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Some Reactions of the 7,8-Dihydrothiazolo[2,3-*i*]purine Ring System

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7,8-Dihydrothiazolo[2,3-*i*]purines react with mercaptide ions to form 1-[2-(substituted-thio)ethyl] derivatives of purine-6(1*H*)-thiones. This reaction explains the unexpected formation of 9-benzyl-1-[2-(9-benzyl-9*H*-purin-6-ylthio)ethyl]-9*H*-purine-6(1*H*)-thione (Va) from the reaction of 9-benzyl-9*H*-purine-6(1*H*)-thione and 1,2-dibromoethane.

Among the derivatives of purine-6(1*H*)-thione that we have prepared for evaluation as anticancer agents are the 6,6′-(alkylenedithio)bispurines (I). 6,6′-(Methylenedithio)bispurine (Ia) and 6,6′-(tetramethylenedithio)bispurine (Ic) were readily prepared by the reaction of two equivalents of purine-6(1*H*)-thione with one equivalent of bromochloromethane and 1,4-dibromobutane, respectively, in *N*,*N*-dimethylformamide (DMF).[2] Both these compounds exhibited the expected absorption in the ultraviolet region of the spectrum, *i.e.*, the absorption maximum typical of 6-(alkylthio)purines around 292 mμ[2] but having twice the intensity.

However, when the reaction conditions described above were applied to 1,2-dibromoethane, no 6,6′-(ethylenedithio)bispurine (Ib)[3] appeared to be formed. Although the ultraviolet spectral data obtained on aliquots were equivocal, the chromatographic data definitely established that the reaction mixture contained only unchanged purine-6(1*H*)-thione and 7,8-dihydrothiazolo[2,3-*i*]purine (II).[5] The addition of a second equivalent of dibromoethane converted the remaining purine-6(1*H*)-thione to II. This result caused us to investigate the reaction of 1,2-dibromoethane with 9-benzyl-9*H*-purine-6(1*H*)-thione (IV),[6] as the dihydrothiazolo-[2,3-*i*]purine could not form in this case.[7] An examination of aliquots from this reaction mixture by means of ultraviolet spectroscopy and chromatography (see Fig. 1) revealed that the mixture contained two products in addition to unchanged 9-benzyl-9*H*-purine-6(1*H*)-thione. The addition of a second equivalent of 1,2-dibromoethane caused a shift in the ultraviolet spectrum as shown in Fig. 1 (1). The appearance of an absorption maximum at 325 mμ (at pH 13) indicated the presence of an N_1-substituted derivative of purine-6(1*H*)-thione.[8] A paper chromatogram of this mixture [Fig. 1 (2)] developed in a mixture of isopropyl alcohol and dilute ammonium hydroxide[9] showed two ultraviolet absorbing spots. The ultraviolet spectra of the two spots A and B were determined: the spectrum of the faster traveling spot (A) showed a single maximum at 297 mμ (shoulder at 288 mμ) and indicated that this material was 6,6′-(ethylenedithio)bis(9-benzyl-9*H*-purine) (Id); the spectrum of the second spot (B) showed two maxima, one at 288 mμ (shoulder at 296 mμ) and one at 340 mμ. This type of spectrum indicates the presence of both a thiol and a thione group attached to a purine ring and thus pointed to the unsymmetrical structure Va, 9-benzyl-1-[2-(9-benzyl-9*H*-purin-6-ylthio)ethyl-9*H*-purine-6(1*H*)-thione, for B.

To explain the formation of the product Va we examined more closely the reaction of 1,2-dibromoethane and IV, and found that if the reaction is carried out at 40° instead of 60°, a good yield of 3-benzyl-7,8-dihydro-3*H*-thiazolo-[2,3-*i*]purinium bromide (VIII) can be isolated. This compound was identified by its elemental analyses and ultraviolet spectrum. The ionic nature of the bromine was established by a Volhard titration in the cold. Furthermore, if one equivalent of VIII is allowed to react with one equivalent of 9-benzyl-9*H*-purine-6(1*H*)-thione (IV) at 70°, a 98% yield of Va is obtained. This outcome must be the result of an attack by the mercaptide ion of IV on C_8 of the dihydrothiazole ring of VIII. A similar ring opening by the attack of the ethoxide ion on 2,3-dihydrooxazolo[2,3-*a*]isoquinolinium chlorate and 2,3,5,6-tetrahydrooxazolo[2,3-*a*]isoquinolinium perchlorate has been described.[10]

Altering the conditions of the original reaction by adding one equivalent of 1,2-dibromoethane dropwise to a solution of two equivalents of IV in DMF

(1) This work was supported by funds from the C. F. Kettering Foundation and from the Cancer Chemotherapy National Service Center, National Cancer Institute, National Institutes of Health, Contract No. SA-43-ph-1740.

