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Synthesis of 2,4-Dithiouridine and 2-Thiocytidines

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Starting from 2,4-dithio-1-methyluracil (II) various 1-methylpyrimidines were obtained through the intermediate pyrimidine (III). Some comparisons were made on the methylation of oxo- and thio-pyrimidines. A protected 4-thiouridine (XIII) afforded 2,4-dithiouridine (XV) by the direct thiation with phosphorus pentasulfide at elevated temperature and subsequent deblocking. 2-Thiocytidines were prepared from XV and the possible tautomeric form of 2-thiocytidine was discussed by the UV spectral behaviors.

Studies on replacement reactions of the oxo-group with thio-group in purine and pyrimidine moieties of nucleosides have attracted a great deal of interest since 6-thiopurines have been found as effective antitumor agents.[2] Furthermore, recent findings on the existence of 4-thiouridine[3] and a 2-thiouracil derivative[4] in some transfer ribonucleic acids have added the importance of the studies on the synthesis and chemical characteristics of thio-nucleosides.

This paper deals with the synthesis of hitherto unreported types of thio-derivatives of naturally occurring pyrimidine nucleosides, 2,4-dithiouridine and 2-thiocytidines. The preliminary account of this work has been appeared.[5]

Among the methods introducing thio function in C-4 of uridine the direct thiation of protected uridines with phosphorus pentasulfide in pyridine has been useful.[6,7] In these cases no formation of 2,4-dithio or 2-thio derivatives was observed. For the introduction of thio group in place of oxo function in C-2 of uridines two main approaches have been applied. Cleavage of the anhydro linkage of O^2-5′-cyclo-2′,3′-O-isopropylideneuridine by hydrogen sulfide and triethylamine in dimethylformamide gives the 2-thiouridine derivative with the concomitant formation of 5′-deoxy-5′-mercapto-2′,3′-O-isopropylidineuridine.[8] Recent reports showed that O^2,2′-cyclouridines also gave the arabinosyl-2-thiouracils.[9,10]

The second method involves the ring closure to 2-thiouracils. Reaction of 2,3,5-tri-O-benzoyl-D-ribosylamine with β-ethoxyacryloylisothiocyanate affords 2-thiouridine derivatives[11]

1) Location: *Kita-12, Nishi-6, Sapporo.*
2) J.J. Fox, I. Wempen, A. Hampton, and I.L. Doerr, *J. Am. Chem. Soc.*, **80**, 1669 (1958), and references therein.
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7) *a*) I. Wempen, R. Duschinsky, L. Kaplan, and J.J. Fox, *J. Am. Chem. Soc.*, **83**, 4755 (1961); *b*) F. Sorm, J. Smrt, and V. Cereneckij, *Experientia*, **17**, 64 (1961); *c*) M. Ikehara, T. Ueda, and K. Ikeda, *Chem. Pharm. Bull.* (Tokyo), **10**, 767 (1962).
8) *a*) D.M. Brown, D.B. Parihar, A.R. Todd, and S. Varadarajan, *J. Chem. Soc.*, **1958**, 3023; *b*) R.W. Chambers and V. Kurkov, *J. Am. Chem. Soc.*, **85**, 2160 (1963).
9) W.V. Ruyle and T.Y. Shen, *J. Med. Chem.*, **10**, 331 (1967).
10) T. Sekiya and T. Ukita, *Chem. Pharm. Bull.* (Tokyo), **15**, 1498 (1967).
11) G. Shaw, R.N. Warrener, M.H. Maguire, and R.K. Ralph, *J. Chem. Soc.*, **1958**, 2294.

and similar ring closure from a ribosyl thiourea and β-ethoxyacryloyl chloride gives 2-thiouridines.[12]

A different approach which could be applied to nucleoside level has been reported by Brown and Harper[13] in which the direct thiation of 1-methyl- or 1,3-dimethyl-uracil with phosphorus pentasulfide in tetralin at elevated temperature is applied to form the 2,4-dithio derivatives.

