Purine Thioglycosides. I. *S*-Glycosides of 6-Mercaptopurine[1]

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The preparation of a series of *S*-glycosides of 6-mercaptopurine is described. Two routes of synthesis were employed, the reaction of a 6-halogenopurine with a thioglycose derivative, and the reaction of 6-mercaptopurine with a halogenoacylated sugar, followed by deacylation. Thioglycoside configurations were assigned by analogy with known alkyl and aryl thioglycosides. The purine thioglycosides are readily oxidized to sulfones. 6-Purinyl β-D-glucothiopyranoside is hydrolyzed in acid solution, and in neutral solution by a previously unrecognized thioglycosidase which is widely distributed in plant and animal tissues. Several other purinyl thioglycosides also are substrates for this enzyme.

The present studies, involving the synthesis and biochemical properties of purine thioglycosides, were undertaken as part of a program concerned with the presentation of 6-mercaptopurine (6-MP) in modified or masked forms. The purine thioglycosides described in this paper have reduced toxicity as compared with the aglycone; moreover, the aglycone can be liberated either chemically or enzymatically. The preparation of 6-purinyl β-D-glucothiopyranoside (MPG) (Scheme I, V) resulted in the detection of a previously unrecognized thioglycosidase, widely distributed in plant and animal tissues including tumors, which catalyzed the hydrolysis of several members of the series.[2] Members of this series also serve as substrates for myrosin, the thioglycosidase of the mustard plant, and for almond emulsion.[2]

The *S*-glucuronide of 6-MP also was of interest in view of the wide distribution of uronic acid derivatives in nature and the reported high glucuronidase activity of certain tumors.[3] This compound, however, was not a substrate for bacterial β-glucuronidase although it was a moderately good substrate for the mammalian thioglycosidase.

In view of the ease with which MPG can liberate 6-MP it is difficult to determine the extent to which it, *per se*, possesses antitumor activity. When the drug is administered orally, splitting is extensive due to the high thioglycosidase activity of intestinal secretions, and its activity and toxicity resemble those of free 6-MP. This difficulty may not be overcome unequivocally by giving the drug by the intraperitoneal route, since it could reach the intestinal tract *via* biliary secretion or by the ingestion of excreted drug (it is rapidly cleared by the kidney[4]). However, MPG has significant antitumor activity and low toxicity when given parenterally.[5-7]

Starting with the work of Fischer[8] who prepared the first synthetic thioglycosides, a variety of routes have been developed for the synthesis of thioglycosides. Of these, the following general reactions, where R represents the 6-purinyl radical and G the glycofuranosyl or pyranosyl group, represent the methods (A,[8] B,[8c] C,[9] and D[10]) investigated in the present report for the synthesis of purine thioglycosides (Scheme II).

Two of the earlier methods (A and B, Scheme II) used for the synthesis of alkyl and aryl thioglycosides were found unsuitable for the purine series. In attempting route A, formation of the required mercaptal by acid catalysis did not occur, presumably because of the low solubility of 6-MP in concentrated HCl and the highly polar character of the purinyl sulfhydryl group. Only hypoxanthine and unreacted 6-MP were isolated.

Due to the poor solubility and low reactivity of the silver mercaptide of 6-MP, route B resulted in very low yields of purine thioglycosides.

We had used the general routes C and D previously[11] for the synthesis of aliphatic and aromatic thioethers of 6-MP and thioguanine. In C the anion of 6-MP is treated with an appropriate alkyl or aralkyl halide in alkaline solution resulting in *S*-alkylation. In D the high reactivity of the 6-purinyl halogen[12] makes possible a nucleophilic attack by the anion of a thiosugar on the 6-purinyl carbon atom. Route D was the method of choice for the synthesis of purine thioglycosides, and was generally employed unless the appropriate thiosugars could not be prepared. Reaction rates and yields were high. The products were relatively free from by-products or from 6-MP and could be readily purified by recrystallization.

A vexing problem in the preparation of purine thioglycosides, especially by route C, was the persistent presence of small residues of free 6-MP. Various techniques were necessary to eliminate the trace contaminants: ion-exchange resins, paper and column chromatography, precipitation of traces of 6-MP as heavy metal mercaptides, and countercurrent extraction.

(1) (a) The authors conducted the major part of this research at The Wellcome Research Laboratories as a part of a program in Cancer Chemotherapy. (b) The authors wish to acknowledge their indebtedness to Samuel Blackman for analyses, to Edward Bresnick for help with enzyme kinetics, and to Gertrude B. Elion for advice, suggestions, and help in many ways. (c) The senior author is the recipient of Career Scientist Award (I-260) of the New York Health Research Council and of a grant from the National Science Foundation (GB-3210).

(2) (a) I. Goodman, J. B. Fouts, E. Bresnick, R. S. Menegas, and G. H. Hitchings, *Science*, **130**, 450 (1959); (b) I. Goodman, G. B. Elion, and G. H. Hitchings, *Fed. Proc.*, **14**, 219 (1955); (c) I. Goodman, *ibid.*, **18**, 236 (1959).

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(8) (a) E. Fischer, *Ber.*, **27**, 673 (1894); (b) E. Fischer and K. Delbruck, *ibid.*, **42**, 1476 (1909); (c) E. Fischer and B. Helferich, *ibid.*, **47**, 210 (1914).