(2) T. P. Johnston, L. B. Holum, and J. A. Montgomery, *J. Am. Chem. Soc.*, **80**, 6265 (1958).

(3) This compound has been prepared by the reaction of 1,2-ethanedithiol with 6-chloropurine.[4]

(4) R. K. Robins, personal communication.

(5) R. W. Balsiger, A. L. Fikes, T. P. Johnston, and J. A. Montgomery, *J. Org. Chem.*, **26**, 3446 (1961).

(6) J. A. Montgomery and C. Temple, Jr., *J. Am. Chem. Soc.*, **83**, 630 (1961).

(7) This supposition proved to be not entirely true, as 9-benzyl-6-(2-bromoethylthio)-9*H*-purine exists only as the quaternary purinium bromide VIII. The chlorine of the corresponding chloro compound, 9-benzyl-6-(2-chloroethylthio)-9*H*-purine, was found to be largely in the covalent state.

(8) G. B. Elion in G. E. W. Wolstenholme, and C. M. O'Conners, eds., *The Chemistry and Biology of Purines* (A Ciba Foundation Symposium), J. and A. Churchill, Ltd., London, 1957, p. 39.

(9) R. Markham and J. D. Smith, *Nature*, **168**, 406 (1951).

(10) W. Schneider and B. Müller, *Chem. Ber.*, **93**, 1579 (1960).

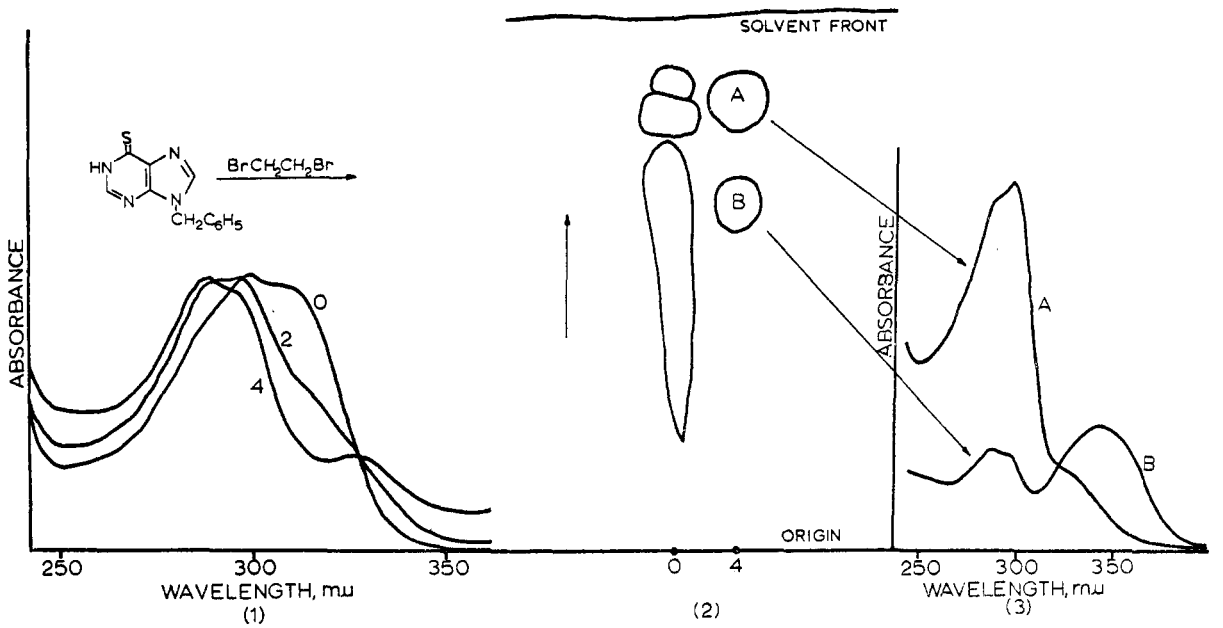


Fig. 1. Study of the reaction of 1,2-dibromoethane with 9-benzyl-9*H*-purine-6(1*H*)-thione. (1) Ultraviolet absorption spectra at pH 13 of reaction aliquots at: 0—"zero" time, 2—two hours, 4—four hours, with the addition of a second equivalent of 1,2-dibromoethane. (2) Paper chromatogram of reaction aliquots 0 and 4 above developed in a mixture of isopropyl alcohol and dilute ammonium hydroxide. (3) Ultraviolet absorption spectra of spots A and B.

held at 80° changed the course of the reaction entirely, and a 60% yield of 6,6′-(ethylenedithio)-bis(9-benzyl-9*H*-purine) (Id) was obtained with no evidence for the formation of any Va. Apparently, the low temperature, 40°, favors the formation of the purinium bromide VIII to the exclusion of Id, whereas the addition of 1,2-dibromoethane to a large excess of IV at the high temperature, 80°, favors the formation of Id to the exclusion of VIII. At the intermediate temperature, 60°, at which the reaction was first carried out, a mixture of Va (from the purinium bromide) and Id, in a much larger amount, is obtained.

