1.0 g; 50% ether in benzene, 1.7 g; and 75% ether in benzene, 0.1 g. Substances (2.0 g) with appropriate mobility on tlc were eluted by the last 400 ml of 20% ether, by 50% ether, and by 75% ether. Saponification yielded neutrals (1.55 g) and a crystalline acid (526 mg). Recrystallization from ethyl acetate, methanol–water (containing a little ethyl acetate), and ethyl acetate gave **6** (245 mg, 3.3%), mp 130° with subsequent recrystallization and remelting at 174–175°, α_D +47°.

Anal. Calcd for $C_{27}H_{46}O_4$: C, 74.6; H, 10.7. Found: C, 74.6; H, 11.0.

The methyl ester, crystallized from methanol, melted at 92–94°.

3α-Hydroxy-5β-25L-cholestanoic Acid (7).—Electrolysis of lithocholic acid (6.5 g, Nutritional Biochemicals Corp., Cleveland, Ohio) with L half-ester (2.5 g) and Na (40 mg) for 4 hr yielded neutrals (7.1 g), which were dissolved in benzene (10 ml), diluted with petroleum ether (20 ml), and applied to a column of alumina (150 g, deactivated with 6% water) prepared in petroleum ether. Eluting solvents (1.0 l. each) and residues follow: 33% benzene in petroleum ether, 1.9 g; 50% benzene in petroleum ether, 0.8 g; and benzene, 1.2 g. Solvents and residues of further elution follow: 5% ether in benzene (0.4 l.), 0.4 g; 20% ether in benzene (0.6 l.), 0.8 g; and ether (0.4 l.), 0.4 g. Substances (750 mg) with the mobility (tlc) expected of methyl monohydroxycholestanoate were eluted by the first 400 ml of benzene. Saponification yielded no neutrals but an acid (723 mg) which crystallized from ether. Several recrystallizations from ether gave **7** (278 mg, 3.9%), mp 169–170°, α_D +43°.

Anal. Calcd for $C_{27}H_{46}O_3$: C, 77.5; H, 11.1. Found: C, 77.7; H, 11.8.

The methyl ester, crystallized from ether–petroleum ether, melted at 112–113°.

3α-Hydroxy-5β-25D-cholestanoic Acid (8).—A similar electrolysis with lithocholic acid (recrystallized), Na, and D half-ester for 3.75 hr yielded neutrals (6.8 g) and traces of acid. After chromatography the product emerged with the first 800 ml of benzene. Saponification yielded crystalline neutrals (556 mg) and an acid (940 mg) which crystallized on standing with ether–petroleum ether. Recrystallization from methanol–water, ether, and methanol–water gave **8** (392 mg, 5.4%), mp 155–157°, α_D +26°.

Anal. Calcd for $C_{27}H_{46}O_3$: C, 77.5; H, 11.1. Found: C, 77.7; H, 11.6.

The methyl ester, crystallized from ether–petroleum ether, melted at 108–109°.

Registry No.—**1**, 23047-29-2; **1** methyl ester, 23740-21-8; **2**, 23740-14-9; **2** methyl ester, 23740-22-9; **3**, 23740-15-0; **4**, 23740-16-1; **5**, 23740-17-2; **6**, 23740-18-3; **6** methyl ester, 23740-23-0; **7**, 23740-19-4; **7** methyl ester, 23740-24-1; **8**, 23740-20-7; **8** methyl ester, 23829-36-9.

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The Reaction of 5-Bromouracil Derivatives with Sulfur Nucleophiles, and a Novel Synthetic Route to 5-Sulfur-Substituted Uracils and Nucleotides[1a,b]

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1-Methyl-5-bromouracil (**1**) reacts with sodium hydrosulfide in dimehtyl sulfoxide at room temperature to give 1-methyl-5-mercaptouracil (**2**, isolated as the disulfide **3**) and 1-methyluracil (**4**). Deuterium-exchange studies are consistent with 1,4 addition of the reagent to **1**, followed by tautomerization, to give two stereoisomeric adducts which, after nucleophilic displacement of the bromine by another anion of the reagent, undergo *trans* elimination reactions leading to **2** and **4**, respectively. Based on this mechanism, a useful, novel synthetic route was developed for the introduction of a 5-sulfur substituent into the pyrimidine ring of uracil derivatives *via* addition of methyl hypobromite to the 5,6 double bond, followed by reaction of the adduct with sodium disulfide. By use of this method, **3** was synthesized in 74% yield, and the disulfide of the nucleotide 5-mercapto-2′-deoxyuridine 5′-phosphate (**15**) was synthesized in an overall yield of 68%.