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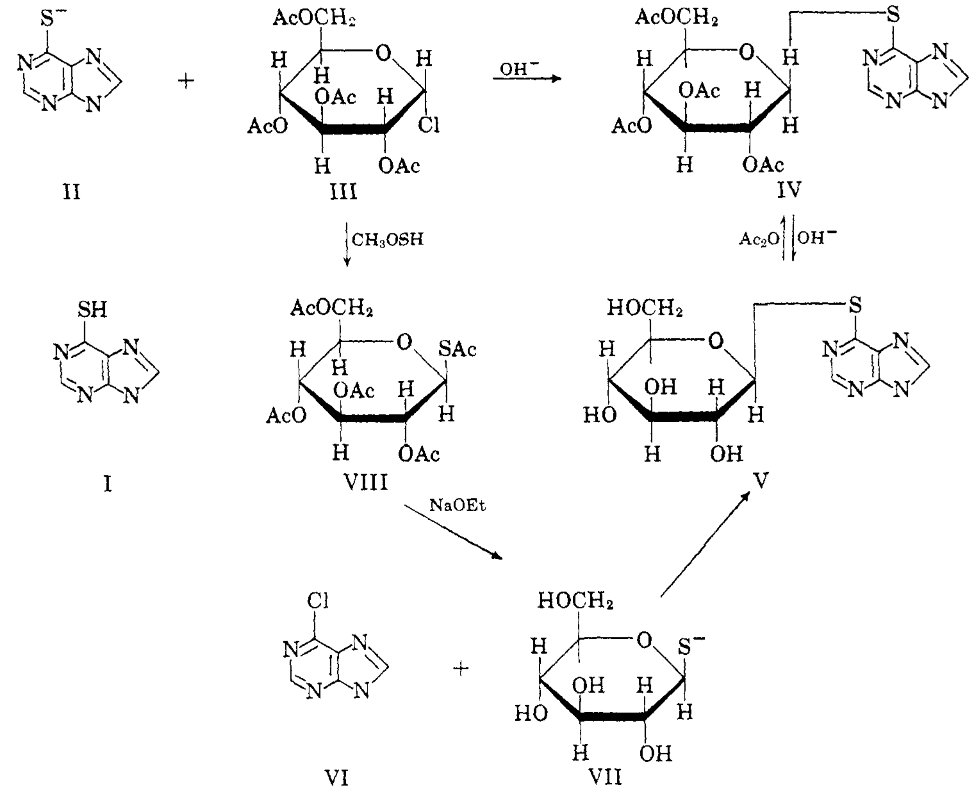
SCHEME I



SCHEME II
GENERAL METHODS FOR THE SYNTHESIS OF THIOGLYCOSIDES

A $R_1SH + R_2CO \xrightarrow[\text{HCl}]{\text{H concd}} \underset{\text{mercaptal}}{R_2CH(SR_1)_2} \xrightarrow[\text{HgCl}_2]{\text{H}^+} \underset{[\text{O}]}{R_2CSR_1}$

B $RSH + Ag^+ \xrightarrow{\text{NH}_4\text{OH}} RSAg \xrightarrow[\text{halide}]{\text{acetoglycosyl}} RSG$

C $RSH + \text{acetoglycosyl halide} \xrightarrow{\text{OH}^-} RSG$

D $RCl + NaSG \longrightarrow RSG$

Ion-exchange resins were helpful in some cases, but these were frequently found to catalyze thioglycoside hydrolysis. Purifications by cellulose column chromatography or by continuous countercurrent extraction procedures were used successfully. But the most useful procedure for removal of traces of 6-MP with a minimum of loss of thioglycoside was the mercaptide precipitation method. 6-MP forms insoluble salts with Ag^+, Cu^{2+}, Zn^{2+}, Pb^{2+}, and other cations. Treatment of aqueous or ethanolic solutions of purine thioglycosides with PbO_2 or HgO effectively removed 6-MP residues. Because of its solubility properties, however, lead diacetate was found the most suitable reagent for removing traces of 6-MP by mercaptide formation.

For the preparation of thiosugars the method of Schneider, *et al.*,[13] involving the condensation of acetoglycosyl halides with ethyl xanthogenate was satisfactory. However, the thioacetyl method[14] was most versatile and resulted in the highest yields of desired intermediate sugars.

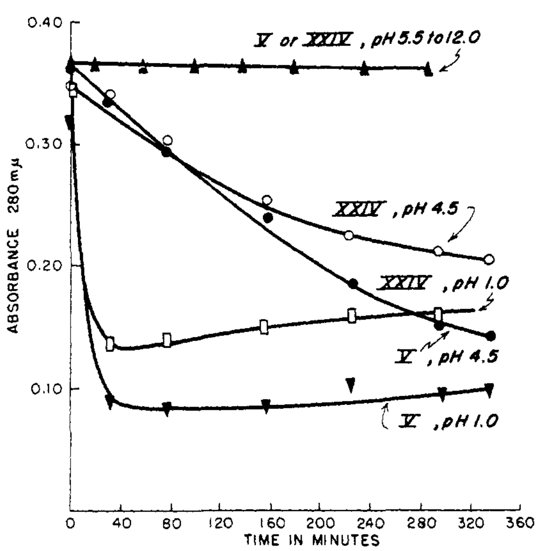


Figure 1.—Acid-catalyzed hydrolysis of 6-purinyl β-D-glucothiopyranoside (V) and 6-purinyl β-D-glucothiopyranuronide amide (XXIV) at 25°; concentration, 2.30×10^{-2} μmole/ml.

The thioglycosides prepared in the present series were optically active, and configuration was assigned by analogy with known alkyl and aryl thioglycosides.[15]

In contrast to the alkyl and arylthioglycosides[8b,15–17] the 6-purinyl thioglycosides undergo facile acid-catalyzed or enzyme-catalyzed hydrolysis (Figures 1 and 2). The purine thioglycosides are readily oxidized to form sulfones and may be converted to stable acyl derivatives (Scheme III).

