Synthesis and Biological Activity of Certain 8-Mercaptopurine and 6-Mercaptopyrimidine S-Nucleosides[1]

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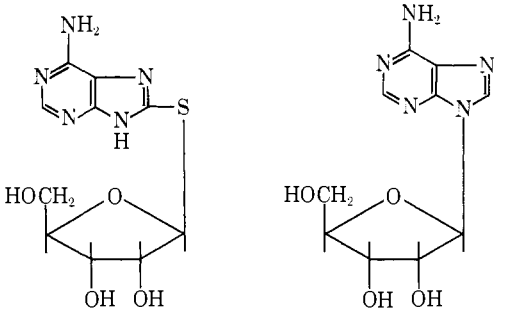
Received February 3, 1969

S-Nucleosides were prepared by the reaction of acylglycosyl halides and the anions of certain 8-mercaptopurines and 6-mercaptopyrimidines. Among these, the adenosine analog 6-amino-8-(β-D-ribofuranosyl)thiopurine showed biological activity in the three test systems examined. It inhibited the *in vitro* growth of *Escherichia coli* by 50% at 5×10^{-6} *M*, and that of leukemia L1210 and Ehrlich ascites at 1 and 3×10^{-4} *M*, respectively. Comparative studies carried out in the *E. coli* systems with the S-nucleoside and with 8-mercaptoadenine and 8-mercaptoadenosine indicate that, in these cells, the S-glycoside linkage of the S-nucleoside remains intact. Further studies employing a partially purified preparation of adenosine deaminase revealed that the compound is not subject to deamination. An inhibition analysis showed that the inhibition of the growth of *E. coli* by the adenine S-riboside is partially preventable by the natural pyrimidines and to a greater extent by aspartate, but *not* by purines.

Molecular models demonstrate the spatial similarity between 6-amino-8-(β-D-ribofuranosyl)thiopurine (**1**) and adenosine (**2**) as well as between 6-(β-D-ribofuranosyl)thiouracil (**3**) and uridine (**4**). It was therefore desirable to prepare these unknown ribofuranosyl S-nucleoside analogs (**1** and **3**) and to compare their biological activity with the activity of the corresponding 8-mercaptopurines or 6-mercaptopyrimidines. Such a comparison was of particular interest since the existence of a mammalian thioglycosidase capable of cleaving certain 6-mercaptopurine thioglycosides has been



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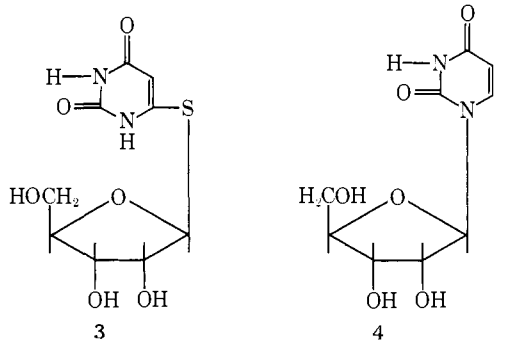

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demonstrated.[3] Several S-glucopyranoside analogs and their acetylated derivatives have been prepared also and are included in the comparison.

A number of thioglucopyranosides of various mercapto-substituted aromatic heterocycles have been reported recently[4] including those of 2-mercaptopyrimidine,[5] 4-mercaptopyrimidine,[6] and 6-mercaptopurine.[3,7] However no thioglycosides of the natural bases have so far been prepared and no D-ribofuranose S-glycosides have been described.

Three general methods have in the past been used to prepare thioglycosides of mercapto derivatives of N heterocycles:[4] (A) reaction of the anion of a thio-substituted base with a glycosyl halide, (B) reaction of a 1-thioglucose derivative with a halogen-substituted heterocycle, (C) reaction of a heavy metal salt of a mercapto-substituted base with a glycosyl halide. Several modifications of method A were used in this study as reported in the Experimental Section. Method B was eliminated from consideration, since nucleophilic replacement of the 8-halogen of purines[8] or the 6-halogen of pyrimidines[9] under anionic conditions requires severe conditions. Method C was investigated but the attempted use of Ag salts of 8-mercaptoadenine and 6-mercaptouracil gave little or no S-nucleoside as noted recently in the case of 6-mercaptopurine.[7]

A pronounced hypsochromic shift was observed between the uv spectra of the thiopurine or thiopyrimidine and their respective S-glycosides (see Table I). This shift is parallel to that found in the S-methyl derivatives *vs.* the mercapto heterocycle (see Table I) and serves as a convenient means of monitoring the S-glycosidation reaction. It is of interest that the attachment of an electronegatively inductive sugar produces, in most cases, a greater hypsochromic shift than does the methyl group.

(1) This investigation was supported by Public Health Service Research Grant No. CA-08109 from the National Cancer Institute and by Grant T-436 from the American Cancer Society. Reported before the Medicinal Chemistry Division, 155th National Meeting of the American Chemical Society, San Francisco, Calif., April 1968, No. N15.

