solution was made strongly basic by adding a solution of sodium methoxide in methanol. Most of the methanol was removed by distillation, and the residue was taken up in ether (500 ml) and extracted with two 50-ml portions of water. The ether solution was dried over magnesium sulfate and then concentrated to 100 ml for vpc analysis.

In experiments with sodium methoxide as the electrolyte, sodium (2.3 g, 0.1 g-atom) was allowed to react with methanol (125 ml). 2,5-Dimethylfuran (25 ml, 22.2 g, 0.231 mol) was added to the solution which was then electrolyzed at 2 A until 43,200 coulombs of charge had passed through the solution. The solution was taken up in ether (500 ml) and extracted with two 100-ml portions of water. The ether solution was dried over magnesium sulfate and concentrated to 100 ml for vpc analysis.

Decomposition of Benzoyl Peroxide in 2,5-Dimethylfuran.—Benzoyl peroxide (4.84 g, 0.02 mol) in 2,5-dimethylfuran (175 ml) was maintained at 75–80° for 24 hr. The solution was taken up in ether (500 ml) and extracted with two 50-ml portions of a solution of sodium bicarbonate (8.2 g) in water (100 ml). Acidification of the bicarbonate extract yielded 1.4 g of benzoic acid, mp 120–122°.

The ether solution was dried over magnesium sulfate, and the ether was removed, yielding 6 g of an oil. This oil was distilled in three portions in a short-path still and yielded first a solid which adhered to the condenser and then a liquid. The total yield of solid, which proved to be the known 2,5-bis(benzoyloxymethyl)furan,[10] was 1.8 g, mp 77–78° after two crystallizations from hexane. The yield of liquid, almost certainly 2-methyl-5-benzoyloxymethylfuran, was 2.1 g. After redistillation it had $n^{24}D$ 1.5345.

Anal. Calcd for $C_{13}H_{12}O_3$: C, 71.94; H, 5.95. Found: C, 71.02; H, 5.56.

Registry No.—2,5-Dimethylfuran, 3710-43-8; I, 18801-74-6; II, 18801-75-7; 2,5-bis(methoxymethyl)-furan, 18801-76-8; 2-methyl-5-benzoyloxymethylfuran, 18801-77-9.

(10) F. H. Newth and L. F. Wiggins, *Research* (London), **3**, 50 (1950).

Nucleosides. LV. Synthesis of a Sulfur-Bridged Thymine Anhydro Nucleoside and Derivatives[1,2]

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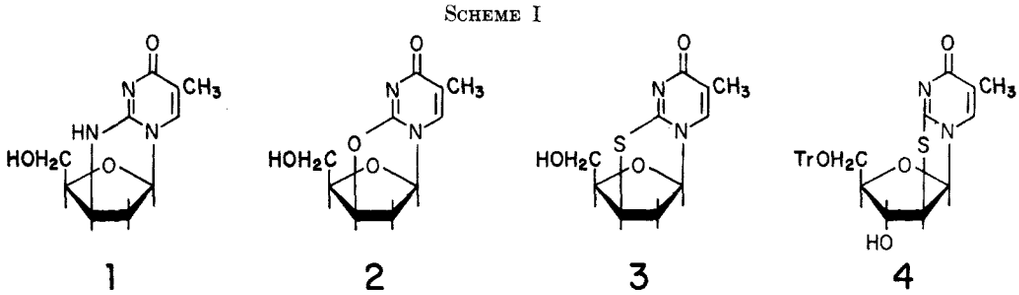
Treatment of 2-*O*-methyl-3′-*O*-mesylthymidine (**13**) in dimethylformamide with hydrogen sulfide afforded (*S*)-2,3′-anhydro-1-(2,3-dideoxy-β-D-*threo*-pentofuranosyl)-2-thiothymine (**3**). Compound **3** was cleaved in alkali to yield the relatively stable mercapto derivative, 1-(2,3-dideoxy-3-mercapto-β-D-*threo*-pentofuranosyl)-thymine (**14**). Acid-catalyzed treatment of **14** with acetone gave the oxathiolane derivative, 1-[2,3-dideoxy-3,5(*S,O*)-isopropylidene-β-D-*threo*-pentofuranosyl]thymine (**15**). Compound **14** was oxidized to the intermolecular disulfide, **17**. The sulfur-bridged anhydro nucleoside (**3**) was also obtained by treatment of 2,5′-anhydro-3′-*O*-mesylthymidine (**5**) in dimethylformamide with hydrogen sulfide. From the latter reaction a second product was obtained and identified as the internal disulfide of 1-(trideoxy-3,5-dimercapto-β-D-*threo*-pentofuranosyl)thymine (**8**). An alternate and preferred synthesis of **8** from di-*O*-mesylthymidine (**11**) is described. The ultraviolet absorption spectrum of the 3′-mercapto derivative (**14**) as a function of pH revealed the presence of *two* dissociations with apparent pK_a values of 10.2 and ~8.4. The higher of these is due to ionization of the N-3 proton. The ionization associated with $pK_a \sim 8.4$ is interpreted as a reflection of the rupture of a weak hydrogen bond between the 3′-mercapto group and the 2-carbonyl.

Previous studies[3] on the use of anhydropyrimidine nucleosides as intermediates for the alteration of the sugar moiety led to the synthesis of nitrogen-bridged isosteres (**1**) of the 2,3′-anhydro nucleoside (**2**) (Scheme I). An obvious corollary to this work would be the introduction of sulfur as the bridge atom, such as **3**. Such "(*S*)-2,3′-anhydro" or epithio structures could serve as precursors for the preparation of 2′,3′-dideoxy-3′-mercapto nucleosides of the *xylo* (*lyxo*) configuration and possibly could lead by reduction to nucleosides devoid of the 2-oxo substituent in the aglycon.