(13) W. Schneider, R. Gille, and K. Eisfeld, *Ber.*, **61**, 1244 (1928).
(14) (a) M. Gehrke and W. Kohler, *ibid.*, **64**, 2696 (1931); (b) M. Černy, J. Vrkoč, and J. Staněk, *Chem. Listy*, **52**, 311 (1958).
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(16) I. Goodman, J. R. Fouts, and G. H. Hitchings, *Fed. Proc.*, **17**, 232 (1958).
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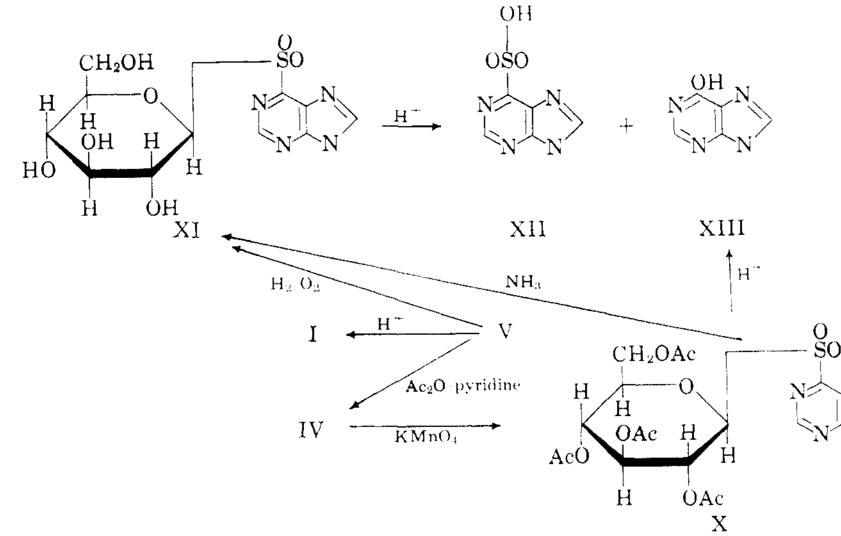
SCHEME III



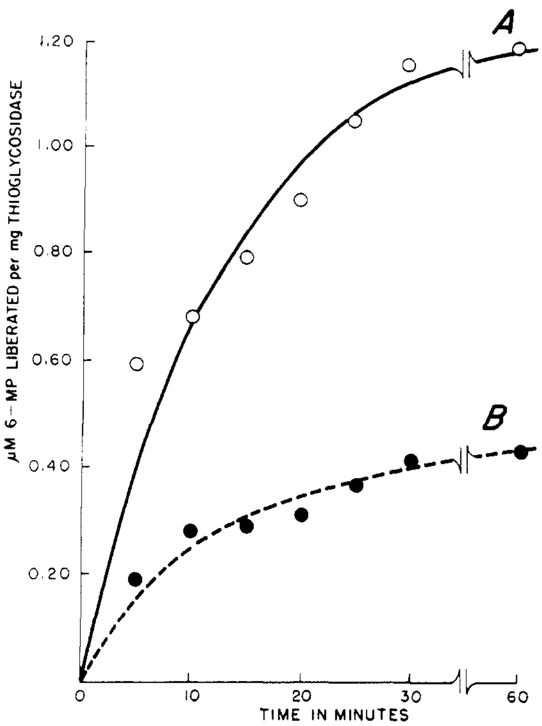


Figure 2.—Hydrolysis of MPG by hog liver thioglycosidase. Hog liver thioglycosidase was purified by $(NH_4)_2SO_4$ fractionation of Me_2CO powder. The preparation used was a dialyzed solution of the 20–55% (of saturation) fraction. Reaction flasks containing 10 mg of enzyme protein in 1 ml, 40 mg of MPG (6 μmole/ml) in curve A or 20 mg of MPG (3 μmole/ml) in curve B, and 20 ml of acetate buffer (0.1 mole), pH 5.8, were incubated at 38°. Parallel flasks containing boiled enzyme solution were carried as controls. Aliquots (2.0 ml) were withdrawn at 5-min intervals, diluted to 10 ml with absolute EtOH, centrifuged, and read on the spectrophotometer at 325 mμ.

Experimental Section

General.—Melting points were determined on the Kofler micro melting point apparatus and are reported uncorrected. Uv absorption spectra were determined on the Beckman D.U., the Beckman recording, and the Cary Model 11 spectrophotometers. Radioactivity was measured by the infinitely thin plating technique using an internal gas flow counter. Counting was continued for times sufficient to give a probable error no greater than 10%. No coincidence corrections were required. Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements were within ±0.4% of the theoretical values.

Purine Thioglycosides.[18] **6-Purinyl β-D-Glucothiopyranoside (V) (Method C) (Schemes I and II).**—6-MP monohydrate I[19] (75 g, 0.44 mole) was dissolved in 600 ml of concentrated NH_4OH (28%) at 25°. To this solution was added a solution containing 180 g (0.49 mole) of 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl chloride (III)[20] in 500 ml of absolute EtOH. A 15° rise in temperature of the reaction mixture was noted within 15 min. The mixture was allowed to stand for 17 hr at 25° and filtered, and the precipitate was washed with 95% EtOH. Simultaneous glycosylation and deacylation took place yielding 87 g of impure product. A second precipitate (25 g) formed in the filtrate within 10 min after filtration. This material, a white, crystalline, odorless solid, was pure V·hydrate. It has mp 158–160° and $[\alpha]^{22}D$ −36.8° (*c* 1, pyridine); the uv absorption spectra are shown in Table I.