This method was adapted to the thiation of 1-methyl-4-thiouracil and the substitution reactions of 2- and 4-thio groups were studied first. Since 1-methyluracil has been converted directly to 2,4-dithio-1-methyluracil,[13] 1-methyl-4-thiouracil (I)[6] should likewise give the dithiouracil (II) and it was found to be the case. Methylation of II with methyl iodide in the presence of alkali afforded 1-methyl-4-methylthio-2-pyrimidinethione (III). The presence of excess methyl iodide converted III to IV which was also prepared from 2,4-bismethylthiopyrimidine (V) and methyl iodide. The structure of IV was confirmed by its conversion to II by the treatment with sodium hydrogen sulfide in methanol. In this conversion the formation of III was observed as the intermediate. Compound (III) was subjected to acid hydrolysis to yield 1-methyl-2-thiouracil (VI),[12,14] the structure confirmed by its ultraviolet (UV) spectral resemblance with those for 1-alkyl-2-thiouracils and not for 1-alkyl-4-thiouracils or 1,3-dialkyl-2,4-dithiouracils,[16,17] indicating that the methylation of II had occurred at S^4 rather than N^3 or S^2.

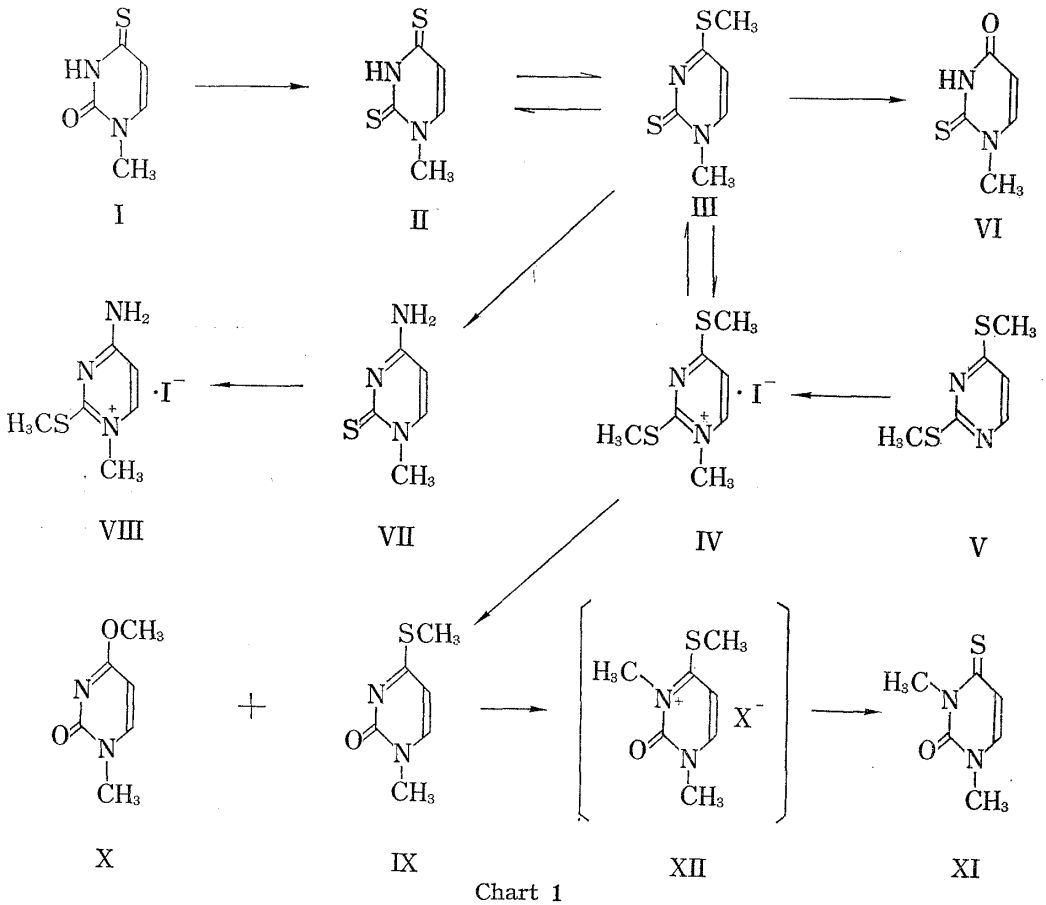

Chart 1

12) M. Sano, *Chem. Pharm. Bull.* (Tokyo), **9**, 709 (1961); **10**, 308 (1962).
13) D.J. Brown and J.S. Harper, *J. Chem. Soc.*, **1961**, 1298.
14) G. Shaw and R.N. Warrener, *J. Chem. Soc.*, **1958**, 153.
15) F.H.S. Curd and D.N. Richardson, *J. Chem. Soc.*, **1955**, 1853.
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The facile substitution of 2–methylthio group to 2–thione group on the pyrimidinium salt had been observed in 4–amino–1,6–dimethyl–2–methylthiopyrimidinium iodide.[15] The 4–methylthio group in III, an intermediate of IV to II, was further substituted by mercapto group under the reaction condition used. Similar substitution was also noticed in the conversion of 1–methyl–4–methylthio–2–pyrimidinone to 1–methyl–4–thiouracil by the treatment with sodium hydrogen sulfide. The treatment of III with methanolic ammonia afforded 1–methyl–2–thiocytosine (VII). Compound (VII) was converted to the known pyrimidinium salt (VIII)[18] which had been prepared by the methylation of 4–amino–2–methylthiopyrimidine. The UV spectral measurement of VIII in alkaline solution showed that VIII was converted to 1–methylcytosine.[19]

It is to be noted that there is a distinct difference between the oxopyrimidines and thiopyrimidines in the reactivity toward nucleophilic alkylation reaction.[20] Whereas the oxopyrimidines, such as uracil or cytosines, give the N–methylated derivative with methyl iodide (or dimethyl sulfate), the thiocounterparts always give the S–alkylated derivatives. Thus 1–methylcytosine gives 1,3–dimethylcytosine and the thiocounterpart (VII) gives VIII under milder condition. 1–Methyl–4–methylthio–2–pyrimidinone (IX),[21] which was also obtainable from IV by treatment with aqueous alkali or with silver carbonate in methanol (in the latter case compound (X) was also formed), afforded 1,3–dimethyl–4–thiouracil (XI)[13,22] by the methylation with dimethyl sulfate in dimethylformamide followed by the treatment with sodium hydrogen sulfide in methanol. The intermediate in this reaction, therefore, should be N–methylated cmpound (XII). The thiocounterpart (III) gave S–methylated compound (IV) as have been described.