The first synthesis of a sulfur-bridged anhydro nucleoside was achieved by Shaw and Warrener[4] starting from preformed 2-thioribofuranosylthymine which gave, subsequently, the (*S*)-2,2′-anhydro nucleoside derivative **4**. Later, several studies[5] described the

SCHEME I



(1) This investigation was supported in part by funds from the National Cancer Institute, National Institutes of Health, U.S. Public Health Service (Grant No. CA 08748).

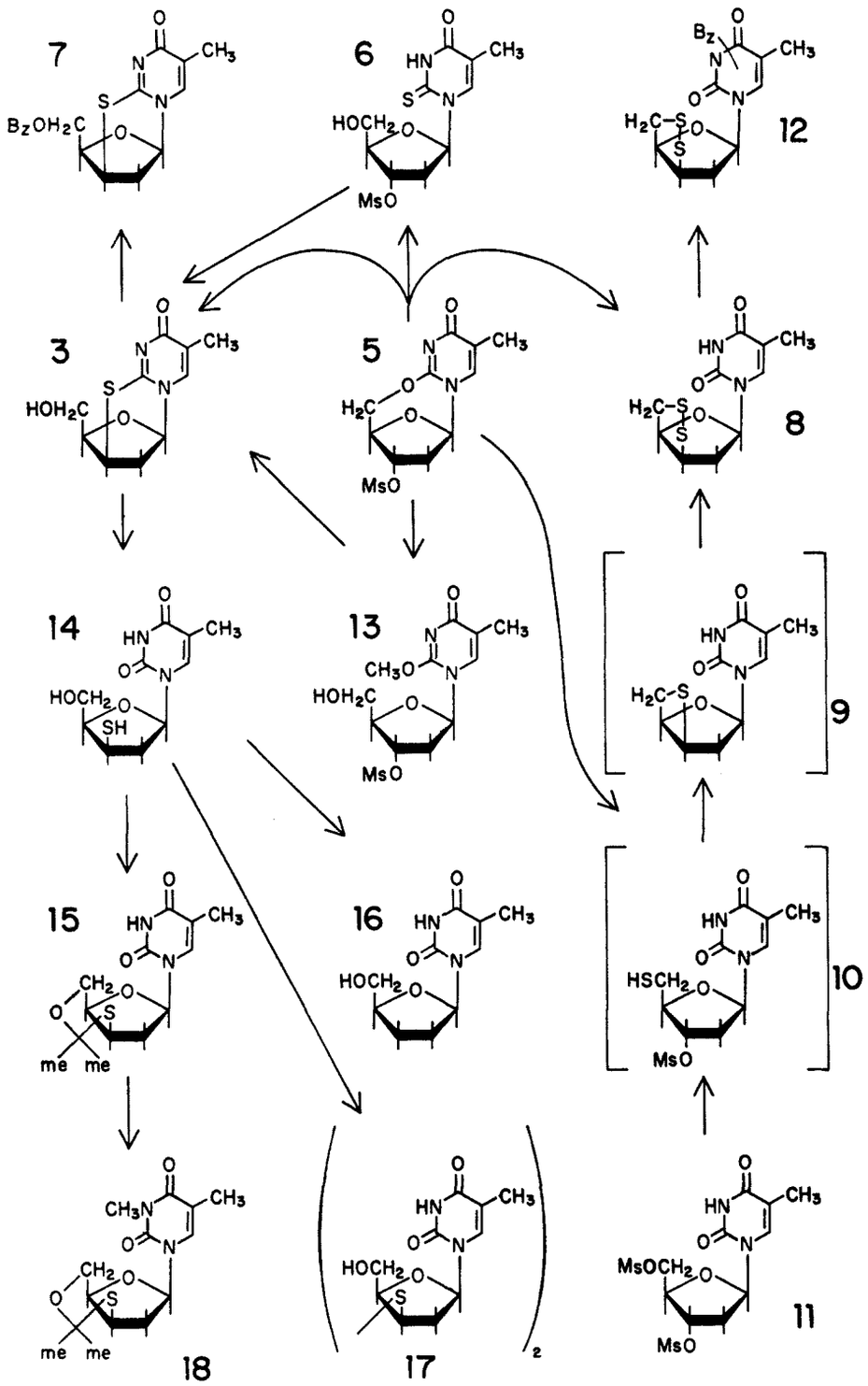
(2) Presented in part at the 21st *Intern. Congr. Pure Appl. Chem., Prague, 1967*, in press.

(3) I. L. Doerr, R. J. Cushley, and J. J. Fox, *J. Org. Chem.*, **33**, 1592 (1968).

(4) G. Shaw and R. N. Warrener, *J. Chem. Soc.*, 50 (1959).

(5) B. Bannister and F. Kagan, *J. Amer. Chem. Soc.*, **82**, 3363 (1960); N. K. Kochetkov, E. I. Budowsky, V. N. Shibaev, G. I. Yeliseeva, M. A. Grachev, and V. P. Demushkin, *Tetrahedron*, **19**, 1207 (1963); R. W. Chambers and V. Kurkov, *J. Amer. Chem. Soc.*, **85**, 2160 (1963); E. J. Reist, A. Benitez, and L. Goodman, *J. Org. Chem.*, **29**, 554 (1964).

SCHEME II



syntheses of 5′,6-epithio derivatives of uridines from 5′-mercapto derivatives of ribosyl- and certain 2′-deoxyribosyluracils.