TABLE I

pH	λ_{max}, mμ	λ_{min}, mμ	$\epsilon_{max} \times 10^3$
1	285	240	13.1
7	280	240	16.0
11	288	250	14.0

V forms stable hydrates with from 1 to 6 moles of H_2O. It is hygroscopic in the anhydrous form. The crystalline solid is readily soluble in H_2O, DMF, glacial AcOH, MeOH, and pyridine. It is very slightly soluble in dioxane, lutidine, and dimethylaniline. It may be recrystallized from absolute MeOH, *i*-PrOH, or from 95% EtOH, but undergoes partial hydrolysis upon recrystallization from H_2O; yield of hydrate, after recrystallization from MeOH, 50 g (33%).

Anal. Calcd for $C_{11}H_{14}N_4O_5S \cdot 1.5H_2O$: C, 38.7; H, 5.02; N, 16.4. Found: C, 39.0; H, 4.85; N, 16.6.

Method D-1.—1-Acetylthio-2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranose (VIII)[14] (1 g, 0.0025 mole) was dissolved in 20 ml of absolute MeOH. To this solution was added a solution of 0.38 g (0.0025 mole) of 6-chloropurine (VI)[21] in 25 ml of absolute MeOH. The mixture was filtered and left at 25° for 16 hr. The solid residue was removed by filtration and the filtrate was concentrated to a syrup *in vacuo*. The syrup was treated with 25 ml of absolute EtOH. The EtOH-insoluble precipitate was separated by filtration. The product, 6-purinyl β-D-glucothio-

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(21) A. Bendich, P. J. Russell, and J. J. Fox, *J. Am. Chem. Soc.*, **76**, 6073 (1954).

pyranoside, was identical with an authentic sample of V previously prepared by method C.

Method D-2.—Sodium thioglucose dihydrate (VII)[22] (5 g, 0.02 mole) was dissolved in 40 ml of H_2O[23] at room temperature. To this solution was added a solution of 3 g (0.02 mole) of 6-chloropurine (VI) in 20 ml of absolute EtOH. The clear solution was stirred for 2 hr at 25°. The precipitate which formed was removed by filtration and washed with 5 cc of cold H_2O, yielding 5.4 g of dry product. A second crop weighing 1.3 g (total yield, 92%) was obtained from the filtrate after standing at 0° for 3 days. The uv absorption characteristics were identical with those of an authentic sample of V prepared by method C (from 6-MP). *Anal.* ($C_{11}H_{14}N_4O_5S \cdot 3H_2O$) N.

The Synthesis of 6-Purinyl β-D-Glucothiopyranoside Labeled with ^{35}S (V) (Scheme I).—For metabolic studies MPG was synthesized with ^{35}S at the glycosyl-purine bridge. Reactions involved in this synthesis are essentially those described in method D-2 and are summarized in Scheme I.

1-[^{35}S]Acetylthio-2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranose (VIII).—^{35}S-Thioacetic acid (IX) (Volk Chemical Co.) (570 mg, specific activity 2.1 mCi/mmole) was distilled *in vacuo* into a solution made from 0.8 g (0.035 g-atom) of Na and 67 ml of absolute EtOH. To this solution was added 2 ml of unlabeled thioacetic acid making a total of 0.034 mole. To the ethanolic solution was added 12.0 g (0.0327 mole) of 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl chloride (III). The mixture was heated under reflux for 1 hr with magnetic stirring. After cooling to 0° the NaCl was removed by filtration. H_2O (150 ml) was added to the filtrate which was then kept at 4° for 20 hr. The product (VIII), a white crystalline solid, was washed (H_2O) and dried; mp 120°, lit.[14b] mp 121°, yield 9 g (67%) (8.3×10^4 cpm/μmole).

Sodium 1-β-D-Glucopyranosyl[^{35}S]thiol (VII).—VIII (9 g, 0.022 mole) was dissolved in 50 ml of absolute EtOH. To this, after cooling to 0°, was added a cold solution (0°) made from 0.77 g (0.034 g-atom) of Na and 70 ml of absolute EtOH. A copious precipitate formed at once. After standing 2 hr at 25°, the solid sodium thioglucose (VII) was collected by filtration and washed with absolute EtOH; yield 4.0 g (83%) (2.96×10^5 cpm/μmole).

[^{35}S]6-Purinyl β-D-Glucothiopyranoside (V).—Sodium [^{35}S]-thioglucose (IX) (4.0 g, 0.183 mole) was treated with 3.45 g (0.0195 mole) of 6-chloropurine (VI) using the method described for V (D-2); yield 2.6 g (43%). Uv absorption curves and paper chromatography demonstrated purity above 99%; activity, 2.1×10^5 cpm/μmole.

6-Purinyl 2',3',4',6'-Tetra-*O*-acetyl-β-D-glucothiopyranoside (IV).—6-Purinyl β-D-glucothiopyranoside monohydrate (V) (10 g, 0.030 mole) was dissolved in 100 ml of pyridine and 35 ml (0.317 mole) of Ac_2O was added. The temperature of the mixture reached 55° within 30 min, then returned to room temperature. After 2 hr at 25°, the reaction mixture was poured into H_2O (300 ml) with stirring. A copious white precipitate was formed; yield 11.0 g (22%), λ_{max}^{EtOH} 286 mμ (ε 14.7×10^3), mp 102–104°. *Anal.* ($C_{19}H_{22}N_4O_9S \cdot H_2O$) C, H, N.

6-Purinyl 2',3',4',6'-Tetra-*O*-acetyl-β-D-glucothiopyranoside Sulfone (X) (Scheme III).—IV (2 g, 0.004 mole) was dissolved in 50 ml of glacial AcOH. To this solution was added 40 ml (0.01 mole) of 4% aqueous $KMnO_4$. After stirring 3 hr at 25° the brown solution was decolorized by adding 25 ml 0.5 *M* Na_2SO_3. The solution was concentrated to 10 ml and chilled. The precipitate formed was recrystallized from MeOH; yield 1 g (48%) of white crystals, λ_{max}^{EtOH} 284 mμ (ε 15.0×10^3), mp 173–175°.