The synthesis of 5-mercapto-2′-deoxyuridine, a structural analog of thymidine, was recently reported.[2] This compound was found to be an effective antimetabolite in various test systems[3] in which it was apparently converted into its 5′-phosphate (**17**). It appeared of interest to synthesize the nucleotide **17** and some of its derivatives; this prompted the investigation of the feasibility of introducing a thiol group at the 5 position of 1-substituted uracil derivatives, to provide a relatively simple method applicable for the preparation of various 5-sulfur-substituted pyrimidine nucleotides. Although 5-halogenopyrimidines are characterized by low reactivity of the halogen atom,[4] Roth and Hitchings reported[5] that thiophenol salts react readily with with 5-bromouracil in ethylene glycol at 150° to give 40–50% yield of 5-arylthiouracils, in addition to uracil and diaryl disulfides obtained as by-products. The observed side reaction was attributed to electron transfer, resulting in the reductive removal of the halogen.[5]

1-Methyl-5-bromouracil (**1**) was selected as a model compound for the determination of the optimal conditions for the substitution reaction. Preliminary experiments indicated that **1** reacted with excess sodium hydrosulfide only at high temperature when ethylene glycol[5] was used as the solvent, but the reaction proceeded readily at room temperature in dimethylacetamide (DMAA) or dimethyl sulfoxide (DMSO). Although in the latter case the reaction appeared to be essentially complete in 1 hr, the presence of both 1-methyl-5-mercaptouracil (**2**) and its disulfide (**3**) in the reaction

(1) (a) This work was supported by U. S. Public Health Service Research Grant R01-CA06695 from the National Cancer Institute, National Institutes of Health, Bethesda, Md. (b) A preliminary report of this work was presented at the 158th National Meeting of the American Chemical Society, New York, N. Y., Sept 1969. (c) To whom inquiries should be directed.

(2) (a) T. J. Bardos, M. P. Kotick, and C. Szantay, *Tetrahedron Lett.*, 1759 (1966); (b) M. P. Kotick, C. Szantay, and T. J. Bardos, *J. Org. Chem.*, in press.

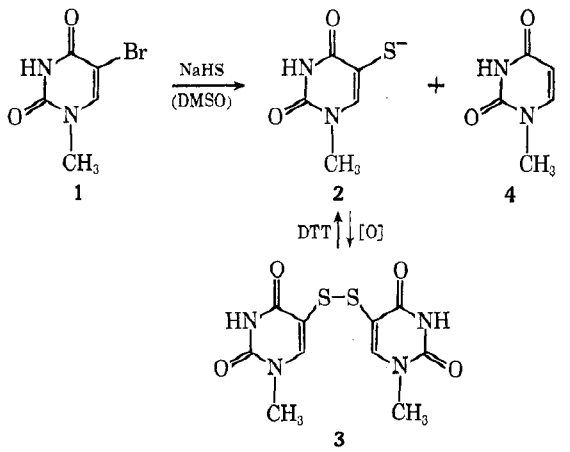
(3) (a) K. Baranski, T. J. Bardos, A. Bloch, and T. I. Kalman, *Biochem. Pharmacol.*, **18**, 347 (1969); (b) T. I. Kalman and T. J. Bardos, *Fed. Proc.*, **27**, 650 (1968).

(4) G. W. Kenner and A. Todd, "Heterocyclic Compounds," Vol. 6, R. C. Elderfield, Ed., J. Wiley & Sons, Inc., New York, N. Y., 1957, p 301.