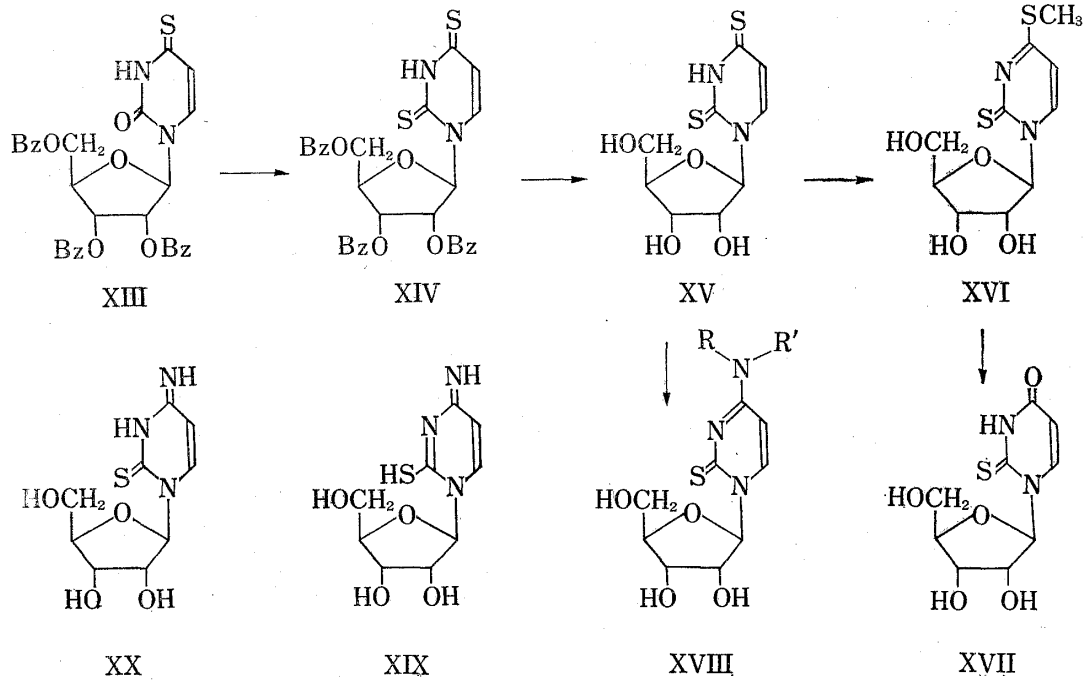

Chart 2

18) D.J. Brown and N.W. Jacobsen, *J. Chem. Soc.*, **1962**, 3172.
19) J.J. Fox and D. Shugar, *Biochim. Biophys. Acta*, **9**, 369 (1952).
20) D.J. Brown and S.F. Mason in "The Pyrimidines," Interscience Publishers, N.Y., 1962, pp. 282—359.
21) L. Wheeler and T.B. Johnson, *Am. Chem. J.*, **42**, 30 (1909).
22) G.B. Elion, and G.H. Hitchings, *J. Am. Chem. Soc.*, **69**, 2138 (1947).
23) In a preliminary series[5] the reaction was carried out at 170—180° for seven hours. Now it was found that the yield was increased in the reaction at lower temperature.

The thiation reaction of 4–thiouridines was next udertaken. 2′,3′,5′–Tri–O–benzoyl–4–thiouridine (XIII)[6] was treated with phosphorus pentasulfide and potassium sulfide in tetralin at 155° and the course of the reaction was followed by thin–layer chromatography.[23] The appearance of two main spots was observed during five hours. After purification by column chromatography of alumina or silica gel the two components could be separated. The slower eluting component (XIV) afforded 2,4–dithiouridine (XV) on debenzoylation. The structure of XV was confirmed by elemental analysis, UV spectral similarity to II and partial methylation to XVI and subsequent acid hydrolysis to 2–thiouridine (XVII).[8,11,12] The compound eluting ahead of XIV also gave XV on deblocking, indicating that the difference between the two components exists in the blocking group (s). The lower ratio of A_{230}/A_{284} in the faster eluting component (s) compared to that of XIV is indicative of partial thiation of carbonyl in benzoyl goup of XIV. The overall yield of XV from XIII was *ca.* 35%.

