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Nucleosides and Nucleotides. VII. Synthesis of 2-Thiocytidine by the Extended Hilbert-Johnson Procedure[1]

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Treatment of 4-amino-2-methylthiopyrimidine with 2,3,5-tri-O-benzoyl-D-ribosyl chloride afforded the ribosylpyrimidinium chloride (VI), which, on treatment with hydrogen sulfide, gave 2',3',5'-tri-O-benzoyl-2-thiocytidine (VII) and 2-thiocytidine (VIII) by further de-benzoylation. Methylation of VIII afforded 2-methylthiopyrimidinium derivative (IX). Treatment of IX (or VI) with methanolic ammonia gave 2,4-diamino-1-(β-D-ribofuranosyl)-pyrimidinium salt. With alkali IX (or VI) gave cytidine. This condensation can be regarded as an extended Hilbert-Johnson procedure for pyrimidine nucleoside synthesis. Relationships between stability of ribosylpyrimidinium salts and structure of their pyrimidines are discussed. The mechanism of the Hilbert-Johnson reaction was established therefrom.

The Hilbert-Johnson procedure for pyrimidine nucleoside synthesis[3] involves the condensation of 2,4-dialkoxypyrimidine (I) with a protected ribosyl chloride to form a 1-ribosyl-2-pyrimidinone (III)[4] *via* a postulated pyrimidinium intermediate (II) as exemplified (Chart 1).

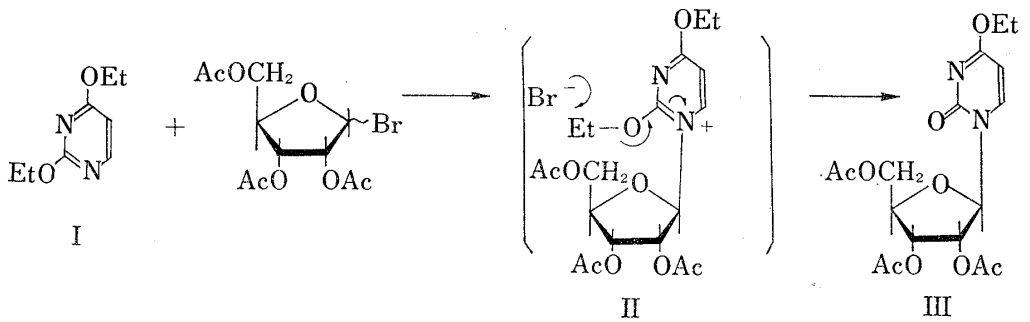

Chart 1

In a model study we have disclosed[5] that 1-methyl-2,4-dialkoxypyrimidinium salt did form by the methylation of I but it was very unstable and converted to a 2-pyrimidinone even in a crystalline state. Other 2,4-disubstituted pyrimidines such as dimethylthio-, diphenoxy-, amino-methylthio-, and amino-alkoxy-pyrimidines gave rather stable 1-methylpyrimidinium salts. If one could obtain an intermediate, such as II, in a stable form this method should have wide uses as the synthetic method of various 2-substituted pyrimidine nucleosides unrestricted to 2-pyrimidinones. Results of the studies of methylation reaction of various pyrimidines, and of their substitution reactions, as the model study of nucleoside synthesis,[5] led us to select certain pyrimidines, namely, 4-amino-2-methylthio-, 2,4-dimethylthio-, and 4-ethoxy-2-methylthio-pyrimidines, as the most promising starting pyrimidines in terms of

1) Part VI: K. Miura and T. Ueda, *J. Biochem.* (Tokyo), in press.
2) Location, *Kita-12, Nishi-6, Kita-ku, Sapporo.*
3) G.E. Hilbert and T.B. Johnson, *J. Am. Chem. Soc.*, 52, 2001, 4489 (1930).
4) G.A. Howard, B. Lythgoe and A.R. Todd, *J. Chem. Soc.*, 1052 (1947).
5) T. Ueda and H. Ohtsuka, *Chem Pharm. Bull.* (Tokyo), 21, 1451 (1973).

the stability of the pyrimidinium salts and the ease of replacement at position 2. The preliminary account of some of the results has appeared.[6]

