continued. When the temperature rose to 15–20°, all of the insoluble material went into solution. The clear solution was poured into 500 ml of $H_2O$ and the mixture was adjusted to pH 5. The product was removed by filtration, washed with deionized $H_2O$, and dried overnight at 70°. The crude product melted at 219–220° dec.

**Bis(7-fluoro-5-nitro-8-quinolinolato)copper(II) (IIa).**—Two solutions containing 0.41 g (0.0018 mole) of 7-fluoro-5-nitro-8-quinolinol in 10 ml of MeOH and 0.21 g (0.0010 mole) of $Cu(OAc)_2 \cdot H_2O$ in 20 ml of 75% aqueous MeOH were mixed and stirred for 1 hr. The product was removed by filtration, boiled in DMF several times, and the insoluble material was washed with acetone and dried under vacuum at 100° overnight. The compound was sufficiently pure for analysis.

**7-Chloro-5-nitro-8-quinolinol (Ib).**—To 400 ml of $H_2O$ was added 5.7 g (0.028 mole) of 5-nitro-8-quinolinol[4] and 1.8 g (0.026 mole) of 85% KOH. Solution was effected by stirring and heating. After cooling to 30°, 50 ml of NaOCl (5.25%) was added and stirring was continued 1 hr longer. The mixture was brought to pH 5 with 25 ml of AcOH. The product was obtained by filtration, washing with deionized $H_2O$, and drying at 70° overnight.

## 6,6′-Dithiobispurinyl Nucleosides[1]

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A number of nucleosides of purine-6-thiol (**1**) and 2-aminopurine-6-thiol (**2**) have been prepared in an effort to modify the toxicity of these clinically useful anticancer agents and to provide drugs for use against **1**- and **2**-resistant cancer strains.[2] A serious problem which has presented itself in the use of these new drug candidates is their rapid elimination and metabolism by oxidative pathways to therapeutically inactive products. The metabolism of 6-mercaptopurine and some of its derivatives has been the subject of a recent review.[3]

In an attempt to provide a "reservoir" source of **1** and **2** as well as their β-D-ribofuranosides, Doerr, *et al.*,[4] prepared the disulfides of these compounds using an adaptation of the iodine-buffer procedure of Miller, *et al.*[5] The disulfides could be envisioned as reverting to the corresponding thiol and sulfenic acid through chemical means by the attack of base,[4] or alternately an enzymatic system such as that regulating the oxidation and reduction of glutathione could prevail to reduce the disulfide to the thiol.

The earlier paper[4] did not record any metabolic data to support directly the "reservoir" hypothesis but did note that the activity of the disulfide of thioinosine in the Sarcoma 180 system was greater than that of **1** while the corresponding thiol was inactive. The thioguanosine disulfide was also found to be a more potent inhibitor on a molar basis than either **2** or thioguanosine.

Despite these rather intriguing results there does not appear to have been any further study upon the effects of disulfide formation on biological activity in 6-thiopurinyl nucleosides.

In an effort to explore this aspect further we have prepared the disulfides of several 6-thiopurinyl nucleosides. The candidates chosen for conversion to their disulfides were α- and β-9-(2-deoxy-D-*erythro*-pentofuranosyl)-9H-purine-6-thiol[2] (**3**), α- and β-2-amino-9-(2-deoxy-D-*erythro*-pentofuranosyl)-9H-purine-6-thiol[6] (**4**), 9-β-D-arabinofuranosyl-9H-purine-6-thiol[7] (**5**), and 2-amino-9-(3-deoxy-β-D-*erythro*-pentofuranosyl)-9H-purine-6-thiol[8] (**6**). All of the compounds chosen with the exception of α-**3** have previously demonstrated significant activity in the leukemia L1210 test system[9] (Table I).

TABLE I
ACTIVITY OF STARTING NUCLEOSIDES AND DISULFIDES IN THE L1210 SCREEN

| Compound | —SH[a,b]— Dose, mg/kg | ILS, % | —S–S[b]— Dose, mg/kg | ILS, % |
|---|---|---|---|---|
| α-3 | | | 400 | 16 |
| β-3 | 200 | 56 | 4–600 | 30 |
| α-4 | 15 | 95 | 400 | 47 |
| β-4 | 20 | 99 | 150 | 32 |
| β-5 | 400 | 63 | 400 | 40 |
| β-6 | 200 | 89 | 75–150 | 33 |

[a] Optimal dose. [b] Optimal dose not determined; administered intraperitoneally in saline suspension.