Anal. Calcd for $C_{19}H_{22}N_4O_{11}S$: C, 44.5; H, 4.31; S, 6.25. Found: C, 44.9; H, 4.14; S, 6.10.

When X was heated at 100° with 0.1 *N* HCl for 10 min, hypoxanthine was formed. V treated in the same manner liberates chiefly 6-MP as the aglycone.

X (100 mg) was dissolved in 15 ml of absolute EtOH saturated with anhydrous NH_3. After 16 hr at 25°, the ammoniacal solution was concentrated *in vacuo* to 5 ml. A white crystalline product was obtained; uv, λ_{max} 256 and 290 mμ in H_2O. These peaks shifted showing rapid decomposition to hypoxanthine. Hydrolysis to hypoxanthine is accelerated when X was dissolved in 0.1 *N* NaOH (Scheme III).

6-Purinyl β-D-glucothiopyranoside (V) may be oxidized readily to the sulfone XI and to purine 6-sulfonic acid (XII) (Scheme III). MPG (5 g) was dissolved in a solution containing 2 ml of concentrated NH_4OH in H_2O (75 ml). To this was added 50 ml of 30% H_2O_2. After 20 hr at 25° the solution was concentrated *in vacuo* to 30 ml. Crystallization occurred after standing at 0° for 1 hr; yield 1 g of colorless needles (18%). The uv absorption spectrum was nearly identical with that of V but its melting point was 170–172°, R_f 0.66 (*i*-PrOH–$(NH_4)_2SO_4$–H_2O, 5:5:90), $\lambda_{max}^{pH\ 7.0}$ 280 mμ (ε 15.9×10^3).

Anal. Calcd for $C_{11}H_{14}N_4O_7S \cdot H_2O$: N, 15.4. Found: N, 15.5.

An identical product was obtained using benzoyl peroxide as the oxidizing agent. XI is unstable in H_2O, dilute acid, and alkali and is cleaved to hypoxanthine and purine-6-sulfonic acid.

As by-products of the oxidation of V, the hydrolysis products XII and hypoxanthine (XIII) (Scheme III) were formed.

Purine-6-sulfonic acid,[24] isolated in the benzoyl peroxide or peracetic acid oxidation of MPG, was converted to a cyclohexylamine salt: mp 230–231°; λ_{max} pH 1 265 mμ (ε 8.13×10^3), pH 11 275 mμ (ε 7.33×10^3).

Anal. Calcd for $C_{11}H_{17}N_5O_3S$: C, 44.2; H, 5.69; N, 23.4. Found: C, 44.4; H, 5.13; N, 23.3.

6-Purinyl α-L-Arabinothiopyranoside (XIV).—6-MP monhydrate (7.75 g, 0.046 mole) was dissolved in 150 ml of liquid NH_3. To this solution was added 15 g (0.051 mole) of 1-chloro-2,3,4-tri-*O*-acetyl-β-L-arabinopyranosyl chloride[25] while stirring. The NH_3 was allowed to distil at 25° (about 1 hr). The viscous residue was dissolved in 150 ml of absolute MeOH and the solution was filtered. The filtrate was concentrated to a syrup *in vacuo*, and NH_4Cl was removed by sublimation at 50° (0.1 mm). The viscous residue was dissolved in 25 ml of absolute EtOH and left at 0° for 16 hr. A copious deposit of crystals formed but was found to be a mixture of thioglycoside with 6-MP. Removal of the 6-MP was effected by treating an aqueous solution of the mixture with Rohm and Haas weak-base ion-exchange resin XE-162; $\lambda_{max}^{pH\ 7.0}$ 280 mμ (ε 14.9×10^3), mp 113–115°. XIV was also prepared by reaction of 6-MP with 2,3,4-tri-*O*-actyl-β-L-arabinopyranosyl chloride as described for V (method C). *Anal.* ($C_{10}H_{12}N_4O_4S \cdot H_2O$) C, H, N.

6-Purinyl β-D-Galactothiopyranoside (XV).—6-MP monohydrate (5 g, 0.029 mole) was treated with 12 g (0.033 mole) of 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl chloride[26] as described for V (method C); recrystallized from MeOH; yield 2.8 g (29%); λ_{max} pH 1 280 mμ, pH 7.0 280 mμ (ε 15.5×10^3), pH 11 285 mμ; mp 145–150° dec. *Anal.* ($C_{11}H_{14}N_4O_5S \cdot H_2O$) N.

Method D.—Sodium thiogalactose (XXVI) (2.50 g, 0.0114 mole) was dissolved in 20 ml of H_2O. The aqueous solution was added to a solution of 1.77 g (0.0114 mole) of 6-chloropurine in 50 ml of absolute EtOH. After 48 hr at 25° the color had changed from light yellow to amber. Upon adding Me_2CO (5 ml) a white precipitate was formed. The solid was removed by filtration and the filtrate was concentrated to dryness *in vacuo*. The solid residue was extracted with hot MeOH (three 5-ml portions). To the MeOH solution was added Me_2CO (15 ml). After 3 hr at 0° the white crystalline product was collected and recrystallized (MeOH); yield 1.7 g (45%). Uv spectra and chromatography demonstrated that the product, identical with XV above, was free of 6-MP and 6-chloropurine.

