phy using Woelm silica gel (250 × 10 mm) and ethyl acetate as solvent. The column was eluted with 100 ml of *n*-hexane–isopropyl ether (2:1) and then with 50 ml of pure isopropyl ether. The latter eluate on evaporation give 40 mg (32%) of slightly yellow product, mp 162–164°. The chromatographic purification was repeated in the same manner, giving 30 mg of colorless crystals, mp 163–164°.

A portion was recrystallized for analysis from *n*-hexane, giving colorless needles: mp 163.5–164.5°; $[\alpha]^{23}D$ 94° (*c* 1.2, $CHCl_3$); ir (KBr) 1690 and 1705 (*S*-acetate C=O), 1750 cm^{-1} (*O*-acetate C=O); nmr ($CDCl_3$) δ 2.07 (s, 6) and 2.32 (s, 6), *O*-acetate methyl and *S*-acetate methyl, respectively.

Anal. Calcd for $C_{18}H_{26}O_8S_4$: C, 43.36; H, 5.26; S, 25.72. Found: C, 43.42; H, 5.20; S, 25.50.

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Registry No.—**1**, 51051-69-5; **2**, 24808-13-7; **3**, 51051-70-8; **4**, 51051-71-9; **5**, 51051-72-0; **6**, 51051-73-1; **7**, 51051-74-2; **8**, 51051-75-3; **9**, 51051-76-4; **10**, 51051-77-5; **11**, 51051-78-6; **12**, 51051-79-7; **13**, 51051-80-0; **14**, 51051-81-1; 3-*O*-benzoyl-1,2;5,6-di-*O*-isopropylidene-D-mannitol, 51051-82-2; 3-*O*-benzoyl-4-*O*-methanesulfonyl-1,2;5,6-di-*O*-isopropylidene-D-mannitol, 51051-83-3.

References and Notes

(1) Presented in part to the Division of Organic Chemistry at the 159th National Meeting of the American Chemical Society, Houston, Tex., Feb 1970, and to the Division of Carbohydrate Chemistry at the 160th National Meeting of the American Chemical Society, Chicago, Ill., Sept 1970.

(2) (a) For preceding publication on thio carbohydrates, see G. E. McCasland, A. K. M. Anisuzzaman, S. R. Naik, and L. J. Durham, *J. Org. Chem.*, **37**, 1201 (1972). (b) For preceding publication on alicyclic carbohydrates, see J. Shapira, T. Putkey, A. Furst, and G. E. McCasland, *Carbohyd. Res.*, **25**, 535 (1972); see also Y. Sanemitsu, N. Kurihara, M. Nakajima, G. E. McCasland, L. F. Johnson, and L. C. Carey, *Agr. Biol. Chem.*, **36**, 845 (1972).

(3) (a) To whom any communications should be addressed, at the University of San Francisco; (b) Stanford University.

(4) Reported preparations of compounds containing more than three mercapto groups include (a) 1,2,3,4-butanetetrathiol, see C. G. Overberger and A. Drucker, *J. Org. Chem.*, **29**, 360 (1964); (b) tetra(mercaptomethyl)methane, M. W. Farlow and F. K. Signaigo, *Chem. Abstr.*, **40**, 5763 (1946).

(5) (a) The preparation of a tetrathiohexitol was reported by S. M. Iqbal and L. N. Owen, *J. Chem. Soc.*, 1030 (1960). It had the L-ido configuration, and the sulfur groups were at positions 1, 2, 5, and 6 (isolated as hexaacetate, mp 133–135°). It should be noted that the tetrathiohexitols now reported by us have the D (ido or manno) configuration, and the sulfur groups are at positions 1, 3, 4, and 6.[5b] (b) This article describes a new series of stereoisomers (hexitol sulfur analogs). It is almost certain that the compounds in this series all have the *same* configuration (D-manno or D-ido), because the reactions employed would not invert position 2 or 5, and would retain or produce a threo configuration at positions 3 and 4.

Optical rotation studies further indicate, in the author's opinion, that the series configuration is D-manno (not D-ido), with a *very high probability.*

The reader is cautioned, however, that this rotation-based configurational assignment (like many similar assignments in the field of carbohydrates) *cannot be regarded as absolutely certain.* Accordingly (as suggested by a referee) we recommend that the D-manno assignments in this article be considered tentative until more rigorous evidence, *e.g.*, X-ray or neutron diffraction studies, is available. (Nmr spectra were recorded for these compounds, but could not be interpreted in terms of configuration.)

(6) J. M. Sugihara and G. U. Yuen, *J. Amer. Chem. Soc.*, **79**, 5780 (1957).

(7) (a) B. R. Baker and A. H. Haines, *J. Org. Chem.*, **28**, 442 (1963); (b) *ibid.*, **28**, 438 (1963).

