[CONTRIBUTION FROM THE DEPARTMENT OF PHARMACOLOGY, YALE UNIVERSITY SCHOOL OF MEDICINE]

Potential Antiradiation Agents. β-Thioethylamino Derivatives of Nucleic Acid Constituents[1,2]

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As part of a program involving the synthesis of potential antiradiation agents to be localized in cell nucleic acids, a group of β-thioethylamino derivatives of adenine and uracil was prepared.

Since the discovery in 1949 that animals could be partially protected against the effects of ionizing radiation by prior administration of cysteine or reduced glutathione,[3] large numbers of thio compounds have been screened as potential antiradiation drugs. Among these, cysteamine (β-mercaptoethylamine) remains one of the most effective agents.

In the hope of increasing the specificity of this action and of protecting the genetic material of the cell which is peculiarly sensitive to radiation damage[4] the synthesis of a group of compounds has been undertaken in which cysteamine is attached through its amino group to a series of purine and pyrimidine components of nucleic acids. It had previously been shown that *N*-substitution of cysteamine can be compatible with radio-protective activity.[5] Thus, the possibility exists that compounds of the type proposed might localize protection from ionizing radiation in the ribonucleic and deoxyribonucleic acids of the cells to be shielded.

As according to Kornberg[6] any nucleotide to be incorporated into the double helix of deoxyribonucleic acid (DNA) must be capable of forming hydrogen bonds with preexisting deoxyribonucleic acid, bulky extraneous groups would have to be attached at a point of the carrier molecule where neither hydrogen bonding nor ribotidation or deoxyribotidation would be interfered with. For this reason the 5- position of pyrimidines and the 8- position of purines would seem to be well suited for the attachment of groups to be introduced into nucleic acids.[7,8]

In addition to the preparation of *N*-cysteaminyl derivatives it seemed of interest to investigate the introduction of the bis(β-thioethyl)amino group into suitable carrier molecules to yield double-armed cysteamines. While attempts to isolate bis-(β-mercaptoethyl)amine have thus far been unsuccessful, the oxidized form of this compound, 1,2-dithia-5-azepane can be prepared in good yield.[9] Thus, the preparation of *N*-substituted 1,2-dithia-5-azepanes appeared feasible.

The uracilyl compounds reported in this study were prepared by the reaction sequences shown.

β-Benzylthioethylamine, prepared by the reaction of ethylene imine with α-toluenethiol, was found to react with 5-bromouracil to yield 5-(β-benzylthioethylamino)uracil (I). This compound could also be obtained by the addition of α-toluenethiol to 5-(β-chloroethylamino)uracil ("one-armed uracil mustard").[10] Debenzylation with sodium in liquid ammonia[11] in the usual fashion resulted in the formation of 5-(β-mercaptoethylamino)uracil. Attempts to isolate this compound as such were unsuccessful; however, oxidation readily yielded its disulfide form (II).

Bis(β-benzylthioethyl)amine[12] reacted with 5-bromouracil to form 5-bis(β-benzylthioethylamino)-uracil (IV). An alternative synthesis of this compound involved the addition of the sodium salt of benzyl mercaptan to 5-bis(β-chloroethylamino)-uracil ("uracil mustard").[13] Reduction of the bis-benzylthio compound (IV) with sodium in liquid ammonia followed by air oxidation yielded *N*-(5-uracilyl)-1,2-dithia-5-azepane (V). Attempts to prepare this compound directly by the reaction of 5-bromouracil with 1,2-dithia-5-azepane[9] were unsuccessful.

N,N'-Bis(8-adeninyl)cystamine (X) was prepared in analogous fashion from 8-(β-benzylthioethylamino)adenine (IX), which in turn was ob-

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(2) This work was supported, in part, by a contract (DA-49-193-MD-2106) from the U. S. Army Medical Research and Development Command, Office of the Surgeon General.

(3) For a review of this field the reader is referred to A. Pihl and L. Eldjarn, *Pharmacol. Revs.*, **10**, 437 (1958).