The thiation of 2′,3′,5′–tri–O–acetyl–4–thiouridine, which was obtained as a crude form afforded XV in much reduced yield and the extensive degradation was noticed during the thiation. The thiation of 3′,5′–di–O–benzoyl–4–thiothymidine[6] resulted in the formation of 2,4–dithiothymine,[24,25] indicating the cleavage of glycosylic linkage under the reaction condition used (160—180°).

Treatment of XV with methanolic ammonia at 100° for 18 hours furnished 2–thiocytidine (XVIII: R=R′=H). The UV spectral behavior is very similar to that for 1–methyl–2–thiocytosine (VII) as expected. Methylation and subsequent alkaline hydrolysis afforded cytidine. Similar treatment of XV with methylamine or dimethylamine gave (XVIII: R=Me, R′=H) and (XVIII: R=R′=Me) respectively and these were converted to the cytidines[7a] by methylation and subsequent hydrolysis.

The UV spectral behaviors of these 2–thiocytidines need some comments. The spectral patterns and λ_{max} of these three 2–thiocytidines differ significantly (see Table I). Whereas (XVIII: R=R′=H) has λ_{max} at 248 mμ and a shoulder at 270 mμ, (XVIII: R=R′=Me) has single maximum at 270 mμ and (XVIII: R=Me, R′=H) has broad maximum at 263 mμ in

Table I. Ultraviolet Absorption of 2–Thiocytidines

	in H_2O				in 1 N HCl			
	λ_{max}	ε	λ_{min}	ε	λ_{max}	ε	λ_{min}	ε
1–Methyl–2–thiocytosine	222 shoulder	11900			227	18300	246	7770
	246	25000	254	22400	273.5	22100		
	266	23300			309	7180	302	7100
2–Thiocytidine	220 shoulder	10300			229	17000	213	10400
	248	22300			276	17400	250	8000
	270 shoulder	17100			310 shoulder	5160		
N^4–Methyl–2–thiocytidine	224	14900	236	12300	238	18500	216	9030
	262.5	27400			276	18050	257	11900
					310 shoulder	6400		
N^4–Dimethyl–2–thiocytidine	217	7950	233	5750	247	18700	219	4900
	270	34900			277	20800	260.5	16200
					313 shoulder	7670		

24) H.L. Wheeler and D.F. McFarland, *Am. Chem. J.*, **43**, 19 (1910).
25) G.B. Elion, W.S. Ide, and G.H. Hitchings, *J. Am. Chem. Soc.*, **68**, 2137 (1946).