(8) In 1960 L. N. Owen and S. M. Iqbal by a similar reaction of 3,4-anhydro-1,2;5,6-di-*O*-isopropylidene-D-talitol obtained a trithiocarbonate (reported mp 108–110°) and reduced it to a dithiol. We now believe that each of these products was a mixture of the D-ido and D-manno diastereomers. See *J. Chem. Soc.*, 1030 (1960).

(9) For an example of the formation of a mercaptodithiolane diketal in the cyclitol series, see G. E. McCasland, S. Furuta, A. Furst, L. F. Johnson, and J. N. Shoolery, *J. Org. Chem.*, **28**, 459, 462 (1963).

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(11) For discussion of the chemistry of trithiocarbonates and dithiocarbonates, see D. S. Breslow and H. Skolnik, "Multi-Sulfur and Sulfur and Oxygen Five and Six-Membered Heterocycles," Part I, A. Weissberger, Ed., Interscience, New York, N. Y., 1966, Chapter 5, Part II.

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(13) J. Kuszman and L. Vargha have recently reported the preparation of a 1,2;5,6-diepithio derivative of L-iditol, and many related intermediates, which may be of interest in connection with the work now reported by us. See *Carbohyd. Res.*, **11**, 165 (1969).

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(16) Although D-mannitol and its thio analogs and derivatives are dissymmetric and optically active, the molecules of such compounds here discussed nearly all contain a twofold simple symmetry axis (point group C_2). This causes nmr chemical-shift equivalence of the protons connected directly or indirectly to carbon atoms 1 and 6, 2 and 5, and 3 and 4 in the hexitol chain.

(17) In March 1970, Leroy F. Johnson at Varian Associates, Palo Alto, Calif., recorded the carbon-13 nmr spectrum of a sample of the D-manno trithiocarbonate diketal, mp 117°, provided by us. The spectrum was recorded with a Varian HA-100 spectrometer at 25.15 MHz, using benzene as solvent (400 mg/ml, 8-mm tube), 25 scans, noise decoupled. The spectrum was consistent with the proton spectrum, but as yet has not yielded additional structural information. Details of this work will be reported elsewhere.

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Scission of the Sulfur–Sulfur Bond in Dipurinyl and Dipyrimidinyl Disulfides by Cyanide[1,2]

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Bis(1-β-D-ribofuranosyl-4-thiouracil) disulfide, its methyl analog, and bis(9-methyl-6-thiopurine) disulfide are decomposed quantitatively into the corresponding thiocyanato and thio derivatives by CN^- buffered at pH 7. 4-Thiocyanatouridine and its methyl analog decompose quantitatively in alkali to the corresponding thio and oxo compounds in 2:7 and 1:1 ratio, respectively. 9-Methyl-6-thiocyanatopurine decomposes in alkali to 9-methyl-6-thiopurine. The reaction of the three above-mentioned disulfides in unbuffered CN^- apparently proceeds through the intermediate formation of the thio and thiocyanato derivatives, the latter decomposing *in situ* under alkaline conditions in the same manner. Synthesis and properties of 4-thiocyanatouridine, its methyl analog, 9-methyl-6-thiocyanatopurine, and bis(1-methyl-4-thiouracil) disulfide are described.

The extreme susceptibility of the disulfide bond in bis(1-β-D-ribofuranosyl-4-thiouracil) disulfide and its methyl analog to nucleophilic attack by OH^- reported earlier from this laboratory[3] led us to extend this study to the

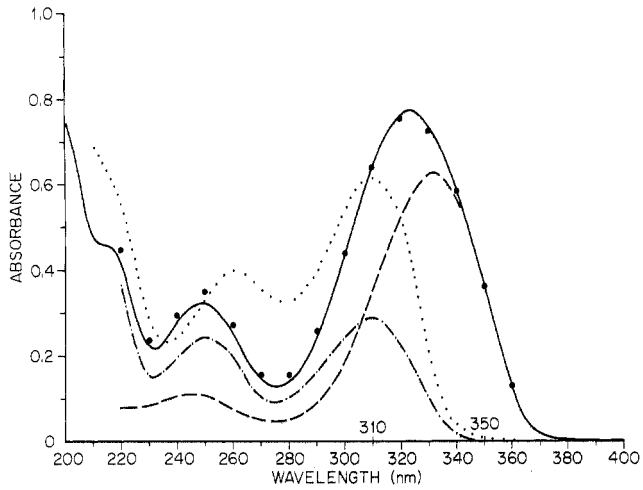


Figure 2. Decomposition of bis(1-β-D-ribofuranosyl-4-thiouracil) disulfide (Ir) by NaCN at pH 7. The uv absorption spectra of Ir at pH 7 (···), Ir after treatment with NaCN at pH 7 (——), IIr at pH 7 normalized at 350 nm (– – –), and IIIr at pH 7 normalized at 310 nm (– · –). Addition spectrum of IIr and IIIr (●).