(2) (a) University of Utah. (b) Roswell Park Memorial Institute.

(3) I. Goodman, J. R. Fouts, E. Bresnick, R. Menegas, and G. H. Hitchings, *Science*, **130**, 450 (1959).

(4) See, for example, G. Wagner and D. Heller, *Arch. Pharm.*, **299**, 481 (1966); G. Wagner and H. Frenzel, *ibid.*, **300**, 421, 433 (1967); G. Wagner and D. Heller, *ibid.*, **299**, 768 (1966); **300**, 783 (1967); G. Wagner and R. Schmidt, *ibid.*, **298**, 481 (1965); H. Zinner and K. Peseke, *Chem. Ber.*, **98**, 3515 (1965); G. Wagner and H. Pischel, *Arch. Pharm.*, **296**, 576 (1963); P. Nuhn and G. Wagner, *ibid.*, **301**, 186 (1968).

(5) G. Wagner and F. Suss, *Z. Chem.*, **6**, 340 (1966); *Chem. Abstr.*, **66**, 55673 (1967).

(6) G. Wagner and F. Suss, *Arch. Pharm.*, **300**, 1027 (1967).

(7) I. Goodman, L. Salce, and G. H. Hitchings, *J. Med. Chem.*, **11**, 516 (1968).

(8) R. K. Robins in "Heterocyclic Compounds," Vol. 8, R. C. Elderfield, Ed., John Wiley and Sons, Inc., New York, N. Y., 1967, pp 284–288.

(9) D. J. Brown, "The Pyrimidines," John Wiley and Sons, Inc., New York, N. Y., 1962, pp 13, 205.

TABLE I

Compound	$\lambda_{max}^{pH\ 1}$, mμ (ε × 10⁻³)	$\lambda_{max}^{pH\ 10-11}$, mμ (ε × 10⁻³)
Adenine 8-SH[17]	310, 242 (24.7, 12.0)	301, 229 (23.4, 18.4)
Adenine 8-SCH_3[17]	290 (20.6)	286, 228 (21.0, 21.0)
Adenine 8-*S*-glucose tetraacetate (**7a**)	277 (18.9)	281, 228 (19.4, 18.9)
Adenine 8-*S*-glucose (**10a**)	278 (20.2)	281, 226 (20.1, 18.1)
Adenine 8-*S*-ribose tribenzoate (**9**)[a]	275, 232 (18.7, 41.8)	282, 231 (18.7, 39.4)
Adenine 8-*S*-ribose (**1**)	281 (21.5)	282, 225 sh (21.3, 20.0)
Hypoxanthine 8-SH[17]	290, 234 (27.2, 8.7)	290, 234 (18.8, 17.6)
Hypoxanthine 8-SCH_3[17]	275 (16.6)	280, 225 (16.7, 18.0)
Hypoxanthine 8-*S*-glucose tetraacetate (**7b**)	267 (16.2)	275, 225 sh (17.8, 12.0)
Hypoxanthine 8-*S*-glucose (**10b**)	269 (18.0)	274 (20.2)
Uracil 6-SH[19]	306 (19.6)	302 (25.2)
Uracil 6-SCH_3[b]	277 (15.8)	284 (14.2)
Uracil 6-*S*-glucose tetraacetate (**12b**)	275 (10.7)	288, 229 (11.6, 11.4)
Uracil 6-*S*-glucose (**13**)	276 (11.0)	287 (11.8)
Uracil 6-*S*-ribose tribenzoate (**14**)[a]	274, 231 (13.0, 26.0)	284, 232 (9.15, 35.4)
Uracil 6-*S*-ribose (**3**)[a]	275 (11.6)	287, 226 sh (12.4, 12.8)
Cytosine 6-SH[19]	317, 242 (40.5, 7.6)	303 (27.5)
Cytosine 6-SCH_3[19]	295 (13.3)	285 (11.9)
Cytosine 6-*S*-glucose tetraacetate (**12a**)[a]	290 (15.2)	287 (9.5)

[a] These samples were dissolved in ethanol and diluted 1:10 by volume with appropriate aqueous pH solution. [b] M. W. Winkley and R. K. Robins, *J. Chem. Soc., C*, 791 (1969).

TABLE II

EFFECT OF *S*-NUCLEOSIDES ON THE GROWTH OF VARIOUS *in Vitro* TEST SYSTEMS[a]

Compd	Molar concn for 50% growth inhib.		
	E. coli	Leukemia L1210	Ehrlich ascites
8-(β-D-Ribofuranosyl)thioadenine (**1**)	5×10^{-6}	1×10^{-4}	3×10^{-4}
8-Thioadenosine	2×10^{-5}	$>10^{-3}$	$>10^{-3}$
8-Thioadenine	2×10^{-5}	$>10^{-3}$	$>10^{-3}$
6-(β-D-Ribofuranosyl)thiouracil (**3**)	$>10^{-3}$	1×10^{-3}	2×10^{-3}
6-Thiouracil	$>10^{-3}$	$>10^{-3}$	$>10^{-3}$

[a] *E. coli* was grown in the synthetic medium of Gray and Tatum.[20] The tumor cells were grown in a synthetic medium (RPMI 1630) containing 5% calf serum.