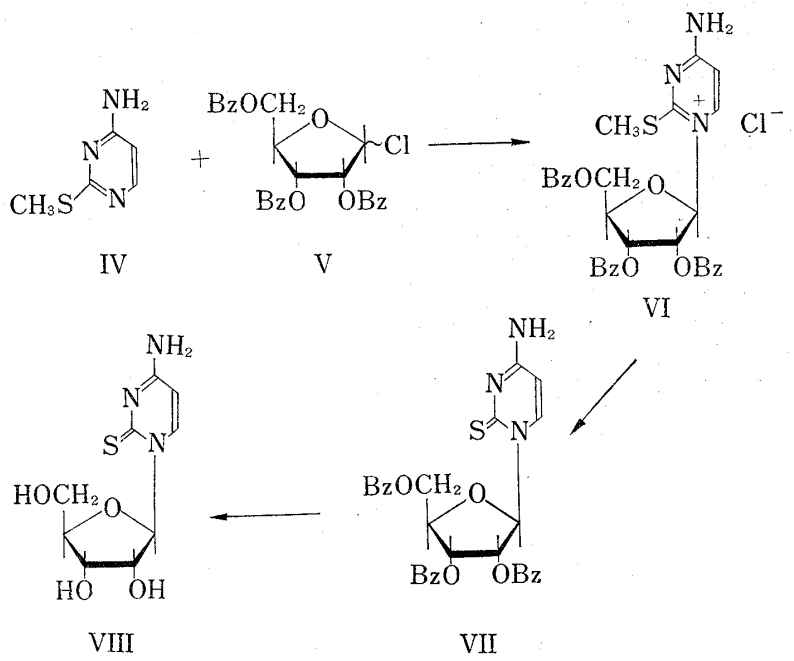

Chart 2

4-Amino-2-methylthiopyrimidine (IV) was brought into reaction with 2,3,5-tri-O-benzoyl-D-ribosyl chloride (V) in anhydrous acetonitrile for ten days at room temperature. After the removal of separated hydrochloride salt of IV the ether insoluble material, which is tailing from the origin on TLC as indicative of the pyrimidinium salt (VI), was dissolved in pyridine and hydrogen sulfide was bubbled through for 2 hr. From the yellow colored reaction mixture 2′,3′,5′-tri-O-benzoyl-2-thiocytidine (VII) was obtained in a crystalline form. Debenzoylation with methanolic ammonia gave β-2-thiocytidine (VIII), mp 218—219°, identical with the authentic specimen[7] in every respect including optical properties.[8] Methylation of VIII with methyl iodide afforded 4-amino-2-methylthio-1-(β-D-ribofuranosyl)pyrimidinium salt (IX) as expected from the model studies.[7b] Treatment of IX with methanolic ammonia gave 2,4-diamino-1-(β-D-ribofuranosyl)pyrimidinium iodide (X) in a crystalline form. The amination of VI also gave X directly but IX was found to be a better intermediate than VI to handle with. Both IX and X migrates on paper electrophoresis as monocation in neutrality. In alkaline medium IX was rapidly con-

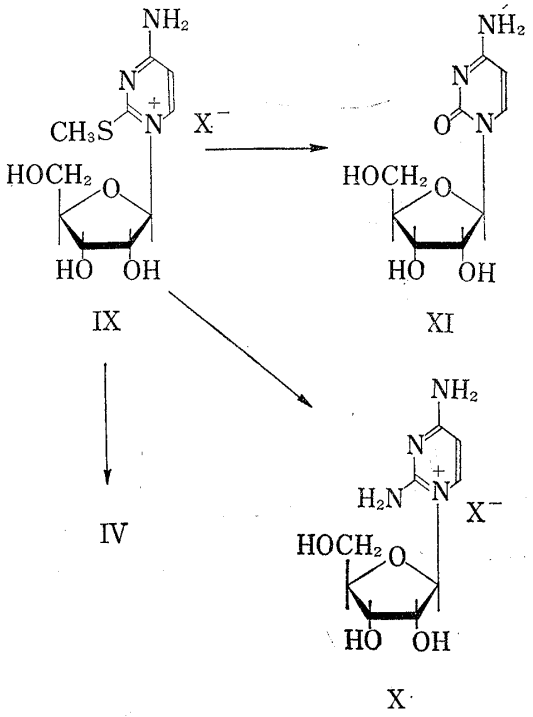

Chart 3

6) T. Ueda and H. Nishino, *J. Am. Chem. Soc.*, **90**, 1678 (1968).
7) *a*) T. Ueda, Y. Iida, K. Ikeda and Y. Mizuno, *Chem. Pharm. Bull.* (Tokyo), **14**, 666 (1966); *b*) *Idem*, *ibid.*, **16**, 1788 (1968).
8) T. Ueda and H. Nishino, *Chem. Pharm. Bull.* (Tokyo), **17**, 920 (1969).

verted to cytidine (XI). Acid treatment of IX gave the starting pyrimidine (IV). Compound X was rather resistant to acid or alkaline treatment which finally gave 2,4-diaminopyrimidine as the sole degradation product.