(5) B. Roth and G. H. Hitchings, *J. Org. Chem.*, **26**, 2770 (1961).

mixture was indicated by the characteristic absorption maximum of the thiolate ion at 335 mμ and by the increase of the absorbancy at this wavelength upon addition of the reducing agent dithiothreitol (DTT).[6] Therefore, the reaction was continued for 3 days, to permit complete oxidation of 2. Two major products were isolated: 1-methyl-5-mercaptouracil disulfide (**3**), in 54% yield, and 1-methyluracil (**4**), in 41% yield (Scheme I).

SCHEME I

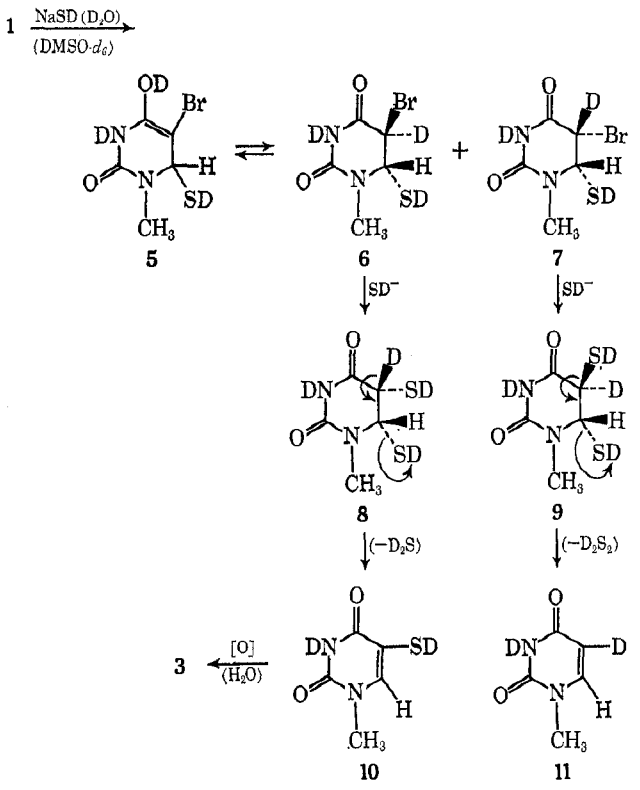


To avoid the reductive replacement of the 5-bromo substituent with hydrogen, leading to the unwanted 1-methyluracil (**4**), the use of other sulfur-containing nucleophilic reagents was attempted, such as potassium thiolacetate, thiourea, sodium disulfide, sodium sulfide, potassium thiocyanate, etc. However, in spite of the effectiveness of these reagents in the preparation of mercaptans from aliphatic and aromatic halides in general, only potassium thiolacetate reacted with **1**, and **4** was obtained also in this case as the second major product.

Recent studies of several investigators[7-9] have demonstrated that in D_2O or MeOD solution a base-catalyzed deuterium exchange occurs at the 5 position of 1-substituted uracil derivatives. It was postulated that the mechanism of this exchange most likely involves a 1,4 addition across the α,β-unsaturated carbonyl system, initiated by a nucleophilic attack of the anion of the base at C-6, followed by an enol–keto tautomeric shift, and then elimination of the C-5 hydrogen atom and the nucleophile.[7] An alternative mechanism, involving the anionic attack at the C-5 position and a 1,2 addition across the 5,6 double bond of the uracil nucleus, was proposed to explain the observed deuterium exchange at the C-6 position in the case of 5-fluorouracil.[9] It occurred to us that the mechanism of the displacement of bromine at C-5 of **1** with sodium hydrosulfide or potassium thiolacetate (or with thiophenol salts in the case of 5-bromouracil[5]) might involve either a 1,2 or a 1,4 addition of the reagent as the necessary first step; this would saturate the 5,6 double bond and thus activate the bromine for nucleophilic displacement by the anion of a second molecule of the sulfur-containing reagent. Such a mechanism would explain the lack of reaction with the other reagents which do not add to the double-bond system.

