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SYNTHESIS OF AMIDINE DERIVATIVES OF ALKYLTHIOSULFURIC ACIDS AS POTENTIAL ANTIRADIATION AGENTS*

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Abstract—Two methods were used to prepare a series of amidine derivatives of alkylthiosulfuric acids to be used as potential antiradiation agents. In one method an equimolar mixture of an amidine and an aminothiosulfuric acid was reacted in boiling methanol to give the corresponding substituted N-(alkylthiosulfuric acid) amidine. In the other method an amidate was reacted with an aminoalkylthiosulfuric acid. The following compounds were prepared:

$RC(NH)NH(CH_2)_2S_2O_3H$	$R = CH_3$, C_2H_5, n-C_3H_7, C_6H_5
$CH_3C(NR')NH(CH_2)_2S_2O_3H$	$R' = C_2H_5$, $CH_3(CH_2)_5$, $CH_3(CH_2)_6$
$RC(NH)NH(CH_2)_3S_2O_3H$	$R = CH_3$, n-C_3H_7, C_6H_5

IN CONTINUING efforts to obtain a compound which will provide protection against lethal doses of radiation, many classes of compounds have been synthesized for biological investigation.[1] To date, the most effective agents are the derivatives of 2-mercaptoalkylamines, aminoalkylisothiuronium salts, and guanidine derivatives obtained by rearrangement of aminoalkylisothiuronium compounds.

As an extension of these earlier studies, we first attempted to synthesize amidine derivatives of mercaptoalkylamines. Three approaches were used. The first was based on the work of Reynolds *et al.*,[2] who mercaptoethylated amines by reaction with ethylene monothiolcarbonate or ethyl 2-mercaptoethylcarbonate. Our attempts to mercaptoethylate acetamidine by reaction with ethylene monothiolcarbonate were unsuccessful; acetamidine and a polymeric ethylene sulfide were recovered. The second approach was through direct reaction of 2-mercaptoethylamine with an amidine. This approach was also unsuccessful, due to the bifunctionality of 2-mercaptoethylamine. The third approach was through reaction of 2-mercaptoethylamine with an imidate. The reaction of 2-mercaptoethylamine hydrochloride with ethyl n-butyrimidate resulted in compounds which were believed to be the hydrochlorides of 2-propylthiazoline and recovered 2-mercaptoethylamine. The reaction of 2-mercaptoethylamine with ethyl n-butyrimidate as free bases gave an unstable oil which could not be identified. These negative results indicate that synthesis of amidine derivatives of 2-mercaptoethylamine is difficult if not impossible, due to the bifunctionality of mercaptoethylamine.

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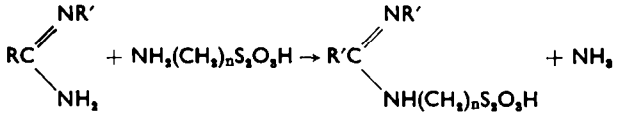
[1] *Symposium on Radiation-Protective Agents, Amer. Chem. Soc.* 141*st* meeting, pp. 28N–39N, Washington, D.C. (1962).

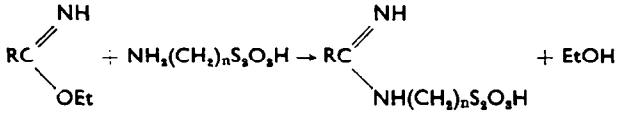
[2] D. D. Reynolds, M. K. Massad, D. L. Fields and D. L. Johnson, *J. Org. Chem.* **26**, 5109–5130 (1961).

The emphasis was then shifted to the synthesis of amidine derivatives of alkylthiosulfuric acids. We hypothesized that these derivatives might be metabolized *in vivo* to yield amidine derivatives of alkyl mercaptans and thus afford protection against radiation or that the amidine derivatives of alkylthiosulfuric acids themselves might give direct protection.[3]

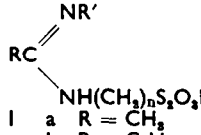
Two general methods for the synthesis of N-(alkylthiosulfuric acid) amidines were developed. In one method an equimolar mixture of an amidine and an aminoalkylthiosulfuric acid reacts in boiling methanol to yield the corresponding substituted N-(alkylthiosulfuric acid) amidine.