Because of the method of synthesis,[7,10] the β configuration was anticipated for all the *S*-nucleosides prepared in this work. The nmr peak corresponding to the anomeric proton of the *S*-glucopyranosides was split into a doublet with $J_{1',2'} = 8$–10 Hz. This corresponds to a *trans* diaxial[11] arrangement of H_1–$H_{2'}$ and confirms the β assignment for these compounds. The couplings of the anomeric protons of the *S*-ribofuranosides were of intermediate value and the tentative assignment of their configuration as β rests on the analogous method of preparation and their negative optical rotations.

The acylated *S*-glycosides were all characterized chemically and evaluated in biological test systems. The corresponding free *S*-nucleosides were obtained by alcoholic NH_3-catalyzed deacylation except for the cytosine *S*-glucoside. Treatment of 6-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)thiocytosine (**12a**) (Scheme I) with methanolic NH_3 at room temperature led to extensive decomposition and liberation of 6-thiocytosine. Some cleavage of 6-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)thiouracil (**14**) was also observed in alcoholic NH_3. Compound **14** was heat sensitive and was not obtained crystalline. The 6-thiopyrimidine *S*-nucleosides also exhibited some light sensitivity especially when impure. With these exceptions, the *S*-nucleosides prepared in this study were stable to recrystallization, deblocking, etc., and all the purified samples remain pure and colorless after several months at room temperature.

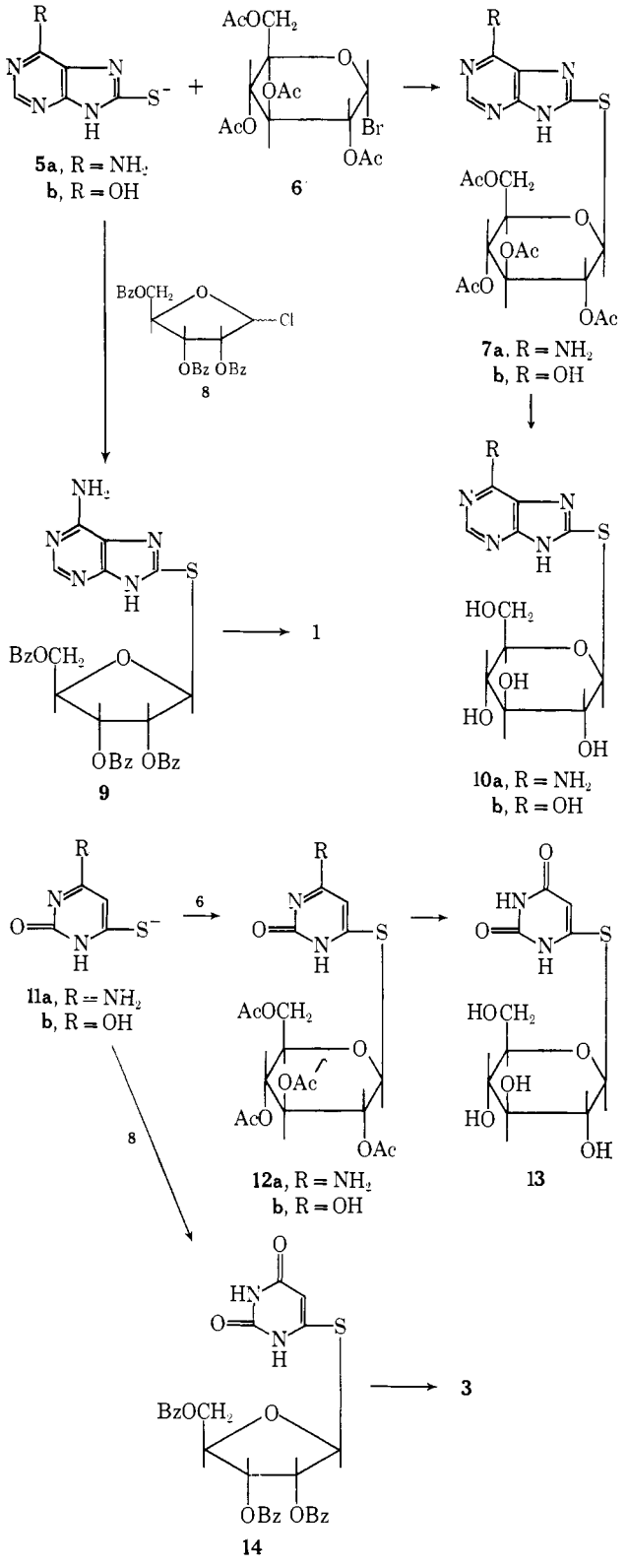
Biological and Biochemical Effects.—In view of the spatial similarity between the natural nucleosides adenosine and uridine and their respective thioglycosides **1** and **3**, it was of interest to examine their biological activity. This activity, as measured by inhibition of cell growth, is summarized in Table II. While the adenosine analog was a quite effective inhibitor of the growth of *Escherichia coli*, the uridine derivative was essentially inactive. In the tumor systems too, the adenine *S*-ribose (A-S-R) was moderately active, whereas the uracil *S*-riboside was inhibitory only at the relatively high concentrations of $1\text{–}2 \times 10^{-3}$ *M*. In all these test systems none of the other compounds, whose synthesis is also reported in this paper, showed any significant inhibitory effect.