To study the mechanism of this reaction, **1** was treated with deuterated sodium hydrosulfide in deuterated DMSO as the solvent. Although an intermediate addition product could not be isolated, the final disulfide product (**3**) obtained in this experiment showed no evidence of deuterium exchange at the C-6 position, as indicated by integration of its undiminished C-6 hydrogen peak in the nmr spectrum. Thus, if the reaction did proceed *via* primary addition of the reagent to the double-bond system, then the initial nucleophilic attack by the SD^- anion appears to have occurred at the C-6 position *via* the initial formation of a 1,4 adduct (**5**), as shown in Scheme II. This, then could tautomerize to

SCHEME II



the more stable 5,6-saturated adducts, **6** and **7**, in which the bromine (α to the carbonyl group) is readily displaced by the nucleophilic attack of another SD^- anion, with inversion of configuration at C-5, to give **8** and **9**, respectively. *trans* elimination of a molecule of D_2S from **8** would give the 5-mercaptouracil derivative **10** which ionizes to the thiolate anion and undergoes autoxidation to the disulfide. *trans* elimination of D_2S_2 from **9** would give the "reduced" by-product, 1-methyluracil, with deuterium at C-5 (**11**). This product was also isolated from the reaction mixture and showed in its nmr spectrum a singlet for the C-6 hydrogen and no absorption peak for a hydrogen at C-5.

While this proposed mechanism (Scheme II) may not be the only one possible, it certainly provides a satisfactory explanation for the obtained results. If the first reaction step had been a 1,2 addition of sodium deuteriosulfide to the 5,6 double bond of **1**, then (a) in the case that the SD^- anion reacted at C-5, the C-6 posi-

(6) T. J. Bardos, and T. I. Kalman, *J. Pharm. Sci.*, **55**, 606 (1966).
(7) S. R. Heller, *Biochem. Biophys. Res. Commun.*, **32**, 998 (1968).
(8) D. V. Santi, and C. F. Brewer, *J. Amer. Chem. Soc.*, **90**, 6236 (1968).
(9) R. J. Cushley, S. R. Lipsky, and J. J. Fox, *Tetrahedron Lett.*, 5393 (1968).

tion would have been deuterated, and (b) in the case that the SD^- anion reacted at C-6, the expected *trans* addition would give only adduct **7** which according to Scheme II would lead to **11** as the only reaction product. Although the formation of **3** by some different mechanism cannot be excluded, a 1,2-addition mechanism for this reaction is not supported by the results, while a 1,4-addition mechanism could entirely explain the ready formation of both reaction products, **3** and **11**.

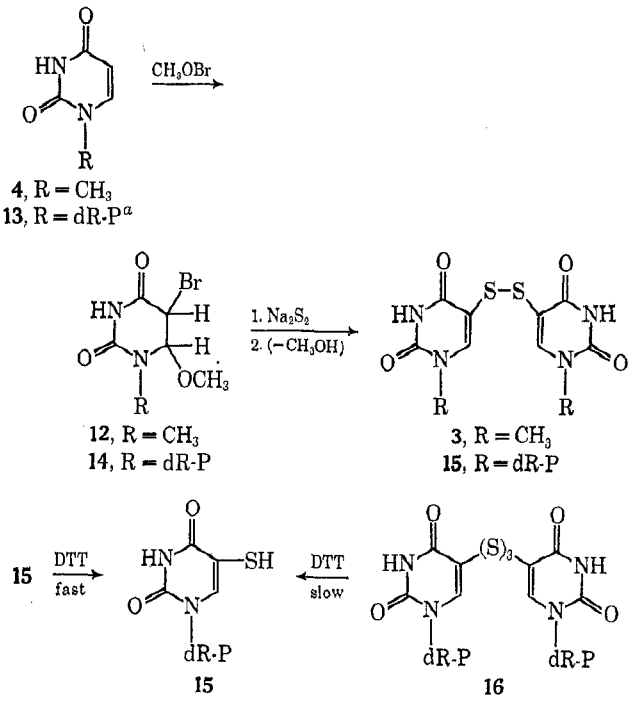
That the intermediate adducts shown in Scheme II could not be isolated is not surprising in view of the expected reactivity of **6** and **7** with a sulfur nucleophile and the understandable lability of the dithiol adducts **8** and **9**. Since the reaction proceeds at room temperature and requires a large excess of the reagent, it is reasonable to assume that the first step is rate limiting, while the nucleophilic displacement and subsequent elimination reactions proceed quite rapidly. Although the "reduced" product **11** could also be formed by direct reduction of either **6** or **7** with sodium deuteriosulfide, this seems unlikely in the presence of the strongly nucleophilic SD^- anion which could most effectively displace the bromine.[10] It is significant that **11** is obtained in nearly the same yield as **3**.