6-Purinyl β-D-Mannothiopyranoside (XVI).—6-MP monohydrate (1 g, 0.0059 mole) was treated with 1.8 g (0.0049 mole) of 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl chloride[27] as described for V (methods C or D); $\lambda_{max}^{H_2O}$ 280 mμ (ε 15.0×10^3), yield 0.6 g (30%), mp 282–285°. *Anal.* ($C_{11}H_{14}N_4O_5S \cdot H_2O$) N.

6-Purinyl α-L-Rhamnothiopyranoside (XVII).—6-MP monohydrate (5 g, 0.029 mole) was treated with 9 g (0.029 mole) of 2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl chloride[28] as described for V (method C); yield 2.1 g (22%); λ_{max} 282 mμ in H_2O, 280 mμ at pH 11; $[\alpha]^{25}D$ −172° (*c* 1, pyridine); mp 165–170° dec. *Anal.* ($C_{11}H_{14}N_4O_4S \cdot H_2O$) N.

(22) Sodium thioglucose was prepared by the method of M. Gehrke, *Ber.*, **61**, 1244 (1928), and was also purchased from the Schering Corp.

(23) In the condensation of 6-chloropurine with sodium thioglucose, formamide was found to be an excellent solvent. It has the advantage of good solubility for both reactants and avoids the hydrolytic cleavage associated with aqueous solutions.

(24) I. L. Doerr, I. Wempen, D. A. Clarke, and J. J. Fox, *J. Org. Chem.*, **26**, 3401 (1961).

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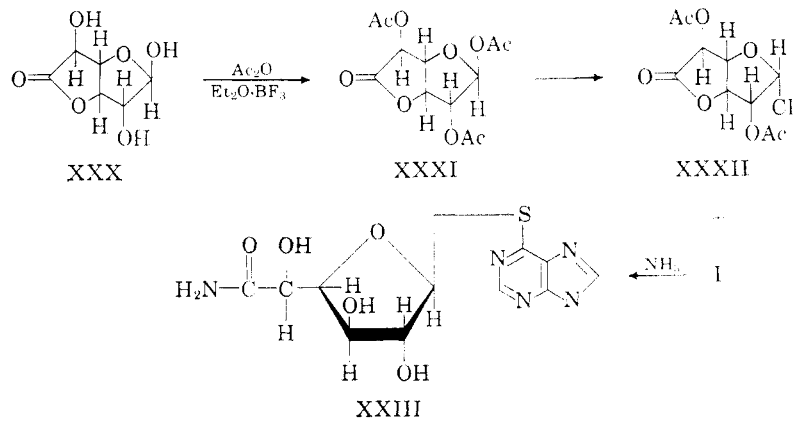
SCHEME IV



Sodium 6-Mercaptopurine (XVIII).—6-MP monohydrate (55 g, 0.323 mole) was suspended in H_2O (100 ml). To this suspension was added a solution of 20 g (0.5 mole) of NaOH in H_2O (100 ml). After filtration the solution was concentrated to 75 ml *in vacuo* and chilled to 0°. The solution became nearly solid with crystals in 1 hr. To recrystallize, the solid was dissolved in 600 ml of 95% EtOH by heating to the boiling point. A gummy insoluble residue was removed by filtration and the filtrate was chilled to 5° for 16 hr. The product (II, Na^+) crystallized as white needles and was washed (95% EtOH). It does not melt but turns yellow at 300°; yield 90%. Uv absorption was identical with that of 6-MP at any given pH. *Anal.* ($C_5H_3N_4SNa \cdot 2H_2O$) N.

6-Purinyl β-D-Ribothiopyranoside (XIX).—6-MP monohydrate 2.95 g, 0.017 mole) was treated with 5.0 g (0.017 mole) of 2,3,4-tri-*O*-acetyl-α-D-ribopyranosyl chloride (XXIX) as described for XIV; after recrystallization (MeOH), yield 1.0 g (19%), $\lambda_{max}^{pH\ 7.0}$ 280 mμ (ε 15.5 × 10^3), mp 106–109°. *Anal.* ($C_{10}H_{12}N_4O_4S \cdot H_2O$) N.

6-Purinyl 2,3,4-Tri-*O*-acetyl-β-D-ribothiopyranoside (XX).—2,3,4-Tri-*O*-acetyl-α-D-ribopyranosyl chloride (XXIX) (1 g, 0.003 mole) was dissolved in 25 ml of DMF. To this solution was added 0.9 g (0.004 mole) of the sodium salt of 6-MP (XVIII). The mixture was heated on the steam bath for 3 hr with stirring. The solvent was removed *in vacuo* on the steam bath. The black residue was dissolved in $CHCl_3$ and the $CHCl_3$ was washed (two 100-ml portions of cold H_2O, two 100-ml portions of 5% Na_2CO_3, two 200-ml portions H_2O). After drying the $CHCl_3$ solution (Na_2SO_4), the solvent was removed by distillation *in vacuo*. The syrup was dissolved in absolute EtOH and decolorized with charcoal. After adding anhydrous pentane to incipient cloudiness the solution was left at 5° for 18 hr. The thioglycoside formed white crystals. On recrystallization from EtOH–pentane, the product had λ_{max}^{EtOH} 285 mμ (ε 15.2 × 10^3), mp 79–82°. *Anal.* ($C_{16}H_{18}N_4O_7S \cdot C_2H_5OH$) N.

6-Purinyl β-D-Xylothiopyranoside (XXI).—Using the procedure described for V (method D-2), 6-chloropurine (1.6 g, 0.010 mole) was treated with 2 g (0.010 mole) of sodium thio-D-xylose which was prepared from 2,3,4-tri-*O*-acetyl-α-D-xylopyranosyl chloride[26] by an adaptation of the method of Gehrke and Kohler;[14a] $\lambda_{max}^{pH\ 7.4}$ 280 mμ (ε 16.1 × 10^3). After continuous extraction with ethyl acetate to remove 6-MP, the yield was 1.3 g (25%), mp 183–187°. *Anal.* ($C_{10}H_{12}N_4O_4S$) C, H, N.