Compounds Ia through j were prepared by this method. In the other method an aminoalkylthiosulfuric acid reacts with an imidate to yield the corresponding N-(alkylthiosulfuric acid) amidine.

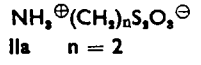


Compounds Ib and f were synthesized by this method.



I	a	$R = CH_3$	$R' = H$	$n = 2$
	b	$R = C_6H_5$	$R' = H$	$n = 2$
	c	$R = CH_3$	$R' = (CH_2)_5CH_3$	$n = 2$
	d	$R = CH_3$	$R' = C_6H_5$	$n = 2$
	e	$R = n\text{-}C_3H_7$	$R' = H$	$n = 2$
	f	$R = C_2H_5$	$R' = H$	$n = 2$
	g	$R = CH_3$	$R' = (CH_2)_9CH_3$	$n = 2$
	h	$R = CH_3$	$R' = H$	$n = 3$
	i	$R = C_6H_5$	$R' = H$	$n = 3$
	j	$R = n\text{-}C_3H_7$	$R' = H$	$n = 3$

Various methods were used to prepare the starting materials. 2-Aminoalkylthiosulfuric acid (IIa) was synthesized by three different methods.



IIa	$n = 2$
b	$n = 3$

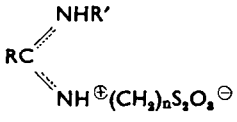
In one method sodium thiosulfate pentahydrate and 2-bromoethylamine hydrobromide were ground in a small amount of water and then heated to 50°, whereupon an exothermic reaction set in. The temperature of the reaction mixture was not allowed to rise above 65°. The product (IIa) was extracted in a Soxhlet apparatus with methanol. In the second method a mixture of anhydrous sodium thiosulfate and 2-bromoethylamine hydrobromide was heated in boiling methanol for several hours. The

[3] N. A. Rosenthal, *Symposium on Radiation-Protective Agents, Amer. Chem. Soc.* 141*st* meeting, p. 29N, Washington, D.C. (1962).

inorganic products were removed by filtration and the methanolic solution concentrated to give the product (IIa). The best quality of product was obtained by the thallous thiosulfate method,[4] which requires an extra step for the preparation of thallous thiosulfate from thallous formate and sodium thiosulfate. The thallous thiosulfate reacted with 2-bromoethylamine smoothly at room temperature to give compound IIb.

The amidines were obtained commercially or were prepared by Pinner's method.[5] The imidates were prepared by Pinner's method also. n-Butyronitrile was condensed with ethyl alcohol and an equimolar amount of anhydrous hydrogen chloride to give ethyl n-butyrimidate hydrochloride, m.p. 75–77°, in 82% yield. This result differs from that of Drozdov and Bekhli,[6] who reported an 18% yield, m.p. 64–65°.

The new products were easily soluble only in water and methanol and were practically insoluble in other common solvents. Since the impurities have similar solubilities, purification of the products was difficult. Products Ic, f, and h could not be purified satisfactorily for elemental analysis despite persistent efforts. Although no work was carried out to show the structure of the new products, we believe that they are zwitterionic by nature, and might be represented by the following general resonance form (III).



III

The new compounds were submitted to the Walter Reed Army Medical Center, Washington, D.C., for biological tests. The results to date indicate that these compounds provide some protection against lethal doses of radiation.