Based on the above mechanistic interpretation of the reaction between **1** and sodium hydrosulfide, we sought to design a better method for the introduction of a sulfur substituent at the 5 position of the uracil nucleus. If the above mechanism is correct, then an adduct of the type **6** should readily react also with those sulfur-containing reagents that did not react with **1** in our initial experiments (presumably because of their inability to form an addition product), and, in the absence of a type **7** stereoisomer, a higher yield of the desired 5-sulfur-substituted product should result. Methyl hypobromite is known to form isolable adducts with various uracil and cytosine derivatives,[11] presumably by *trans* addition to the 5,6 double bond. Thus, these adducts could be expected to have a configuration similar to **6**.

Consequently, methyl hypobromite was treated with **4** according to the general method of Duschinsky, *et al.*,[11] and the adduct **12** was isolated and characterized. It readily reacted at 0° in dimethylacetamide solution with sodium disulfide (one of the reagents that were unreactive toward **1**), to give an overall yield of 75% (based on 1-methyluracil, **4**) of the pure disulfide **3** (Scheme III, R = CH_3). A small amount (12%) of **4** was also isolated; this was probably due to the reversibility of the addition reaction under basic conditions.[11]

This approach was readily applicable also to the synthesis of the desired nucleotide, 5-mercapto-2′-deoxyuridine 5′-phosphate (**17**). The corresponding disulfide, **15**, was obtained from 2′-deoxyuridine 5′-phosphate (**13**) by the above procedure (Scheme III, R = dR-P) in an overall yield of 68% (as the analytically pure barium salt). In addition, a small amount of another sulfur-containing nucleotide was isolated which analyzed for the trisulfide **16**. Both **15** and **16**

SCHEME III



[a] dR-P = 2-deoxy-β-D-ribofuranosyl 5-phosphate.

can be reduced to the 5-mercapto nucleotide **17** with dithiothreitol;[6] however, the reduction proceeds much more slowly in the case of the trisulfide **16** than in the case of **15** or other 5-uracilyl disulfides.[6]

Further possible applications of the new synthetic method for the introduction of sulfur substituents at the C-5 position of uracil and cytosine rings of nucleoside triphosphates, oligonucleotides, and nucleic acids are under investigation.

Experimental Section

All melting points were taken on a Mel-Temp apparatus and they are corrected. Infrared spectra were recorded on a Perkin-Elmer Infracord or Beckman IR-8 employing potassium bromide disks. Nmr spectra were recorded on a Varian Model A-60 spectrophotometer in the indicated solvent with TMS as an internal standard. Ultraviolet spectra were obtained on a Beckman DB recording spectrophotometer. Optical rotations were measured in a 1-dm tube using a Perkin-Elmer Model 141 automatic polarimeter at 589 mμ. Elemental analyses were performed by Galbraith Laboratories, Knoxville, Tenn.