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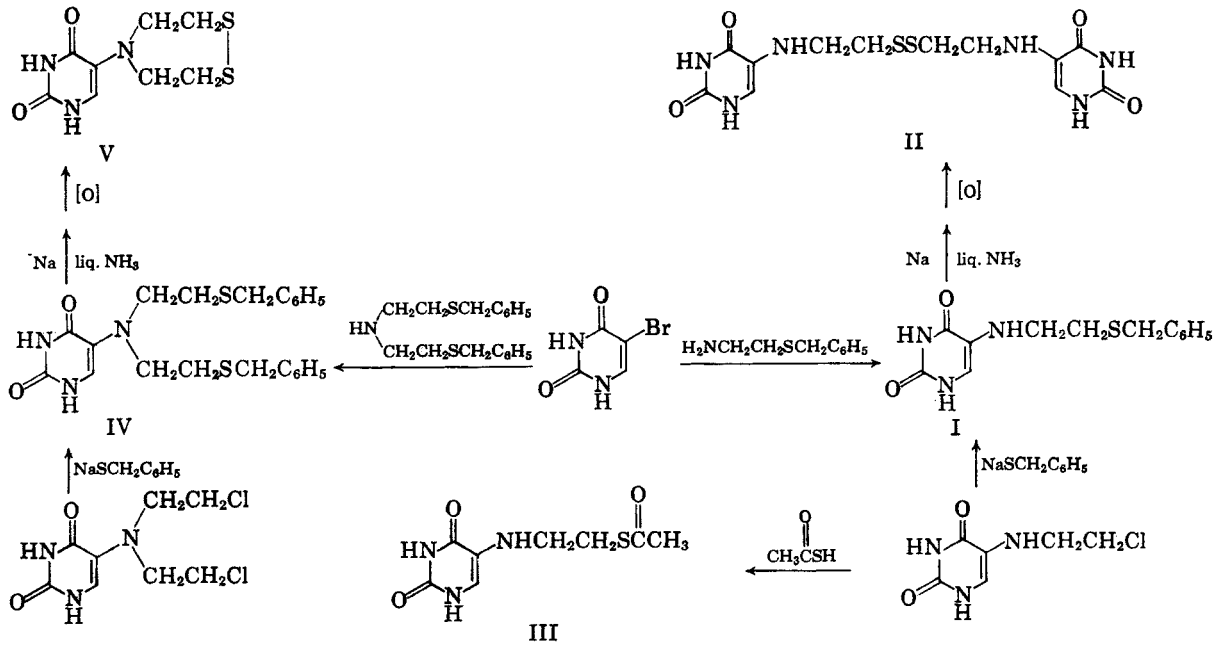
(9) W. H. H. Günther and H. G. Mautner, *J. Am. Chem. Soc.*, **82**, 2762 (1960).

(10) A. Benitez, L. O. Ross, L. Goodman, and B. R. Baker, *J. Am. Chem. Soc.*, **82**, 4585 (1960).

(11) R. S. Sifferd and V. du Vigneaud, *J. Biol. Chem.*, **108**, 757 (1935).

(12) W. H. H. Günther and H. G. Mautner, unpublished data.

(13) D. A. Lyttle and H. G. Petering, *J. Am. Chem. Soc.*, **80**, 6459 (1958).