In this report, we describe the direct synthesis of an (*S*)-2,3′-anhydro nucleoside from a 2,5′-anhydro precursor (Scheme II). It was envisioned that cleavage of 2,5′-anhydro-3′-mesylthymidine (**5**)[3] with hydrogen sulfide would result in a 2-thiothymidine derivative which should then undergo *in situ* an intramolecular displacement of the 3′-mesylate by the 2-thione to form the (*S*)-2,3′-bridged nucleoside (**3**). Treatment of **5** at room temperature in dimethylformamide with hydrogen sulfide produced a low yield of an unstable product (**6**). The ultraviolet absorption properties of this material were similar to those for 1-β-D-glucopyranosyl-2-thiothymine[6] and its elemental analysis was also consistent with **6**. Reaction of **6** with ethanolic triethylamine afforded a high-melting crystalline solid (**3**). The absence of the mesyloxy function was confirmed by examination of the infrared spectrum and by elemental analysis. Most revealing was the ultraviolet absorption spectrum of **3** which was similar to

(6) M. Sano, *Chem. Pharm. Bull.* (Tokyo), **10**, 320 (1962).

that of 2-ethylthio-1-methyl-4-pyrimidinone.[7] The presence of a free hydroxyl function in the sugar moiety was shown by the preparation of the 5′-*O*-benzoyl derivative (7). Neither compound **3** nor **7** was sufficiently soluble for pmr analyses.

When the above-mentioned hydrogen sulfide treatment of **5** was carried out at 70°, compound **6** was not isolable. From this reaction, **3** was isolated in ~20% yield along with a second compound (**8**, 19%) for which the elemental analysis and molecular weight determination showed the presence of two sulfur atoms in the molecule. The absence of both mesyl and mercapto groups was confirmed by the absence of bands characteristic for such functions in the infrared spectrum. The ultraviolet absorption spectrum of **8** was similar to that of thymidine. These data indicate that the sulfur atoms in **8** are located exclusively on the sugar moiety in an intramolecular disulfide linkage. The isolation of **8** and **3** from the same reaction mixture might suggest that **3** was an intermediate in the formation of **8**. Treatment of **3** with hydrogen sulfide under identical reaction conditions (with or without the addition of methanesulfonic acid)[8,9] did not produce **8**. Most of compound **3** was recovered along with some 2-thiothymine and thymine. An alternate route for the preparation of **8** in good yield was achieved by reaction of di-*O*-mesylthymidine (**11**) with hydrogen sulfide and dimethylformamide at 70°. The (*S*)-2,3′-anhydro nucleoside (**3**) was not formed in the course of this reaction.

Attempts to determine the pmr spectrum of **8** were not successful due to its insolubility. Benzoylation of **8** gave monobenzoyl **12**.[10,11] The pmr spectrum[12] of the more soluble **12** in pyridine-d_5 was consistent with a 3′,5′-disulfide formulation. By application of double resonance, the H-3′–H-4′ coupling was found to be 6.6 Hz—a value which does not give a clear choice between a *cis* or *trans* relationship. The possibility that ring opening of episulfide **9** occurred by nucleophilic attack on C-3′ resulting in the eventual formation of a 3′,5′-"down" dithiolane is, however, considered unlikely. Examination of molecular models indicates that such a *trans*-fused five-membered ring system would be highly strained. We conclude, therefore, that the structure of **8** shown in Scheme II is the most plausible.

A most probable mechanism for the formation of the internal disulfide **8** from the reaction of **5** → **3** is as follows. Attack by sulfide ion on 5′ of **5** would produce the 5′-deoxy-5′-mercaptothymidine **10**. Displacement of the 3′-mesylate by the 5′-thiol would then give the 3′,5′-episulfide derivative **9**. Attack by sulfide ion on C-5′ of **9** would produce a 3′,5′-dimercapto derivative which should be in a favorable position for the formation of the internal disulfide **8**.

An alternate and preferred synthetic route to **3** involved the use of 2-*O*-methyl-3′-*O*-mesylthymidine (**13**) (prepared from **5**)[3] as starting material. The advantage of this route is that the sulfide nucleophile attacks only at C-2 of the aglycon. Treatment of **13** with hydrogen sulfide in dimethylformamide–triethylamine for 20 hr at 70° gave directly pure **3** in ~50% yield. The mother liquors from this reaction did not contain any epidisulfide (**8**), but did show the presence of at least four minor components among which was 3′-*O*-mesylthymidine. (The formation of this minor component may arise from traces of moisture present in the reaction mixture.) The other minor components were not further investigated.

The (*S*)-2,3′-anhydro bridge of **3** was considerably more stable to alkali than the corresponding oxygen analog **2**, but less stable than the 2,3′-imino analog **1** which is completely inert to alkaline hydrolysis.[3] Alkaline cleavage of the sulfur bridge was accomplished by treatment of **3** with 1 *N* sodium hydroxide at 70° for 1 hr and afforded 1-(2,3-dideoxy-3-mercapto-β-D-*threo*-pentofuranosyl)thymine (**14**) in good yield. Nucleoside **14** exhibited a band at 3.9 μ (thiol) in the infrared spectrum.[13] When **14** was treated with acetone in the presence of methanesulfonic acid, the 3′,5′-oxathiolane derivative **15** was formed. Desulfurization of **14** with activated Raney nickel yielded the known[14] 2′,3′-dideoxy nucleoside **16**. Air oxidation of a basic solution of **14** gave the intermolecular disulfide **17**, which differed in properties from the known ribo (down) isomer.[13] The molecular weights of compounds **15** and **17** were confirmed by mass spectrum fragmentation analyses. The conversion of **14** into **15** and **17** is consistent with the "up" configuration for the 3′-thiol in **14**. Acid hydrolysis of **3** resulted in extensive glycosyl cleavage.