1-Methyl-5-mercaptouracil Disulfide (3).—To a solution of 1-methyl-5-bromouracil[12] (1, 0.60 g, 2.9 mmol) in 10 ml of DMSO, NaSH (0.66 g, 11.8 mmol) was added, and the mixture was stirred for 3 days at room temperature. The progress of the conversion of **1** to the mercapto compound **2**, and of the oxidation of **2** to the disulfide **4**, was followed by tlc and by observing the changes in the uv and nmr spectra. EtOH (5 ml) and Et_2O (100 ml) were then added, and the precipitate was separated by filtration, dissolved in 5 ml of water, and acidified with 10% HCl. The white crystals were filtered, washed with water, and recrystallized from DMF–EtOH, to give 0.25 g (54%) of the product **3**: mp 302–303° dec; uv (pH 7.2) λ_{max} 282 mμ (ε 17,310) and λ_{min} 239 mμ (ε 10,710) [upon addition of DTT, the spectrum changes to that of the thiolate ion[6] (**2**): λ_{max} 335 mμ (ε 10,930) and λ_{min} 291 (ε 4330)]; nmr (DMSO-d_6) δ 3.27 (6 H, s, NCH_3) and 8.15 ppm (2 H, s, 6-CH).

Anal. Calcd for $C_{10}H_{10}N_4O_4S_2$: C, 38.21; H, 3.20; N, 17.82; S, 20.40. Found: C, 38.16; H, 3.20; N, 17.72; S, 20.23.

1-Methyluracil (4).—The DMSO–EtOH–Et_2O mother liquor was evaporated *in vacuo*, and the residue was recrystallized from

(10) We believe that the previously reported reduction of 5-bromo-5-fluoro-6-substituted 5,6-dihydrouracil derivatives with thiols[11] may also proceed *via* nucleophilic displacement of the bromine by the thiolate anion and subsequent displacement of the thiol group by reaction with another molecule of the thiol (disulfide formation). This would explain the observed retention of configuration,[11] as the result of double inversion at C-5.

(11) R. Duschinsky, T. Gabriel, W. Tautz, A. Nussbaum, M. Hoffer, E. Grundberg, J. H. Burchenal, and J. J. Fox, *J. Med. Chem.*, **10**, 47 (1967).

(12) A. Benitez, L. O. Ross, L. Goodman, and B. R. Baker, *J. Amer. Chem. Soc.*, **82**, 4585 (1960).

MeOH to give 0.15 g (41%) of 1-methyluracil (**4**), which was identified by uv spectra and mixture melting point with an authentic sample:[13] nmr (DMSO-d_6) δ 3.25 (3 H, s, NCH_3), 5.55 (1 H, d, J = 7.9 Hz, 5-CH), and 7.60 ppm (1 H, d, J = 7.9 Hz, 6-CH).

Reaction of 1 with NaSD in DMSO-d_6.—A solution of NaSH (0.40 g, 7.14 mmol) in D_2O (5 ml) was evaporated *in vacuo* to dryness, and this procedure was repeated four times with four 5-ml portions of D_2O. To the residue, DMSO-d_6 (4 ml) and **1** (0.30 g, 1.46 mmol) were added with stirring at room temperature. Work-up of the reaction products was the same as in the reaction described above. In one experiment, the 5-sulfur-substituted product (0.10 g, 43%), isolated after 2 days, showed in the nmr spectrum two distinct singlets at δ 8.32 and 8.15 ppm for the C-6 hydrogen of the thiol (**2**) and of the disulfide (**3**), respectively. On prolonged standing in the nmr tube, the δ 8.32 peak disappeared and the simultaneously increased 8.15 peak gave an integrated ratio of 1:3 relative to the NCH_3 peak (3.28). In another experiment, the stirring of the reaction mixture was continued for 4 days, and only the disulfide **3** was isolated in pure form.

The second product (**11**) was also obtained in 43% yield (0.08 g) and had the same melting point and uv spectrum as **4** but its nmr spectrum showed absence of the C-5 proton absorption and the C-6 doublet of **4** was replaced by a singlet: nmr (DMSO-d_6) δ 3.25 (3 H, s, NCH_3) and 7.63 ppm (1 H, s, 6-CH).