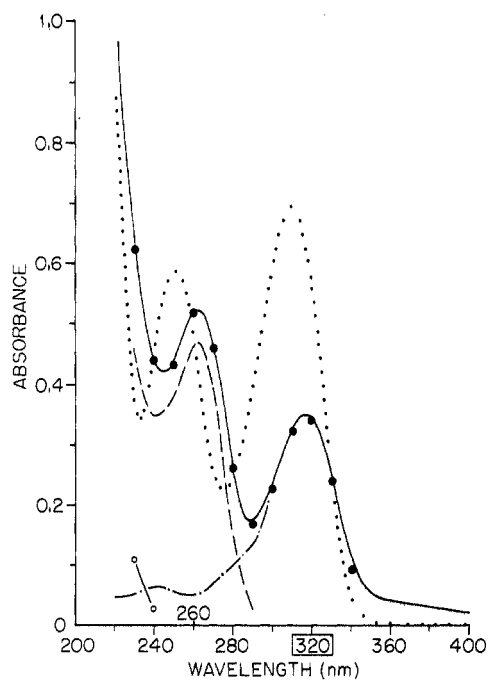


Figure 5. The uv absorption spectra of 4-thiocyanatouridine (IIIr) at pH's 2 and 7 (···) and 12 (——) (decomposes), and spectral correlation of its hydrolysis products at pH 12. The uv absorption spectra of IIr at pH 12 normalized at 320 nm (– · –), IVr at pH 12 normalized at 260 nm (– – –), and thiocyanate at pH 12 normalized at 230 nm (O - O). Addition spectrum of the last three (IIr + IVr + SCN^-) (●).

cleavage of the disulfide bond in these compounds and bis(9-methyl-6-thiopurine) disulfide by CN^-. The first step in the reaction of all three purine and pyrimidine disulfides with CN^- was very similar. All three quantitatively decomposed into the thiocyanato and thio derivatives when the reaction was carried out with NaCN buffered at pH 7. In each case, the spectra of the disulfides treated with CN^- can be shown to be the addition spectra of the two products formed in stoichiometric amounts (Figures 1-3).[4] In unbuffered CN^-, however, the intermediate thiocyanato derivatives were degraded owing to alkaline conditions.

In order to demonstrate that the reaction with unbuffered CN^- proceeded through the intermediate formation of the thiocyanato derivatives, we synthesized all three of them (IIIr, IIIm, and VII) independently and studied their spectral properties and decomposition in alkali (Table I, Figures 4-6). The spectra of all three thiocyanates are essentially the same at pH 2 and pH 7. Compounds IIIm and IIIr decomposed in alkali to IIm and IVm, and IIr and IVr, in 1:1 and 2:7 ratio, respectively (Figures 4 and 5). The decomposition of the thiocyanates IIIm and IIIr takes place according to the following equations.

Table I
Spectral Properties

Compd	pH or solvent	Spectral characteristics, λ, nm (ε × 10^{-3}) Maxima	Minima
Ir	7[a]	309 (19.60)	279 (10.50)
		261 (11.70)	236 (7.70)
	Ethanol[b]	311 (18.15)	280 (9.46)
		262 (12.38)	237.5 (7.70)
Im	7	307.5 (18.12)	277 (8.70)
		257 (12.10)	236 (8.30)
V	7	290 (26.10)	287 (25.48)
		283 (25.69)	239 (5.94)
		215 (28.60)	208 (28.13)
IIr[c]	6.5	331 (21.00)	274 (1.65)
		245 (4.00)	225 (2.60)
	11.8	316 (19.70)	268 (2.40)
IIm	2, 6	334 (20.19)	278 (0.90)
		242.5 (4.04)	225 (2.31)
	12	315 (17.05)	257 (2.65)
VI[d]	0	326 (18.5)	
	5.1	321 (26.1)	
		229 (12.7)	
	11.1	309 (21.4)	
		234 (13.0)	
IIIr[e]	2, 6	309 (8.41)	275 (2.74)
		251 (6.83)	233 (4.15)
IIIm	2, 6	307 (7.79)	271.5 (1.99)
		245 (6.35)	232 (4.56)
		217.5 (12.72)	
	Ethanol[f]	312 (6.5)	
		245.5 (5.4)	
VII	2, 6	277 (12.73)	237.5 (2.40)
IVm	2, 6	267 (9.48)	232 (1.32)
	12	264 (6.74)	241 (3.43)
NaCNS	2	ϵ_{240} 250, ϵ_{230} 1500, ϵ_{220} 2950, ϵ_{210} 3350	
	12	ϵ_{240} 250, ϵ_{230} 1500, ϵ_{220} 4000	

[a] Reported max 309, 261; min 278, 236 (ref 12). [b] Reported max 320 (29.75), 260 (6.50); min 280 (5.40) (ref 7). [c] Data from N. K. Kochetkov, E. I. Budowsky, V. N. Shibaev, and M. A. Grachev, *Biochim. Biophys. Acta*, **59**, 749 (1962). [d] Data from J. H. Lister in "Fused Pyrimidines," Part II, D. J. Brown, Ed., Wiley-Interscience, New York, N. Y., 1971, p 485. [e] Reported max 310 (8.00), 250 (7.80); min 280 (4.00) in ethanol (ref 7). [f] Reported, in ethanol, max 318.5 (7.78), 256 (6.7) (ref 6).