The general nature of the dihydrothiazole ring opening was then established by the reaction of VIII with purine-6(1*H*)-thione and with *p*-toluenethiol to give 9-benzyl-1-[2-(purin-6-ylthio)ethyl]-9*H*-purine-6(1*H*)-thione (Vb) and 9-benzyl-1-[2-(*p*-tolylthio)ethyl]-9*H*-purine-6(1*H*)-thione (Vc), respectively.

To investigate these ring opening reactions further and firmly establish the identity of the proposed products Va, Vb, and Vc, we turned our attention to the reactions of 7,8-dihydrothiazolo-[2,3-*i*]-purine (II) itself. Reaction of II with purine-6(1*H*)-thione gave 1-[2-(purin-6-ylthio)-ethyl]purine-6(1*H*)-thione (VIb), whereas a similar reaction of II with *p*-toluenethiol gave 1-[2-(*p*-tolylthio)ethyl]purine-6(1*H*)-thione (VIc) and with hydrogen sulfide gave 1-(2-mercaptoethyl)purine-6(1*H*)-thione (VId). The structure of VIc was then established by an unequivocal synthesis. Inosine (X) was allowed to react[11] with 2-chloroethyl *p*-tolyl sulfide and the resulting 1-[2-(*p*-tolylthio)-ethyl]inosine (XI) hydrolyzed in ethanolic hydrochloric acid to give the corresponding hypoxanthine (XII), which was thiated with phosphorus pentasulfide in tetralin[8] to give VIc. This latter sample was identical in all respects (spectra, chromatographic behavior, and melting point) with the original material prepared by the *p*-toluenethiol ring opening.

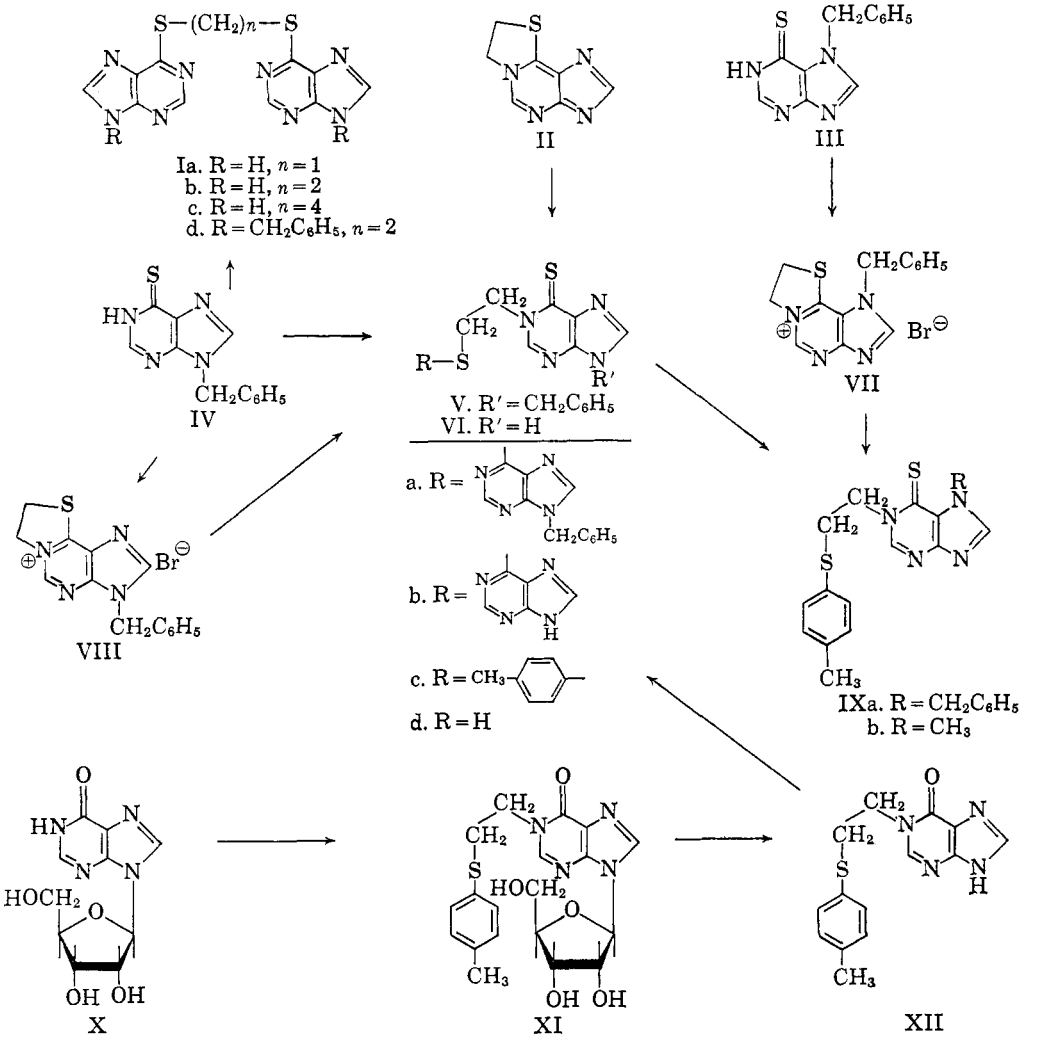
Benzylation of 1-[2-(*p*-tolylthio)ethyl]purine-6-(1*H*)-thione (VIc) with α-chlorotoluene gave 7-benzyl-1-[2-(*p*-tolylthio)ethyl]-7*H*-purine-6(1*H*)-thione (IXa) rather than the expected 9-isomer. The position of benzylation was established by preparing the same compound from 7-benzyl-7*H*-purine-6(1*H*)-thione (III)[6] via 1-benzyl-7,8-dihydro-1*H*-thiazolo[2,3-*i*]purinium bromide (VII). Methylation of VIc gave 7-methyl-1-[2-(*p*-tolylthio)ethyl]-7*H*-purine-6(1*H*)-thione (IXb).