Because of the marked sensitivity of *E. coli* to inhibition by A-S-R and to learn whether the activity of this compound is exerted following its cleavage to the base analog, two related compounds, 8-thioadenine (A-SH) and 8-thioadenosine (AR-SH) were examined for biological activity. Both of these analogs inhibited 50% of the growth of *E. coli* at a concentration of

(10) C. D. Purves, *J. Am. Chem. Soc.*, **51**, 3619 (1929).

(11) For discussion of the configuration and conformation of pyranosides see R. U. Lemieux, R. K. Kullnig, H. J. Berstein, and W. G. Schneider, *ibid.*, **80**, 6098 (1958); R. U. Lemieux and J. W. Lown, *Can. J. Chem.*, **41**, 889 (1963); R. U. Lemieux and A. R. Morgan, *ibid.*, **43**, 2205 (1965).

SCHEME I



2×10^{-5} M, whereas they were quite inactive against the tumors. This difference in activity served as one indication that, in the cell, the S-glycoside linkage remains intact. This indication was confirmed by the observation that combinations of A-S-R with either A-SH or AR-SH produced the same extent of synergistic inhibition, whereas the effect of A-SH in combination with AR-SH was additive (Figure 1). This implies, of course, that the adenine S-riboside affects at least

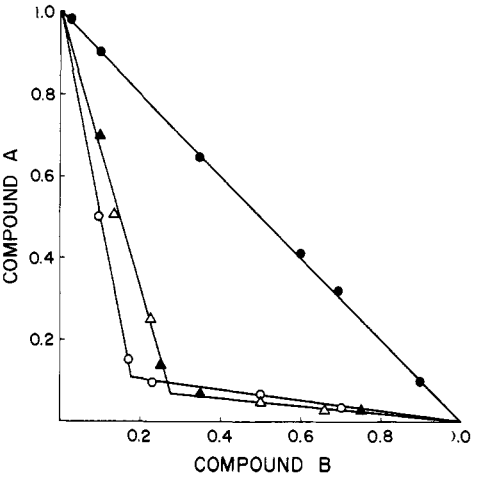


Figure 1.—Effects of combinations of adenine-8-S-ribose (A-S-R), 8-thioadenine (A-SH), 8-thioadenosine (AR-SH), and adenosine (AR) on the growth of *E. coli* in terms of fractional inhibitory concentrations [concentration of inhibitor present in the combination divided by concentration of inhibitor required to give the same degree of inhibition by itself: G. B. Elion, S. Singer, and G. H. Hitchings, *J. Biol. Chem.*, **208**, 477 (1954)] at 50% growth inhibition: ●, A-SH + AR-SH; ▲, AR-SH + A-S-R; △, A-SH + A-S-R; ○, AR + A-S-R.

one metabolic site different from the ones acted upon by the 8-mercapto analogs, both of which appear to affect the same site.

To gain some information concerning the metabolic site possibly affected by A-S-R, an inhibition analysis was performed. The results summarized in Table III

TABLE III

EFFECT OF PURINES, PYRIMIDINES, AND ASPARTATE ON THE INHIBITION OF THE GROWTH OF *E. coli* BY 8-(β-D-RIBOFURANOSYL)THIOADENINE[a]

Metabolite (10^{-3} M) added to assay medium	Molar concn of 8-(β-D-ribofuranoysl)-thioadenine for 50% growth inhib
None	5.0×10^{-6}
2′-Deoxycytidine	6.5×10^{-5}
2′-Deoxyuridine	5.7×10^{-5}
Cytidine	4.8×10^{-5}
Uridine	3.2×10^{-5}
Thymidine	1.0×10^{-5}
Cytosine	6.5×10^{-6}
Uracil	6.0×10^{-6}
Thymine	5.5×10^{-6}
Guanosine	5.2×10^{-6}
Xanthosine	5.2×10^{-6}
Adenosine (at 1×10^{-7} M)	1.0×10^{-6}
Inosine (at 1×10^{-7} M)	1.8×10^{-6}
Aspartic acid	$>10^{-3}$