neutral solution. In the case of cytidines such large shift of maximum was not observed[7a] by the introduction of methyl group on N^4. Therefore the dissimilarity of the absorption pattern in XVIII is probably due to the differences of tautomeric forms between these compounds and not to the simple red shift resulting from the substitution of hydrogen with methyl group. Since (XVIII: R=R'=Me) represents a 4–amino–2–thione tautomeric form, the tautomeric structure of 2–thiocytidine itself may not be the structure described as (XVIII: R=R'=H) and some contributions of the other possible tautomeric form such as XIX or XX may be present. The UV spectra of the protonated species of XVIII are all similar and quite different to those for VIII, which may be the reflection of protonation on N^3 and not on S^2 in XVIII, provided that the substitution of hydrogen with methyl group in sulfhydryl group exhibits no appreciable spectral change. Thus it follows that the predominant tautomeric forms present in 2–thiocytidine may be both XVIII and XX. However nore precise studies are neccessary to clarify this aspect.

In this regard 2–thiocytidine may possess interesting biochemical properties.

2,4–Dithiouridine was converted to the 5'–phosphate by the standard method of phosphorylation[26] *via* its 2',3'–O–isopropylidene derivative. During the step of phosphorylation or work–up some degradation to 2–thiouridine 5'–phosphate was observed.

Experimental

1-Methyl-2,4-dithiouracil (II)——A) A mixture of 1–methyl–4–thiouracil (I)[6] (1.0 g) and phosphorus pentasulfide (1.5 g) in 20 ml of tetralin was heated at 180° in oil bath temperature for 4 hr, under stirring. After cooling, the solution was removed from the precipitates by decantation and the precipitate was washed with small amount of ether and dissolved in 5 N NH_4OH and reprecipitated by acidification with 10% HCl. The precipitate was collected and recrystallized from H_2O to give 0.27 g(23%) of (II),[13] mp 258°.

B) Compound IV (0.37 g) was dissolved in methanolic sodium methoxide (90 mg of Na in 8 ml) previously saturated with hydrogen sulfide, and the refluxed for 1.5 hr, during which H_2S was bubbled. After cooling the yellow needles separated was collected by filtration and washed with methanol to yield II, 0.15 g, 80%, mp 255—257° (darken). This gave correct analysis and identified by UV spectra with the authentic material.[13]

2,4-Dimethylthio-1-methylpyrimidinium Iodide (IV)——2,4–Dimethylthiopyrimidine (V),[13] 1 g, was dissolved in 10 ml of methyl iodide and set aside for 2 days at room temperature. After evaporation the residue was recrystallized from EtOH to yield 1.5 g of IV, mp 144—145° (decomp.), *Anal.* Calcd. for $C_7H_{11}N_2IS_2 \cdot \frac{1}{2}H_2O$: C, 26.01; H, 3.71; N, 8.67; I, 39.30; S, 19.82. Found: C, 26.17; H, 3.67; N, 8.66; I, 39.44; S, 19.44. UV $\lambda_{max}^{H_2O}$ mμ; 229, 265, 295, 310 shoulder; $\lambda_{min}^{H_2O}$ mμ; 251, 275.

Treatment of (IV) with Ag_2CO_3 in MeOH——The iodide IV, 0.35 g, and 0.4 g of Ag_2CO_3 in 5 ml of MeOH was stirred for 4 hr. After filtration with the aid of celite the filtrate was concentrated to leave the residue which was dissolved in hot benzene. The separated crystal (X), mp 139—140.5°, 0.1 g, has lower mp compared to that of the authentic[27] (149—150°), it has correct analysis and UV spectra. From the mother liquor IX, mp 121—122°, was obtained which showed on depression of mp on admixture with the authentic material.[21]

Treatment of (IV) with Alkali——The UV spectra of IV in 0.1 N NaOH solution changed within 10 hr to those for (IX). $\lambda_{max}^{H_2O}$ mμ; 304, 270 shoulder: $\lambda_{min}^{H_2O}$ 241 mμ; λ_{max}^{3NHCl} mμ; 332, 270; λ_{min}^{3NHCl} mμ; 288, 242.