(IIIm) $2R'SCN + 4OH^- =$
$R'O^- + R'S^- + SCN^- + OCN^- + 2H_2O$

(IIIr) $9R'SCN + 18OH^- =$
$7R'O^- + 2R'S^- + 7SCN^- + 2OCN^- + 9H_2O$

Titration of IIIm with alkali to pH 11 indicated the consumption of 2 equiv of alkali per mole of IIIm. The amounts of $R'O^-$, $R'S^-$, and SCN^- formed were calculated from the spectra; the spectral correlation is shown in Figures 4 and 5. We were unable to obtain any direct evidence for the formation of CNO^-; its extinction in the ultraviolet range is negligible at the concentrations used in these experiments. The compound VII, on the other hand, was transformed in alkali to VI to the extent[5] of 85% with no evidence for the formation of any oxo compound, for VI could be recovered completely by treatment of the reaction mixture with Na_2S. This indicates that the stability

Table II[a]
Cleavage of the Thiocyanates and Disulfides by H^+, OH^-, HS^-, and CN^-

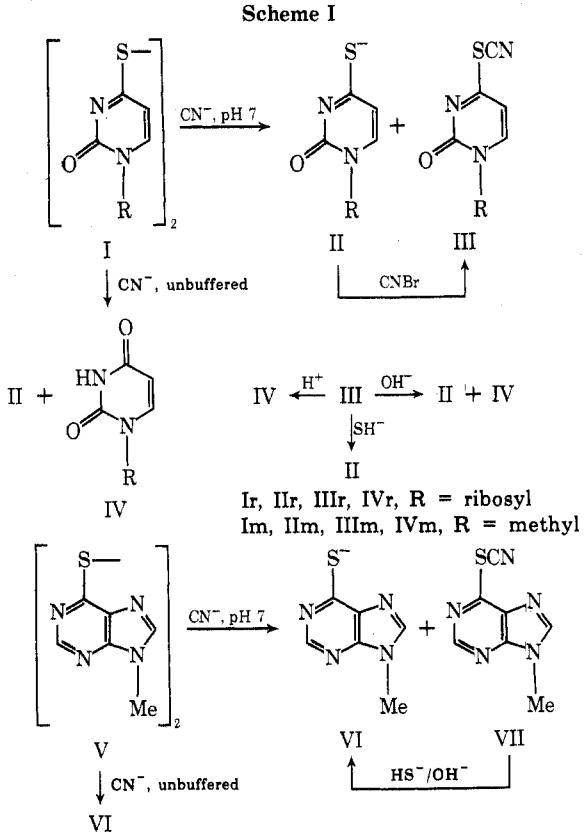
Compd	Reaction conditions	Reagent	Products
Ir (31.4)	1 hr	CN^- (pH 7)	IIIr (34), IIr (29.7)
Ir (35.7)	2 hr	CN^- (unbuffered)	IVr (26.2), IIr (43.3), CNS^- (26.2)
Im (33.7)	3 hr	CN^- (pH 7)	IIIm (33), IIm (36)
Im (33.9)	2 hr	CN^- (unbuffered)	IVm (18.1), IIm (51.8), CNS^- (18.1)
V (33.3)	3.5 hr	CN^- (pH 7)	VI (32.6), VII (31.4)
V (32)	72 hr	CN^- (unbuffered)	VI (61.8)
IIIr (82)	Immediate	OH^-	IIr (18), IVr (64), CNS^- (64)
	48 hr	H^+	IVr (81)
	Immediate	HS^-	IIr (82)
IIIm (59)	Immediate	OH^-	IIm (29), IVm (29), CNS^- (29)
	48 hr	H^+	IVm (59)
	Immediate	HS^-	IIm (59)
VII (71.6)	5 days	OH^-	VI (60.8)
	1 day	H^+	No reaction
	Immediate	HS^-	VI (71)

[a] Numbers in parentheses indicate one-third the number of nanomoles of the compounds in a 3-ml cuvette. All reactions were carried out at 25°.

of the ring-S bond in these compounds decreases in the order VII > IIIm > IIIr. Compounds IIIm and IIIr are converted into IVm and IVr quantitatively in acid, while VII is unaffected in acid. All three thiocyanato derivatives are readily reduced to thio compounds by SH^-. The decomposition of the thiocyanato derivatives in alkali is parallel to the decomposition of the thiocyanato derivatives formed *in situ* as a result of the nucleophilic attack of the unbuffered CN^- (pH 10.2) on the disulfides. The amounts of products formed were in exactly the same ratio as predicted on the basis of the formation of the thiocyanates as intermediates (Table II, Figures 7-9).