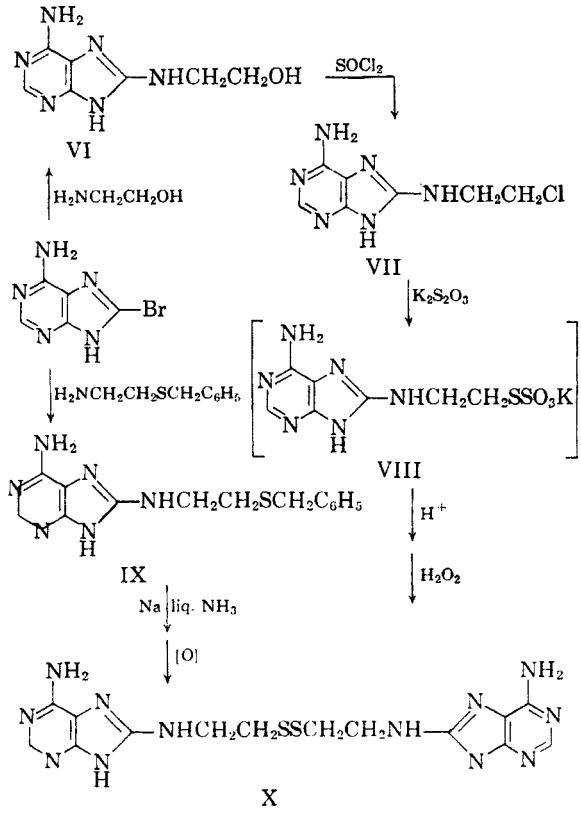


tained by the reaction of 8-bromoadenine with β-benzylthioethylamine.

Bisadeninylcystamine gave satisfactory carbon and hydrogen, but variable nitrogen analyses. Therefore, this compound was made by an alternative synthesis. 8-(β-Chloroethylamino)adenine) ("single-armed adenine mustard") (VII) was prepared and converted to the Bunte salt (VIII), acid treatment of which yielded the disulfide (X), which proved to be identical with the material obtained from 8-(β-benzylthioethylamino)adenine (IX). These reactions are summarized in VI–X.

Throughout the synthetic sequences reported considerable difficulties were encountered in isolating mercaptans as such, because of the rapidity of air oxidation. Some radioprotective substances retain activity on being oxidized, presumably because the disulfides formed are readily reduced *in vivo*.[14] With other mercapto compounds, cysteine, for instance, oxidation to the disulfide abolishes antiradiation activity completely.

The synthesis of solid mercaptan derivatives, stable to oxidation, but capable of being hydrolyzed within the host to be protected against ionizing radiation, is, therefore, of interest. In one approch to this problem the synthesis of thioacyl derivatives is being investigated. Acyl derivatives of coenzyme A, in which acyl groups are attached to the sulfur of the terminal cysteamine residue of the coenzyme, are fairly stable under physiological conditions, enabling them to be transported to sites where enzymes involved in transacylation reactions can attack them.[15] It is



hoped that thioacyl derivatives of other *N*-substituted cysteamines might behave similarly. As a model compound *S*-acetyl-*N*-(5-uracilyl)cysteamine (IV) was prepared by the reaction of 5-(β-chloroethylamino)uracil with thioacetic acid.

The compounds described have been submitted to the Walter Reed Army Institute of Research for biological testing.

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EXPERIMENTAL

β-Benzylthioethylamine. A solution of 9.6 g. (0.223 mole) of ethyleneimine in 80 ml. of absolute ethanol was added dropwise to a stirred solution of 25 g. (0.201 mole) of α-toluenethiol in 400 ml. of absolute ethanol. The mixture was heated to reflux for 5 hr. Excess solvent was removed under reduced pressure and the product obtained in 81% yield after distillation; b.p. 78–80° (0.15 mm.),[16] $n_D^{25.4}$ 1.5740.

5-β-Benzylthioethylaminouracil (I). *Method A.* A mixture of 5.0 g. (0.026 mole) of 5-bromouracil and 10.0 g. (0.06 mole) of β-benzylthioethylamine in 20 ml. of ethylene glycol monoethyl ether was heated to reflux for 1 hr. After addition of 50 ml. of diethyl ether, the white, crystalline product was removed by filtration and washed with water and ether. Recrystallization from ethylene glycol yielded 5.2 g. (71%) of product melting at 228–230°.[17]

Anal.[18] Calcd. for $C_{12}H_{13}N_3O_2S$: C, 56.30; H, 5.45; N, 15.15; S, 11.56. Found: C, 56.55; H, 5.66; N, 15.15; S, 11.56.

Method B. A solution of 0.17 g. (0.074 g.-atom) of sodium, 0.454 g. (0.0037 mole) of α-toluenethiol, and 0.7 g. (0.0037 mole) of 5-(β-chloroethylamino)uracil[10] in a mixture of 30 ml. of absolute ethanol and 25 ml. of ethylene glycol monoethyl ether was heated to reflux for 5 hr. The solvent was removed under suction and the residue washed with water and ether. Recrystallization from ethylene glycol monomethyl ether yielded 0.2 g. (24%) of product which showed no melting point depression when mixed with material prepared by method A.