1-Methyl-4-methylthio-2-pyrimidinethione (III)——Compound (II) (148 mg, 1 mmole) was dissolved in 1 ml of 1 N NaOH and to this solution was added 1 ml of EtOH containing 0.055 ml of methyl iodide, stirred for 40 min at room temperature. The precipitated material was filtered and the filtrate was concentrated to a small volume and separated precipitate was collected and combined with the former precipitate and recrystallized from benzene to yield yellow prisms (III), 97 mg, 60%; mp 150°; *Anal.* Calcd for $C_6H_8N_2S_2$: C, 41.77; H, 4.73; N, 16.26. Found: C, 41.57; H, 4.94; N, 16.02. UV $\lambda_{max}^{50\%EtOH}$ mμ, (ε); 286 (34800), 330 shoulder (3150): $\lambda_{min}^{50\%EtOH}$ 238 (1580); λ_{max}^{2NHCl} 250 shoulder (5350), 278 (27700), 365 (3150); λ_{min}^{2NHCl} 229 (2360), 340 (2680). Treatment of III in a solution of 1 N HCl at 100° for 45 min resulted in precipitation of (VI), mp 225° from EtOH, in 70% yelid: UV λ_{max}^{1NHCl} mμ: 217, 266, 290 shoulder; λ_{max}^{1NNaOH} mμ; 235, 266; identical with those reported.[14,16,17]

1-Methyl-2-thiocytosine (VII)——Compound (III), 85 mg, was dissolved in 15 ml of methanolic ammonia and heated for 3 hr at 100° in a sealed tube. The residue after evaporation of the solvent was decolorized

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27) G.E. Hilbert and T.B. Johnson, *J. Am. Chem. Soc.*, **52**, 2001 (1930).

by charcoal and recrystallized from water to give white needles (VII), 50 mg, mp >240°. *Anal.* Calcd. for $C_5H_7N_3S$: C, 42.53; H, 4.99; N, 29.76. Found: C, 42.32; H, 4.95; N, 29.95; UV see Table I.

Treatment of (IX) with Sodium Hydrogen Sulfide——Three hundred miligrams of IX in sodium methoxide solution previously saturated with hydrogen sulfide was refluxed for 3 hr and after cooling the separated crystals were collected by filtration and recrystallized from aqueous EtOH to yield 1-methyl-4-thiouracil; 150 mg, mp 193° identical with the authentic sample.[6]

Methylation of 1-Methylpyrimidines——A) Compound (III), 100 mg, was dissolved in 3 ml of methyl iodide in a sealed tube and kept for 3 days at room temperature. After evaporation of the solvent the residual crystalline mass was washed with C_6H_6 to leave 100 mg of IV identical with that from V.

B) Compound (VII), 100 mg, was likewise treated with methyl iodide at 60° for 3 hr to yield 120 mg of VIII, mp 224—225°, identical with that reported.[18] UV ($\lambda_{max}^{H_2O,H^+}$ at 242 mμ) changed in alkaline solution to $\lambda_{max}^{1N\,NaOH}$ 273 mμ; λ_{max}^{NHCl} 283 mμ, which are identical with those for 1-methylcytosine.[19]

C) Compound (IX), 600 mg, was dissolved in 10 ml of dimethylformamide and treated with 0.6 ml of dimethyl sulfate at 100° for 4 hr. The reaction mixture was poured into NaHS-MeOH solution and H_2S was bubbled for 3 hr and left to stand for overnight. Concentration under reduced pressure left the residue which was taken up in $CHCl_3$, the small insoluble material filtered off, and concentrated to a small volume and kept in refrigerator. The separated crystals were washed with small amount of ether and dried to leave (XI),[13,22] 150 mg, 29%, mp 128—130°.