Ultraviolet Studies.—The ultraviolet absorption spectrum of (*S*)-2,3′-anhydro nucleoside **3** is similar to that of 2-ethylthio-1-methylpyrimidinone-4,[7] as expected, exhibiting a high extinction in the 237-mμ region without any evidence for ionization between pH 3 and 12. Above pH 12, slow cleavage of the (*S*)-2,3′-anhydro bridge begins. Below pH 3, protonation of the aglycon occurs which is almost completed[15] in 1 *N* hydrochloric acid giving a spectrally determined "apparent" pK_a of ~1.2. 2-Methylthio-3,6-dimethylpyrimidinone-4,[7] an analogous compound, also exhibits a basic pK_a (~0.9) in this pH region.

The spectra of compounds **8**, **15**, and **17** resemble, as expected, those for thymidine in the 1–12 pH region giving a spectrally determined apparent pK_a of 9.7 ± 0.1 (thymidine = 9.8).[16] In these three derivatives, the sulfur atom is bound in disulfide or oxathiolane groupings. An almost identical spectrum is also

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

(8) For example, the opening of the anhydro bridge of 2,3′-anhydro-1-(2-deoxy-β-D-*threo*-pentofuranosyl)thymine with sodium benzoate–dimethylformamide reagent was facilitated by the presence of free benzoic acid in the reaction mixture.[9]

(9) J. J. Fox and N. C. Miller, *J. Org. Chem.*, **28**, 936 (1963).

(10) Benzoylation of the aglycon of thymidine or uridine with benzoyl chloride in pyridine has already been documented.[11] The position of the benzoyl residue on the aglycon is not known. The pmr spectrum of **12** shows the absence of the N-3 proton.

(11) J. J. Fox, D. van Praag, I. Wempen, I. L. Doerr, L. Cheong, J. E. Knoll, M. L. Eidinoff, A. Bendich, and G. B. Brown, *J. Amer. Chem. Soc.*, **81**, 178 (1959).

(12) These data were kindly supplied by Dr. R. J. Cushley of Yale University School of Medicine using a Bruker HFX-3 spectrometer operating at 90 MHz.

(13) The fact that compound **14** was obtained as a free thiol was unexpected. Previous studies [N. Miller and J. J. Fox, *J. Org. Chem.*, **29**, 1772 (1964)] on the synthesis of the "down" (*ribo*) isomer of **14** showed that it could not be isolated as the free thiol but rather formed a disulfide *in situ*.

(14) K. E. Pfitzner and J. G. Moffatt, *J. Org. Chem.*, **29**, 1508 (1964); J. P. Horwitz, J. Chua, M. A. DaRooge, M. Noel, and I. L. Klundt, *ibid.*, **31**, 205 (1966).

(15) In 6 *N* hydrochloric acid a slow decomposition of **3** was observed spectrally.

(16) J. J. Fox and D. Shugar, *Biochim. Biophys. Acta*, **9**, 369 (1952).

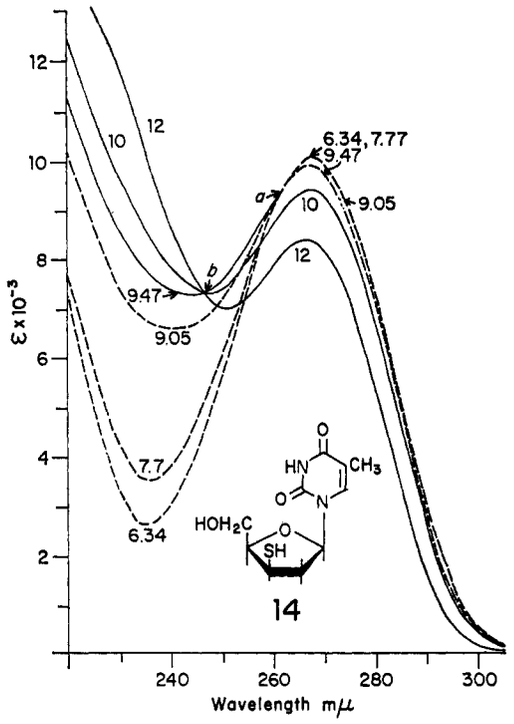


Figure 1.—Curves shown were run in aqueous solutions at pH values indicated. The italicized letters *a* and *b* refer to isosbestic points for the two dissociations (pK_{a1} = 8.4; pK_{a2} = 10.2) exhibited by compound **14**. Point *a* is delineated by curves for pH 0 and 6.34 to 9.47. Point *b* comprises curves for pH 9.47 to 12. Because of overlapping pK_a's, the lower pK_a value (8.4) was determined at 247 mμ where isosbestic *b* was taken as the end curve.

exhibited by the disulfide of the "2'-deoxyribo" isomer, 1-(2,3-dideoxy-3-mercapto-β-D-*erythro*-pentofuranosyl)thymine.[13] Indeed, this latter nucleoside as well as **8**, **15**, **17** and thymidine give ratios of maximum/minimum of 1.6–1.7 for the monoanionic species (pH 12).

