methanol gave a solid (0.228 g, 91%): mp 248° (slow decomposition); $[\alpha]^{25.0}D$ −30.6° (*c* 0.50, *N*,*N*′-dimethylformamide).

Anal. Calcd for $C_{78}H_{120}N_{14}S_8O_{20}$ (1670.13): C, 56.01; H, 7.24; N, 11.74; S, 5.76. Found: C, 56.02; H, 7.19; N, 11.79; S, 5.52.

Registry No.—III, 31025-11-3; VII, 31025-12-4; VIII, 31025-13-5; X, 31025-14-6; XI, 31025-15-7; XIII, 31020-53-8; XVII, 31025-16-8; XVIII, 31025-17-9; XX, 2212-76-2; XXI, 31025-19-1; XXII, 31025-20-4; XXIV, 31020-54-9; XXV, 31020-55-0; XXVII, 31020-56-1; XXVIII, 31020-57-2; XXIX, 31020-58-3; XXX, 31020-59-4.

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Synthesis of 2-Thiouridine and 2-Thioisouridine by Mercuri Procedure[1,2]

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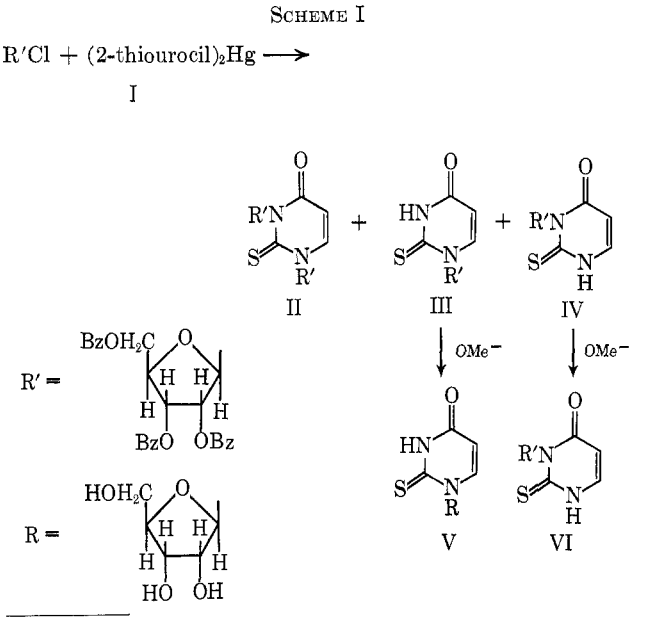
Contrary to an earlier report, (2-thiouracil)$_2$Hg (I) can be obtained from 2-thiouracil and $HgCl_2$. The compound I on treatment with 2,3,5-tri-*O*-benzoyl-D-ribofuranosyl chloride formed one disubstituted (II) and two monosubstituted products (III and IV). The compounds III and IV on debenzoylation with sodium methoxide formed 2-thiouridine (V) and 2-thioisouridine (VI), respectively. Compounds V and VI were converted into uridine and isouridine, respectively, by cyanogen bromide. The pK_a's of both V and VI are 8.1.

The facile formation and cleavage of disulfide bonds involving 2-thio- and 4-thiopyrimidines in tRNA has been invoked on several occasions to explain biochemical mechanisms.[3-6] Although 2-thiouracil[7] and 4-thiouridine[8,9] form disulfides readily after iodine treatment, a similar oxidation of 2-thiouridine derivatives was not observed by at least two groups of workers.[10,11] It is possible that such oxidation may take place easily when 2-thiouridine moieties are parts of a macromolecule. In order to study the properties of 2-thiouridines, particularly the formation of a disulfide on oxidation, the chemical synthesis of the compound was undertaken. Of the five different methods of synthesis of 2-thiouridine,[12-16] the one reported by Lee and Wigler[16] based on mercuri condensation was reinvestigated. Several discrepancies were observed, and the characterization and properties of the intermediate blocked nucleosides and the final products are reported.