EXPERIMENTAL*

Attempted synthesis of amidine derivatives of mercaptoalkylamines

2-Mercaptoethylamine hydrochloride (11·36 g, 0·10 mole) and ethyl n-butyrimidate hydrochloride (15·16 g, 0·10 mole) were stirred in 300 ml absolute ethanol for 24 hr at room temp. The mixture was heated at 50° for 1 hr and cooled. The ammonium chloride which precipitated was removed by filtration, and the solvent was evaporated from the filtrate. The oily product was treated with chloroform. The insoluble material appeared to be 2-mercaptoethylamine hydrochloride. (Found: C, 2·084; H, 7·07; S, 27·38; N, 13·78; calc. for C_2H_8NSCl: C, 21·14; H, 7·10; S, 28·22; N, 12·33%). The chloroform-soluble material, m.p. 90–100°, appeared to be a hydrochloride of 2-n-propylthiazoline. (Found: C, 41·85; H, 7·42; N, 9·15; S, 20·02; Cl, 21·55. $C_6H_{12}NSCl$ requires: C, 43·49; H, 7·30; N, 8·46; S, 19·35; Cl, 21·40%).

The reaction of 2-mercaptoethylamine with ethyl n-butyrimidate as free bases gave only an unstable oil which could not be identified.

Synthesis of amidine derivatives of alkylthiosulfuric acid by using an amidine

N-(2-*Ethylthiosulfuric acid*) *acetamidine* (Ia). A solution of acetamidine hydrochloride (10·0 g, 0·105 mole) in 50 ml absolute methanol was treated with a solution of sodium methoxide (0·1 mole) in

* Mps are uncorrected.

[4] H. Z. Lecher and E. M. Hardy, *J. Org. Chem.* **20**, 475 (1955).

[5] Pinner, *Die Imidoäther und ihre Derivate*. R. Oppenheim (Gustav Schmidt), Berlin (1892). This book contains detailed descriptions of the amidine and imidate preparations which were used in the present work.

[6] N. S. Drozdov and A. F. Bekhli, *Zh. Obschi. Khim.* **14**, 280 (1944).

50 ml absolute methanol. The mixture was refluxed in a Soxhlet apparatus for 48 hr, during which time the 2-aminoethylthiosulfuric acid (15·7 g, 0·10 mole) contained in the thimble was gradually extracted and ammonia was evolved. Sodium chloride was removed from the mixture by filtration and the solvent was removed from the filtrate. The viscous oil obtained was dissolved in the minimum quantity of methanol and placed in a freezer overnight. The N-(2-ethylthiosulfuric acid) acetamidine which crystallized was filtered, dried, and recrystallized from absolute methanol. The yield, m.p. 173–174° (dec), was 12 g (61% of theoretical). (Found: C, 24·05; H, 5·27; N, 13·70; S, 32·35. $C_4H_{10}N_2S_2O_3$ requires: C, 24·23; H, 5·05; N, 14·13; S, 32·34%).

N-(2-*Ethylthiosulfuric acid*) *benzamidine* (Ib). Sodium methoxide (0·02 mole in 50 ml methanol) was added with stirring to a solution of benzamidine hydrochloride (2·02 g, 0·02 mole) in 50 ml absolute methanol. The sodium chloride which precipitated was removed by filtration, and 2-aminoethylthiosulfuric acid (3·14 g, 0·02 mole) was added to the filtrate. The mixture was refluxed for 24 hr, during which time ammonia gas was evolved. Upon cooling the mixture, N-(2-ethylthiosulfuric acid) benzamidine precipitated and was removed by filtration. The filtrate was concentrated, and a second crop of crystals was obtained. The solids were combined and recrystallized from absolute methanol. The total yield of product, m.p. 223–225° (dec), was 2·8 g (54% of theoretical). (Found: C, 41·19; H, 4·72; S, 24·52; N, 10·56. $C_9H_{12}S_2N_2O_3$ requires: C, 41·52; H, 4·65; S, 24·63; N, 10·76%).

N-(n-*Hexyl*-N′-2-*ethylthiosulfuric acid*) *acetamidine* (Ic). N-Hexylamine (10·12 g, 0·10 mole) was added to a solution of N-(2-ethylthiosulfuric acid) acetamidine (19·83 g, 0·10 mole) in 50 ml absolute methanol. The mixture was refluxed for 24 hr, during which time ammonia was evolved. Then the solvent was evaporated, and a yellow oil obtained. Chromatography of this material on silica gel proved ineffective in purification. However, chromatography on cellulose powder and elution with 40% ethyl acetate–methanol yielded 15 g (53% of theoretical) of product as an amber-colored nondistillable oil which appeared to be contaminated with 4% ammonium thiosulfate. (Found: C, 40·48; H, 7·41; N, 10·55; S, 23·78. Found corrected for 4% impurity of ammonium thiosulfate: C, 42·10; H, 7·51; N, 10·19; S, 22·96. $C_{10}H_{22}N_2S_2O_3$ requires: C, 42·52; H, 7·85; N, 9·92; S, 22·71%).