The thiols were smoothly converted to disulfides in good yields upon treatment in the iodine-buffer system.[4,5]

With the exception of α- and β-**3** all of the nucleoside disulfides proved to be quite water insoluble and were recovered by filtration of the reaction mixture and conveniently purified by recrystallization from DMF–$H_2O$ (method A). The 2-deoxynucleoside disulfides (α- and β-**3**) had significant water solubility and were best recovered by lyophilization of the oxidation mixture and recrystallization of the residues from a minimum of $H_2O$ (method B). The physical constants of the new compounds are listed in Table II. All of the disulfides exhibited the characteristic uv spectra described previously.[4,10]

**Biological Testing.**—The preliminary screening results in the L1210 system for the new disulfides are listed in Table I along with the optimal doses for the parent thiols.[9] It would appear from these data that all of the

(1) Most of this work was performed under the auspices of the Cancer Chemotherapy National Service Center, National Institutes of Health, Public Health Service, under Contract PH-43-66-904. The views expressed in this paper are those of the authors and do not necessarily reflect those of the CCNSC.

(2) See R. H. Iwamoto, E. M. Acton, and L. Goodman, *J. Org. Chem.*, **27**, 3949 (1962), and leading references therein for a discussion of the rationale for the preparation of these compounds.

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(8) G. L. Tong, K. J. Ryan, W. W. Lee, E. M. Acton, and L. Goodman, *ibid.*, **32**, 859 (1967).

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(10) The uv spectra of the nucleoside disulfides showed the decomposition in base described previously.[4] Two peaks were evidenced in the base spectrum of **5**-S–S, one at ~312 mμ previously[4] attributed to the chromophore **5** and the second at ~355 mμ assigned[4] to an unknown component. Recent work on the base treatment of bis(4-thiouridine) disulfide [B. C. Pal, M. Uziel, D. G. Doherty, and W. E. Cohn, Abstracts, 156th National Meeting of the American Chemical Society, Atlantic City, N. J., Sept 1968, Paper 213] makes it appear likely that the long-wavelength absorption is due to the presence of the nucleoside sulfenic acid.

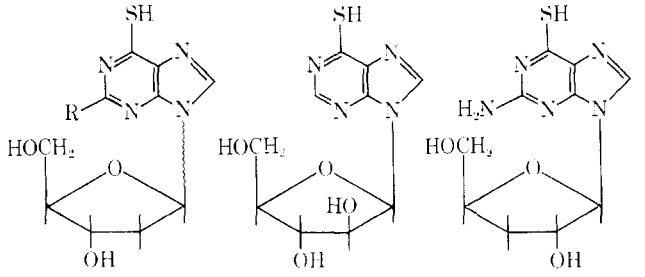
TABLE II
PHYSICAL CONSTANTS FOR NUCLEOSIDE DISULFIDES

| Starting thiol | Mp, °C | $[\alpha]_D$, deg | Yield, % | Method | Disulfides $\lambda_{max}$, mμ ($\epsilon \times 10^{-3}$)[d] | Formula[c] |
|---|---|---|---|---|---|---|
| α-3 | 140–150 (eff) | +54.7[a] | 32 | A | 290 (29.6) | $C_{20}H_{22}N_8O_8S_2 \cdot H_2O$ |
| | | | 74 | B | | |
| β-3 | ~100 (eff) | −23.4[b] | 80 | B | 289 (29.7) | $C_{20}H_{22}N_8O_8S_2 \cdot 1.33H_2O$ |
| α-4 | 208–209 | +110[b] | 61 | A | 322 (21.4) | $C_{20}H_{24}N_{10}O_6S_2 \cdot H_2O$ |
| β-4 | 214–217 (eff) | −33[a] | 81 | A | 322 (21.1) | $C_{20}H_{24}N_{10}O_6S_2 \cdot 1.67H_2O$ |
| β-5 | 213–216 | +13.5[b] | 84 | A | 288 (28.3) | $C_{20}H_{22}N_8O_8S_2$ |
| β-6 | 219–221 (eff) | −40[b] | 39 | A | 322 (20.9) | $C_{20}H_{24}N_{10}O_6S_2$ |

[a] $H_2O$. [b] DMF. [c] All compounds analyzed correctly for C, H, N, O, S. [d] In 1% DMSO–EtOH.

thiols with significant activity in this screen (25% increase in life span) retain activity but show a loss in potency upon conversion to the disulfides.