N,N'-Bis(5-uracilyl)cysteamine (II). To a mixture of 6.0 g. (0.0216 mole) of 5-β-benzylthioethylaminouracil and 150 ml. of liquid ammonia, which was cooled in a Dry Ice–acetone bath, 1.92 g. (0.0834 g.-atom) of sodium was added in small portions with stirring. When the addition was completed the blue solution was treated with 5.0 g. of amomnium chloride and stirring continued. When most of the ammonia had evaporated the residue was dissolved in cold water and the resulting mixture filtered. The filtrate was neutralized with glacial acetic acid. The precipitate was collected and washed with water and ether. The product was dissolved in dilute sodium hydroxide and reprecipitated with acetic acid. A yield of 2.2 g. (27%) was obtained. The product was recrystallized from ethylene glycol monomethyl ether.

Ultraviolet spectrum. pH 10, λ_{max} 290 mμ, ϵ_{max}, 8300.

Anal. Calcd. for $C_{12}H_{16}N_6O_4S_2$: C, 38.70; H, 4.33; N, 22.57; S, 17.22. Found: C, 38.82; H, 4.97; N, 22.86; S, 16.97.

5-(β-Acetylthioethylamino)uracil (III). A suspension of 1.5 g. (0.008 mole) of 5-(β-chloroethylamino)uracil[10] in 40 ml. of water was treated with 0.60 g. (0.008 mole) of thioacetic acid and 0.67 g. (0.008 mole) of sodium bicarbonate. The mixture was heated to reflux temperature under nitrogen for 16 hr. After filtration the solution was evaporated to dryness. The residue was dissolved in water and poured through a Darco column. After all sodium chloride had been removed, the product was eluted with ethanol. Evaporation of the ethanol fraction yielded 0.3 g. (16%) of product which was recrystallized from water; m.p. 325–327°.

Anal. Calcd. for $C_6H_{11}N_3O_3S$: C, 41.91; H, 4.83; N, 18.33. Found: C, 42.01; H, 4.57; N, 18.21.

5-Bis(β-benzylthioethylamino)uracil (IV). *Method A.* To 30 ml. of absolute ethyl alcohol were added 0.3 g. (0.013 g.-atom) of sodium, 0.78 g. (0.00634 mole) of α-toluenethiol, and 0.8 g. (0.00317 mole) of 5-bis(β-chloroethyl)aminouracil.[10] The mixture was refluxed for 5 hr. and the solvent removed under vacuum. The residue was dissolved in water and neutralized with acetic acid. Addition of ether caused the precipitated oil to crystallize. Recrystallization from methyl alcohol yielded 0.3 g. (31%) of product, m.p. 158–160°.

Anal. Calcd. for $C_{22}H_{28}N_4O_2S_2$: C, 61.79; H, 5.88; N, 9.82; S, 14.96. Found: C, 61.39; H, 5.90; N, 9.87; S, 14.99.

Method B. A mixture of 1.5 g. (0.00785 mole) of 5-bromouracil and 4.0 g. (0.0126 mole) of bis(β-benzylthioethyl)amine[12] in 30 ml. of ethylene glycol monoethyl ether was heated to reflux for 17 hr. and the solvent removed under reduced pressure. The residue was washed with water and allowed to crystallize from ethyl ether. Recrystallization from methanol yielded 0.4 g. (12%) of product, showing no melting point depression when mixed with a sample prepared by method A.

N-(5-Uracilyl)1,2-dithia-5-azepane (V). A mixture of 0.4 g. (0.0009 mole) of 5-bis(β-benzylthioethyl)aminouracil and 30 ml. of liquid ammonia in a Dry Ice–acetone bath was treated with 0.1 g. (0.00434 g.-atom) of sodium. To the blue solution 0.5 g. of ammonium chloride was added and stirring continued until all the ammonia had evaporated. The residue was dissolved in cold water and filtered. The filtrate was neutralized with acetic acid. The white precipitate was collected, washed with water and ether, and crystallized from methyl alcohol. A yield of 0.1 g. (56%) of product melting at 190–193° was obtained.

