($Rf=0.35$) and a product presumably corresponding to triacetyluridine ($Rf=0.75$) which gave uridine by alkaline hydrolysis. The ratio of two compounds determined by UV spectra was 2: 1. The formation of compound II was not detected.

2′-Deoxy-2′-acetylthio-3′,5′-di-O-acetyluridine (II)——a) A solution of 4.40 g of I in 25 ml of DMF and 25 ml of thioacetic acid was heated at 110° for 6 hr in a steel container. The reaction mixture was evaporated *in vacuo* and the residue was chromatographed on a column (43×4.0 cm) of 250 g of silica gel in $CHCl_3$. Elution with each 800 ml of $CHCl_3$ and 2% ethanol-$CHCl_3$ afforded no nucleoside derivative. The latter fraction contained a by-product,[26] which was identical with authentic dimethylthioformamide.[27] Additional elution with 600 ml of 4% ethanol-$CHCl_3$ afforded compound II, which was contaminated with dimethylthioformamide. The crude product was purified by rechromatography on the same system and II was crystallized from ethanol to yield 1.64 g (30%), mp 124—126°. UV $\lambda_{\max}^{H_2O}$ nm (ε): 259.5 (9800), $\lambda_{\min}^{H_2O}$ nm (ε): 229 (6400). PMR ($CDCl_3$) δ: 2.14 and 2.19 (6H, each s, two acetoxyls), 2.34 (3H, s, CH_3COS), 4.1—4.4 (4H, m, 5′-H_2, 4′-H, 2′-H), 5.26 (1H, dd, $J_{2',3'}=5.4$ Hz, $J_{3',4'}=1.2$ Hz, 3′-H), 6,25 (1H, d, $J_{1',2'}=9.2$ Hz, 1′-H). Mass Spectrum m/e: 275 (S^+), 113 ($B^+ + 2$). *Anal.* Calcd. for $C_{15}H_{18}O_8N_2S$: C, 46.63; H, 4.69; N, 7.25; S, 8.30. Found: C, 46.39; H, 4.68; N, 7.17; S, 8.34.

Finally, elution with each 800 ml of 8% and 16% ethanol-$CHCl_3$ gave an unidentified uridine derivative (9200 OD units at 260 nm, paper chromatography: $Rf=0.58$) and 0.22 g of starting material (I) respectively.

b) A solution of 4.4 g of I in 25 ml of dioxane and 25 ml of thioacetic acid was heated at 110° for 6 hr in a steel container. After evaporation of the solvent, the residue was chromatographed on a silica gel column as mentioned above. II was crystallized from the concentrate of the eluates without rechromatography. The yield was 3.55 g (65%), mp 124—126°. The last eluate gave 0.28 g (6%) of starting material (I).

---

25) Y. Furukawa and M. Honjo, *Chem. Pharm. Bull.* (Tokyo), **16**, 2286 (1968).

26) UV $\lambda_{\max}^{\text{ethanol}}$ nm: 268. The silica gel thin-layer chromatogram (solvent: 5% ethanol-$CHCl_3$) revealed a spot of $Rf=0.55$.

27) G.R. Pettit and L.R. Garson, *Canad. J. Chem.*, **43**, 2640 (1965).

2′-Deoxy-2′-mercaptouridine (III)——A solution of 3.1 g (8.0 mmol) of triacetate (II) in 120 ml of 1N KOH (solvent; H_2O: EtOH, 1: 1) was kept at 10° for 30 min, neutralized with 100 ml of Dowex 50 W (H^+) resin. The resin was filtered and washed with 100 ml of water. The combined filtrates were evaporated in vacuo to leave a white solid which gave a single spot of *Rf* 0.48 on a paperchromatogram. Recrystallization of the crude solid from aqueous ethanol gave 2.03 g (97%) of III as plates, mp 171—174°. UV $\lambda_{\max}^{\mathrm{pH}\,4.2}$ nm (ε): 260 (9800), $\lambda_{\min}^{\mathrm{pH}\,4.2}$ nm (ε): 229.5 (2400), $\lambda_{\max}^{\mathrm{pH}\,12}$ nm (ε): 259 (7300), $\lambda_{\min}^{\mathrm{pH}\,12}$ nm (ε): 253 (7200). IR $\nu_{\max}^{\mathrm{KBr}}$ cm^{-1}: 2560 (SH). PMR (DMSO-d_6) δ: 2.38 (1H, d, $J_{\mathrm{SH},2'}=7.1$ Hz, SH), 3.47 (1H, m, 2′-H), 3.56 (2H, d, $J_{4',5'}=3.6$ Hz, 5′-H_2), 3.92 (1H, m, $J_{3',4'}=1.6$ Hz, 4′-H), 4.05 (1H, dd, $J_{2',3'}=5.5$ Hz, 3′-H), 5.89 (1H, d, $J_{1',2'}=8.6$ Hz, 1′-H). PMR (D_2O) δ: 3.64 (1H, dd, $J_{2',3'}=5.3$ Hz, 2′-H), 3.82 (2H, d, $J_{4',5'}=3.8$ Hz, 5′-H_2), 4.19 (1H, m, $J_{3',4'}=2.1$ Hz, 4′-H), 4.32 (1H, dd, $J_{2',3'}=5.3$ Hz, 3′-H), 6.01 (1H, d, $J_{1',2'}=8.4$ Hz, 1′-H). Mass Spectrum *m/e*: 260 (M^+), 149 (S^+), 112 ($B^+ + 1$). *Anal.* Calcd. for $C_9H_{12}O_5N_2S$: C, 41.54; H, 4.65; N, 10.67; S, 12.32. Found: C, 41.49; H, 4.76; N, 10.74; S, 12.22.