2,4-Dithiouridine (XV)——2′,3′,5′-Tri-O-benzoyl-4-thiouridine,[6] 1.15 g, P_2S_5, 0.9 g, and powdered potassium sulfide, 0.2 g, were suspended in 45 ml of tetralin and heated to 155° (inner temperature) under vigorous stirring. The course of the reaction was followed by thin-layer chromatograph (TLC) with C_6H_6–AcOEt 9:1 as the solvent. During 5 hr 7 spots were revealed having *Rf* of 0.0, 0.13, 0.33 (starting material), 0.42, 0.52, 0.62 and 0.82. The spots 0.52 and 0.62 became predominant. Most of the solvent was distilled off under reduced pressure and the resulted dark red residue was taken up in C_6H_6, the insoluble material filtered off, and applied on a column of alumina or silica gel (30 g) and eluted with C_6H_6, then with 15% AcOEt in C_6H_6. The fraction containing material having *Rf* of 0.52 and 0.62 were collected and concentrated to a syrup (880 mg). This was taken up in NaOEt-EtOH (50 mg of Na in 50 ml of EtOH) and warmed up to 50° to dissolve the syrup and kept for 3 hr. To this solution was added ion exchange resin (Dowex 50, H^+ form) to its neutrality and filtered. The filtrate was concentrated to a syrup, taken up in 50 ml of $CHCl_3$ and 20 ml of H_2O. The water layer was separated and washed once with $CHCl_3$, concentrated to a small volume during which XV was separated as yellow needles. The crystals were collected by filtration and recrystallized from H_2O containing small amount of EtOH to yield 0.17 g of XV mp 166—167°, 35% from XIII: *Anal.* Calcd. for $C_9H_{12}O_4N_2S_2$: C, 39.13; H, 4.38; N, 10.14. Found: C, 39.23; H, 4.39; N, 10.35. UV $\lambda_{max}^{pH5.8}$ mμ, (ε): 283 (22500), 350 shoulder (12900); λ_{max}^{pH9} 280 (16900) 320 (24800): pK_a; 7.4 (measured by UV spectra); *Rf* of paper chromatography; 0.52 in BuOH–H_2O, 86:14; 0.37 in iso-PrOH-conc. NH_4OH–H_2O, 7:1:3.

Purification of Tribenzoylated Intermediate (XIV)——Thiation reaction starting from 20 g of XIII was carried out at 165° for 1.5 hr and after the work-up the mixture was applied onto the column of 500 g of alumina, eluted with benzene containing increasing amount of AcOEt. Fractions A (7 g, *Rf* 0.62 in TLC, $A_{230}/A_{284}=0.97$) and B (4.5 g, *Rf* 0.52, $A_{230}/A_{284}=1.53$) were roughly separated. After several recrystallizations of the fraction B from PrOH yellow powder (XIV), mp 97—100° was obtained; *Anal.* Calcd. for $C_{30}H_{24}O_7N_2S_2$: C, 61.23; H, 4.11; N, 4.77. Found: C, 61.20; H, 4.38; N, 4.67. Both A and B fractions gave XV on deblocking in *ca.* 50—60% yield.

Thiation of 2′,3′,5′-Tri-O-acetyluridine——Triacetyluridine, 5.6 g, and 2.0 g of P_2S_5 were dissolved in 100 ml of pyridine and refluxed for 3 hr under stirring. Pyridine layer was collected by decantation and concentrated to a syrup which was taken up in $CHCl_3$ and washed with H_2O, dried over Na_2SO_4, and concentrated to leave a syrup, 8.1 g. One-fourth of this syrup was subjected to column chromatography (alumina, 30 g; C_6H_6–AcOEt, 1:1) to afford 1.1 g of yellow powder. UV spectra are very close to those for 4-thiouridine.[6]

This powder was subjected to the second thiation at 155° for 45 min as described above to give 0.11 g of dithio-derivative which was deacetylated to yield 38 mg of XV.

Methylation and Hydrolysis of XV——Compound (XV), 100 mg, was dissolved in 2.2 ml of MeOH containing 0.022 ml of methyl iodide and to this solution was added 0.36 ml of 1 N NaOH and stirred for 1 hr at room temperature during which the solution became neutral. On evaporation of the solvent the residual syrup was applied to paper chromatography, (solvent, iso-PrOH-conc. NH_4OH–H_2O, 7:1:3). Two spots were revealed at *Rf* 0.37 and 0.64, former was that for the starting material (XV), and the latter has UV $\lambda_{max}^{H_2O}$ 287 mμ and 340 mμ, similar to those of III, indicating the structure of XVI. The syrup containing (XVI) was dissolved in EtOH–H_2O, 8:2, and 1 N HCl was added to pH 2 and kept for 3 hr at 60°, then applied to paper chromatography (solvent: BuOH–H_2O, 86:14) which showed three spots, *Rf* 0.50, 0.24, and 0.14. The UV measurements of these showed that the compound having *Rf* 0.50 was XV, 0.24, the main component, was 2-thiouridine (XVII)[8,11,12] and 0.14 unidentified material.