Lee and Wigler[16] were unable to prepare (2-thiouracil)$_2$Hg (I) from 2-thiouracil and $HgCl_2$ in aqueous solution by the method of Fox, *et al.*[17] In my hands, however, 2-thiouracil did form with mercuric chloride the desired (2-thiouracil)$_2$Hg in 2:1 stoichiometry and in a quantitative yield. It is noteworthy that (2-methylthiouracil)$_2$Hg was previously obtained in high yield directly from 2-methylthiouracil by Scannell and Allen.[18] However, Lee and Wigler[16] claim to have prepared I in 83% yield by prior acetylation of 2-thiouracil followed by mercuric chloride treatment: mp 282–286° dec, λ_{max} (ethanol) 294 nm. On the other hand, I have been unable to prepare 2-thiouracil·HgCl salt using equimolar amounts of 2-thiouracil and $HgCl_2$; elemental analysis of the product indicated the formation of mixtures. The addition of mercuric bromide in this mercuri condensation reaction was not essential and did not improve the yield of 2-thiouridine.

The treatment of I with 2,3,5-tri-*O*-benzoyl-D-ribofuranosyl chloride yielded a mixture of blocked nucleosides II, III, and IV separable by chromatography on silicic acid (Scheme I). On debenzoylation with sodium methoxide in methanol, IV gave 2-thioisouridine (VI), identified by the similarities of its uv absorption spectra

SCHEME I



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(2) This work was presented at the 160th National Meeting of the American Chemical Society, Chicago, Ill., Sept 13–18, 1970, Abstracts, BIOL-25.

(3) J. A. Carbon, L. Hung, and D. S. Jones, *Proc. Nat. Acad. Sci. U. S.*, **53**, 979 (1965).

(4) N. Sueoka and T. Kano-Sueoka, *Prog. Nucl. Acid Res. Mol. Biol.*, **10**, 23 (1970); *Proc. Nat. Acad. Sci. U. S.*, **52**, 1535 (1964).

(5) B. Goehler and R. H. Doi, *ibid.*, **56**, 1047 (1966).

(6) J. Carbon and H. David, *Biochemistry*, **7**, 3851 (1968).

(7) W. H. Miller, R. O. Roblin, Jr., and E. B. Astwood, *J. Amer. Chem. Soc.*, **67**, 2201 (1945).

(8) J. J. Fox, D. Van Praag, I. Vempen, I. L. Doerr, L. Cheong, J. E. Knoll, M. L. Eidinoff, A. Bendich, and G. B. Brown, *ibid.*, **81**, 178 (1959).

(9) M. N. Lipsett and B. P. Doctor, *J. Biol. Chem.*, **242**, 4072 (1967).

(10) W. V. Ruyle and T. Y. Shen, *J. Med. Chem.*, **10** (3), 331 (1967).

(11) R. W. Chambers and V. Kurkov, *J. Amer. Chem. Soc.*, **85**, 2160 (1963).

(12) D. B. Strominger and M. Friedkin, *J. Biol. Chem.*, **208**, 663 (1954).

(13) D. M. Brown, D. B. Parihar, A. Todd, and S. Varadarajan, *J. Chem. Soc.*, **3028** (1958).

(14) G. Shaw, R. N. Warrener, M. H. Maguire, and R. K. Ralph, *ibid.*, **2294** (1958).

(15) Y. Ueda, Y. Iida, K. Ikeda, and Y. Mizuno, *Chem. Pharm. Bull.*, **14** (6), 666 (1966).

(16) H-J. Lee and P. W. Wigler, *Biochemistry*, **7**, 1427 (1968).

(17) J. J. Fox, N. Yung, J. Davoll, and G. B. Brown, *J. Amer. Chem. Soc.*, **78**, 2117 (1956).

(18) J. P. Scannell and F. W. Allen, *J. Org. Chem.*, **25**, 2143 (1960).