N-*Ethyl*-N′-(2-*ethylthiosulfuric acid*) *acetamidine* (Id). A solution of N-(2-ethylthiosulfuric acid) acetamidine (9·9 g, 0·05 mole) in 50 ml absolute methanol was added to a solution of ethylamine (70%, 50 ml) in absolute methanol. The mixture was refluxed for 24 hr, and then the solvent was evaporated. The yellow oil obtained was chromatographed on cellulose powder. The product was eluted with 40% ethyl acetate–methanol. The yield of product, which could not be distilled without decomposition, was 9·4 g (83% of theoretical). (Found: C, 32·10; H, 7·12; N, 12·84; S, 27·53. $C_6H_{14}N_2S_2O_3$ requires: C, 31·84; H, 6·23; N. 12·38; S, 28·34%).

N-(2-*Ethylthiosulfuric acid*) *n-butyramidine* (Ie). Sodium methoxide (0·05 mole) in methanol was added to a solution of n-butyramidine hydrochloride (6·13 g, 0·05 mole). The sodium chloride which formed was removed by filtration, and 2-aminoethylthiosulfuric acid (7·9 g, 0·05 mole) was added to the filtrate. This mixture was refluxed for 72 hr, during which time ammonia was emitted. The solvent was evaporated, and 12·15 g of a yellow glasslike material was obtained. This material was chromatographed on activated charcoal (40–65 mesh) and eluted with 10% benzene-methanol. The oily product, m.p. 125–130°, solidified on standing. (Found: C, 32·64; H, 6·52; N, 12·31; S, 28·68. $C_6H_{14}N_2S_2O_3$ requires: C, 31·84; H, 6·23; N, 12·38; S, 28·34%).

N-(2-*Ethylthiosulfuric acid*) *propionamidine* (If). Sodium methoxide (0·10 mole) in absolute methanol was added to a solution of propionamidine hydrochloride[5] (9·8 g, 0·10 mole) in absolute methanol. The sodium chloride which precipitated was removed by filtration, and 2-aminoethylthiosulfuric acid (15·7 g, 0·10 mole) was added to the filtrate. The mixture was refluxed for 24 hr, during which time ammonia was emitted. The mixture was filtered and the solvent was evaporated from the filtrate. The yellow viscous oil obtained was chromatographed on activated charcoal (40–65 mesh). Elution with absolute methanol and 10% benzene-methanol gave 10·45 g (49% of theoretical) of a viscous yellow oil which contained 11% ash. Since no further purification could be achieved, the sample was analyzed by combustion. (Found: after deduction of ash content C, 28·02; H, 5·73; N, 12·1; S, 30·35. $C_5H_{12}N_2S_2O_3$ requires: C, 28·28; H, 5·70; N, 13·20; S, 30·21%).

N-*n-Decyl*-N′-(2-*ethylthiosulfuric acid*) *acetamidine* (Ig). n-Decylamine (7·87 g, 0·05 mole) and N-(2-ethylthiosulfuric acid) acetamidine (9·9 g, 0·05 mole) were added to 50 ml of absolute methanol. The mixture was stirred and refluxed for 48 hr, during which time ammonia was evolved. The

mixture was cooled and filtered, and the solvent was evaporated from the filtrate. The cloudy orange oil obtained (16·83 g) was chromatographed on activated charcoal (40–65 mesh). The I.R. spectrum indicated that the yellow oil obtained was an amidine containing the thiosulfuric acid group. (Found: C, 50·10; H, 8·83; N, 7·9; S, 18·57. $C_{14}H_{30}N_2S_2O_8$ requires: C, 49·67; H, 8·93; N, 8·28; S, 18·95%).