EXPERIMENTAL

The melting points below 260° were determined on a Kofler Heizbank and are corrected; those above 260° were determined in a capillary in an aluminum block and are uncorrected. The ultraviolet spectra were determined in aqueous solution with a Beckman DK-2 (optical densities at the maxima with a Beckman DU) or a Cary Model 14. In the case of the paper chromatograms, the middle portion of the spots was cut out and taped to the cuvette carrier of the Cary Model 14 in a manner which allowed the light from the monochrometer to pass through the paper.

6,6′-(Methylenedithio)bispurine (Ia). Bromochloromethane (0.99 ml., 15 mmoles) was added to a well stirred mixture of purine-6(1*H*)-thione monohydrate (5.00 g., 29.4 mmoles), anhydrous potassium carbonate (4.06 g., 29.4 mmoles), and 45 ml. of DMF. After the spontaneous reaction subsided, the mixture was heated at 50–60° for 2.5 hr., then cooled and poured into cold water (300 ml.) The white precipitate that

(11) E. Shaw, *J. Am. Chem. Soc.*, 80, 3899 (1958).



formed was collected by filtration, washed with water and then acetone, and dried. This material was recrystallized twice from acetic acid, the acetic acid removed from the product by digestion with warm saturated bicarbonate solution, and the resulting crystalline solid washed with water and dried *in vacuo* at 110°; yield 2.39 g. (51%); m.p. 269° (dec.); λ_{max} in mμ ($\epsilon \times 10^{-3}$): *p*H 1—296 (31.6); *p*H 7—295 (33.8); *p*H 13—295 (28.8); C_2H_5OH— 294 (37.0).

Anal. Calcd. for $C_{11}H_8N_8S_2$: C, 41.75; H, 2.55; N, 35.41. Found: C, 41.55; H, 2.70; N, 35.23.

6,6'-(Tetramethylenedithio)bispurine (Ic). 1,4-Dibromobutane (0.52 ml., 4.4 mmoles) was added to a well stirred mixture of purine-6(1*H*)-thione monohydrate (1.50 g., 8.82 mmoles), potassium carbonate (1.28 g., 9.25 mmoles), and 15 ml. of dimethyl sulfoxide. After the exothermic reaction subsided, the mixture was heated with stirring for 2 hr. between 50 and 60°. Pouring the cooled mixture into cold water (90 ml.) gave a white precipitate which was collected by filtration, washed with water, and dried; yield 1.43 g. The material was recrystallized from DMF (20 ml.) and dried *in vacuo* at 110°; yield 1.1 g. (71%); m.p. 290–291°; λ_{max} in mμ ($\epsilon \times 10^{-3}$): *p*H 1—295 (26.5); *p*H 7—287 (27.7); *p*H 13—292 (27.9).

Anal. Calcd. for $C_{14}H_{14}N_8S_2$: C, 46.91; H, 3.94; N, 31.26. Found: C, 47.12; H, 4.29; N, 30.94.