From the above results it can be stated that IV is superior as the starting pyrimidine for the synthesis of 2-substituted (oxo, thioxo and amino) pyrimidine nucleosides.

The reaction of 2,4-dimethylthiopyrimidine (XII) with V, on the other hand, gave no nucleosidic material and the products were hydrochloride salt of XII and an unidentified compound derived from the halosugar. This is also the case in the reaction with 4-ethoxy-2-methylthiopyrimidine and V. These results may be explained as follows: the once formed pyrimidinium intermediate (XV) is highly unstable because of the localization of a positive charge on N-1 and, in addition, the 2-substituent is not susceptible to the cleavage, which is in contrast to the case in the conversion of II to III, thus makes the whole pyrimidinium moiety strong eliminating group. Another possibility is that these pyrimidines simply acted as base for the elimination of hydrochloric acid from the acylated halosugar. To investigate the lability of the postulated intermediate, XV, another approach to synthesize XV was deviced.

2,4-Dithiouridine (XIII)[7] was treated with excess of methyl iodide and equivalent amount of ethoxide in ethanol and course of the reaction was followed by thin-layer chromatography (TLC) and ultraviolet (UV) spectra. Methylation of XIII at S-4 occurred rapidly to give XIV[7] which was followed by the formation of two spots on TLC. The spot travelling faster (*Rf* 0.77, UV λ_{max}^{EtOH} 250, 292 nm) was corresponded to that of XII, the other tailing from the origin (*Rf* 0.06, UV λ_{max}^{EtOH} 230, 288, 310 sh. nm) was assigned to that of XV though not isolated. On duration of the reaction XII became major spot and a non UV-absorbing spot, which was derived from the ribose moiety but different from ribose, appeared concomitantly.

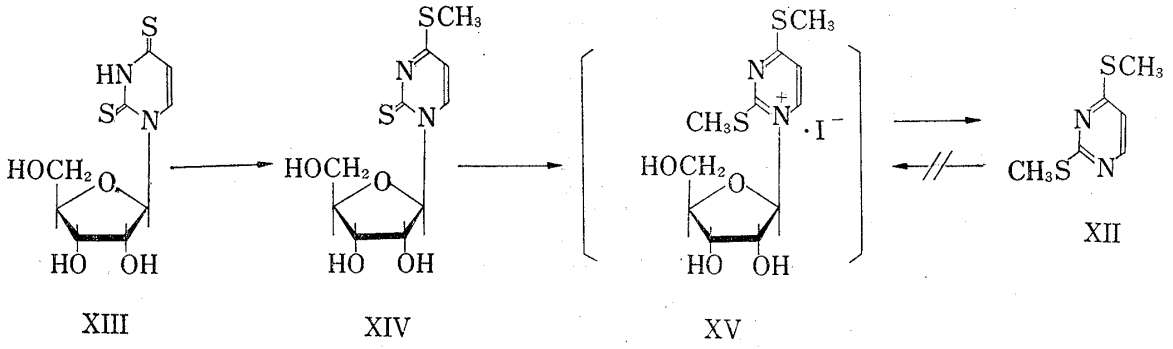

Chart 4

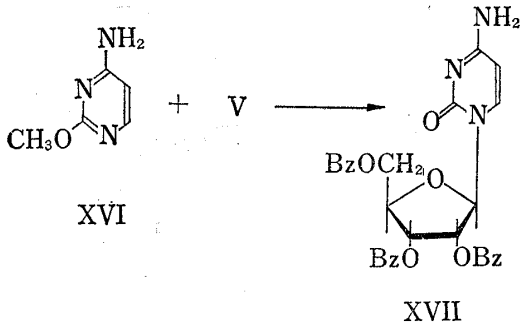

Chart 5

From these results together with the successful synthesis of VI from IV it can be concluded that, in 1-glycosylpyrimidinium salts their stabilities will depend on the extent of the delocalization of the positive charge on a pyrimidine moiety. Introduction of amino group makes the pyrimidinium structure more stable since amino group is better electron donating group than alkoxy or alkylthio group. In fact the stability of the ribosylpyrimidinium salts are in the decreasing order of X>IX>II and XV.