The compounds IIIm and VII were prepared, in good yield, by the action of CNBr on the solution of IIm and VI in equivalent amounts of alkali. We were unable to prepare IIIm by the published method.[6] The spectral properties of the compound prepared by us also do not agree with the data reported earlier[6] (*cf.* Table I). The preparation of IIIr, however, was difficult owing to its lability. Although reports on the preparation of this compound have appeared in the literature,[6,7] it has not been characterized rigorously. We found that the best way to prepare this compound is to use the cyanide cleavage reaction of the disulfide Ir. In the first step, the disulfide Ir was treated with NaCN buffered at pH 7. When completion of the reaction was indicated by the uv absorption spectrum of the reaction mixture, the solution was treated with CNBr to convert the thiol IIr to the thiocyanato derivative IIIr (Scheme I). This method was also successful with the methyl analog (Figures 10 and 11).

Scheme I



Degani and Patchornik[8] used a similar method for the synthesis of 2-nitro-5-thiocyanatobenzoic acid from 5,5′-dithiobis(2-nitrobenzoic acid). Cyanogen bromide has previously been shown to oxidize thiols to disulfides when 0.5 mol of reagent is used per mole of the thiol.[9] Compound IIr has also been reported to form a disulfide when treated with 0.5 mol of CNBr per mole of reactant. In addition, we found that IIm, on treatment with even 1.1 mol of CNBr in ethanol containing 1 mol of triethylamine, forms the disulfide Im instead of the expected thiocyanato derivative IIIm. Paralleling the experience of Degani and Patchornik,[8] we found that, to obtain a quantitative yield of the thiocyanate from the disulfide by cyanide cleavage (step 1) followed by cyanogen bromide treatment (step 2), the presence of cyanide is necessary in the second step to decompose any disulfide that may be formed in this step. The disulfide formation from the thiouridine by cyanogen bromide probably proceeds *via* the intermediate sulfenyl bromide.[7] We have examined and eliminated the alternate possibility involving the intermediate formation of thiocyanate, since attempts to prepare Im by reaction of IIIm with IIm were not successful. The thiocyanate IIIr was characterized by its quantitative conversion to IVr in acid and to IIr in sodium bisulfide (Figure 12). Moreover, the similarity of its uv absorption spectrum with that of its methyl analog characterizes IIIr to be 4-thiocyanatouridine (Figures 4 and 5).

Contrary to earlier observation,[7] we found that the spectra of Ir and IIIr are very similar (Figures 2 and 5). Both of them have their higher absorbancy peak at 309 nm, although the lower absorption peak position of IIIr shows a comparative blue shift of about 10 nm. However, the two can be distinguished by their spectra in alkali. In alkali, Ir forms sulfenic acid[3] which shows an absorption maximum at 360 nm, whereas IIIr in alkali does not show appreciable absorbance at 360 nm (Figure 5). The previously reported spectral data of Ir do not agree with our

results[7] (Table I). The use of commercial preparations without purification might have led to this anomaly.

We have thus established that the disulfides Ir, Im, and V are initially cleaved by the cyanide to form the thiocyanato and thio derivatives quantitatively in 1:1 ratio, the former undergoing further degradation under alkaline conditions. Thiocyanatopyrimidines IIIr and IIIm form IIr and IVr, and IIm and IVm, in 2:7 and 1:1 ratio, respectively. Thiocyanatopurine VII, however, is quantitatively converted into VI resembling the behavior of 6-thiopurine itself.[11] Our results support the findings of Walker and RajBhandary[10] that 4-thiocyanatouridine, like its methyl analog, is degraded under alkaline conditions partly to thiouridine and partly to uridine, instead of its complete degradation to uridine as earlier reported.[6]

Experimental Section

Melting points were observed in a Thomas-Hoover apparatus and are uncorrected. Thin layer chromatography was carried out by use of E. Merck tlc plates and Cellulose F and with the following solvent systems: A, 1-butanol-water, 86:14 (v/v); B, isobutyric acid-ammonia-water, 66:1:33 (v/v/v); and C, 0.1 *M* phosphate buffer (pH 6.8)-ammonium sulfate-1-propanol, 100:60:2 (v/w/v). Bis(1-β-D-ribofuranosyl-4-thiouracil) disulfide,[12] its methyl analog,[3] and 1-methyl-4-thiouracil[12] were synthesized by published methods. 9-Methyl-6-thiopurine was obtained from Cyclo Chemical Corp., Los Angeles, Calif. All other chemicals were reagent-grade commercial products. The uv absorption spectra at different pH's were recorded on a Cary recording spectrophotometer Model 14 PM on the same solution in the same cuvette using small amounts of acid, alkali, or buffer solutions to alter the pH. The ir spectra were recorded on a Perkin-Elmer 257 grating spectrophotometer with KBr disks. Elemental analyses were by Galbraith Laboratories, Knoxville, Tenn.