6,6'-(Ethylenedithio)bis(9-benzyl-9H-purine) (Id). 1,2-Dibromoethane (0.20 ml., 2.3 mmoles) was added dropwise over a period of 1 hr. to a well stirred mixture of 9-benzyl-9*H*-purine-6(1*H*)-thione[6] (1.00 g., 4.13 mmoles), anhydrous potassium carbonate (0.571 g., 4.13 mmoles), and 15 ml. of DMF at 80°, and the mixture was kept at that temperature for 3 hr. The cooled reaction mixture was poured into ice water and this mixture refrigerated overnight. The white precipitate that formed was collected by filtration, washed with water, and air-dried. Recrystallization from 2-methoxyethanol gave fine light-yellow needles, which were dried *in vacuo* over phosphorus pentoxide for 4 hr. at 60°; yield 0.88 g. (60%); m.p. 204°; λ_{max} in mμ ($\epsilon \times 10^{-3}$): *p*H 1—287 (25.8), 292 sh. (25.2); *p*H 7—289 (25.2), 293 sh. (25.1); *p*H 13—290 (26.8), 296 sh. (26.7).

Anal. Calcd. for $C_{26}H_{22}N_8S_2$: C, 61.17; H, 4.34; N, 21.95. Found: C, 61.17; H, 4.49; N, 21.93.

9-Benzyl-1-[2-(9-benzyl-9H-purin-6-ylthio)ethyl]-9H-purine-6(1H)-thione (Va). A mixture of 3-benzyl-7,8-dihydro-3*H*-thiazolo[2,3-*i*]purinium bromide (1.00 g., 2.86 mmoles), 9-benzyl-9*H*-purine-6(1*H*)-thione[6] (0.690 g., 2.86 mmoles), and anhydrous potassium carbonate (0.394 g., 2.86 mmoles) in 15 ml. of DMF was heated for 1 hr. at 70° with stirring. The cooled reaction mixture was poured into ice water and this mixture refrigerated overnight. The white precipitate that formed was collected by filtration, washed with water, and air-dried. Recrystallization from a mixture of methyl alcohol, DMF, and water gave light-yellow needles; yield 1.43 g. (98%); m.p. 190°; λ_{max} in mμ ($\epsilon \times 10^{-3}$): *p*H 1—288 sh. (18.3), 293 (19.4), 326 (20.2); *p*H 7—288 sh. (18.5), 293 (19.4), 325 (22.4); *p*H 13—289 sh. (19.3), 294 (20.7), 324 (20.8).

Anal. Calcd. for $C_{26}H_{22}N_8S_2$: C, 61.17; H, 4.34; N, 21.95. Found: C, 60.78; H, 4.51; N, 21.92.

9-Benzyl-1-[2-(purin-6-ylthio)ethyl]-9H-purine-6(1H)-thione (Vb). A well stirred mixture of 3-benzyl-7,8-dihydro-3*H*-thiazolo[2,3-*i*]purinium bromide (500 mg., 1.43 mmoles), purine-6(1*H*)-thione monohydrate (245 mg., 1.43 mmoles), potassium carbonate (197 mg., 1.43 mmoles), and 5 ml. of DMF was heated for 24 hr. at 75–85°. The reaction mixture was then cooled to room temperature and poured into 20 ml. of cold water. The white solid that formed was collected by filtration, recrystallized from aqueous DMF, and then dried over phosphorus pentoxide at 100° for 18 hr. *in vacuo;* yield 121 mg. (20%); m.p. 232°; λ_{max} in mμ ($\epsilon \times 10^{-3}$): *p*H 1—290 (17.1), 324 (16.0); *p*H 7—290 (12.8), 325 (16.4); *p*H 13—295 sh. (14.1), 302 (15.4), 321 (15.6).

Anal. Calcd. for $C_{19}H_{16}N_8S_2 \cdot {}^3/_4\ H_2O$: C, 52.70; H, 4.18; S, 14.82. Found: C, 52.73; H, 4.40; S, 14.85.

9-Benzyl-1-[2-(p-tolylthio)ethyl]-9H-purine-6(1H)-thione (Vc). A mixture of 3-benzyl-7,8-dihydrothiazolo[2,3-*i*]-purinium bromide (500 mg., 1.43 mmoles), *p*-toluenethiol (266 mg., 2.14 mmoles), potassium carbonate (197 mg., 1.43 mmoles), and 5 ml. of DMF was heated at 75–85° for 4 hr. with stirring. The mixture, cooled to room temperature, was poured into 15 ml. of cold water, a yellow solid being precipitated. Recrystallization of the crude solid from methyl alcohol afforded tiny yellow crystals, which were collected, dried over phosphorus pentoxide *in vacuo* at 60° for 2 hr.; yield 412 mg. (74%); m.p. 156°; λ_{max} in mμ ($\epsilon \times 10^{-3}$): *p*H 1—329 (16.3); *p*H 7—328 (20.3); *p*H 13—328 (20.0).