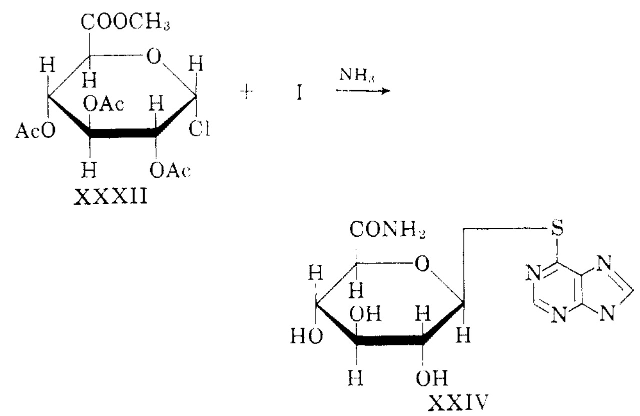
6-Purinyl Hepta-*O*-acetyl-β-D-thiolactoside (XXII).—1-Thioacetylhepta-*O*-acetyl-β-D-lactose (XXVII) (5 g, 0.0072 mole) was treated with 1.1 g (0.0071 mole) of 6-chloropurine using the procedure for V (D-1); yield 2.5 g (45%), mp 122–124°. XXII was also prepared by method C (Scheme II) from hepta-*O*-acetyl-α-D-lactosyl chloride[29] and 6-MP; λ_{max}^{EtOH} 280 mμ (ε 15.2 × 10^3). *Anal.* ($C_{31}H_{38}N_4O_{17}S$) N.

6-Purinyl-β-D-glucothiofuranosiduronic Acid Amide (XXIII, Scheme IV).—6-MP monohydrate (5 g, 0.029 mole) was dissolved in 200 ml of liquid NH_3. 1-Chloro-2,5-di-*O*-acetyl-α-D-glucofururonolactone (XXXII) (8 g, 0.029 mole) was added to the NH_3 solution. The product was isolated as the amide, using the procedure described for V; yield 3.2 g (32%), mp 188–190°, $\lambda_{max}^{pH\ 7}$ 280 mμ. *Anal.* ($C_{11}H_{13}N_5O_5S \cdot H_2O$) N.

(29) C. S. Hudson and A. Kunz, *J. Am. Chem. Soc.*, **47**, 2052 (1925).

6-Purinyl-β-D-glucothiopyranosiduronic Acid Amide (XXIV Scheme V).—Using the procedure described for XIV, 6-MP monohydrate (5 g, 0.029 mole) was treated with 10.6 g (0.030 mole) of methyl 1-chloro-2,3,4-tri-*O*-acetylglucopyranuronate

SCHEME V



prepared from methyl 1,2,3,4-tetra-*O*-acetylglucopyranouronate.[30] The product, isolated as the amide, was recrystallized from MeOH; yield 1.8 g (18%), $\lambda_{max}^{pH\ 7.0}$ 280 mμ, mp 205–207°. *Anal.* ($C_{11}H_{13}N_5O_5S \cdot H_2O$) N.

Carbohydrate Intermediates. 1-*S*-Acetyl-2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranose (XXV).—2,3,4,6-Tetra-*O*-acetyl-α-D-galactopyranosyl chloride[26] (10 g, 0.027 mole) was dissolved in *i*-PrOH (50 ml). This was added to a solution made from 0.621 g (0.027 g-atom) of Na and 2.05 g (0.027 mole) of thioacetic acid in *i*-PrOH (50 ml). The mixture was refluxed on the steam bath for 1 hr, cooled, and filtered to remove NaCl. Water was added to the alcoholic filtrate until cloudy. On standing at 0° for 48 hr, a mixture of oil with crystals had formed. The mixture was recrystallized from absolute MeOH; yield 2.8 g (25%), mp 114–115°. *Anal.* ($C_{16}H_{22}O_{10}S$) C, H.

Sodium Thiogalactose (XXVI).—1-Acetylthio-2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranose (XXV) (7.20 g, 0.076 mole) was dissolved in 50 ml of absolute MeOH. To this solution was added a solution made from 0.41 g (0.076 g-atom) of Na and 15 ml of absolute MeOH. After 1 hr at 25°, 25 ml of absolute EtOH was added. The white crystalline product which formed instantaneously was filtered and washed (Me_2CO); yield 3.33 g (87%), mp 172–175° dec. *Anal.* ($C_6H_{11}O_5SNa \cdot 0.5C_2H_5OH$) C, H.

1-*S*-Acetylhepta-*O*-acetyl-β-D-lactose (XXVII).—Using a procedure similar to that described by Gehrke and Kohler[14a] for monosaccharides, 13 g (0.198 mole) of hepta-*O*-acetyl-α-D-lactosyl chloride, prepared by the method of Hudson and Kunz,[29] was added to a solution made from 0.58 g (0.025 g-atom) of Na, 50 ml of absolute EtOH, and 1.7 g (0.025 mole) of thioacetic acid. The mixture was heated under reflux on the steam

(30) (a) Methyl 1,2,3,4-tetra-*O*-acetyl-δ-D-glucopyranuronate was supplied courtesy of Corn Products Co., Chicago, Ill. (b) W. F. Goebel and F. H. Babers, *J. Biol. Chem.*, **111**, 347 (1935). (c) K.-C. Tsou and A. Seligman, *J. Am. Chem. Soc.*, **74**, 5605 (1952).

bath for 2 hr with protection from atmospheric moisture. The NaCl was removed by filtration and H_2O was added to the filtrate until turbitidity appeared. An amber oil formed within 20 min, which, on scratching, crystallized. The crystals were washed (H_2O) and recrystallized from 50% EtOH; mp 87–89°, yield 11.4 g (83%). *Anal.* ($C_{28}H_{38}O_{18}S \cdot H_2O$) C, H.