with those of 3-ethyl-2-thiouracil.[19] On treatment with cyanogen bromide, VI was converted into isouridine. Cyanogen bromide reaction presumably goes through a thiocyanate intermediate and appears to be superior to other methods in terms of yields and mild conditions.[20] The earlier observation[16] that, unlike 2-thiouridine, 2-thioisouridine cannot be converted into isouridine by chloroacetic acid has been confirmed; this treatment results in the cleavage of the glycosyl linkage. 2-Thioisouridine has been assigned the β configuration on the basis of the "trans rule" proposed by Baker, *et al.*[21] This assignment has now been confirmed by X-ray crystallography of the compound by Einstein, *et al.*[22] The compound III, on debenzoylation with sodium methoxide in methanol, yields 2-thiouridine, identified spectrophotometrically by the published spectral data on the compound synthesized by other methods[13-15] (Figure 1). An X-ray crystallographic analysis of 2-thiouridine has been completed and will be reported shortly by Hawkinson, *et al.*, of this laboratory. Compound II has been tentatively identified as 1,3-di(2′,3′,-5′-tri-*O*-benzoyl-β-D-ribofuranosyl)-2-thiouracil. Attempted hydrolysis of II by sodium methoxide in methanol led to a mixture of products among which 2-thiouridine and 2-thiouracil have been identified. No S-substituted or N-3-substituted derivative could be identified. Disubstitution on N-1 and O^4 in the case of II was eliminated as no 2-thiocytidine could be found on treatment of II with ammonia. The infrared absorption spectra of all three compounds are very similar except in the region of 1400–1550 cm^{-1}.

The total yield of blocked nucleosides by this procedure is 32% of theory based on the halogenose. This is comparable with the 30–50% yield of blocked ribothymidine by the same method.[23] For the preparation of 2-thiouridine, the recently announced silyl procedure of Vorbrüggen, *et al.*[24] appears to be the method of choice (yield 70%). The mercuri procedure has its merit in the fact that it yields also the 2-thioisouridine, which is otherwise inaccessible.

The pK_a of 2-thiouridine has been spectrophotometrically determined to be 8.1 (lit.[16] 8.8) following the procedure described by Albert and Sarjeant.[25] The same value of 8.1 was also obtained with a sample of 2-thiouridine synthesized by the silyl procedure[24] (a generous gift from Dr. Vorbrüggen, Hauptlaboratorium der Schering AG, Berlin, Germany). Lee and Wigler's preparation of 2-thiouridine may not have been pure as indicated by the considerably low value of optical rotation reported by them[16] (see Experimental Section). This may be the reason for the higher pK_a value obtained by them. In the case of 2-thiouridine, 2-thioisouridine, and uridine, there is a lowering of about

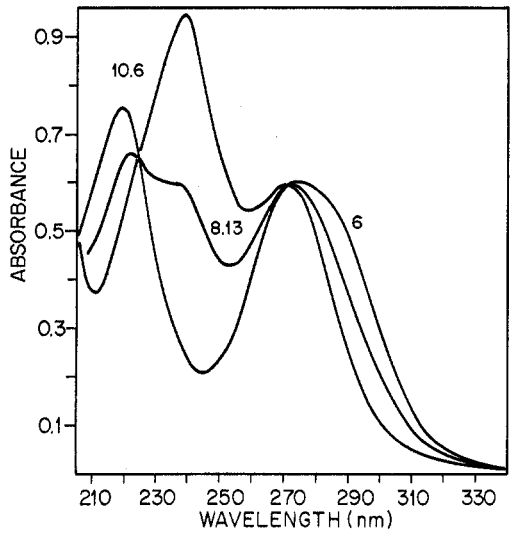

Figure 1.—Uv absorption spectra of 2-thiouridine at pH 6, 8.13, and 10.6.

0.5 in the pK_a values of the ribosyl compounds compared with their alkyl analogs, indicating the acid-strengthening effect of the ribosyl group, whereas in the case of 4-thiouridine there is practically no difference. These differences have sometimes been attributed to the possible interaction between the sugar (2′ or 5′) hydroxyl group and the O^2 of the pyrimidine ring.[26,27] However, the X-ray crystallography of 2-thiouridine and 2-thioisouridine does not shed any light on this problem.