Anal. Calcd. for $C_{21}H_{20}N_4S_2$: C, 64.27; H, 5.14; N, 14.28; S, 16.31. Found: C, 64.46; H, 5.04; N, 14.11; S, 16.42.

1-[2-(Purin-6-ylthio)ethyl]purine-6(1H)-thione hydrate (VIb). To a well stirred mixture of purine-6(1*H*)-thione monohydrate (483 mg., 2.80 mmoles), potassium carbonate (387 mg., 2.80 mmoles), and 5.0 ml. of DMF was added 500 mg. (2.80 mmoles) of 7,8-dihydrothiazolo[2,3-*i*]purine.[5] The resulting mixture was stirred at room temperature for 10 min. and then heated at 110–115° for 4 hr., a white solid being formed. The reaction mixture was cooled to room temperature and poured into ice water (20 ml.). The aqueous mixture was made neutral by the addition of a few drops of concentrated hydrochloric acid and refrigerated overnight. The white solid that formed was collected by filtration, recrystallized from ethanolic DMF, and dried over phosphorus pentoxide at 60° *in vacuo* for 4 hr.; yield 800 mg. (86%); m.p. 270°; λ_{max} in mμ ($\epsilon \times 10^{-3}$): *p*H 1—292 (14.4), 325 (16.4); *p*H 7—291 (13.0), 326 (16.2); *p*H 13—302.5 (16.0), 328 (18.5).

Anal. Calcd. for $C_{12}H_{10}N_8S_2 \cdot H_2O$: C, 42.59; H, 3.25; N, 33.07; S, 18.90. Found: C, 42.67; H, 3.63; N, 32.56; S, 18.43.

1-[2-(p-Tolylthio)ethyl]purine-6(1H)-thione (VIc). *A.* A mixture of 7,8-dihydrothiazolo[2,3-*i*]purine[5] (500 mg., 2.80 mmoles), *p*-toluenethiol (348 mg., 2.80 mmoles), potassium carbonate (388 mg., 2.80 mmoles), and 5 ml. of DMF was stirred for 3 hr. at 40–45°. The cooled reaction mixture was poured into 25 ml. of cold water and refrigerated overnight. The white solid that formed was collected by filtration and recrystallized from methyl alcohol. When the aqueous DMF filtrate was adjusted to *p*H 5 with hydrochloric acid, additional product precipitated. This material was also recrystallized from methyl alcohol and the two samples combined; yield 520 mg. (60%); m.p. 210°; λ_{max} in mμ ($\epsilon \times 10^{-3}$): *p*H 1—245 (sh.) (9.0), 329 (14.4); *p*H 7—241 (12.3), 329 (16.2); *p*H 13—241 (13.4), 329 (20.8).

Anal. Calcd. for $C_{14}H_{14}N_4S_2$: C, 55.62; H, 4.67; S, 21.17. Found: C, 55.51; H, 4.84; S, 21.26.

B. A mixture of 1-[2-(*p*-tolylthio)ethyl]hypoxanthine (XII) (500 mg., 1.75 mmoles) and phosphorus pentasulfide (1.31 g., 5.85 mmoles) in tetralin (12 ml.) was heated for 5 hr. at 180–185° with stirring. The reaction mixture was then cooled to 0° and filtered. The insoluble residue was first washed with petroleum ether to remove adhering solvent and then extracted with boiling benzene (5 × 10 ml.). The combined extracts were evaporated *in vacuo*. Recrystallization of the yellowish-white residue from methyl alcohol with charcoal treatment gave white crystals, which were dried *in vacuo* over phosphorus pentoxide at 60° for 4 hr.; yield 210 mg.; m.p. 210°. This material was identical with that prepared in *A* above; a mixed melting point was undepressed.

1-(2-Mercaptoethyl)purine-6(1H)-thione (VId). A stirred mixture of 7,8-dihydrothiazolo[2,3-*i*]purine[5] (500 mg., 2.80 mmoles), potassium carbonate (387 mg., 2.80 mmoles), and 5 ml. of DMF was heated to 50°, and hydrogen sulfide was slowly bubbled through the mixture for 1 hr. The reaction mixture, cooled to room temperature, was poured into 25 ml. of cold water and refrigerated overnight. The white solid that formed was collected and extracted with boiling propyl alcohol (5 × 10 ml.), and the combined extracts evaporated to dryness under reduced pressure. Recrystallization of the residue from ethyl alcohol gave white crystals, which were dried over phosphorus pentoxide *in vacuo* at 60° for 4 hr.; yield 120 mg. (20%); m.p. 188–190°; nitroprusside test for thiol—positive; λ_{max} in mμ ($\epsilon \times 10^{-3}$): *p*H 1—228 (11.2), 326 (16.7); *p*H 7—236 (10.4), 325 (18.9); *p*H 13—234 (17.4), 325 (21.5); $\bar{\nu}^{KBr}_{max}$ (cm.$^{-1}$) 2490 (SH).