It has been reported several decades ago[9] that the reaction of 4-amino-2-methoxy-pyrimidine (XVI) with acetobromoglucose did not

9) G.E. Hilbert, *J. Am. Chem. Soc.*, **56**, 190 (1934).

give the nucleoside. In the light of the findings described here this result should be explained by the high susceptibility of dehydrobromination of acetobromoglucose with base. Therefore it is well expected that the reaction of XVI with V might give the nucleoside, cytidine.

Treatment of XVI with V in acetonitrile for six days resulted in a formation of a crystalline precipitate, which was not the pyrimidinium salt but the hydrochloride salt of 2′,3′,5′-tri-O-benzoylcytidine (XVII). Debenzoylation of XVII afforded cytidine and overall yield from V was around 50%.

It is also conceivable that in the original Hilbert-Johnson reaction the intermediate II should be present at least in a small amount. To attest this the reaction mixture of I and V was treated directly with methanolic ammonia and, on successive debenzoylation, X was obtained in a yield of ~2%, together with cytidine. This fact establishes the conclusive proof of the proposed mechanism of the Hilbert-Johnson reaction, since X should have been derived only from the intermediate II.

Finally, 2-ethoxy-4-methylthiopyrimidine was treated with V, to see wether it gives the intermediate or not, and the results were exactly similar to those of the reaction of I with V, yielding 2′,3′,5′-tri-O-benzoyl-4-methylthiouridine.

Experimental

4-Amino-2-methylthio-1-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)pyrimidinium Chloride (VI) and 2′,3′,5′-tri-O-Benzoyl-2-thiocytidine (VII)——4-Amino-2-methylthiopyrimidine (IV, 2.13 g, 15 mmoles) and 2,3,5-tri-O-benzoyl-D-ribosyl chloride (V, 5.09 g, 1 mmoles) were dissolved in freshly distilled acetonitrile (40 ml) and kept for 10 days at 30° in a sealed flask with few pieces of molecular sieve. After removal of the separated crystals of HCl salt of IV the solvent was concentrated to a small volume, ether was added to the residue, and the solution was kept in a refrigerator overnight. The precipitate was collected and washed several times with ether. TLC of the precipitate (VI) (silica gel, $CHCl_3$: EtOH=35: 5) showed a tailing spot at origin. This gummy precipitate was dissolved in 60 ml of pyridine and dry H_2S was bubbled through for 2 hr. The solution became yellow and TLC showed a spot of 0.5, positive for periodate-benzidine spray test. The solvent was removed in vacuo and co-evaporated with EtOH to furnish a mass which was crystallized from hot MeOH to yield 2.54 g (44.5%) of VII, mp 190—191° (decomp), $[\alpha]_D = -26.6°$ (c, 1.0, $CHCl_3$). *Anal.* Calcd. for $C_{30}H_{25}O_7N_3S$: C, 63.03; H, 4.41; N, 7.35. Found: C, 62.95; H, 4.60; N, 7.43.

2-Thiocytidine (VIII) from VII——Compound VII (2.76 g) was suspended in a solution of sat. NH_3 in MeOH, 140 ml, and kept overnight under shaking in a sealed flask. After evaporation, the residue was taken up in H_2O and extracted with $CHCl_3$. The water layer was concentrated to dryness and the residue was crystallized from water to afford white plates (VIII), 1.16 g (92.7%), mp 218—219°.[10] Optical properties of VIII are in good agreement with those reported.[7,8] NMR (d_6–DMSO) δ ppm: 6.66 (b.s., 1′–H), 6.11 (d, 5–H), 8.18 (d, 6–H), $J_{5,6}$=7 Hz, 7.63 (b. 4–NH_2). *Anal.* Calcd. for $C_9H_{13}O_4N_3S$: C, 41.70; H, 5.05; N, 16.21; S, 12.35. Found C, 41.71; H, 4.88; N, 16.22; S, 12.20.