The ultraviolet spectrum of the mercapto derivative **14** was unexpectedly different (Figure 1). Not only did it show a ratio of maximum/minimum of 1.2 for the pH 12 curve, but, more important, nucleoside **14** exhibited *two overlapping equilibria with two sets of isosbestic points* in the pH 0–12 region, with apparent pK_a values of 8.4 and 10.2. The lower pK_a value must represent ionization of the 3'-mercapto to the 3'-sulfide. Evidence for the assignment of this ionization to the 3'-mercapto group was obtained as follows. The 3',5'-oxathiolane derivative **15** was methylated with methyl iodide to **18**. The spectrum of **18** also resembled that for 1,3-dimethylated uracil or 3-methylthymidine,[17] and showed no spectral shifts between pH 7 and 12. When an aqueous solution of nucleoside **18** was treated with acid in the cuvette, the oxathiolane ring was hydrolyzed and a *single* spectral pattern associated with the lower pK_a was observed in the pH 6–12 region. These data assign the lower pK_a in **14** to the free thiol and, concomitantly, establish the position of methylation in **18** on N-3. (A 4-alkoxy derivative would be labile to acid.) The formation of the 3'-sulfide (pK = ~8.4) in **14** would serve to depress ionization of the N-3 hydrogen in the aglycon. Indeed, the pK_a of N-3 dissociation in **14** is ~10.2, whereas that in thymidine and in **8**, **15** and **17** is 9.7 ± 0.1.

It remains to be explained why the equilibrium involved in sulfide ion formation in the sugar moiety is visualized in the ultraviolet spectrum of **14**. Alteration of spectra due to interaction between the sugar hydroxyl groups and the aglycon of pyrimidine nucleosides has been observed previously[16] and these were interpreted[18] as a reflection of rupture of hydrogen bonding in the high alkaline region (pH 12–14) between the 2-carbonyl and the sugar moiety. Similar interactions have been noted recently[19] with certain 2-oxo-4-imidazoline-4-carboxylic acid nucleosides.

The spectral behavior of **14** is also consistent with the hypothesis that below pH ~6 the 3'-mercapto group is hydrogen bonded to the 2-carbonyl. An examination of molecular models shows that such bonding is eminently feasible. Such an –SH···O hydrogen bond should be weaker than the corresponding –OH···O type.[20] As the pH of the medium is increased above 6, rupture of this weak hydrogen bond occurs and is completed at pH 9–10. It is the rupture of this H bond between the aglycon and 3'-thiol of the sugar which leads to the spectral shifts associated with the lower pK_a of ~8.4. This explanation would also account in part for the relatively high resistance of the 2'-deoxyxylo-3'-mercapto nucleoside (**14**) to air oxidation to disulfide under neutral conditions when compared to the 2'-deoxyribo-3'-mercapto analog.[13] In the latter isomer, intramolecular hydrogen bonding of the type described above cannot exist.

Experimental Section[21]

(*S*)-2,3'-Anhydro-1-(2,3-dideoxy-β-D-*threo*-pentofuranosyl)-2-thiothymine (3). A. From Compound 13.—A solution of 2.6 g (7.8 mmol) of **5** in 60 ml of dry dimethylformamide containing 2 ml of triethylamine was treated with a stream of hydrogen sulfide for 10 min. The reaction mixture was sealed in a bomb and heated at 70–75° for 20 hr. The bomb was cooled, opened, the contents were filtered and the precipitate was washed with dry ether. Shining crystals [470 mg, 26%, mp 290–291° (eff)[22]] were obtained. The residual hydrogen sulfide was removed from the filtrate by a stream of nitrogen. A small amount of solid was filtered, 360 mg, (20%), mp 277–279° (eff). The filtrate was evaporated *in vacuo* to a syrup which was treated with ethanol and reconcentrated. The resulting semisolid was triturated with cold methanol and the solid filtered, 100 mg (6%), mp 222–232° (eff). The two impure crops were combined and recrystallized from hot water. Needles (380 mg, mp 294–295°[22]) were obtained. The average yield of pure **3** from this reaction is *ca.* 50%. The infrared spectrum showed the absence of the 3'-mesyloxy function at 1170 cm^{-1}: ultraviolet absorption properties, $\lambda_{max}^{pH\ 6.34}$ 237 mμ (ε 29,100) and shoulder at 270 mμ; $\lambda_{max}^{1N HCl}$ 232 and 278 mμ (12,500 and 8850), shoulder at 260 mμ; λ_{min}

(17) D. Shugar and J. J. Fox, *Biochim. Biophys. Acta*, **9**, 199 (1952); R. E. Beltz and D. W. Visser, *J. Amer. Chem. Soc.*, **77**, 736 (1955).

(18) J. J. Fox, L. F. Cavalieri, and N. Chang, *ibid.*, **75**, 4315 (1953); J. J. Fox, J. F. Codington, N. C. Yung, L. Kaplan, and J. D. Lampen, *ibid.*, **80**, 5155 (1958).

(19) B. A. Otter, E. A. Falco, and J. J. Fox, *J. Org. Chem.*, **33**, 3593 (1968).

(20) L. J. Bellamy in "Organic Sulfur Compounds," Vol. I, N. Kharasch, Ed., Pergamon Press, New York, N. Y., 1961, p 57.

(21) Microanalyses were performed by Galbraith Laboratories, Inc., Knoxville, Tenn., and by Spang Microanalytical Laboratory, Ann Arbor, Mich. Mass spectral analyses were performed by Morgan-Schaffer Corp., Montreal, Canada. Tlc was carried out on silica gel GF 254 using the following systems: a, benzene–methanol (5:1); b, chloroform–methanol (10:1); c, *n*-butyl alcohol–ethanol–water (40:11:19). Except where noted, pmr spectra were obtained using a Varian A-60 spectrometer. Ultraviolet absorption data were determined using a Cary Model 15 recording spectrophotometer.

(22) A melting characteristic of this compound is an efflorescence of each crystal at 198–200°.

242 mμ (6090); pK_a ~ 1.21 (spectrophotometrically determined).

Anal. Calcd for $C_{10}H_{12}N_2O_3S$: C, 49.98; H, 5.04; N, 11.66; S, 13.34. Found: C, 50.05; H, 4.99; N, 11.65; S, 13.49.