Di-(2′-deoxy-2′-uridinyl)-disulfide (IV)——To a solution of 110 mg (0.42 mmol) of III in 10 ml of 50% aqueous ethanol was added dropwise 4.2 ml of 0.1 mol ethanolic iodine solution under stirring at room temperature. To the reaction mixture, 2 ml of Dowex 1 (HCO_3^-) resin was added. The resin was filtered and washed 5 ml of water. After evaporation of combined filtrates, the residue gave a single UV-absorbing spot (*Rf* 0.22) on a paper chromatogram. The product was purified through cellulose column chromatography and crystallized from 95% ethanol to obtain 54 mg of IV (yield 50%), mp 161—164°. UV $\lambda_{\max}^{H_2O}$ nm (ε): 260.5 (17700), $\lambda_{\min}^{H_2O}$ nm (ε): 230.5 (5000); $\lambda_{\max}^{\mathrm{pH}\,12}$ nm (ε): 261.5 (12600), $\lambda_{\min}^{\mathrm{pH}\,12}$ nm (ε): 245.5 (10500). PMR (D_2O) δ: 3.71 (1H, dd, 2′-H), 3.78 (2H, d, $J_{4',5'}=4.2$ Hz, 5′-H_2), 4.09 (1H, m, 4′-H), 4.41 (1H, dd, $J_{3',4'}=2.5$ Hz, $J_{2',3'}=5.8$ Hz, 3′-H), 6.24 (1H, d, $J_{1',2'}=7.9$ Hz, 1′-H). *Anal.* Calcd. for $C_{18}H_{22}O_{10}N_4S_2$: C, 41.69; H, 4.28: N, 10.80; S, 12.37. Found: C, 41.51; H, 4.36; N, 10.66; S, 12.16. The infrared spectrum showed the absence of a thiol peak in the 2600 cm^{-1} region.

2′-Deoxy-2′-mercapto-3′,5′-di-O-acetyluridine (V)——To a solution of 500 mg (1.45 mmol) of II in 25 ml of EtOAc was added 0.6 ml (6 mmol) of *n*-butylamine. After 1 hr at room temperature, 3.0 g of dry Dowex 50 W (H^+) resin suspension in EtOAc was added, and the mixture was stirred for 2 hr. The reaction mixture showed no starting material by TLC with 10% ethanol in $CHCl_3$; there were a main spot due to V and a minor spot due to an unknown product (*Rf* 0.43 and 0.14). The resin was filtered and washed with two 10 ml portions of EtOAc. Combined filtrates were evaporated, and the residual glass was dissolved in 5 ml of $CHCl_3$, added to a chromatographic column (21 × 1.9 cm) of 28 g of silica gel in $CHCl_3$. Elution with 100 ml portion of 4% ethanol in $CHCl_3$ afforded 338 mg (76%) of V, as a glass. A portion was reprecipitated from ethanol-H_2O to give an analytical sample. UV $\lambda_{\max}^{\mathrm{ethanol}}$ nm (ε): 258 (9800). IR $\nu_{\max}^{\mathrm{KBr}}$ cm^{-1}: 2550 (SH). PMR ($CDCl_3$) δ: 1.87 (1H, d, $J_{\mathrm{SH},2'}=10.1$ Hz, SH), 2.14 and 2.20 (6H, each s, two acetoxyls), 3.55 (1H, m, 2′-H), 4.34 (3H, br. s, 4′-H, 5′-H_2), 5.24 (1H, dd, $J_{3',4'}=1.5$ Hz, $J_{2',3'}=5.8$ Hz, 3′-H), 6.04 (1H, d, $J_{1',2'}=8.3$ Hz, 1′-H). *Anal.* Calcd. for $C_{13}H_{16}O_7N_2$: C, 45.35; H, 4.68; N, 8.14; S, 9.31. Found: C, 45.38; H, 4.78; N, 7.88; S, 9.12.