Bis(9-methyl-6-thiopurine) Disulfide (V). 9-Methyl-6-thiopurine (VI), 166.2 mg (1 mmol), was brought into solution in 25 ml of water by adding 1 *N* NaOH to a pH of 10.5. The solution was cooled in ice water and treated with 1 ml of 1 *N* iodine solution. The pH fell to 9.5. The precipitate was filtered and washed with water, crude yield 136 mg. It was washed with dilute ammonia in the cold to remove traces of starting material, and recrystallized from 50% ethanol. Yield of chromatographically homogeneous material was 90 mg (27% of theory), mp 232–233°.

Anal. Calcd for $C_{12}H_{10}N_8S_2$: C, 43.62; H, 3.05; N, 33.92. Found: C, 43.41; H, 3.09; N, 33.78.

Bis(1-methyl-4-thiouracil) Disulfide (Im). A suspension of 1-methyl-4-thiouracil, 142.2 mg (1 mmol), in 10 ml of ethanol was treated with triethylamine (1 mmol) and cyanogen bromide (1.1 mmol) at 25° with stirring. A white precipitate was formed in about 10 min. The reaction mixture was chilled in ice and filtered, yield, 100 mg (71% of theory). It was identified as the disulfide by comparing its melting point, uv spectra, and tlc in solvents A and B with those of an authentic specimen.

9-Methyl-6-thiocyanatopurine (VII). A solution of 9-methyl-6-thiopurine, 83.1 mg (0.5 mmol), in a mixture of 0.5 ml of 1 *N* NaOH and 2.5 ml of water was treated with 1 *M* ethanolic CNBr, 0.55 ml, at 25°. A white precipitate appeared almost immediately. The reaction mixture was allowed to stand at room temperature for 30 min, then cooled in ice, filtered, washed with ice-cold water, and dried *in vacuo* over P_2O_5: yield 81 mg (85% of theory); mp 181.5–182°; ir 2180 cm^{-1} (-SCN).

Anal. Calcd for $C_7H_5N_5S$: C, 43.97; H, 2.64; N, 36.63. Found: C, 43.72; H, 2.48; N, 36.45.

1-Methyl-4-thiocyanatouracil (IIIm). A solution of 1-methyl-4-thiouracil, 142 mg (1 mmol), in 5 ml of 0.2 *N* NaOH was treated at once with 1.1 ml of 1 *M* ethanolic CNBr while stirring at room temperature. The reaction mixture was allowed to stand for 1 hr, cooled in ice, filtered, and washed with a little ice-cold water: yield 110 mg (66% of theory); white, glistening plates; mp 145–146° dec; chromatographically homogeneous (solvents A, C).

Anal. Calcd for $C_6H_5N_3OS$: C, 43.10; H, 3.10; N, 25.13. Found: C, 43.19; H, 2.93; N, 25.22.

4-Thiocyanatouridine (IIIr). One milliliter of freshly prepared 1 *M* NaCN was added dropwise to a suspension of bis(1-β-D-ribofuranosyl-4-thiouracil) disulfide (Ir), 5.2 mg (0.01 mmol), in 5 ml of 0.05 *M* phosphate buffer (pH 7). The pH of the reaction mixture was maintained by simultaneous addition of 0.5 *M* KH_2PO_4 in a pH stat. The disulfide gradually went into solution. After standing for 1.5 hr, the A_{325}/A_{275} was found to be 5.9. It is necessary to attain this ratio to ensure complete conversion of the disulfide. One milliliter of 1 *M* ethanolic solution of CNBr was added and the reaction mixture was allowed to stand for 10 min. It was then run through a column of Sephadex G-10 (25 × 2 cm), eluting with oxygen-free water and collecting 5-ml fractions. The appropriate fractions were combined and evaporated in a rotary evaporator under high vacuum at room temperature to half the original volume. The yield of chromatographically homogeneous 4-thiocyanatouridine was practically quantitative. The aqueous solution deteriorated slowly on standing, as judged by its uv spectrum.