N-(3-*Propylthiosulfuric acid*) *acetamidine* (Ih). Sodium methoxide (0·05 mole) in methanol was added to a solution of acetamidine hydrochloride (4·73 g, 0·05 mole) in 50 ml absolute methanol. The precipitated sodium chloride was removed by filtration, and 3-aminopropylthiosulfuric acid was added to the filtrate. The mixture was refluxed for 48 hr, during which time ammonia was evolved. The solvent was evaporated at red. press. and the residue was recrystallized from 50% ethyl acetate-methanol. The yield, m.p. 178–180° (dec), was 9·4 g (88·5% of theoretical). This solid was shown by combustion to contain 15·5% ash. (Found: C, 28·85; H, 5·81; N, 13·00. $C_5H_{12}N_2S_2O_8$ requires: C, 28·30; H, 4·70; N, 13·20%).

N-(3-*Propylthiosulfuric acid*) *benzamidine* (Ii). Sodium methoxide (0·05 mole) in absolute methanol was added to a solution of benzamidine hydrochloride (7·8 g, 0·05 mole) in absolute methanol. The precipitated sodium chloride was filtered, and 2-aminopropylthiosulfuric acid (8·5 g, 0·05 mole) was added to the filtrate. This mixture was refluxed for 48 hr, during which time ammonia was evolved. The solvent was evaporated at red. press., and the residue was recrystallized from absolute methanol to yield 8·9 g, m.p. 215–217°. (Found: C, 43·50; H, 5·60; N, 9·36; S, 22·86. $C_{10}H_{14}N_2S_2O_8$ requires: C, 43·77; H, 5·14; N, 10·20; S, 23·38%).

N-(3-*Propylthiosulfuric acid*) *butyramidine* (Ij). Sodium methoxide (0·05 mole) in absolute methanol was added to a solution of n-butyramidine hydrochloride (6·13 g, 0·05 mole) in absolute methanol. The precipitated sodium chloride was filtered, and 2-aminopropylthiosulfuric acid (8·5 g, 0·05 mole) was added to the filtrate. The mixture was refluxed for 48 hr, during which time ammonia was evolved. The solvent was evaporated at red. press. The cloudy oil obtained was dissolved in absolute methanol and passed through a column of activated charcoal. The inorganic material eluted first was discarded. Successive elutions with absolute methanol yielded 6·83 g (56·6% of theoretical) of an oily product which, on standing, yielded a solid, m.p. 134–136°. (Found: C, 35·09; H, 6·73; N, 11·53; S, 26·75. $C_7H_{16}N_2S_2O_8$ requires: C, 34·98; H, 6·71; N, 11·66; S, 26·68%).

Synthesis of amidine derivatives of alkylthiosulfuric acid by using an imidate

N-(2-*Ethylthiosulfuric acid*) *benzamidine* (Ib). Sodium methoxide (0·02 mole) in 50 ml absolute methanol was added to a solution of ethyl benzimidate hydrochloride[5] (3·70 g, 0·02 mole), m.p. 122°, in 50 ml absolute methanol. 2-Aminoethylthiosulfuric acid (3·14 g, 0·02 mole) was added to this mixture. The resulting suspension was stirred at room temp for 24 hr. The mixture was neutralized with methanolic hydrogen chloride and filtered. The filtrate was evaporated, and a yellow solid was recrystallized from methanol. The yield of product, m.p. 220–225°, was 2·41 g (46·3% of theoretical). I.R. spectra showed that the material was identical with the N-(2-ethylthiosulfuric acid) benzamidine obtained by reaction of benzamidine with 2-aminoethylthiosulfuric acid (p. 1316).

N-(2-*Ethylthiosulfuric acid*) *propionamidine* (If). A solution of sodium methoxide (0·05 mole) in methanol was added to a solution of ethyl propionimidate hydrochloride[5] (6·9 g, 0·05 mole) in absolute methanol. The sodium chloride which precipitated was removed by filtration, and 2-aminoethylthiosulfuric acid (7·86 g, 0·05 mole) was added to the filtrate. The mixture was stirred at 50° for 17 hr. The solvent was then evaporated stepwise while solid ammonium chloride was removed by filtration. The cloudy yellow oil obtained was chromatographed on cellulose powder, and 7·33 g (69% of theoretical) of a yellow nondistillable oil was obtained. The I.R. spectrum of this oil was identical with that of the product obtained by the amidine method.

