Potential Antiradiation Agents. I. Primary Aminoalkanethiosulfuric Acids

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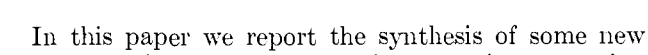
A series of primary aminoalkanethiosulfuric acids, prepared by the reaction of the appropriate bromoalkylamine hydrobromide with sodium thiosulfate, was tested for potential radioprotective activity. In addition, the Bunte salts, (2-amino-2-thiazolin-5-yl)methylthiosulfuric acid and 2-amino-4-thiazolylmethylthiosulfuric acid, and 2-aminoethaneselenosulfuric acid were synthesized and screened similarly. The antiradiation activities of the corresponding aminothiols, where available, were determined and compared to the related thiosulfates.

The search for antiradiation agents was prompted by the need to provide biological systems with a chemical means of mininizing the deleterious effects of ionizing radiation. As man's activities increase in areas of potential radiation hazard, the development of suitable prophylactic agents becomes progressively more desirable.

Cysteine[1] and its decarboxylated form, 2-mercaptoethylamine (MEA)[2], were found to prolong the life of mice subjected to lethal radiation. Eldjarn and Pihl[3] ascribe the protective activity of these and related compounds to their ability to form transitory mixed disulfides with protein sulfhydryl groups. Later, Sörbo[4] found that the aminoalkanethiosulfates, 2-aminoethanethiosulfuric acid (**1**) and sodium S-sulfocysteine (**9**), also gave mixed disulfides with protein SH groups

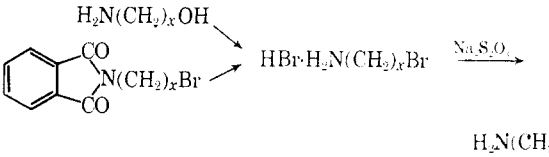
1 **9**

and he suggested that thiosulfates, because of this property, might be active antiradiation agents. Compound **1** was found[5] to prolong the life of mice when administered prior to their exposure to lethal radiation. The material offers the advantage over MEA·HCl in being less toxic, of not being subject to air oxidation in solution, and of being essentially neutral in solution. We have shown that the sodium salt of **1**, when treated with mercaptides, can be used to synthesize mixed disulfides containing the aminoethyl moiety.[6]



In this paper we report the synthesis of some new alkylthiosulfuric acids possessing a primary amino group (Table I). The antiradiation test data are presented for these compounds and related compounds (Table II). When an aminothiol corresponding to an aminothiosulfuric acid was available to us, we have included the test data for purposes of comparison.

Chemistry.—A series of α,ω-aminoalkanethiosulfuric acids was prepared in which the number of CH_2 groups separating the functional groups was 2–6 and 10. These compounds were synthesized by the reaction of sodium thiosulfate with the appropriate bromoalkylamine hydrobromides. The latter were obtained *via* the concentrated HBr treatment of either the appropriate bromoalkylphthalimide or amino alcohol. It is of interest that attempts to prepare aminomethanethiosulfuric acid have, thus far, been unsuccessful.[7]



2-Aminopropane-1-thiosulfuric acid (**7**) was made by the thiosulfate ring cleavage of 1-methylaziridine while its isomer, 1-aminopropane-2-thiosulfuric acid (**8**) was synthesized by the bisulfite scission of the corresponding disulfide.[8]

2-Amino-5-(bromomethyl)-2-thiazoline hydrobromide, used in the preparation of (2-amino-2-thiazolin-5-yl)-methylthiosulfuric acid (**10**), was prepared by the addition of bromine to 1-allyl-2-thiourea in ethanol by the method of Dixon.[9] The intermediate 1-(2,3-dibromopropyl)-2-thiourea cyclized *in situ* without the recommended heating period.