1-Methyl-5-bromo-6-methoxy-5,6-dihydrouracil (12).—A cold solution of MeOBr, freshly prepared from 0.43 g (5.5 mmol, 0.14 ml) of Br_2 according to the procedure of Duschinsky, *et al.*,[11] was filtered through a Celite filter directly into an ice-cold solution of 1-methyluracil (**4**, 0.25 g, 2 mmol) in 6 ml of MeOH. The stirring and cooling was continued at 0° for 1 hr; then the solution was kept at room temperature for another hour. After addition of Et_2O (50 ml) to the solution, white crystals deposited which were filtered, washed with cold Et_2O, and dried *in vacuo:* yield 0.42 g (90%); mp 144.5°; uv spectrum end absorption only; nmr (CD_3COCD_3) δ 3.18 (3 H, s, NCH_3), 3.55 (3 H, s, $-OCH_3$), 4.66 (1 H, d, J = 2.1 Hz), and 4.92 ppm (1 H, d, J = 2.1 Hz).

Conversion of 12 into 1-Methyl-5-mercaptouracil Disulfide (3).—Crude (unrecrystallized) addition product (**12**, 0.42 g) and $Na_2S_2 \cdot 5H_2O$[14] (0.40 g, 2 mmol) were dissolved in 3.5 ml of ice-cold dimethylacetamide, and the solution was stirred for 2 hr at 0° and then at room temperature for 24 hr. EtOH (2 ml) and Et_2O (50 ml) were added to the solution and the precipitated solids were filtered and then redissolved in 8 ml of water. After the solution stood for 3 hr in the refrigerator, the deposited white crystals were filtered, washed with water, and dried *in vacuo* to yield 0.23 g of pure **3** [74%, based on 1-methyluracil (**4**) as starting material]. The mixture melting point, with a sample of the product **3** obtained previously by the reaction of **1** with sodium hydrosulfide, was not depressed, and the uv, ir, and nmr spectra of the two samples were identical.

After the evaporation of the DMAA–EtOH–Et_2O mother liquor, 0.03 g (12%) of 1-methyluracil (**4**) was isolated and identified.

N_1-(2′-Deoxy-β-D-ribofuranosyl)-5-bromo-6-methoxy-5,6-dihydrouracil 5′-Phosphate (14) Monosodium Salt.—A cold solution of MeOBr, freshly prepared from 0.77 g (9.82 mmol, 0.25 ml) of Br_2 according to the procedure of Duschinsky, *et al.*,[11] was filtered through a Celite filter directly into an ice-cold suspension of 2′-deoxyuridine 5′-phosphate (**13**) disodium salt ($2.5H_2O$) (1.30 g, 3.27 mmol) in 13 ml of MeOH. Stirring and cooling at 0° was maintained for 3 hr; then to the light yellow solution 300 ml of Et_2O was added. The deposited white crystals were filtered and washed with cold Et_2O and recrystallized from MeOH–Et_2O to give 1.40 g of **14** (96%). The uv spectrum showed only end absorption.

Anal. Calcd for $C_{10}H_{16}N_2O_9BrPNa$: C, 27.22; H, 3.42; Br, 18.12; Found: C, 26.61; H, 3.79; Br, 18.21.

N_1-(2′-Deoxy-β-D-ribofuranosyl)-5-mercaptouracil 5′-Monophosphate Disulfide (15) and N_1-(2′-Deoxy-β-D-ribofuranosyl)-5-mercaptouracil 5′-Monophosphate Trisulfide (16).—To a solution of 1.40 g of crude (unrecrystallized) addition product (**14**) in 16 ml of dimethylacetamide, $Na_2S_2 \cdot 5H_2O$[14] (1.08 g, 5.4 mmol) was added at 0°, with stirring. The reaction mixture was then stirred for 5 hr at 0° and at room temperature for 12 hr, during which time a white precipitate formed. Ether (25 ml) was added to the mixture and the precipitate was filtered and washed with cold EtOH and Et_2O. The precipitate was then dissolved in 40 ml of water, and a solution of 0.76 g of $BaCl_2 \cdot 2H_2O$ in 5 ml of water was added. The pH of the solution was adjusted to 8.5 with dilute NH_4OH. Then 30 ml of EtOH was added which precipitated the Ba salt of **15** (1.10 g, 63%).