Preparation of aminoalkylthiosulfuric acids

2-*Aminoethylthiosulfuric acid* (IIa). *Method A.* 2-Bromoethylamine hydrobromide (20·3 g, 0·1 mole) and sodium thiosulfate pentahydrate (24·8 g, 0·1 mole) were ground in a mortar, and distilled water (15 ml) was added. The solution was transferred to a 50-ml round-bottomed flask, and the mortar was rinsed with 5 ml water. The flask was heated gently in a water bath to 50°, whereupon the temp rose spontaneously to 65°. The mixture was removed from the bath, and its pH was adjusted to 6–7 with solid sodium hydrogen carbonate. The mixture was left overnight at room temp, and then carbon black was added. The mixture was filtered and the solvent was removed *in vacuo* at 50° in a rotating evaporator. The dry residue was extracted in a Soxhlet apparatus with 300 ml of absolute

methanol, the methanolic extract was evaporated, and the residue was recrystallized from ethanol. The yield of the first crop was 9·55 g (61% of theoretical), m.p. 180–195° (dec). This material yielded no ash upon burning and contained no sodium. The second crop (4·1 g), a less pure material, was isolated from the mother liquor.

Method B. A mixture of 2-bromoethylamine hydrobromide (4·10 g, 0·02 mole) and anhydrous sodium thiosulfate (6·5 g, 0·04 mole) was heated in boiling methanol (100 ml). The solids were removed by filtration and extracted with 100 ml methanol in a Soxhlet apparatus. The mixture of filtrate and extract was concentrated to give compound IIa (2·4 g, 82%).

Method C. Thallous thiosulfate (92·6 g, 0·178 mole) was added to a solution of 2-bromoethylamine hydrobromide (36·5 g, 0·178 mole) in water (180 ml). The mixture was stirred for 48 hr at room temp. Thallous chloride was then removed by filtration and washed with water. The combined washings and filtrate were concentrated *in vacuo* at 50°. The remaining solid, 26 g (97% of theoretical), m.p. 195° (dec), was crystallized from methanol.

The I.R. spectra of the products prepared by the three methods were identical.

3-Aminopropylthiosulfuric acid (IIb). Thallous thiosulfate (104 g, 0·2 mole) was suspended in a solution of 3-bromopropylamine hydrobromide (43·8 g, 0·2 mole) in 200 ml water. The mixture was stirred at room temp for 24 hr. The yellow thallous bromide was removed by filtration, and the solvent was evaporated from the filtrate. The white solid obtained was recrystallized from 50% aqueous methanol, and 26·3 g (76·9% of theoretical), m.p. 182–183° (dec.), was obtained.

Preparation of ethyl n-butyrimidate hydrochloride[5]

n-Butyronitrile (69·11 g, 1·0 mole) was dissolved in absolute ethanol (46·06 g, 1·0 mole), and anhydrous hydrogen chloride (approx. 1 mole) was added as the solution cooled in an ice bath. The mixture was allowed to stand at room temp overnight. No precipitation took place, even when anhydrous ethyl ether was added. The solvent was removed at red. press. without the application of heat. The slurry obtained was filtered, and the solid obtained was washed free of excess hydrogen chloride with anhydrous ethyl ether. The yield of dry product, m.p. 75–77° (lit.[6] 64–65°) was 124·0 g (82% of theoretical).

Acknowledgments—We are indebted to Dr. E. Baltazzi, of Armour Research Foundation, for many useful discussions and for Method A for the preparation of 2-aminoethylthiosulfuric acid, and Mr. W. Saschek, of the University of Chicago, for microanalyses. We are grateful to the Union Carbide Chemical Company for samples of ethylamine, n-hexylamine, and acetonitrile.