The mother liquor from the isolation of crude **3** contained at least four other components. Only the major one was isolated and identified as 3'-mesylthymidine by a mixture melting point with an authentic sample[9] and a comparison by tlc in system b.

B. From Compound 5.—A suspension of **5** (4.0 g, 13 mmol) in 100 ml of dry dimethylformamide containing 4 ml of triethylamine was cooled slightly and treated with a stream of hydrogen sulfide for 30 min. The reaction mixture was then sealed in a bomb and heated at 65° for 20 hr. The bomb was cooled, opened and the white precipitate filtered and washed with ether. This solid (**3**, 740 mg, 24%), recrystallized from boiling water, yielded large prisms, mp 288–290° (eff).[22] This product was identical with that obtained from A when compared by spectrophotometric and chromatographic methods. The dimethylformamide mother liquor (labeled **5** → **5** filtrate) from the above reaction was retained for the isolation of compound **8** (*vide infra*).

C. From Compound 5 with Isolation of 1-(2-Deoxy-3-*O*-mesyl-β-D-*erythro*-pentofuranosyl)-2-thiothymine (6).—A solution of 500 mg (1.65 mmol) of **5** in 80 ml of dry dimethylformamide containing 5 ml of triethylamine was treated with a stream of hydrogen sulfide at room temperature. After 2 hr the reaction flask was stoppered and allowed to stand overnight. The hydrogen sulfide was driven out of the reaction mixture with a stream of dry nitrogen and the pale yellow solution evaporated *in vacuo* to a syrup. Trituration of the syrup with ethanol yielded a precipitate which was filtered and proved to be recovered starting material, 280 mg (56%). The ethanolic filtrate was evaporated to a syrup which solidified on addition of water. The precipitate was filtered and air dried, 320 mg (solvated), dec pt 160°. This crude product (**6**) could not be recrystallized without further reaction to form some **3** (ultraviolet absorption data, $\lambda_{max}^{pH\ 1}$ 218 and 273 mμ, λ_{min} at 242 mμ; addition of one drop of 10 *N* sodium hydroxide to the neutral solution gave a strong absorption maximum at 240 mμ with a shoulder at 265–270 mμ).

Anal. Calcd for $C_{11}H_{16}N_2O_6S_2 \cdot 1H_2O$: C, 37.27; H, 5.12; N, 7.91; S, 18.09. Found: C, 37.18; H, 5.23; N, 8.10; S, 18.50.

The 2-thio intermediate (**6**) was treated with a threefold excess of ethanolic triethylamine (1 *M*) and was stirred at room temperature overnight. The precipitate was filtered and washed with ether. The product, recrystallized from hot water, yielded prisms, mp 286–288° (eff).[22] The properties of this compound agreed with those of **3** detailed in A.

Synthesis of Compound 8. A. From 3',5'-Di-*O*-mesylthymidine.—A solution of **11** (500 mg, 1.26 mmol) in 20 ml of dry dimethylformamide containing triethylamine (2.5 mmol) was treated with a stream of hydrogen sulfide for 10 min. The reaction mixture was then sealed in a glass-lined bomb and heated at 65–70° for 20 hr. The bomb was cooled and the contents were filtered. The filtrate was aerated with dry nitrogen to remove residual hydrogen sulfide and the solvent evaporated *in vacuo*. The resulting syrup was triturated with ethanol and the precipitate filtered. The yield of crude product **8** was 200 mg (59%), mp 228° dec with slow sinter from 220°. Recrystallization of the crude solid by solution in 1 *N* sodium hydroxide and reprecipitation with 2 *N* acetic acid yielded needles, mp 259–263° dec. This compound gave a negative nitroprusside test and the infrared spectrum showed the absence of both a mesyloxy function at 1170 cm^{-1} and a thiol band in the 2600 cm^{-1} region: ultraviolet absorption properties, $\lambda_{max}^{pH\ 0\text{-}6.34}$ 267 mμ (ε 9670); λ_{min} 234 mμ (2630); $\lambda_{max}^{pH\ 12}$ 267 mμ (7640), shoulder at 225 mμ; λ_{min} 244 mμ (4670); pK_a 9.62 ± 0.1 (spectrophotometrically determined).

Anal. Calcd for $C_{10}H_{12}N_2O_3S_2$: C, 44.10; H, 4.44; N, 10.29; S, 23.55; mol wt, 272. Found: C, 44.17; H, 4.43; N, 10.37; S, 23.50; mol wt, 272 (mass spectrum).

B. Isolation of Compound 8 from 5 → 3 Filtrate.—The dimethylformamide filtrate from the reaction of **5** to **3** was treated with a stream of dry nitrogen until the hydrogen sulfide was gone and solvent was evaporated under high vacuum. The residue was triturated thoroughly with cold ethanol, filtered and washed with ether. Examination of this solid (1.48 g) by tlc in system a showed some of compound **3** together with another major and several minor components. The mixed solids were leached with boiling water and the undissolved (*S*)-2,3'-anhydro compound **3** was removed. The aqueous solution was evaporated *in vacuo* to a syrup which was triturated with ethanol. The resultant solid recrystallized as in A gave crystalline **8** (900 mg, 25%, mp 260–262° dec) identical in chromatographic and spectrophotometric properties with that obtained in A.