[a] The assays were carried out in the synthetic medium of Gray and Tatum.[20] The figures given are averages of two experiments, calculated by linear interpolation between experimentally determined values.

show that the inhibition exerted by this compound can be reversed by various pyrimidines but *not* by purines. Indeed, adenosine and inosine enhance the A-S-R inhibition about three- to fivefold when present at 1×10^{-7} M, a concentration at which they, themselves, are without effect. The inhibition of the growth of *E. coli* by A-S-R is, however, reversed most extensively by aspartic acid, an amino acid involved in the bio-

synthesis of the pyrimidines. This observation indicates that the effective reversal of A-S-R inhibition by the pyrimidine nucleosides is not likely ascribable to their interference with the cellular uptake of the analog. Furthermore, it shows that adenosine, which inhibits the growth of *E. coli* by 50% at 7×10^{-7} *M*, acts, at least in part, in a manner different from A-S-R, since its inhibitory effect is reversed by the pyrimidines, but not by aspartate. A difference in site of action is also demonstrated by the finding that combinations of A-S-R and adenosine inhibit the growth of *E. coli* synergistically (Figure 1). On the other hand, the reversal pattern for A-S-R parallels that for N^6-(Δ^2-isopentenyl)adenosine (IPAR), whose interference with the growth of *E. coli* is partially prevented by pyrimidines and by aspartic acid.[12]

In view of the indication that in the strain of *E. coli* used the *S*-glycoside linkage of A-S-R remains intact, the question arises whether the compound acts at the nucleoside stage, or is phosphorylated to the nucleotide. Such information would be of particular interest since A-S-R is not subject to deamination by adenosine deaminase.

Considering the useful chemotherapeutic effect which IPAR exerts upon certain tumors,[13,14] and the similarity between the reversal patterns of IPAR and A-S-R, the further *in vivo* evaluation of the *S*-nucleoside appears promising.

Experimental Section[15]

8-(2,3,5-Tri-*O*-benzoyl-β-D-ribofuranosyl)thioadenine (9).—To a solution of 9.62 g (0.02 mol) of 2,3,5-tri-*O*-benzoylribofuranosyl chloride (**8**)[16] in 50 ml of DMF (dried over 4-A molecular sieves) was added 3.82 g (0.02 mol) of 8-mercaptoadenine[17] sodium salt with stirring under N_2. The resulting solution was allowed to stand for 1.5 hr at room temperature under N_2 and then an additional 0.96 g (0.005 mol) of sodium 8-mercaptoadeninate was added. The solution was stirred for 0.5 hr and then evaporated to a dark residue which was partitioned between $CHCl_3$ and H_2O. The organic phase was washed with saturated aqueous $NaHCO_3$ and H_2O, dried (Na_2SO_4), filtered, and evaporated to give a semisolid residue. This product was dissolved in a minimum volume of $CHCl_3$ and applied to a silica gel column (500 g, packed in Et_2O and washed with $CHCl_3$). The column was washed with 10 l. of $CHCl_3$ and the yellow material eluted with this wash was discarded. The product was eluted with EtOAc to yield 2.71 g (20%) of white solid after solvent evaporation. A small sample was recrystallized twice from Me_2CO to give crystals of **9**, mp 237.5–238.5° dec, $[\alpha]^{26}D$ −5.9° (*c* 1, DMF). *Anal.* ($C_{31}H_{25}N_5O_7S$) C, H, N.

8-(β-D-Ribofuranosyl)thioadenine (1).—Compound **9** (0.36 g, 0.00058 mol) was dissolved in 100 ml of MeOH presaturated with NH_3 at −8°. This solution was sealed and allowed to stand 4 days at room temperature, then evaporated to dryness, and 100 ml of H_2O was added. The mixture was extracted with four 100-ml portions of $CHCl_3$ and the aqueous phase was evaporated to dryness. This residue was crystallized from EtOH–H_2O to give 0.16 g (91%) of white needles. One recrystallization from the same solvent pair gave crystals of **1**, mp 196–198° dec, $[\alpha]^{25}D$ −13.8° (*c* 1, DMF). *Anal.* ($C_{10}H_{13}N_5O_4S$) C, H, N.

8-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)thioadenine (7a).—To a solution of 10.4 g (0.025 mol) of acetobromoglucose (**6**)[18] in 100 ml of absolute EtOH was added 5 g of $CaCO_3$ and the suspension was stirred for 5 min. A suspension of 3.4 g (0.018 mol) of 8-mercaptoadenine[17] sodium salt in 200 ml of absolute EtOH was added and the resulting mixture was stirred for 24 hr at room temperature with exclusion of moisture. The pH (pHydrion paper) was adjusted to pH 8 periodically with methanolic NaOMe during this 24-hr period and the mixture was allowed to stand an additional 48 hr. The suspension was evaporated to dryness and the residue was stirred with 700 ml of $CHCl_3$ for 2 hr and filtered, and the filtrate was evaporated to dryness. This product was dissolved in 600 ml of hot MeOH and cooled for 2 days at 0°. The crystalline product (4.94 g, 55%) was filtered, mp 266–268° dec, and a small sample was recrystallized from MeOH for analysis, mp 271–273° dec, $[\alpha]^{25}D$ −33.4° (*c* 1, DMF), nmr (DMSO-d_6) δ 5.8 (d, 1, *J* = 10 Hz, $H_{1'}$). *Anal.* ($C_{18}H_{23}N_5O_8S$) C, H, N.