Thiation of 3′,5′-Di-O-benzoyl-4-thiothymidine——The compound[6] was treated with P_2S_5 in tetralin at 160—180° and the course of the reaction was followed by TLC. Only new spot obtained was that of 2,4-

dithiothymine identified by the direct comparison of TLC with the authentic material[24] and by its UV spectra.[25]

2-Thiocytidine (XVIII, R=R′=H)——Compound (XV), 500 mg, was treated with 20 ml of methanolic ammonia at 100° for 18 hr in a sealed tube. After evaporation of the solvent the residue was recrystallized from EtOH containing small amount of H_2O to yield 230 mg of (XVIII, R=R′=H), mp 208—209°. *Anal.* Calcd. for $C_9H_{13}O_4N_3S$: C, 41.70; H, 5.06; N, 16.21. Found: C, 41.68; H, 4.86; N, 16.41.

N^4-Methyl-2-thiocytidine (XVIII, R=Me, R′=H) and N^4-Dimethyl-2-thiocytidine (XVIII, R=R′=Me)—— By the similar procedure (XV), 90 mg, was converted to (XVIII, R=Me, R′=H), 38 mg, mp 189—190°. *Anal.* Calcd. for $C_{10}H_{15}O_4N_3S$: N, 15.38. Found: N, 15.37. and 150 mg of XV afforded (XVIII, R=R′=Me), 80 mg, 52%, recrystallized from EtOH, mp 180—181°. *Anal.* Calcd. for $C_{11}H_{17}O_4N_3S$: C, 45.99; H, 5.92; N, 14.63. Found: C, 46.11; H, 6.06; N, 14.67.

Methylation and Hydrolysis of 2-Thiocytidines——About 10 mg of XVIII and excess of methyliodide in ab. MeOH were heated at 60° for 3 hr and the solvent was removed, the residue was treated with NaOH solution. The UV spectra of methylated and hydrolyzed compounds are as follows; 2-thiocytidine, 247 mμ and 271 mμ; N^4-methyl-2-thiocytidine, 251 mμ and 272 mμ; N^4-dimethyl-2-thiocytidine, 260 mμ and 280 mμ respectively. The UV spectra of the latters are in good accordance with those for the corresponding cytidines,[7a] indicating the methylation occurring at S^2 and subsequent hydrolysis of the methylthio group to form cytidines.

2′,3′-O-Isopropylidene-2,4-dithiouridine and 2,4-Dithiouridine 5′-Phosphate——Compound (XV), 0.38 g, and 2,2-dimethoxypropane, 3 ml, were dissolved in acetone, 25 ml, and 0.2 ml of 60% $HClO_4$ was added and stirred for 1 hr at room temperature. The solution was neutralized by the addition of powdered $KHCO_3$, filtered and the filtrate was concentrated to a syrup to which water was added and evaporated to leave a yellow mass. On recrystallization from H_2O–EtOH, 3:1, yellow needles, 2′,3′-O-isopropylidene derivative of XV, 220 mg, were obtained; mp 177—177.5°. *Anal.* Calcd. for $C_{12}H_{16}O_4N_2S_2$: C, 45.26; H, 5.07; N, 8.80. Found: C, 45.58; H, 5.15; N, 9.04. The phosphorylation was carried out by the method of Tener[26] starting from 200 mg of the isopropylidene derivative and 1.25 mmole of 2-cyanoethyl phosphate and 1.0 g of dicyclohexylcarbodiimide. After the work-up and purification by DEAE-cellulose ion exchange chromatography 84 mg of bis(triethylammonium) 2,4-dithiouridine 5′-phosphate was obtained. UV $\lambda_{max}^{H_2O}$ mμ; 284, 350 shoulder; λ_{max}^{1NNaOH} 280, 320 mμ, mobility of paper electrophoresis, 0.05 N NH_4OAc, pH 6.8, 700 V, 1 hr: uridine 4.5 cm, uridine 5′-phosphate 9.0 cm, XV 4.1 cm, 5′-phosphate of XV 8.2 cm. Formation of 2-thiouridine 5′-phosphate was observed in some extent, which was eluted ahead of the 5′-phosphate of XV; UV $\lambda_{max}^{H_2O}$ 275 mμ, $\lambda_{min}^{H_2O}$ 245 mμ; $\lambda_{min}^{0.1NNaOH}$ 239, 271 mμ; $\lambda_{min}^{0.1NNaOH}$ 260 mμ.

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