The above preparation has been spectrophotometrically duplicated (Figure 10). Three milliliters of an aqueous solution of Ir containing 50 μl of 0.5 *M* phosphate buffer, pH 6, was treated with 10 μl of 1 *M* freshly prepared NaCN. The spectra were recorded before, and 2 hr after, addition of NaCN. Then 10 μl of 1 *M* ethanolic CNBr was added and the spectrum of the thiocyanate IIIr formed was recorded. For characterization of the thiocyanate IIIr, the solution was transferred from the cuvette to a flask and weighed. The solution was then evaporated at room temperature in a rotary evaporator under high vacuum to about half its volume to remove excess CNBr. It was then reconstituted by adding water to compensate for the loss in weight, after which it was treated with 10 μl of 1 *M* Na_2S and the spectrum was recorded after addition of 25 μl of 10 *N* NaOH. Another sample of thiocyanate IIIr, prepared in identical manner, was treated with 50 μl of 10 *N* HCl and the spectrum was recorded. The results are shown in Table II and Figure 12. The spectral properties are recorded in Table I. The molar extinction of 4-thiocyanatouridine was calculated on the basis of its quantitative conversion to 4-thiouridine in sodium sulfide.

Action of Acid, Alkali, and Sodium Sulfide on 4-Thiocyanatouridine (IIIr), Its Methyl Analog (IIIm), and 9-Methyl-6-thiocyanatopurine (VII). Three milliliters of a solution of each thiocyanate in oxygen-free water was treated separately with 25 μl of 10 *N* HCl, 25 μl 10 *N* NaOH, and 10 μl of 1 *M* Na_2S at 25°. The results are shown in Table II and Figures 4–6 and 12.

Reaction of Bis(1-β-D-ribofuranosyl-4-thiouracil) Disulfide (Ir), Its Methyl Analog (Im), and Bis(9-methyl-6-thiopurine) Disulfide (V) with Cyanide. Three milliliters of a solution of each disulfide in oxygen-free water was treated with 50 μl of 0.5 *M* phosphate buffer followed by 10 μl of 1 *M* freshly prepared NaCN at 25°. For studying the reactions of unbuffered NaCN, the phosphate buffer was omitted. The results are shown in Table II and Figures 1–3 and 7–9.

Attempted Preparation of Bis(1-methyl-4-thiouracil) Disulfide (Im) from the Thio (IIm) and Thiocyanato (IIIm) Derivatives. A mixture of 1-methyl-4-thiouracil, 2.84 mg (0.02 mmol), 1-methyl-4-thiocyanatouracil, 3.34 mg (0.02 mmol), ethanol, 0.5 ml, and triethylamine (0.02 mmol) was stirred at room temperature for 1 hr. It was centrifuged and, after removal of the supernatant, the residue was taken up in water and the uv absorption spectrum in alkali was recorded. Absence of any appreciable absorbance at 360 nm indicates absence of formation of any disulfide. In alkali, the uv absorption spectrum of Im shows a peak at 360 nm due to the formation of sulfenic acid.[3]

Titration of 1-Methyl-4-thiocyanatouracil (IIIm) with Alkali. Compound IIIm, 11.55 mg, was suspended in 5 ml of water containing 2 drops of thymolphthalein indicator solution [0.04 g/100 ml of ethanol-water (1:1)] and titrated with 0.1 *N* NaOH solution. The amount of alkali consumed was 1.35 ml or 1.95 mol/mol of IIIm.

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Registry No.—Im, 23193-01-3; Ir, 18427-02-6; IIm, 35455-86-8; IIr, 13957-31-8; IIIm, 29401-10-3; IIIr, 51056-62-3; IVm, 615-77-0; V, 51056-63-4; VI, 1006-20-8; VII, 51056-64-5; NaCN, 143-33-9; NaCNS, 540-72-7.

Supplementary Material Available. Figures 1, 3, 4, and 6–12 will appear following these pages in the microfilm edition of this volume of the journal. Photocopies of the supplementary material from this paper only or microfiche (105 × 148 mm, 24× reduction, negatives) containing all of the supplementary material for the papers in this issue may be obtained from the Journals Department, American Chemical Society, 1155 16th St., N.W., Washington, D. C. 20036. Remit check or money order for $3.00 for

photocopy or $2.00 for microfiche, referring to code number JOC-74-1466.

References and Notes

(1) Research supported by the U. S. Atomic Energy Commission under contract with the Union Carbide Corp.
(2) Presented at the 24th Southeastern Regional Meeting of the American Chemical Society, Birmingham, Ala., Nov. 2–4, 1972.
(3) B. C. Pal, M. Uziel, D. G. Doherty, and W. E. Cohn, *J. Amer. Chem. Soc.*, **91**, 3634 (1969).
(4) See paragraph at end of paper regarding supplementary material.
(5) Lack of quantitative conversion of VII to VI may be due to the instability of 6-mercaptopurine in alkali noted earlier. 6-Mercaptopurine is converted to purine 6-sulfinate in better than 60% yield in dilute alkaline solution (30–50 μM) for 96 hr [I. L. Doerr, I. Wempen, D. A. Clarke, and J. J. Fox, *J. Org. Chem.*, **26**, 3401 (1961)].
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Ultraviolet- and γ-Ray-Induced Reactions of Nucleic Acid Constituents. Reactions of Purines with Ethers and Dioxolane

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Ultraviolet- and γ-ray-induced reactions of caffeine, adenine, and guanosine with tetrahydrofuran, tetrahydropyran, dioxane, tetrahydrofurfuryl alcohol, and dioxolane are described. The reactions lead to the appropriate 8-substituted purines in yields of up to 90% when performed in the presence of photoinitiators. A free-radical mechanism is proposed for these reactions.