Benzoylation of Compound 8 to 12.—A solution of **8** (250 mg, 9.2 mmol) in 20 ml of dry pyridine was treated with 3 ml of benzoyl chloride and heated at 65–70° for 20 hr. The solvent was removed *in vacuo* to half volume and poured into a well-stirred water–ice slurry. The oily semisolid was taken up in methylene dichloride, dried over sodium sulfate, and evaporated *in vacuo*. Residual pyridine was removed by repeated evaporations with water. The syrupy residue was triturated with ethanol to obtain a tan solid, 220 mg (64%), mp 186–190°. Recrystallization of the crude solid from hot ethanol gave **12**, mp 190–191°, which migrated as a single spot when examined by tlc (system a): pmr data[11] in pyridine-d_5, 8.23, 7.42 (5, m, phenyl); 7.88 (1, s, H_6); 6.30 (1, Q, $H_{1'}$); 4.97 (1, Q, $H_{4'}$); 4.26 (1, Q, $H_{3'}$); 3.55 (1, d, $H_{5'}$); 2.93 (2, m, $H_{2'}$, $H_{5''}$); 2.17 (1, m, $H_{2''}$); 1.93 (3, s, 5-CH_3). The following coupling constants were obtained by double resonance experiments: $J_{4',5'}$ = 0 Hz, $J_{4',5''}$ = 4.0; $J_{3',4'}$ = 6.6 Hz.

Anal. Calcd for $C_{17}H_{16}N_2O_4S_2$: C, 54.23; H, 4.28; N, 7.44; S, 17.04. Found: C, 54.36; H, 4.33; N, 7.39; S, 16.98.

(*S*)-2,3'-Anhydro-1-(5-*O*-benzoyl-2,3-dideoxy-β-D-*threo*-pentofuranosyl)-2-thiothymine (7).—Compound **3** (0.5 g, 1.95 mmol) in 25 ml of anhydrous pyridine was treated with 0.23 ml (1.95 mmol) of benzoyl chloride and the reaction mixture was heated overnight at 70°. The reaction mixture was filtered hot and the solid (recovered starting material 40%) was washed with ether to remove pyridine. The combined filtrate and washings were evaporated *in vacuo* to a white solid, 310 mg. The crude product was recrystallized from ethanol (*ca.* 25 ml) to yield 220 mg, mp 258–260°.

Anal. Calcd for $C_{17}H_{16}N_2O_4S$: C, 59.28; H, 4.68; N, 8.14; S, 9.31. Found: C, 59.13; H, 4.90; N, 8.07; S, 9.24.

1-(2,3-Dideoxy-3-mercapto-β-D-*threo*-pentofuranosyl)thymine (14).—A suspension of 1.1 g (4.6 mmol) of **3** in 20 ml of 1 *N* sodium hydroxide was heated in an oil bath at 70° until complete solution occurred (~1 hr).[23] The hot solution was filtered, the filtrate was diluted 1:1 with cold water, and glacial HOAc was added dropwise to pH 5. The precipitate was filtered, and washed successively with ice water, cold methanol and cold ether. The product (**14**, 900 mg, 76%, mp 195–197°) is analytically pure. Evaporation *in vacuo* of the mother liquor gave a second crop (140 mg, mp 184–189°) which was purified by recrystallization from hot ethanol containing one drop of 2-mercaptoethanol and cooling the solution under a nitrogen atmosphere. Product **14** appeared as a single component by tlc in solvent systems a and b; it gave a positive nitroprusside test for a free SH group whose presence was confirmed by an infrared absorption peak at 2600 cm^{-1}. The pmr spectra showed the presence of three exchangeable protons: ultraviolet absorption properties, $\lambda_{max}^{pH\ 0\text{-}6.34}$ 267 mμ (ε 9870); λ_{min} 235 mμ (2660); $\lambda_{max}^{pH\ 9.47}$ 267 mμ (10,040); λ_{min} 245 mμ (7275); $\lambda_{max}^{pH\ 12}$ 267 mμ (8365), slight shoulder at 225 mμ; λ_{min} 252 mμ (7010); pK_a values ~8.4 ± 0.1 and 10.21 ± 0.05 (spectrophotometrically determined).

Anal. Calcd for $C_{10}H_{14}N_2O_4S$: C, 46.50; H, 5.46; N, 10.85; S, 12.41. Found: C, 46.37; H, 5.45; N, 10.84; S, 12.31.

1-(2,3-Dideoxy-3,5(*S*,*O*)-isopropylidene-β-D-*threo*-pentofuranosyl)thymine (15).—Compound **14** (1.0 g, 3.9 mmol) in 125 ml of dry acetone containing 5 ml of dimethoxypropane was treated with 385 mg (4 mmol) of methanesulfonic acid and the reaction mixture was refluxed for 40 min. The dark red solution was neutralized with a 1 *M* solution of triethylamine in ethanol and the solvents were removed by evaporation *in vacuo* at a bath temperature of <40°. Addition of methanol to the residual syrup yielded a solid which was filtered and washed with cold methanol and then with ether. The yield of product was 710 mg (61%), mp 178–180°. This compound gave a negative nitroprusside test for a free thiol function: ultraviolet absorption data, $\lambda_{max}^{pH\ 6.34}$ 212 and 268 mμ (ε 9420 and 10,080, respectively); λ_{min} 236 mμ (2850); $\lambda_{max}^{pH\ 12}$ 267 mμ (7960); λ_{min} 243 mμ (4175); pK_a 9.76 ± 0.1 (spectrophotometrically determined).

Anal. Calcd for $C_{13}H_{18}N_2O_4S$: C, 52.33; H, 6.08; N, 9.39;

(23) Cleavage of **3** under milder conditions (0.1 *N* sodium hydroxide) occurred so slowly that competing reactions such as disulfide formation and glycosyl cleavage considerably reduced the yield of **14** and made isolation more difficult.