Anal. Calcd. for $C_7H_8N_4S_2$: C, 39.60; H, 3.80; N, 26.39; S, 30.21. Found: C, 39.60; H, 3.97; N, 26.65; S, 30.17.

A subsequent experiment starting with 6.00 g. of the thiazolopurine gave an 80% yield of the thiol, m.p. 190°.

1-Benzyl-7,8-dihydro-1H-thiazolo[2,3-i]purinium bromide (VII). 1,2-Dibromoethane (0.18 ml., 2.1 mmoles) was added dropwise to a stirred mixture of 7-benzyl-7*H*-purine-6(1*H*)-thione[6] (500 mg., 2.06 mmoles), anhydrous potassium carbonate (285 mg., 2.06 mmoles), and 5 ml. of DMF, and the resulting mixture was heated at 40–50° for 3 hr., a white solid being formed during this period. The reaction mixture was cooled in an ice bath for 2 hr., and then the solid was collected and extracted in a Soxhlet extractor with 35 ml. of acetonitrile. The product, which crystallized from the extract on cooling as tiny white needles, was collected and dried over phosphorus pentoxide *in vacuo* at 100°; yield 210 mg. (30%); m.p. 262°; λ_{max} in mμ ($\epsilon \times 10^{-3}$): *p*H 1—313 (12.2); *p*H 7—313 (12.1); *p*H 13—288 (12.1).

Anal. Calcd. for $C_{14}H_{13}BrN_4S$: C, 48.14; H, 3.75; N, 16.05; S, 9.19. Found: C, 48.43; H, 3.88; N, 16.22; S, 9.20.

3-Benzyl-7,8-dihydro-3H-thiazolo[2,3-i]purinium bromide (VIII). 1,2-Dibromoethane (0.18 ml., 2.1 mmoles) was added dropwise to a stirred mixture of 9-benzyl-9*H*-purine-6(1*H*)-thione[6] (500 mg., 2.06 mmoles), anhydrous potassium carbonate (285 mg., 2.06 mmoles), and DMF (10 ml.), and then the mixture was heated at 40° for 1 hr. The resulting white suspension was filtered and the solid extracted with acetonitrile. Concentration of the combined filtrate and extract *in vacuo* gave a white crystalline solid, which was dissolved in methyl alcohol and then precipitated from solution by the addition of ethyl ether; yield 535 mg. (74%). The analytical sample was obtained by recrystallization from methyl alcohol–ethyl ether; λ_{max} in mμ ($\epsilon \times 10^{-3}$): *p*H 1—302 (13.8); *p*H 7—302 (13.3); *p*H 13—288–289 (7.74); CH_3OH—302 (13.7).

Anal. Calcd. for $C_{14}H_{13}BrN_4S$: C, 48.14; H, 3.75; Br, 22.88; N, 16.04. Found: C, 47.71; H, 3.99; Br, 22.80; N, 15.96.

7-Benzyl-1-[2-(p-tolylthio)ethyl]-7H-purine-6(1H)-thione (IXa). *A.* A mixture of 1-benzyl-7,8-dihydro-1*H*-thiazolo-[2,3-*i*]purinium bromide (VII) (500 mg., 1.43 mmoles), *p*-toluenethiol (266 mg., 2.14 mmoles), potassium carbonate (197 mg., 1.43 mmoles), and 5 ml. of DMF was heated for 24 hr. at 70–80°. The reaction mixture was then cooled to room temperature and poured into 20 ml. of cold water. The cheese-like solid that formed when the mixture was refrigerated for 6 hr. was collected by filtration and recrystallized from methyl alcohol; yield 520 mg. (93%); m.p. 108°; λ_{max} in mμ ($\epsilon \times 10^{-3}$): *p*H 1—247.5 (11.7), 333 (12.8); *p*H 7—247.5 (15.2), 340 (13.8); *p*H 13—246.5 (15.1), 339 (13.6).

Anal. Calcd. for $C_{21}H_{20}N_4S_2 \cdot {}^1/_4\ H_2O$: C, 63.60; H, 5.19; S, 16.15. Found: C, 63.55; H, 5.11; S, 16.04.