The filtrate (A) was treated with more EtOH (15 ml), and the precipitated solids were collected (0.15 g). This consisted of a mixture of the nucleotide disulfide (**15**) and trisulfide (**16**) which were separated by preparative tlc on Cellex-PEI anion-exchange cellulose [2 M CH_3COOH–1 M LiCl (1:1)]: R_f 0.74 for **15**, 0.48 for **16**. After elution and evaporation of the eluates *in vacuo* to dryness, the LiCl was extracted from the residues with MeOH. The residues were dissolved in water and treated with 2 equiv of aqueous $BaCl_2$ and with dilute NH_4OH to adjust the pH to 8.5. This precipitated the Ba salts of the disulfide **15** (0.08 g, 5%; total yield of **15**, 68%) and of the trisulfide **16** (0.04 g, 2.3%), respectively.

From the mother liquor (B), by further addition of EtOH, the Ba salt of deoxyuridine 5′-monophosphate (**13**) was isolated (0.09 g, 7%) and identified by comparison of uv spectra and R_f values on tlc [Cellex-PEI, 2 M CH_3COOH–1 M LiCl (1:1)] with those of an authentic sample.

Barium Salt of the Disulfide 15.—The barium salt of **15** was obtained: uv (pH 7.2) λ_{max} 273 mμ (ϵ 15,260) and λ_{min} mμ 247 (ϵ 11,090) [upon addition of DTT,[6] the spectrum changes to that of the thiolate ion, **17**: λ_{max} 330 mμ (ϵ 8060) and λ_{min} 295 mμ (ϵ 5380)]; $[\alpha]^{25}D$ 106.02° (c 0.46, 0.1 N HCl).

Sodium Salt of the Disulfide 15.—This was prepared by the treatment of the Ba salt with Chelex 100 ion-exchange resin (Na^+ form) in water: uv (pH 7.2) λ_{max} 273 mμ (ϵ 16,020) and λ_{min} 247 (ϵ 11,260) [upon addition of DTT[6] (**17**) the spectrum changes: λ_{max} 330 mμ (ϵ 8560) and λ_{min} 293 (ϵ 5390)]; $[\alpha]^{25}D$ +27.54° (c 0.305, H_2O); nmr (D_2O) δ 6.14 (5, J = 6.5 Hz, 1′-CH nucleosidic proton) and 7.57 ppm (s, 6-CH).

Anal. Calcd for $C_{18}H_{20}N_4O_{16}P_2S_2Na_4 \cdot 5H_2O$: C, 25.23; H, 3.53; N, 6.54; S, 7.48; P, 7.23. Found: C, 25.38; H, 3.11; N, 6.10; S, 7.04; P, 6.77.

Free Acid Form of the Disulfide 15.—This was obtained upon treatment of the sodium salt with Dowex 50-WX8 (H^+ form): $[\alpha]^{25}D$ +121.11° (c 0.28, H_2O); nmr (D_2O) δ 6.18 (t, J = 6.4 Hz, 1′-CH) and 7.91 ppm (s, 6-CH).

Barium Salt of the Trisulfide 16.—The barium salt of **16** was obtained: uv (pH 7.2) λ_{max} 277 mμ (ϵ 13,470) and λ_{min} 249 mμ (ϵ 7450) [after reduction with DTT to **17** the spectrum changed: λ_{max} 332 mμ (ϵ 6880) and λ_{min} 295 mμ (ϵ 4010)].

Anal. Calcd for $C_{18}H_{20}N_4O_{16}P_2S_3Ba_2 \cdot 6H_2O$: C, 19.84; H, 2.96; S, 8.83. Found: C, 19.15; H, 2.66; S, 8.90.

Registry No.—**3**, 23735-47-9; **4**, 615-77-0; **11**, 23735-49-1; **12**, 23735-50-4; **14** monosodium salt, 23735-51-5; **15**, 23735-52-6; **15** barium salt, 23735-53-7; **15** sodium salt, 23735-54-8; **16**, 23735-55-9; **16** barium salt, 23735-56-0.

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