2′-Deoxy-2′-methylthiouridine (VI)——To a solution of 130 mg (0.5 mmol) of III in 30 ml of 0.2M NH_4HCO_3 buffer (pH=7.3) was added 0.15 ml of CH_3I (2.4 mmol) under stirring. After repeated evaporation with water, the residue was purified through cellulose column chromatography. 101 mg of VI was crystallized from ethanol–hexane (yield 70%), mp 124—126°. UV $\lambda_{\max}^{H_2O}$ nm (ε): 261 (9900), $\lambda_{\min}^{H_2O}$ nm (ε): 230 (2900); $\lambda_{\max}^{\mathrm{pH}\,12}$ nm (ε): 261 (7300), $\lambda_{\min}^{\mathrm{pH}\,12}$ nm (ε): 244 (5900). PMR (DMSO-d_6-5% D_2O) δ: 2.03 (3H, s, SCH_3), 3.33 (1H, dd, $J_{2',3'}=5.4$ Hz, 2′–H), 3.58 (2H, br. d, 5′-H_2), 3.89 (1H, m, 4′-H), 4.21 (1H, dd, $J_{3',4'}=2.2$ Hz, 3′-H), 6.05 (1H, d, $J_{1',2'}=8.3$ Hz, 1′-H). Mass Spectrum *m/e*: 162 (S^+-1), 112 ($B^+ + 1$). *Anal.* Calcd. for $C_{10}H_{14}O_5N_2S$: C, 43.79; H, 5.14; N, 10.21; S, 11.69. Found: C, 43.63; H, 5.08; N, 10.22; S, 11.76.

Desulfurization of Di-(2′-deoxy-2′-uridinyl)-disulfide (IV)——To 80 mg of disulfide (IV) dissolved in 20 ml of 85% aqueous ethanol was added activated Raney nickel (0.6 ml of thick slurry), and the reaction mixture was refluxed for 30 min. The nickel was filtered and washed well with water. The combined filtrates showed two UV-absorbing spots due to uracil (*Rf* 0.67) and 2′-deoxyuridine (*Rf* 0.78) by paper chromatography with water (molar ratio; 6: 4). After evaporation of the filtrate, the residue was dissolved in 10 ml of 0.1M NH_4OH and applied to a chromatographic column (25 × 1.8 cm) of Dowex 1 × 2 (HCO_3^-) resin (200—400 mesh). Elution was performed with the use of linear concentration gradient of NH_4HCO_3 buffer (pH 9.3) (1 liter of water in the mixing chamber and 1 liter of 0.1M buffer in the reservoir, one fraction; 20 ml/40 min). The first fraction (tube No. 47—50) was combined and desalted by repeated evaporation with water. Crystallization from ethanol afforded 14 mg of 2′-deoxyuridine (yield 20%, mp 164—166°, undepressed on admixture with the authentic sample). Mass Spectrum *m/e*: 139 ($HB\overset{+}{C}H=CH_2$), 117 (S^+), 112 ($B^+ + 1$). From the second fraction (tube No. 51—55) 10 mg of uracil was obtained (yield 29%). UV $\lambda_{\max}^{\mathrm{pH}\,12}$ nm: 283.

2′-Deoxy-2′,6-epithio-5,6-dihydro-arabinofuranosyluracil (VIIIb)——A solution of 130 mg of (*S*)-2,2′-cyclo-2-thiouridine (VII)[24] in 5 ml of 1N NaOH was left standing at 25° for 5 hr and neutralized with 7 ml of Dowex 50 W (H^+). The resin was filtered and washed with 20 ml of water. The combined filtrates were evaporated in vacuo to dryness. The residue was recrystallized from ethanol to afford 42 mg of VIIIb (yield 30%) as plates, mp 221—223°. PMR (DMSO-d_6) δ: 2.85 (2H, m, 5a-H, 5b-H), 5.15 (1H, dd, $J_{5a,6}=$

9.6 Hz, $J_{5b,6}=6.1$ Hz, 6-H), 6.27 (1H, d, $J_{1',2'}=5.3$ Hz, 1'-H). *Anal.* Calcd. for $C_9H_{12}O_5N_2S$: C, 41.54; H, 4.65; N, 10.67; S, 12.32. Found: C, 41.23; H, 4.65; N, 10.67; S, 11.91.

Acknowledgement This work was supported, in part, by the Grant-in-Aid for Scientific Research from the Ministry of Education and Welfare which is gratefully acknowledged. The PMR, mass, and elemental analyses were performed by the members of the Central Analytical Room of the Faculty of Pharmaceutical Sciences, Hokkaido University, to whom our thanks are due.