8-(β-D-Glucopyranosyl)thioadenine (10a).—To 150 ml of MeOH presaturated with NH_3 at −10° was added 1.1 g (0.0022 mol) of **7a** with stirring. The resulting solution was allowed to stand 24 hr at room temperature and then was evaporated to dryness. The resulting white solid was dissolved in hot MeOH–H_2O and cooled to give white crystals of the monohydrate of **10a**. This product was recrystallized twice from the same solvent pair to give 0.66 g (86%) of **10a**·H_2O which was dried *in vacuo* at 60° over P_2O_5 for 2 days to obtain anhydrous crystals, mp 206–208° dec, $[\alpha]^{24}D$ −76° (*c* 1, DMF), nmr (DMSO-d_6, D_2O) δ 5.3 (d, 1, *J* = 9 Hz, $H_{1'}$). *Anal.* ($C_{11}H_{15}N_5O_5S$) C, H, N, S.

8-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)thiohypoxanthine (7b).—To a suspension of 1.73 g (0.0103 mol) of 8-mercaptohypoxanthine[17] in 50 ml of H_2O was added enough 1 *N* NaOH to effect solution and then 40 ml of Me_2CO was added. The pH was adjusted to 9 (Beckman pH meter, glass electrode) with 1 *N* NaOH and 5.6 g (0.014 mol) of acetobromoglucose (**6**)[18] in 40 ml of Me_2CO was added dropwise with stirring over a 20-min interval with periodic addition of 1 *N* NaOH to maintain pH 9. The pH was held at 9 for an additional 40 min and the solution was stirred an additional 12 hr, then evaporated to a syrup which was treated with 500 ml of $CHCl_3$ and this mixture was extracted with three 100-ml portions of H_2O. The organic phase was dried (Na_2SO_4), filtered, and evaporated to dryness. The residue was dissolved in 30 ml of hot EtOH and this solution deposited crystals upon cooling at 0°. This product was recrystallized three times from EtOH to yield 0.45 g (9%) of crystals, mp 253–254°, $[\alpha]^{25}D$ −19.5° (*c* 1, DMF), nmr (DMSO-d_6) δ 5.7 (d, 1, *J* = 10 Hz, $H_{1'}$). *Anal.* ($C_{24}H_{32}N_4O_{10}S$) C, H, N.

8-(β-D-Glucopyranosyl)thiohypoxanthine (10b).—To 60 ml of MeOH presaturated with NH_3 at −10° was added 0.23 g (0.00046 mol) of **7b**. The resulting solution was sealed and allowed to stand 3 days at room temperature. It was evaporated to dryness, dissolved in 50 ml of H_2O, and extracted with four 50-ml portions of $CHCl_3$. The aqueous phase was filtered and the filtrate was evaporated to dryness. The resulting solid was crystallized once from EtOH–H_2O to yield 0.08 g (53%) of crystals, mp 140–155° dec, when applied to a rapidly heating melting point apparatus at 130°, $[\alpha]^{25}D$ −62.3° (*c* 1, H_2O), nmr (DMSO-d_6, D_2O) δ 5.2 (d, 1, *J* = 9 Hz, $H_{1'}$). *Anal.* ($C_{11}H_{14}N_4O_6S$) C, H, N.

6-(2,3,5-Tri-*O*-benzoyl-β-D-ribofuranosyl)thiouracil (14).—To a solution of 4.8 g (0.01 mol) of 2,3,5-tri-*O*-benzoyl-D-ribofuranosyl chloride[18] in 30 ml of DMF (freshly distilled and dried over 4-A molecular sieves) was added 1.74 g (0.0105 mol) of 6-mercaptouracil[19] sodium salt and the suspension was stirred for 4 hr at room temperature. The mixture was evaporated to dryness at 40° and 30 ml of EtOH was added and again evaporated to dryness. The residue was partitioned between $CHCl_3$ and H_2O and the combined organic phase was dried (Na_2SO_4). This was filtered and the filtrate was evaporated to give 6.4 g of yellow