Results and Discussion

Compound **1**, studied earlier by Holmberg and Sörbo,[5] afforded good radiation protection in mice. When the number of carbon atoms separating the amino and thiosulfate groups was increased to three (**2**), the protective effect decreased by approximately one-half. These results are, in general, in accord with those of Kaluszyner, *et al.*,[10] who found that **1** gave protection comparable to that afforded by MEA·HCl, while **2** was somewhat less effective. Hansen and Sörbo,[11] on the other hand, found that both **1** and **2** were inferior in activity to MEA.

When the number of CH_2 groups separating the amine and thiosulfate functions was increased beyond three, activity was completely lost.

Of the two isomeric aminopropylthiosulfuric acids **7** and **8**, only the former provided radiation protection comparable to **1**. Sodium S-sulfocysteine (**9**) gave only marginal protection in our test system, while Hansen and Sörbo[11] found no protective effect with the latter compound.

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TABLE I

AMINOALKANETHIOSULFURIC ACIDS

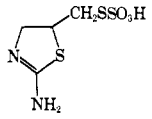
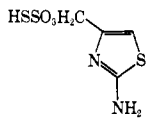
$RSSO_3H$

Compd	R	Formula[a]	Mp, °C dec	% yield	Recryst solvent
3	4-Aminobutyl	$C_4H_{11}NO_3S_2$	184–186	42	H_2O–EtOH
4	5-Aminopentyl	$C_5H_{13}NO_3S_2$	200–201	66	MeOH
5	6-Aminohexyl	$C_6H_{15}NO_3S_2$	213–214	67	H_2O
6	10-Aminodecyl	$C_{10}H_{23}NO_3S_2$	201–205	95	50% EtOH[b]
10	(2-Amino-2-thiazolin-5-yl)methyl	$C_4H_8N_2O_3S_3$	220–225	71	H_2O
11	2-Amino-4-thiazolylmethyl	$C_4H_6N_2O_3S_3 \cdot H_2O$	203–205	50	H_2O

[a] All compounds were analyzed for C, H, N, S. [b] This difficultly recrystallizable compound can be purified satisfactorily by treating it with several portions of hot MeOH.

TABLE II

PROTECTION OF MICE AGAINST X- OR γ RADIATION BY PRIMARY AMINOALKANETHIOSULFURIC ACIDS AND THEIR CORRESPONDING AMINOTHIOLS

Compd	Formula	LD_{50}, mg/kg[a]	Drug dose, mg/kg[a]	Time interval, min[b]	Radiation dose, R[c]	% survival (30-day)
1[d]	$H_2NCH_2CH_2SSO_3H$	450	350	15	800	73
	$C_2H_7NS \cdot HCl$[e]	250	150	15	1000	80
2[f]	$H_2NCH_2CH_2CH_2SSO_3H$	600	500	30	800	40
	$C_3H_9NS \cdot HCl$[g]	175	150	15	825	0
3	$H_2NCH_2(CH_2)_2CH_2SSO_3H$	150	50	15	1000	0
4	$H_2NCH_2(CH_2)_3CH_2SSO_3H$	500	400	30	1000	0
5	$H_2NCH_2(CH_2)_4CH_2SSO_3H$	500	300	15	1000	0
6	$H_2NCH_2(CH_2)_8CH_2SSO_3H$	>1200	1000	30	800	0
7[h]	$CH_3CH(NH_2)CH_2SSO_3H$	450	350	30	975	85
	$C_3H_9NS \cdot HCl$[i]	300	175	15	800	20
8[h]	$CH_3CH(SSO_3H)CH_2NH_2$	>900	800	30	825	7
	$C_3H_9NS \cdot HCl$[g]	425	300	15	800	66
9[j]	$HO_2C–CH(NH_2)CH_2SSO_3Na \cdot 1.5H_2O$	1900	1600	15	825	20
	$C_3H_7NO_2S \cdot HCl$	>1800	1000	15	825	83
10		50	20	15	1000	0
11		500	300	30	825	5
12[k]	$H_2NCH_2CH_2SeSO_3H$	17.5	10	30	1000	0
	$C_2H_7NSe \cdot HCl$[k]	10	5	15	825	0