Experimental Section

Melting points were observed in a Thomas-Hoover apparatus and are uncorrected. Thin layer chromatography was carried out on E. Merck tlc plates and descending chromatography on Whatman paper using the following solvent systems: (A) 3% ammonium chloride; (B) 0.1 *M* phosphate buffer (pH 6.8)–saturated ammonium sulfate–1-propanol (100:60:2 by volume); (C) 2-propanol–concentrated ammonia–water (60:10:30 by volume); (D) 1-butanol–water (86:14 by volume); and (E) benzene–ether (1:1 by volume). Spots on tlc silica gel plates were visualized in iodine vapor. 2-Thiouracil was purchased from Aldrich Chemical Co. and was found to be chromatographically homogeneous (Whatman 3 MM, solvent A, R_f 0.64). The ultraviolet absorption spectra were taken on a Cary recording spectrophotometer Model 14 PM. Spectra were recorded for the same solution in the same cuvette after addition of small amounts of concentrated acid, alkali, or buffer solutions to adjust the pH. Infrared spectra were recorded for 0.5% dispersions of the sample in potassium bromide pellets in a Perkin-Elmer infrared spectrophotometer Model 257. Optical rotations were determined on a Rudolph polarimeter.

Di-2-thiouracilylmercury (I).—2-Thiouracil, 12.81 g (0.1 mol), was dissolved in 100 ml of 1 *N* sodium hydroxide solution and 900 ml of warm water at 50°. A solution of mercuric chloride, 13.6 g (0.05 mol) in 125 ml of ethanol, was added rapidly with stirring. The precipitate was allowed to stand for 1 hr and then filtered with suction. It was washed with water until the filtrate was free from chloride. The residue was dried *in vacuo* over phosphorus pentoxide; the yield of di-2-thiouracilylmercury was quantitative. A sample for analysis was prepared by drying the material over P_2O_5 at 100° and 0.01 mm for 2 hr.

Anal. Calcd for $C_8H_6N_4O_2S_2Hg$: C, 21.12; H, 1.33; N, 12.32. Found: C, 21.14; H, 1.29; N, 12.43.

The compound I melts at 271° dec with darkening between 250–260°. It dissociates completely in 0.1 *N* hydrochloric acid into 2-thiouracil and Hg^{2+}, as evident from its molar extinction

(19) D. Shugar and J. J. Fox, *Bull. Soc. Chim. Belg.*, **61**, 293 (1952).

(20) R. T. Walker and U. L. RajBhandary, *Biochem. Biophys. Res. Commun.*, **38**, 907 (1970); M. Saneyoshi and G. Chihara, *Chem. Pharm. Bull.*, **15**, 909 (1967).

(21) (a) B. R. Baker, J. P. Joseph, R. E. Schaub, and J. H. Williams, *J. Org. Chem.*, **19**, 1786 (1954); (b) B. R. Baker, *Chem. Biol. Purines, Ciba Found. Symp.*, 120 (1957).

(22) J. R. Einstein, Biology Division, Oak Ridge National Laboratory, private communication.

(23) I. Wempen and J. J. Fox, *Methods Enzymol.*, **12A**, 62 (1967).

(24) While this work was in progress, synthesis of 2-thiouridine in high yield by the silyl modification of Hilbert–Johnson procedure was reported by H. Vorbrüggen, P. Strehlke, and G. Schulz in *Angew. Chem., Int. Ed. Engl.*, **8**, 976 (1969).

(25) A. Albert and E. P. Sarjeant, "Ionization Constants of Acids and Bases," Methuen, London, 1962, p 69.

(26) J. J. Fox, L. F. Cavalieri, and N. Chang, *J. Amer. Chem. Soc.*, **75**, 4315 (1953).

(27) K. L. Wierzchowski and D. Shugar, *Biochim. Biophys. Acta*, **25**, 355 (1957).

and uv absorption spectrum: ϵ_{273} in 0.1 *N* hydrochloric acid solution, 28,240 (ϵ_{273} of mercuric chloride in 0.1 *N* HCl is negligible) and ϵ_{273} of 2-thiouracil in 0.1 *N* HCl 14,180. The compound is stable at pH 12. Spectral characteristics: pH 12, max 280 nm, min 262 nm; pH 2, max 273 and 212.5 nm, min 240 nm; in ethanol, max 296 nm, min 262 nm.