S, 10.75; mol wt, 298. Found: C, 52.15; H, 6.04; N, 9.22; S, 10.98; mol wt, 298 (mass spectrum).

1-(2,3-Dideoxy-3,5(*S,O*)-isopropylidene-β-D-*threo*-pentofuranosyl)-3-methylthymine (18).—A solution of **15** (300 mg, 1.0 mmol) in 22 ml of 0.1 *N* sodium hydroxide and 5 ml of methyl iodide was stirred overnight at room temperature. The reaction mixture was evaporated *in vacuo* and the residual syrup triturated with water. This solid was filtered, 160 mg (51%), mp 139–140°. In the pH region of 5.4–12 the ultraviolet absorption maximum was 267 mμ.

Anal. Calcd for $C_{14}H_{20}N_2O_4S$: C, 53.82; H, 6.45; N, 8.97; S, 10.26. Found: C, 53.75; H, 6.41; N, 8.92; S, 10.19.

3'-Deoxythymidine (16).—A suspension of **14** (111 mg, 4.3 mmol) in 10 ml of water was stirred and treated with 1 *N* sodium hydroxide dropwise until complete solution was obtained. Activated Raney nickel (*ca.* 2 ml of thick slurry) was added and the reaction mixture stirred at room temperature for 1 hr. (The optical density at 267 mμ showed a decrease of 43% during this period.) The nickel was filtered and washed well with water. The filtrate was neutralized with dilute acetic acid and the solvent evaporated *in vacuo* to dryness. The residue was reconcentrated three times with ethanol and the resulting white solid triturated several times with boiling acetone. The acetone extracts were combined and evaporated *in vacuo* to a solid. The product (**16**, 36 mg, 37%, mp 145° with sinter at 140°[14]) gave a negative nitroprusside test. The presence of a single product was demonstrated by tlc in system c.

When this reaction was repeated using a much smaller amount of the Raney nickel catalyst, no **16** was formed. The only product was the disulfide **17**, identified by tlc (system c).

Disulfide (17) of Compound 14.—A solution of 100 mg (0.4 mmol) of **14** in 20 ml of 1 *N* sodium hydroxide was aerated for 24 hr. The sodium carbonate was filtered and the filtrate acidified with 1 *N* hydrochloric acid and evaporated to dryness. The residue was recrystallized from hot water. The yield of **17** was 60 mg (59%), mp 252–255°. The infrared spectrum showed the absence of a thiol peak in the 2600-cm^{-1} region: ultraviolet absorption data, $\lambda_{max}^{pH\ 0\text{-}6.34}$ 267 mμ; λ_{min} 235 mμ; $\lambda_{max}^{pH\ 12}$ 212 and 267 mμ, inflection at 225 mμ; λ_{min} 244 mμ; pK_a 9.8 ± 0.05 (spectrophotometrically determined).

Registry No.—**3**, 18634-58-7; **6**, 18634-59-8; **7**, 18634-60-1; **8**, 18634-61-2; **14**, 18634-62-3; **15**, 18634-63-4; **16**, 18634-64-5; **17**, 18634-65-6; **18**, 18634-66-7.

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Purine Nucleosides. XXIII. Direct Amination of Purine Nucleosides[1]

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The direct introduction of an amino group into the purine moiety at positions N-1 and N-7 has been achieved in various purine nucleosides. This is the first reported direct amination of a purine ring nitrogen. Hydroxylamine-O-sulfonic acid converted inosine into 1-aminoinosine (**2**) and guanosine to 1-aminoguanosine (**4**). 6-Amino-9-β-D-ribofuranosyl-8-purinone (**7**) similarly gave 6,7-diamino-9-β-D-ribofuranosyl-8-purinone (**8**). A sequence of stepwise amination procedures with hydroxylamine-O-sulfonic acid provided direct introduction of the amino group at both positions N-1 and N-7 to give 1,2,7-triamino-9-β-D-ribofuranosyl-6,8-purinedione (**12**). These unique nucleoside derivatives provide interesting tools for future biochemical study. Since the N-amino group is capable of acting either as a hydrogen-bond acceptor or donor, these compounds at the nucleotide and polynucleotide level should present unusual possibilities for interaction with protein and nucleic acid.

Although early attempts to prepare certain N-aminopurines were unsuccessful,[2] Montgomery and coworkers prepared 9-N-aminohypoxanthine[3] and 9-amino-6-chloropurine[4,5] by appropriate ring-closure procedures of requisite hydrazinopyrimidine derivatives. Although the synthesis of 9-amino-6-chloro-8-hydroxypurine has been reported by ring closure of 5-amino-4-chloro-6-hydrazinopyrimidine with phosgene,[6] a recent publication[7] has shown this original structural assignment to be in error. The only reported N-amino derivatives of purines where the amino group is attached directly to a ring nitrogen atom are 9-N-aminopurines[3-7] or 9-N-substituted aminopurine derivatives.[5,8] In all instances the N-amino group was introduced into the purine moiety *via* ring-closure procedures of preformed pyrimidine intermediates or in one instance by ring closure of a 1-amino derivative of imidazole.[9]

Of considerable interest would be the synthesis of N-aminopurine nucleosides with an amino group attached at various potentially hydrogen-bonding sites on the heterocyclic base. The amino group is especially attractive for this purpose since this group is capable of acting either as a hydrogen bond acceptor or donor. The 9-N-aminopurines are unsuitable for this study since the position required for nucleoside formation is blocked. Since purine derivatives with an N-amino function on other nitrogen atoms are unknown this problem was viewed from the possibility of *direct* introduction of an amino group into the preformed purine ring of naturally occurring purine nucleosides.

Hoegerle and Erlenmeyer[10] treated 2-pyridone with

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