[a] Intraperitoneal administration. [b] Administration prior to irradiation. [c] ^{60}Co γ irradiation at 975–1000 R (dose rate 100–500 R/min); all other doses were delivered by a 300-kvp X-ray (dose rate 45 R/min). [d] H. Bretschneider, *Monatsh.*, **81**, 372 (1950); D. L. Klayman, W. F. Gilmore, and T. R. Sweeney, *Chem. Ind.* (London), 1632 (1965). [e] Intraperitoneal administration; dosage based on the weight of the free base. [f] Reference 10. [g] Submitted by Dr. R. P. Louthan, Phillips Petroleum Co., Bartlesville, Okla. [h] Reference 8. [i] Submitted by Dr. E. R. Atkinson, Arthur D. Little, Inc., Cambridge, Mass. [j] Reference 4. [k] D. L. Klayman, *J. Org. Chem.*, **30**, 2452 (1965).

The two compounds, **10** and **11**, where the amino group was remotely located on a heterocyclic ring offered no protection to the irradiated mice. Similarly, no activity was obtained from the highly toxic Se analog of **1**, 2-aminoethaneselenosulfuric acid (**12**).

In general, aminoalkanethiosulfuric acids are less toxic than the corresponding thiols. While MEA·HCl is more toxic than **1**, the two compounds are about equally protective at their maximum tolerated doses. The next higher homolog, 3-mercaptopropylamine hydrochloride is more toxic than its thiosulfate form **2** and is totally ineffective. Good protection has been reported by other workers, however, for 3-mercaptopropylamine and it has been claimed to be even slightly superior to MEA·HCl on a molar basis.[12] Still others[10,11] have found 3-mercaptopropylamine to be ineffective in their test systems.

2-Aminopropane-1-thiol, related to the good radioprotective thiosulfate **7**, exhibited poor activity. Conversely, 1-aminopropane-2-thiol, related to the poor radioprotective agent **8**, showed moderately good antiradiation activity. Both of these isomeric aminothiols were reported[13] to afford radioprotection comparable to MEA.

Cysteine protected 83% of the mice while the S-sulfo derivative **9** was effective only to the extent of 20%, as mentioned earlier. 2-Aminoethylselenol hydrochloride, like its Se-sulfo derivative (**12**), was highly toxic and also ineffective as an antiradiation agent.

Thus, it appears that in the series of primary aminoalkanethiosulfuric acids, the optimal spacing between the amino and thiosulfate groups is two carbon atoms. Because the thiosulfate group confers decreased toxicity,

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one may administer greater quantities of potential radioprotective agents possessing this moiety than of the corresponding thiol. However, in going from the thiol to the thiosulfate, the radioprotective activity is not changed in a manner which is entirely predictable.

Experimental Section

Biological Methods.—Female mice of the Walter Reed Bagg Swiss or Inbred Charles River (ICR) strains, 5–6 weeks old and weighing 21–25 g, were randomly assigned to treatment groups. Toxicity estimations were based on a 10-day observation period following intraperitoneal injections of each agent in graded doses.[14] The maximum tolerated dose of each drug was administered 15–30 min before exposure to lethal whole-body irradiation.

The drugs, which were freshly prepared for administration prior to use, were, depending on their solubility, either dissolved in physiological saline solution or homogenized in a glass tissue grinder in a suspending medium of physiological saline solution containing 0.2% methylcellulose (4000 cP) and 0.4% Tween-80. The suspensions were exposed to an ultrasonic apparatus for approximately 2 min to ensure uniform particle size. The pH range of the solutions or suspensions was adjusted to 6–7, if necessary.

Irradiation of the mice was performed utilizing either of two sources: 300-kvp GE Maxitron unit, with radiation factors of 20 mA, hvl 2 mm Cu, tsd 85 cm, dose rate in air 45 R/min or a specially designed ^{60}Co irradiator, which contained 1200 Ci of ^{60}Co, half above and half below the radiation chamber, with a dose rate range from 100–50 R/min over a 5-year period.