Anal. Calcd. for $C_8H_{11}N_3O_2S_2$: C, 39.16; H, 4.51; N, 17.12; S, 26.13. Found: C, 39.14; H, 4.99; N, 17.00; S, 26.31.

8-(β-Hydroxyethylamino)adenine (VI). A mixture of 6.0 g. (0.0263 mole) of 8-bromoadenine and 9.2 g. of monoethanolamine in 60 ml. of ethylene glycol monomethyl ether was heated to boiling over a period of 20 hr. The solvent was removed under vacuum and the residue washed successively with benzene, chloroform, and ether. Recrystallization from hot water yielded 4.2 g. (82%) of crystalline product melting at 262–265°.

Anal. Calcd. for $C_7H_{10}N_6O$: C, 43.29; H, 5.13; N, 43.27. Found: C, 43.18; H, 5.33; N, 43.30.

8-(β-Chloroethyl)aminoadenine dihydrochloride (VII). A mixture of 3.0 g. (0.0154 mole) of 8-(β-hydroxyethylamino)-adenine, 25 ml. of thionyl chloride, and 0.5 ml. of dimethylformamide in 50 ml. of chloroform was heated to reflux for 3 hr. The solvent was removed under vacuum and the residue dissolved in absolute methanol saturated with anhydrous hydrogen chloride. On chilling 2.5 g. (68%) of white product decomposing over a range of 215–224° was obtained.

Ultraviolet spectrum. Methanol, λ_{max} 296 mμ, ϵ_{max} 22,600.

Anal. Calcd. for $C_7H_{11}N_6Cl_3$: C, 29.43; H, 3.91; N, 29.43; total Cl, 37.24; ionic Cl, 24.83. Found; C, 29.53; H, 4.19; N, 29.34; total Cl, 36.47; ionic Cl, 24.80.

8-(β-Benzylthioethylamino)adenine (IX). A mixture of 1.5 g. (0.007 mole) of 8-bromoadenine and 3.5 g. (0.021 mole) of β-benzylthioethylamine in 20 ml. of ethylene glycol monomethyl ether was heated to reflux with stirring for 18 hr. The solvent was removed under reduced pressure. The brownish residue was washed successively with benzene, water, and ether. The product was recrystallized from aqueous methanol. A yield of 1.3 g. (62%) of product melting at 213–215° was obtained.

Anal. Calcd. for $C_{14}H_{16}N_6S$: C, 56.16; H, 5.05; N, 28.07; S, 10.71. Found: C, 56.13; H, 5.66; N, 28.10; S, 11.13.

N,N'-Bis(8-adeninyl)cystamine (X). *Method A.* 8-(β-Benzylthioethylamino)adenine was debenzylated in the same fashion as the analogous uracil derivative. The mercaptan was oxidized by the use of 3% hydrogen peroxide. Product decomposing at 205° was obtained in 35% yield.

Ultraviolet spectrum. pH 10, λ_{max}, 280 mμ, ϵ_{max} 28,100.

Anal. Calcd. for $C_{14}H_{18}N_{12}S_2$: C, 40.18; H, 4.33, S, 15.32. Found: C, 40.32; H, 4.66; S, 14.38.

Method B. A suspension of 1.0 g. (0.0035 mole) of 8-(β-

(16) E. Walton, A. N. Wilson, F. W. Holly, and K. Folkers, *J. Am. Chem. Soc.*, **76**, 1146 (1954), who prepared this compound by a different procedure report b.p. 95–99° (0.6 mm.).

(17) All melting points are uncorrected.

(18) Analyses were carried out at Midwest Microlab, Inc., Indianapolis, Ind.

chloroethylamino)adenine dihydrochloride in 20 ml. of water was treated with 1.0 g. of potassium thiosulfate and heated to reflux for 7 hr. After the addition of 8 ml. of concd. hydrochloric acid the solution was heated for 1 more hour and left to stand at room temperature overnight. The solvent was removed under vacuum and the residue extracted with carbon disulfide. The product was dissolved in water, neutralized with ammonium hydroxide, and treated with 8 ml. of 3% hydrogen peroxide. A yield of 0.15 g. (11%) of disulfide was obtained.