Condensation of Di-2-thiouracilylmercury with 2,3,5-Tri-*O*-benzoyl-D-ribofuranosyl Chloride.—A suspension of 4.549 g (0.01 mol) of finely ground di-2-thiouracilylmercury (I) in 350 ml of dry xylene was placed in a 1-l. three-necked flask fitted with a mechanical stirrer, dropping funnel, and a distillation head. The flask was heated in an oil bath and 100 ml of xylene was distilled off. The ribosyl chloride prepared from 10.09 g (0.02 mol) of 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribose by the method of Thomas, *et al.*,[28] was added through the separatory funnel rapidly with vigorous stirring. The distillation head and the separatory funnel were replaced by a reflux condenser and a stopper, and the reaction mixture was refluxed gently for 1 hr; refluxing longer led to lower yields. The reaction mixture was cooled somewhat and filtered hot with suction. The filtrate was evaporated to about 50 ml in a rotary evaporator at 30° and treated with 500 ml of petroleum ether (bp 40–50°). It was kept at 4° for several hours. The precipitate was collected, dissolved in 50 ml of chloroform, and washed twice with 50 ml of 30% w/w aqueous potassium iodide and twice with 50 ml of water. The chloroform layer was dried with anhydrous sodium sulfate and filtered. The filtrate was evaporated to dryness in a rotary evaporator at 30°; the weight of the residue was 9.2 g.

The crude residue, 4.6 g, was chromatographed on a column of silica gel (70 × 2 cm) using a linear gradient of benzene and ether and collecting 10-ml fractions. Four chromatographically homogeneous (tlc, silica gel, solvent E) fractions were collected.

Fraction 1, tubes 23–26, 1.42 g, R_f 0.94, was devoid of nitrogen and was not examined further.

Fraction 2, tubes 29–31, 0.578 g, R_f 0.88, was characterized as 1,3-di(2′,3′,5′-tri-*O*-benzoyl-β-D-ribofuranosyl)-2-thiouracil (II): mp 99°; $[\alpha]^{22}D$ +19.5° (*c* 1.1%, chloroform); λ_{max}^{EtOH} 230 nm (ε 81,700), 274 (5700); λ_{min}^{EtOH} 210 nm (ε 26,700), 260 (3400); ir (KBr) major bands, 3410, 1725, 1600, 1440, 1370, 1260, 1120, 1090, 710 cm^{-1}.

Anal. Calcd for $C_{56}H_{44}O_{15}N_2S$: C, 66.13; H, 4.36; N, 2.755. Found: C, 66.31; H, 4.47; N, 2.67.

Fraction 3, tubes 36–43, 0.724 g, R_f 0.61, was characterized as 1-(2′,3′,5′-tri-*O*-benzoyl-β-D-ribofuranosyl)-2-thiouracil (III): $[\alpha]^{22}D$ −33° (*c* 2.5%, in chloroform); λ_{max}^{EtOH} 232 nm (ε 47,500), 275 (14,900); λ_{min}^{EtOH} 210 nm (ε 19,900), 253 (6800); ir (KBr) major bands, 3410, 1725, 1600, 1375, 1265, 1120, 1090, 710 cm^{-1}.

Anal. Calcd for $C_{30}H_{24}O_8N_2S$: C, 62.93; H, 4.23; N, 4.89. Found: C, 63.09; H, 4.32; N, 4.65.

Fraction 4, tubes 50–59, 0.461 g, R_f 0.31, was characterized as 3-(2′,3′,5′-tri-*O*-benzoyl-β-D-ribofuranosyl)-2-thiouracil (IV): mp 133°; $[\alpha]^{22}D$ +44° (*c* 3.1%, chloroform); uv λ_{max}^{EtOH} 230 nm (ε 40,760), 272 (14,820), 303 (9300); λ_{min}^{EtOH} 209 nm (ε 21,690), 254 (7140), 289 (7810); ir (KBr) major bands, 3410, 1725, 1510, 1265, 1120, 1090, 710 cm^{-1}.

(28) H. J. Thomas, J. A. Johnson, Jr., W. E. Fitzgibbon, Jr., S. J. Clayton, and B. R. Baker, "Synthetic Procedures in Nucleic Acid Chemistry," Vol. 1, W. W. Zorbach and R. S. Tipson, Ed., Wiley, New York, N. Y., 1968, p 249.

Anal. Calcd for $C_{30}H_{24}O_8N_2S$: C, 62.93; H, 4.23; N, 4.89. Found: C, 63.13; H, 4.16; N, 4.66.