4-Amino-2-methylthio-1-(β-D-ribofuranosyl)pyrimidinium Chloride (IX)——One hundred mg of VIII was suspended in abs. MeOH, 0.2 ml of CH_3I was added, and the solution was stirred for 2 hr. Solvent was removed and the residue was dissolved in 15 ml of EtOH. The solution was stirred with 50 mg of AgCl for 3 hr in the darkness. The insoluble material was removed by filtration and the filtrate was concentrated to dryness. The residue was crystallized from MeOH–ether to leave white crystals (IX), 81 mg (67.8%), mp 155—156° (decomp). $[\alpha]_D = -26°$ (*c*, 1.0, H_2O). UV $\lambda_{max}^{H_2O}$, nm (ε): 246.5 (28300), 275 sh (9000). NMR (d_6-DMSO) δ ppm: 2.71 (s, S-CH_3), 3.75 (b.s, 5′H_2), 5.77 (d, 1′-H), $J_{1',2'}$=3 Hz, 6.74 (d, 5-H), 8.59 (d, 6-H), $J_{5,6}$=8 Hz, 9.07, 9.40 (b.s, 4-NH_2). *Anal.* Calcd. for $C_{10}H_{16}O_4N_3SCl$: C, 38.77; H, 5.21; N, 13.57. Found: C, 38.89; H, 5.13; N, 13.34.

Treatment of IX with Acid or Alkali——When an aqueous solution of IX was made alkaline strong mercaptan odor evolved and the product was identified with cytidine by UV and chromatographic determination. Compound IX was dissolved in 2N AcOH and kept at 90°. After 2.5 hr and 4.5 hr 76 and 88% of IX was converted to IV as measured by UV spectra.

2,4-Diamino-1-(β-D-ribofuranosyl)pyrimidinium Iodide (X)——One hundred mg of IX was dissolved in 15 ml of sat. NH_3–MeOH and kept for 1 hr at room temperature. The solvent was removed, the residue was triturated with ether and decanted. The residue was crystallized from MeOH–ether to yield 140 mg

10) The mp was about ten degrees higher than that reported.[7] We feel that the compound obtained here is of higher purity.

(*ca.* 100%) of X, mp 164.5—165.5° (decomp). $[\alpha]_D = +10°$ (*c*, 1.0, H_2O). NMR (d_6-DMSO) δ ppm: 3.69 (b.s, 5′H_2), 5.58 (d, 1′-H), $J_{1,2}$=6 Hz, 6.2 (d, 5-H), 8.0 (d, 6-H), $J_{5,6}$=8 Hz, 7.9—8.4 (d, 2,4-NH_2). *Anal.* Calcd. for $C_9H_{15}O_4N_4I$: C, 29.20; H, 4.08; N, 15.15. Found: C, 29.13; H, 4.36; N, 15.19. Perchlorate salt of X was obtained by passing through the Dowex-1×8 (perchlorate form) column. The eluate was concentrated and the residue was crystallized from EtOH–ether, mp 178.5° (decomp). UV $\lambda_{max}^{H_2O}$, nm (ε): 210 (25800), 230—237 sh (1299); 267 (7900); $\lambda_{max}^{OH^-}$ nm (ε): 230.5 (21100), 276 sh (3400), 305 sh (1400). *Anal.* Calcd. for $C_9H_{15}O_8N_4Cl$: C, 31.54; H, 4.41; N, 16.35. Found: C, 31.54; H, 4.57; N, 16.13.

Treatment of X with Acid or Alkali——Perchlorate of X is stable in 2N AcOH at 90° for 24 hr. In 1N HCl at room temperature for 5 hr X was converted to 2,4-diaminopyrimidine. In 1N NaOH solution X is partially converted to the pyrimidine within 24 hr.

Methylation of 2,4-Dithiouridine (XIII). Formation of XII——Seventeen mg of XIII was dissolved in 1 ml of EtOH and leq. of 1N NaOEt in EtOH was added. Four eq. of CH_3I in 0.3 ml of EtOH was added into the mixture. An aliquot was applied to TLC (silica gel, $CHCl_3$–EtOH=35: 5) at appropriate intervals. The spots were excised, eluted with EtOH and analyzed spectrophotometrically. After 8 min the spot (*Rf* 0.21) of XIII changed almost completely to that of XIV (same *Rf* value) and two spots began to appear at *Rf* 0.77 (XII, UV λ_{max}^{EtOH} 250, 292 nm) and *Rf* 0.06 (XV, UV λ_{max}^{EtOH} 230, 288, 310 sh nm). The spot of XII continued to increase with the concomitant decrease of XIV and after 4 hr the reaction was practically complete. The spot of XV never became major constituent throughout the reaction. A compound transparent under UV lamp and was visualized by the spray of H_2SO_4 and heating was the final principal sugar derivative (*Rf* 0.14). This spot was positive for periodate–benzidine spray test but different from ribose (*Rf* 0.01).