B. A mixture of 1-[2-(*p*-tolylthio)ethyl]purine-6(1*H*)-

thione (VIc) (500 mg., 1.65 mmoles), potassium carbonate (228 mg., 1.65 mmoles), α-chlorotoluene (0.21 ml., 1.8 mmoles), and 5 ml. of DMF was stirred at 60–65° for 3 hr. The reaction mixture was cooled and poured into 20 ml. of cold water, a yellowish cheese-like solid being formed. After overnight refrigeration, the aqueous solution was decanted from the solid, which was then recrystallized from methyl alcohol to give pale yellow crystals, which were washed with ether and air-dried; yield 540 mg. (83%); m.p. 109–110°; λ_{max} in mμ ($\epsilon \times 10^{-3}$): *p*H 1—247 (11.8), 333 (13.2); *p*H 7—247 (14.6), 335 (14.1); *p*H 13—246 (15.5), 337 (14.1). A mixed melting point with an authentic sample of the 7-isomer was not depressed.

Anal. Calcd. for $C_{21}H_{20}N_4S_2 \cdot {}^1/_4\ H_2O$: C, 63.60; H, 5.19; S, 16.15. Found: C, 63.77; H, 5.12; S, 16.24.

7-Methyl-1-[2-(p-tolylthio)ethyl]-7H-purine-6(1H)-thione (IXb). Iodomethane (0.063 ml., 1.0 mmole) was added dropwise to a stirred mixture of 1-[2-(*p*-tolylthio)ethyl]-purine-6(1*H*)-thione (VIc) (280 mg., 0.92 mmole), potassium carbonate (127 mg., 0.92 mmole), and 3 ml. of DMF. The mixture was heated at 40–50° for 4 hr., and then poured into 20 ml. of cold water. The thick red oil that formed was separated by decantation and crystallized from aqueous methyl alcohol; yield 29 mg. (10%); m.p. 144°; λ_{max} in mμ ($\epsilon \times 10^{-3}$): *p*H 1—247 (9.4), 330 (14.0); *p*H 7—244 (11.4), 330 (16.0); *p*H 13—244 (11.9), 330 (15.7).

Anal. Calcd. for $C_{15}H_{16}N_4S_2$: C, 56.90; H, 5.10; N, 17.73; S, 20.20. Found: C, 56.89; H, 5.49; N, 17.65; S, 19.90.

1-[2-(p-Tolylthio)ethyl]hypoxanthine (XII). To a stirred solution of inosine (1.00 g., 3.76 mmoles) in warm dimethyl sulfoxide (10 ml.) was added potassium carbonate (569 mg.) and 2-chloroethyl *p*-tolyl sulfide[12] (700 mg.). The mixture was heated for 18 hr. at 80–100°, and then cooled to room temperature and poured into 50 ml. of cold water. The volatiles were removed under reduced pressure and the brown residue was refluxed for 2 hr. with a mixture of 5 ml. of concentrated hydrochloric acid and 95 ml. of ethyl alcohol. Again the volatiles were removed under reduced pressure, and the brown syrup that remained dissolved in water. This aqueous solution was treated with charcoal, filtered, adjusted to pH 5 with sodium hydroxide solution, and refrigerated overnight. The brown solid that was deposited was recrystallized from methyl alcohol to give a white solid which was dried at 60° for 4 hr. over phosphorus pentoxide, *in vacuo;* yield 210 mg. (20%); m.p. 168°; λ_{max} in mμ ($\epsilon \times 10^{-3}$): *p*H 1—249.5 (13.4); *p*H 7—253 (12.5); *p*H 13—258 (12.8).

Anal. Calcd. for $C_{14}H_{14}N_4OS$: C, 58.72; H, 4.93; N, 19.57. Found: C, 58.62; H, 4.90; N, 19.36.

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Nitrogen Mustard Analog of Thiocytosine

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2,4-Dimercaptopyrimidine was interacted with diethanolamine in the presence of pyridine to yield *N,N*-bis-(2-hydroxyethyl)thiocytosine, which was subsequently treated with thionylchloride to produce the nitrogen mustard analog. Some preliminary biological studies on the latter compound are indicated.

A number of purine and pyrimidine derivatives which are structurally related to basic chemical structures that are normally present in the cell are known to be effective antitumor agents. The replacement of hydroxy groups has been successful in the production of antitumor agents; for example, the hydroxy group of hypoxanthine has been replaced by the thiol grouping to yield 6-mercaptopurine,[2] an efficient antitumor agent in a number of systems.[3] Alkylating agents containing the nitrogen mustard radical (bis-(2-chloroethyl)-amino-) have also been widely used on a large number of different types of chemical structures.[4]

In the present study an attempt was made to place both of these types of groupings in a single compound, and the structure chosen for the "carrier molecule"[5] was the natural pyrimidine metabolite, cytosine. 4-[Bis-(2-chloroethyl)amino]-2-pyrimidinethiol was accordingly synthesized, and the antitumor activity on an implanted RC mammary adenocarcinoma, as well as some antimicrobial activities, was determined.

The syntheses of only a few pyrimidines containing the nitrogen mustard grouping attached directly to the ring have been described—e.g.,

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