1-β-D-Ribofuranosyl-2-thiouracil (2-Thiouridine) (V).—The benzoyl derivative II, 1.23 g, was treated with 8.6 ml of 1 *N* sodium methoxide in methanol at room temperature overnight. The mixture was evaporated to dryness three times in a rotary evaporator after the addition of 10-ml portions of water. It was finally taken up in 100 ml of water and treated with IR-120 (H^+), 10 g, and filtered. The filtrate was extracted with three 10-ml portions of chloroform and the aqueous layer was evaporated to dryness. The yield of practically pure 2-thiouridine V was 0.46 g (81% of theory). It crystallizes from water in large prisms, mp 208–209° (lit.[18,16] 205–207°, 214°). It is chromatographically homogeneous: R_f 0.55, tlc, solvent B; R_f 0.66, Whatman 1, solvent C; R_f 0.32, Whatman 1, solvent D; $\lambda_{min}^{H_2O}$ 219 nm (ε 17,100), 275 (14,580); $\lambda_{max}^{H_2O}$ 244 nm (ε 4800); $\lambda_{max}^{0.1\ N\ NaOH}$ 239.5 nm (ε 22,600), 271 (14,200); $\lambda_{min}^{0.1\ N\ NaOH}$ 260.5 nm (ε 13,800), 223 (16,200); spectrophotometric pK_a = 8.1 (lit.[16] 8.8); $[\alpha]^{22}D$ +38° (*c* 1%, in water) (lit.[14,16] +39°, +30.8°).

Anal. Calcd for $C_9H_{12}O_5N_2S$: C, 41.53; H, 4.65; N, 10.77. Found: C, 41.32; H, 4.49; N, 10.61.

3-β-D-Ribofuranosyl-2-thiouracil (2-Thioisouridine) (VI).—This was prepared by debenzoylation of IV following the procedure of making 2-thiouridine, yield 80% of theory. The product was crystallized from ethanol and was chromatographically homogeneous: R_f 0.65, tlc, solvent B; $[\alpha]^{22}D$ −24.6° (*c* 0.86%, water); $\lambda_{max}^{H_2O}$ 298 nm (ε 11,200), 271 (9700), 211 (15,700); $\lambda_{min}^{H_2O}$ 280 nm (ε 9300), 241 (3500), 204 (15,600); $\lambda_{max}^{pH\ 12}$ 324 nm (ε 11,700), 258 (6700); $\lambda_{min}^{pH\ 12}$ 281 nm (ε 2100), 244 (5900); spectrophotometric pK_a = 8.11.

Anal. Calcd for $C_9H_{12}O_5N_2S$: C, 41.53; H, 4.65; N, 10.77. Found: C, 41.31; H, 4.64; N, 10.65.

Attempted Debenzoylation of 1,3-Di(2′,3′,5′-tri-*O*-benzoyl-D-ribofuranosyl)-2-thiouracil (II).—(A) Attempted debenzoylation of II by sodium methoxide using the above procedure led to the decomposition of the compound; 2-thiouridine and 2-thiouracil were identified among the products. (B) Debenzoylation of II was also attempted by heating with a saturated solution of ammonia in ethanol in a sealed tube. No 2-thiocytidine could be detected among the products by tlc in solvent systems A or B.

Reaction of 2-Thiouridine V and 2-Thioisouridine VI with Cyanogen Bromide.—The compound V, 4 mg, was dissolved in 5 ml of 0.1 *M* phosphate buffer, pH 8. Cyanogen bromide, 3.26 mg, was added and the reaction mixture was heated on a steam bath for 5 min. It was concentrated in a rotary evaporator and a portion was examined by tlc on cellulose plates (solvent B); the major product (R_f 0.72) (authentic uridine, R_f 0.72) was identified spectrophotometrically as uridine.

The compound VI was also treated with cyanogen bromide in a similar manner. Isouridine, R_f 0.76, tlc, solvent B, was identified spectrophotometrically[18] as one of the products.

Registry No.—I, 12524-88-8; II, 31081-97-7; III, 21052-18-6; IV, 31120-00-0; V, 20235-78-3; VI, 21052-17-5.

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