(12) A. Bloch and C. A. Nichol, *Proc. Am. Assoc. Cancer Res.*, **9**, 6 (1968).
(13) J. T. Grace, M. T. Hakala, R. H. Hall, and J. Blakeslee, *ibid.*, **8**, 23 (1967).
(14) R. Jones, Jr., J. T. Grace, Jr., A. Mittelman, and M. W. Woodruff, *ibid.*, **9**, 35 (1968).
(15) Melting points were taken on a Thomas–Hoover capillary melting point apparatus and are uncorrected. Nmr spectra were determined with a Varian A-60 instrument, uv spectra were obtained on a Beckman DK-2 spectrophotometer, and optical rotations were determined on a Perkin-Elmer Model 141 automatic digital readout polarimeter. All evaporations were accomplished under reduced pressure using a Buchler rotating evaporator. Silica gel (J. T. Baker No. 3405) was used for column chromatography. Thiopurine or thiopyrimidine sodium salts were prepared by suspension of the base in H_2O followed by addition of 1 equiv of NaOH. The resulting solution was lyophilized and the product salt was dried at 75° (1 mm) over P_2O_5. Where analyses are indicated only by symbols of the elements, analytical results obtained for these elements were within ±0.4% of the theoretical values.
(16) R. H. Hall, *J. Am. Chem. Soc.*, **80**, 1145 (1958).
(17) R. K. Robins, *ibid.*, **80**, 6671 (1958).
(18) C. E. Redemann and C. Niemann in "Organic Syntheses," Coll. Vol. III, E. C. Horning, Ed., John Wiley and Sons, Inc., London, 1955, pp 11–14.
(19) H. C. Koppel, R. H. Springer, R. K. Robins, and C. C. Cheng, *J. Org. Chem.*, **26**, 792 (1961).

syrup which was dissolved in a minimum volume of $CHCl_3$ and applied to a column (250 g) of silica gel packed in Et_2O. The column was developed with Et_2O and all fractions having appreciable absorption at 280 mμ and common tlc chromatography were pooled and evaporated to yield a white solid foam (1.88 g, 32%). A small sample of this material for analysis was passed through another column as described above since attempted recrystallization caused decomposition as shown by tlc. The amorphous solid, mp 75–78°, was dried *in vacuo* (P_2O_5) for 2 days at room temperature, $[\alpha]^{24}D$ −19° (*c* 1, DMF). *Anal.* ($C_{30}H_{24}N_2O_9S$) C, H, N.

6-(β-D-Ribofuranosyl)thiouracil (3).—To 150 ml of MeOH presaturated with NH_3 at 0° was added 1.4 g (0.0024 mol) of **14**. The resulting solution was sealed and allowed to stand 3 days at room temperature and then was evaporated to dryness. The residue was treated with 50 ml of H_2O and this was extracted with four 80-ml portions of $CHCl_3$. The aqueous phase was treated with Norit, filtered through a Celite pad, and evaporated to dryness. The residue was dissolved in a minimum volume of EtOAc–*n*-PrOH–H_2O (4:1:2, upper phase) and applied to a silica gel (50 g, packed in Et_2O and then washed with the above solvent) column. The column was eluted with the same solvent and fractions with appreciable absorption at 275 mμ were pooled and evaporated to give 0.135 g (20%) of white solid. This product was crystallized from EtOH and allowed to air dry for 4 days at room temperature while protected from light, mp 119–120°, $[\alpha]^{26}D$ −164° (*c* 1, DMF). *Anal.* ($C_9H_{12}N_2O_6S \cdot 1.25H_2O$) C, H, N, S.

6-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)thiouracil (12b).—To a solution of 0.72 g (0.005 mol) of 6-mercaptouracil[19] and 0.2 g (0.005 mol) of NaOH in 15 ml of H_2O was added 45 ml of Me_2CO and 3.09 g (0.0075 mol) of acetobromoglucose (**6**).[18] The resulting solution was stirred 20 hr at room temperature and then evaporated. The resulting syrup was treated with 200 ml of $CHCl_3$ and this was washed with two 50-ml portions of H_2O (NaCl added to separate emulsion). The organic phase was dried (Na_2SO_4), filtered, and evaporated to a solid foam. This material was dissolved in a minimum volume of $CHCl_3$ and applied to a silica gel (50 g) column packed in Et_2O. Elution with Et_2O and evaporation of pooled fractions gave 0.51 g (22%) of white solid, mp 200–203°. Crystallization of this material from EtOH gave needles, mp 205–207°, $[\alpha]^{26}D$ −3.4° (*c* 1, DMF), nmr (DMSO-d_6) δ 4.9 (d, 1, J = 9.5 Hz, $H_{1'}$). *Anal.* ($C_{18}H_{22}N_2O_{11}S$) C, H, N.