Forty mice were exposed in a perforated Lucite dish 50.8 cm in diameter which rotated continuously during exposure. Equal numbers of control mice injected with only the vehicle used for the particular drug evaluation were irradiated simultaneously with each treated group and thereafter housed jointly eight to a cage.

The mice were given food and water *ad libitum*. The drinking water contained 10–15 ppm of Cl_2 to suppress the growth of *Pseudomonas aeruginosa*.[15] The principles of laboratory animal care as promulgated by The National Society for Medical Research were observed.

Mortality was tabulated for a 30-day period. Under our conditions, approximately 800 R of X and 1000 R of ^{60}Co γ irradiation were equally lethal, all control animals dying between the 10th and 21st days following exposure.

Chemistry.[16] **α-Bromoalkyl-ω-phthalimides** were made essentially by the method of Drake and Garman[17] except that potassium phthalimide (1 mole) was added in one portion, rather than in four, to a stirred and refluxing solution of an α,ω-dibromoalkane (1.8 moles) in 2 l. of acetone. Following stirring and heating for an additional 24 hr, KBr was filtered off and washed with several portions of hot acetone. The combined Me_2CO solutions were evaporated to dryness and the excess dibromide was separated from the residue either by extraction with hexane or by distillation under reduced pressure. The following phthalimides were prepared: N-(4-bromobutyl)phthalimide (63%), mp 79–81°, lit. mp 80.5°,[18] 78–80°;[19] N-(5-bromopentyl)phthalimide (47%), mp 58–60°, lit. mp 61°,[20] 58–60°;[21] N-(6-bromohexyl)phthalimide (50%), mp 52.5–55°, lit. mp 57°,[22] mp 120°;[23] N-(10-bromodecyl)-phthalimide (75%), mp 57–59°, lit. mp 62–63°,[24] mp 57–58°.[25]

Bromoalkylamine Hydrobromides.—2-Bromoethylamine hydrobromide and 2-amino-4-(chloromethyl)thiazole hydrochloride are commercially available while 3-bromopropylamine hydrobromide[26] was prepared by heating 3-aminopropanol with 48% HBr according to the method of Cortese.[27]

The bromoalkylphthalimides, described in the previous section, could be converted to bromoalkylamine hydrobromides by heating them (0.35 mole) under reflux with 125 ml of 48% HBr in 300 ml of AcOH for 48 hr. The hot solution was diluted with 500 ml of H_2O and cooled causing the separation of phthalic acid which was filtered off. The filtrate was evaporated to dryness under reduced pressure and the residue was treated five times with H_2O, each time the solution was taken to dryness. The following bromoalkylamines were prepared: 4-bromobutylamine hydrobromide (39%), mp 157–158°, lit. mp 146–146.5°,[28] 157–158°;[29] 5-bromopentylamine hydrobromide (69%), mp 140–142°, lit. 141–142°;[30] 6-bromohexylamine hydrobromide (49%), mp 138–140°, lit. mp 142–143°;[22] 10-bromodecylamine hydrobromide (59%), mp 143–149°. *Anal.* ($C_{10}H_{23}BrN$) C, H, Br, N (recrystallized from EtOAc, then C_6H_6).

Aminoalkanethiosulfuric Acids.—The bromoalkylamine hydrobromides were heated in H_2O or H_2O–EtOH with 1 equiv of sodium thiosulfate pentahydrate for 0.5–1 hr. The reaction was considered complete when elemental S failed to precipitate from an aliquot which had been strongly acidified. The Bunte salts which crystallized from the cooled and, in some instances, concentrated reaction mixtures were collected and recrystallized until they were free of halide ion. They showed characteristic ir peaks (KBr) near 8.15, 8.40, and 9.80 μ.

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