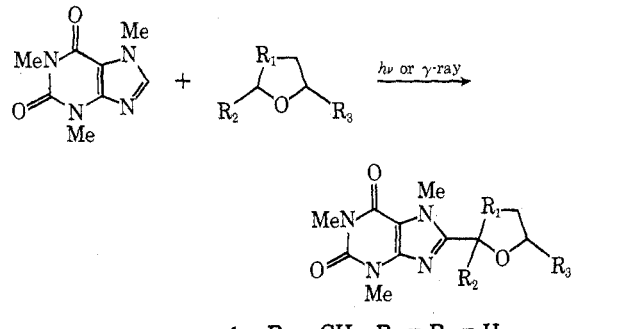
Ultraviolet and γ-ray-induced reactions of purines with alcohols or amines have been described recently.[1] These reactions resulted in the substitution of the appropriate moiety for the 6- or 8-hydrogen atom in the purine system. Thus, in reactions of purines with alcohols the substituent was usually the α-hydroxyalkyl group, while with amines it was the α-aminoalkyl group. The reactions could be induced directly with ultraviolet light (λ >260 nm) or by the use of photosensitizers (with light of λ >290 nm), which increased the yields of the photoproducts.

The aim of the present study is the investigation of the photochemical reactions of purines with a variety of substrates, mainly with those present in living systems. This will contribute to a better understanding of the photochemical reactions of purines, and subsequently to the development of selective photochemical reactions for these moieties in nucleic acids. In addition, it is anticipated that this study will shed further light on the interaction under irradiation of nucleic acids with their environment. The photoreactions of purines with ethers[2] and acetals serve as models for the interaction of purines with sugars and might lead to the discovery of new, so far unknown, irradiation-induced modifications in nucleic acids. The present publication includes full details of the photochemical and γ-ray-induced reactions of purines and purine nucleosides with a variety of ethers, hydroxy ethers, and dioxolane. An attempt was made to carry out the reactions under conditions in which purine moieties in nucleic acids would react selectively; therefore, photosensitizers which have been shown previously to induce selective reactions of purines,[3] *e.g.*, peroxides, were employed.

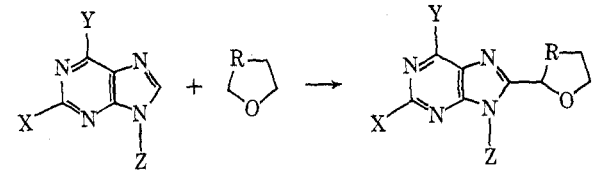
Results and Discussion

Irradiation with ultraviolet light or exposure to γ rays of caffeine, adenine, or guanosine with ethers, hydroxy ethers, or dioxolane led to the substitution of the appropriate moiety for the hydrogen atom at the 8 position of the purine. The site of binding to the purine in the ether moiety is at the carbon atom α to the ether oxygen,[2] whereas with dioxolane it is at the acetalic carbon. The reactions studied can be presented as shown in Scheme I.

Scheme I



1, $R_1 = CH_2$; $R_2 = R_3 = H$
2, $R_1 = O$; $R_2 = R_3 = H$
3a, $R_1 = CH_2$; $R_2 = H$; $R_3 = CH_2OH$
3b, $R_1 = CH_2$; $R_2 = CH_2OH$; $R_3 = H$
4a, $R_1 = CH_2$; $R_2 = H$; $R_3 = CH_2OCOCH_3$
4b, $R_1 = CH_2$; $R_2 = CH_2OCOCH_3$; $R_3 = H$
5, $R_1 = CH_2O$; $R_2 = R_3 = H$
6, $R_1 = (CH_2)_2$; $R_2 = R_3 = H$



X = H; Y = NH_2; Z = H (adenine)
X = NH_2; Y = OH; Z = ribose (guanosine)

7, R = CH_2
8, R = O
9, R = CH_2O
10, R = $(CH_2)_2$
11, R = CH_2
12, R = $(CH_2)_2$

The reactions could be either induced directly by ultraviolet light (λ >260 nm) or through photochemical initiation with peroxides (with light of λ >290 nm) with higher yields of the photoproducts. Products were isolated by column chromatography using a modified "dry column" technique[4] followed by elution with acetone–petroleum ether mixtures for the caffeine derivatives, and methanol–