6-(β-D-Glucopyranosyl)thiouracil (13).—To 100 ml of MeOH presaturated with NH_3 at −8° was added 0.08 g (0.00017 mol) of **12b** and the resulting solution was sealed and allowed to stand 24 hr at room temperature. It was evaporated, 100 ml of H_2O was added to the residue, and this solution was extracted with four 200-ml portions of $CHCl_3$. The aqueous layer was evaporated and the residue was crystallized from EtOH to yield 0.043 g (84%) of product. Recrystallization of this material from MeOH–H_2O gave needles, mp 159–161°, $[\alpha]^{24}D$ −98° (*c* 1, H_2O), nmr (DMSO-d_6, D_2O) δ 4.9 (d, 1, J = 9.0 Hz, $H_{1'}$). *Anal.* ($C_{10}H_{14}N_2O_7S$) C, H, N.

6-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)thiocytosine (12a).—To a solution of 0.96 g (0.0067 mol) of 6-mercaptocytosine[19] and 0.83 g (0.006 mol) of K_2CO_3 in 15 ml of H_2O was added 30 ml of Me_2CO followed by 3.06 g (0.0075 mol) of acetobromoglucose (**6**).[18] The resulting solution was allowed to stand 5 days at room temperature and then was filtered and the filtrate was evaporated to dryness. The syrupy residue was treated with 50 ml of H_2O and then extracted with three 100-ml portions of $CHCl_3$. The combined organic phase was dried (Na_2SO_4), filtered, and evaporated to dryness. This yellow solid was dissolved in a minimum volume of $CHCl_3$ and applied to a silica gel column (75 g, packed in Et_2O and then washed with $CHCl_3$). The column was washed with 1 l. of $CHCl_3$ and 1.5 l. of Et_2O and these washes were discarded. Elution with EtOAc followed by evaporation of the pooled fraction with appreciable absorption at 290 mμ gave 0.82 g (26%) of off-white solid. This product was recrystallized twice from EtOAc–hexane to give crystals, mp 148° dec, when applied to a rapidly heating melting point apparatus preheated to 140°; $[\alpha]^{25}D$ −10° (*c* 1, DMF), nmr (DMSO-d_6) δ 4.9 (d, 1, J = 9 Hz, $H_{1'}$). *Anal.* ($C_{18}H_{23}N_3O_{10}S$) H; C: calcd, 45.66; found, 45.16; N: calcd, 8.87; found, 8.32.

Assay of Antimicrobial Potency and Inhibition Analysis.—The synthetic medium of Gray and Tatum[20] was used for all growth assays involving *E. coli*. The assay technique is essentially that described previously.[21] The assays were carried out by placing 1-ml aliquots of the double-strength medium into 13 × 100 mm culture tubes, and sterilizing them for 6 min at 121°. The thioglycosides, dissolved in water, were sterilized by filtration through a Millipore (0.45 μ) filter, and 1-ml portions of the sterile solution were added to the tubes containing the autoclaved medium. When two compounds were assayed in combination, 0.5 ml of each was added to the sterile medium. The inhibition analyses were performed by adding the metabolites listed in Table III, at concentrations ranging from 10^{-3} to 10^{-7} *M*, to the growth medium containing the drug.

The inocula were prepared from cultures of the test organisms grown in 5 ml of the medium for 20 hr at 37°. Following centrifugation and washing twice with isotonic saline, the cells were resuspended in enough saline to yield an optical density of 0.30 at 470 mμ as measured in a Beckman Model B spectrophotometer. A 1-ml portion of this suspension containing approximately 1.5×10^7 cells was diluted tenfold in saline, and 1 drop of this final dilution was placed in each assay tube. Incubation proceeded for 20 hr at 37°. All assays were carried out by shaking the cultures during incubation. The extent of growth was determined by means of a Klett–Summerson photoelectric colorimeter using a red filter (640–700 mμ).

Assay of Antitumor Activity *in Vitro*.—The assay of the antitumor effect of the *S*-nucleosides was carried out in essentially the same manner as described for the bacteria. The synthetic medium (RMPI 1630) containing 5% calf serum was filter sterilized, as were the analog solutions. The inoculum was prepared by centrifuging a portion of a stock spinner culture and diluting the pellet with enough medium to give a viable cell count of approximately 5×10^5 L1210 cells and 3×10^5 Ehrlich ascites cells/ml of final assay medium. The cultures were shaken gently at 37° for 2 days after which an additional 2 ml of medium containing the analog was added to each tube. After further incubation for 2 days, the number of viable cells was determined with trypan blue.

Susceptibility of A-S-R to Deamination.—Adenosine deaminase, type I, obtained from calf intestinal mucosa (Sigma Chemical Co., Lot 95 B-9022) was used to determine whether A-S-R is subject to deamination. The enzyme was added to 1 ml of 0.05 *M* phosphate buffer, pH 7.5, containing 1×10^{-4} *M* A-S-R. Incubation proceeded for 20 min at 25° and the reaction was monitored at 282 and 270 mμ with a Gilford Model 2000 recording spectrophotometer.[22] The amount of enzyme used was 10, 100, and 2000 times that required to give approximately 50% deamination of 1×10^{-4} *M* adenosine/min.

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