The product showed an infrared spectrum identical with that of the compound obtained by the debenzylation of 8-(β-benzylthioethylamino)adenine. A mixed melting point of the two samples was not depressed.

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[CONTRIBUTION FROM THE DIVISION OF LIFE SCIENCES, STANFORD RESEARCH INSTITUTE]

Potential Anticancer Agents. LXIII.[1] Analogs of Chlorambucil. IX.[2] A Benzylic Analog of Phenoxyacetic Acid Mustard

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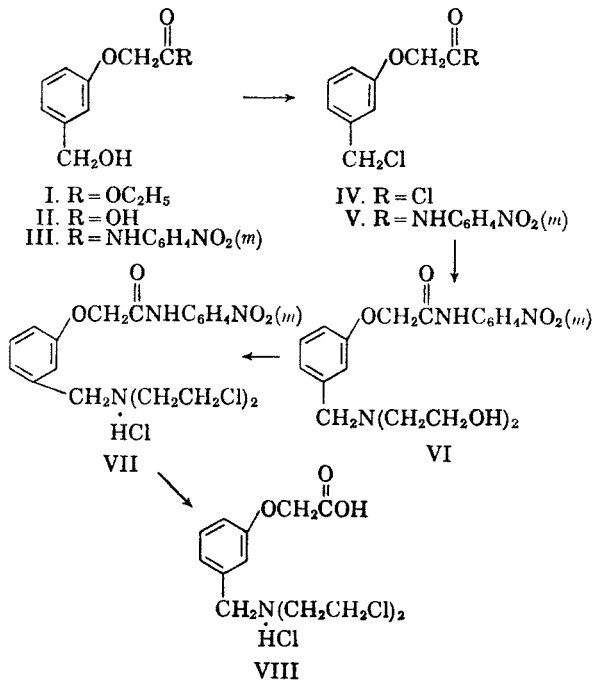
m-[Bis(2-chloroethyl)aminomethyl]phenoxyacetic acid hydrochloride (VIII), a benzylic type analog of phenoxyacetic acid mustard, has been synthesized for anticancer evaluation. In the synthesis *via* *m*-(hydroxymethyl)phenoxyacetic acid, *m*-nitroaniline has been found to be a useful blocking group for the carboxyl function, since the esters were not sufficiently unreactive.

Chlorambucil,[3,4] 4-{*p*-[bis(2-chloroethyl)amino]-phenyl}butyric acid, and phenylalanine mustard[5,6] both have interesting anticancer properties which differ from each other. Although Chlorambucil is highly effective against the Walker rat sarcoma 256, it shows little activity against sarcoma 180, adenocarcinoma 755 or leukemia L-1210 in the mouse. Against the last three tumors, the phenylalanine mustard is effective.

Since aliphatic mustards are chemically more reactive than the corresponding aryl mustards, a change in tumor spectrum or efficiency or both might be anticipated in benzylic type analogs of Chlorambucil and phenylalanine. Benzylic type analogs of Chlorambucil[7] have been prepared, but



unfortunately are, like Chlorambucil, inactive against sarcoma 180, adenocarcinoma 755 or leukemia L-1210 in the mouse. On the other hand, the phenoxyacetic acid mustards[8,9] show an animal tumor spectrum more like phenylalanine mustard than like Chlorambucil. Comparison of the benzylic analogs with the parent arylphenoxyacetic acid mustards against the three mouse tumors is there-

(1) This work was carried out under the auspices of the Cancer Chemotherapy National Service Center, National Cancer Institute, National Institutes of Health, Public Health Service, Contract No. SA-43-ph-1892. The opinions expressed in this paper are those of the authors and are not necessarily those of the Cancer Chemotherapy National Service Center. For the preceding paper in this series see K. A. Hyde, E. M. Acton, W. A. Skinner, L. Goodman, J. Greenberg, and B. R. Baker, *J. Med. Pharm. Chem.*, in press.

(2) For paper VIII on Chlorambucil see K. A. Hyde, E. M. Acton, W. A. Skinner, L. Goodman, and B. R. Baker, *J. Org. Chem.*, **26**, 3551 (1961).

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