Cytidine from 4-Amino-2-methoxypyrimidine (XVI)——4-Amino-2-methoxypyrimidine (XVI, 375 mg, 3 mmoles) and V (1.18 g, 2 mmoles) were dissolved in 10 ml of acetonitrile and kept to stand for 6 days at room temperature with a few pieces of molecular sieve in a sealed flask. The precipitated material was collected by filtration, washed with ether, and crystallized from MeOH to yield 133 mg (11.2%) of hydrochloride of 2′,3′,5′-tri-O-benzoylcytidine (XVII), mp 202°. *Anal.* Calcd. for $C_{30}H_{26}O_8N_3Cl$: C, 60.86; H, 4.43; N, 7.10. Found: C, 60.64; H, 4.72; N, 7.36. The filtrate and washings were combined and applied to a column of silica gel which was developed with $CHCl_3$–EtOH (35: 5). From the eluate XVII was obtained (501 mg, 45.1%), recrystallized from benzene, mp 174.5–175.5°. $[\alpha]_D = -36.°$ (*c*, 1.0, $CHCl_3$). *Anal.* Calcd. for $C_{30}H_{25}O_8N_3$: C, 64.86; H, 4.54; N, 7.57. Found: C, 64.87; H, 4.69; N, 7.44. Compound XVII was treated with NaOEt–EtOH and applied on paper chromatography (H_2O, adjusted to pH 10 with NH_3). The spot coinciding with cytidine was excised, eluted with water, and UV and ORD spectra were measured, which were identical with those for cytidine.

Amination of the Reaction Mixture of I and V Identification of X——Three mmoles of I and 2 mmoles of V were dissolved in 15 ml of acetonitrile and keft to stand for 2 days in a refrigerator. Thirty ml of sat. NH_3–MeOH was added to the solution, which was kept for 2 days at room temperature. After removal of the solvent the residue was redissolved in 50 ml of sat. NH_3–MeOH and kept for 24 hr to complete debenzoylation. The solvent was evaporated and the residue was shaken with $CHCl_3$–H_2O. The aqueous layer was applied to paper chromatography (*n*-BuOH: H_2O=86: 14). The spot, *Rf* 0.1, was eluted and applied to paper electrophoresis (pH 7.0, 700 v, 11.5 mA for 70 min). The spot migrating to cathode was excised and eluted with water. The UV spectral analysis (absorbancy at 267 nm) showed the presence of X in a yield of 1.8%. Cellulose column chromatography (solvent, *n*-BuOH: H_2O=86: 14) of the reaction mixture afforded 16 mg of chloride salt of X.

2′,3′,5′-Tri-O-benzoyl-4-methylthiouridine——2-Ethoxy-4-methylthiopyrimidine[5] was treated with V in acetonitrile for 10 days in a similar manner as described in the synthesis of VI. A formation of the pyrimidinium intermediate was noted by TLC but was not able to isolate. After the work-up the product was recrystallized from benzene. Yield was variable for the runs. $[\alpha]_D = +8°$ (*c*, 1.0, $CHCl_3$). NMR (d_6-DMSO) ppm: 2.51 (s, S-CH_3), 4.68 (5′-H_2, 4′-H), 5.77 (2′-H, 3′-H), 6.08 (b.s, 1′-H), 6.34 (d, 5-H) J=7 Hz, 7.2—7.9 (m. 6-H, $-C_6H_5$). The compound was debenzoylated to 4-methylthiouridine[11] by treatment with sat. NH_3–MeOH at room temperature overnight. 4-Thiouridine, cytidine and uridine were derived from the compound by treatment with NaSH, NH_3–MeOH and NaOH, respectively.

Acknowledgement This work was supported in part by the Grant-in-Aid for Scientific Research from the Ministry of Education to which authors thanks are due.

11) T. Ueda and J.J. Fox, *J. Med. Chem.*, 6, 697 (1963).