2,3,4-Tri-*O*-acetyl-α-D-ribopyranosyl Chloride (XXIX).—1,2,3,4-Tetra-*O*-acetyl-α-D-ribopyranose[31] was converted to XXIX by an adaptation of the Pacsu[20] procedure, yield 61%, mp 93–95°, lit.[31] mp 95°. *Anal.* ($C_{11}H_{15}O_7Cl$) C, H.

1-Chloro-2,5-di-*O*-acetyl-α-D-glucofururonolactone (XXXII).—1,2,5-Tri-*O*-acetyl-α-D-glucofururonolactone[30c] (XXXI) (85 g, 0.28 mole) was dissolved in 500 ml of $CHCl_3$ (U.S.P.) and 44 g (0.23 mole) of $TiCl_4$ was added slowly with stirring. After stirring for 3.5 hr at 25°, the solution was poured into 2 l. of ice water. The $CHCl_3$ layer was washed (5% $NaHCO_3$, H_2O), dried (Na_2SO_4), decolorized with charcoal, and concentrated to a syrup *in vacuo* at 50°. The syrup was dissolved in anhydrous ether and a fine white crystalline product precipitated, yield 20 g (26%), mp 152–157°, $[\alpha]^{25}D$ +240° (*c* 2.0, $CHCl_3$). *Anal.* ($C_{10}H_{11}O_7Cl$) C, H.

Goebel and Babers[30b] prepared XXXII by a different method and reported mp 107.5–108.5° and $[\alpha]^{20}D$ 95.5° (*c* 1.257, $CHCl_3$).

(31) H. Zinner, *Chem. Ber.*, **83**, 153 (1950).

It is possible that this large discrepancy in melting point and optical rotation represents two stereoisomers, although the optical rotation of XXXII reported here is close to that of the analogous 1-bromo-2,4-di-*O*-acetyl-α-D-glucofururonolactone, $[\alpha]^{25}D$ +236°, mp 138–130°.

Hydrolysis of 6-Purinyl Thioglycosides.[32] (a) **Acid Catalysis (Figure 1).**—Stock solutions were prepared to contain 0.23 μmole of thioglycosides/ml in H_2O. One milliliter of stock solution was diluted to 10.0 ml with appropriate buffers. Periodic readings of uv absorption were recorded at 280 mμ and 325 mμ. Decreased absorbance at 280 mμ with simultaneous increase at 325 mμ demonstrated hydrolysis of the thioglycoside with liberation of 6-MP in solutions with pH values below 5. Products of hydrolysis were further identified by paper chromatography. The thioglycosides here described were stable at neutral and alkaline pH values, but were readily hydrolyzed in dilute acid.

(b) **Enzyme Catalysis.**—Hydrolysis of purine thioglycosides is catalyzed by a wide variety of mammalian thioglycosidase.[2a] The action of hog liver thioglycosidase on MPG is illustrated in Figure 2.

(32) Details concerning the kinetics of hydrolysis, the preparation, and the properties of mammalian and other thioglycosidases will appear in a subsequent publication.

The Synthesis and Biological Properties of Hydroxylaminopurines and Related Derivatives[1]

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Syntheses are described for the preparation of substituted hydroxylaminopurines, the related methoxyamino, methylhydroxylamino, methylhydrazino, and methylmercapto derivatives, and some ribonucleosides thereof. These compounds were tested against L1210 mouse leukemia. Two compounds, 6-methoxyaminopurine and 2-hydroxylamino-6-methylmercaptopurine, were active against the parent L1210 line but not against a subline resistant to 6-mercaptopurine, suggesting that they may be converted to active nucleotides by a mechanism similar to that of 6-mercaptopurine.

The marked inhibition of several mouse leukemias by 6-hydroxylamino-9-β-D-ribofuranosylpurine,[2] its 2-amino derivative,[3] and 2,6-dihydroxylaminopurine and its ribosyl derivative[4] indicates that hydroxylamino derivatives of purines or their nucleosides are worthy of further investigation as potential chemotherapeutic agents. We now report the synthesis and biological activity of other substituted hydroxylaminopurines as well as related methoxyamino, methylhydroxylamino, and methylhydrazino derivatives and their nucleosides.

Reaction of 8-methylthiopurine[5] (I) with ethanolic hydroxylamine in the presence of a catalytic amount of chloride ions[3] led to 8-hydroxylaminopurine (II) (Table I). When 2-fluoro-6-mercaptopurine[6] (III) was treated with the hydroxylamine solution, substitution of the 2-fluoro was accompanied by hydrolysis of the mercapto group, leading to the known[7] 2-hydroxylamino-6-hydroxypurine (IV). When 2-fluoro-6-methylthiopurine (V) was similarly treated, 2-hydroxylamino-6-methylthiopurine (VI) was obtained, even in the presence of a catalytic amount of chloride ions. This behavior contrasts with the ease of replacement of a 6-thiomethyl group by hydroxylamino when the C_2 is substituted by NH_2.[3]

Upon reaction with hydroxylamine in the presence of chloride ions, 2,6-dichloropurine[8] (VII) afforded 2-chloro-6-hydroxylaminopurine (VIII). This is analogous to the reported conversion of VII to 2-chloro-6-aminopurine upon aminolysis.[9]

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