One of these compounds **5**-S–S has been tested further in *in vitro* systems at the 1.0-m*M* level and has been found to have negligible effects on inhibition of *de novo* purine biosynthesis (−7%) and adenine phosphoribosyl transferase (13%) and no effect on inosine synthesis by intact human erythrocytes. The compound does show significant inhibition of adenosine kinase (65.3%) and has been designated as warranting further study.[11] The disulfide of **5** was administered (intraperitoneally) to BAFI female mice which were sacrificed after 1 hr and the urine was examined by paper chromatography.[12] The disulfide-treated mice showed a minor spot corresponding to a maximum of 2.7% excretion of **5** as well as several somewhat more intense spots attributable to other metabolites. Mice administered **5** in a companion experiment excreted 37.5% of the unchanged nucleoside during the first hour. Incubation[12] of the disulfide with minces of mouse liver, mouse spleen, and CA755 ascites cells showed a trace conversion to **5** in the spleen mince and no conversion in contact with the other two tissues. It would appear that conversion of the disulfide to the thiol is extremely slow or that **5** is not a major metabolite. The ability of **5** to prolong the life of skin grafts in mice has been recorded previously.[13] A significant increase in the life span of transplanted goldfish scales has also been noted in our laboratories[14] for fish treated with **5**. This activity is retained upon disulfide formation with a lessening in potency (~50%).




3, R = H
4, R = $NH_2$
5
6

(11) The *in vitro* data were provided by Dr. Florence R. White, Head, Biochemistry Section, Drug Evaluation Branch, CCNSC, National Cancer Institute.

(12) Personal communication, Dr. G. A. LePage, Department of Biochemical Oncology, Stanford Research Institute, Menlo Park, Calif. We are greatly indebted to Dr. LePage for making these results available prior to publication.

(13) A. P. Kimball, G. A. LePage, B. Bowman, and S. J. Herriot, *Proc. Soc. Exp. Biol. Med.*, **119**, 248 (1965); A. P. Kimball, S. J. Herriot, and P. S. Allenson, *ibid.*, **126**, 181 (1967).

(14) Experiments were carried out under the direction of Dr. L. Levy, Head, Inflammation Section, Biological Sciences Department.

### Experimental Section

The thionucleosides used as starting materials were all prepared by literature methods.[2,6–8] The new compounds described all proved homogeneous upon tlc (Eastman Kodak silica gel plates–DMF–$CHCl_3$) or upon paper chromatography (*n*-BuOH–$H_2O$). Spots were visualized under uv light. Melting points are capillary melting points in open tubes on the Büchi apparatus and are uncorrected. The disulfides tenaciously retained recrystallization solvents which were evident in their nmr spectra (DMSO-$d_6$).

**6,6′-Dithiobis(9-β-D-arabinofuranosyl)-9H-purine (5-S–S) (Method A).**—Compound **5** (2.84 g, 0.010 mole) was dissolved in warm (45°) pH 7.6 $PO_4^{3-}$ buffer[4] (700 ml) and the solution was cooled to 30°. An "N $I_2$" solution[5] (10 ml, 0.005 mole) was added dropwise with stirring at such a rate that the color was dispersed between each addition. A white precipitate separated which was recovered by filtration, washed twice with $H_2O$ (10 ml) and then EtOH (10 ml), then dried *in vacuo* (2.4 g, 84%), mp 228–231°. A portion of the material (1.9 g) was recrystallized by dissolving in DMF (12 ml) and adding $H_2O$ (10 ml) to give colorless crystals (1.89 g), mp 213–216° dec. (See Table II.)

**6,6′-Dithiobis[9-(2-deoxy-β-D-*erythro*-pentofuranosyl)]-9H-purine Hydrate (β-3-S–S) (Method B).**—β-**3** (0.588 g, 0.0022 mole) was dissolved in warm pH 7.6 $PO_4^{3-}$ buffer[4] (90 ml) and treated dropwise at 38° with a solution of "N $I_2$"[5] (2.2 ml) as in method A. At the conclusion of the addition the faintly yellow solution was shell frozen and lyophilized. The residue was triturated twice with ice–water (2–3 ml), filtered, and vacuum dried (650 mg). The white solid was recrystallized from warm $H_2O$ (5 ml), with chilling. The crystals were recovered by filtration, washed with cold $H_2O$ (1 ml), and vacuum dried (25°) (0.528 g, 80%), mp ~70° softens, ~110° eff (see Table II).

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## Synthesis and Evaluation of Salts of Ethylene–Maleic Acid Copolymers as Antitumor Agents[1]

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Copolymers of ethylene and maleic acid inhibit significantly the growth of Sarcoma 180 in mice.[3]

(1) Presented at the Southwest Regional Meeting of the American Chemical Society, Little Rock, Ark., Dec 1967. Abstracted from the M.S. thesis of J. S. H., Oklahoma State University, May 1966.

(2) To whom inquiries should be addressed.

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