TABLE I

Deriv of fluorene[a]	Mp, °C[b]	KB cell test, ED_{50},[d] μg/ml	Effect[c] T/C	Effect[c] mg/kg	Lethality[d] No. killed	Lethality[d] mg/kg
9-(4-Methylaminobenzylidene)-	135–136[e]	30	0.6	1600		
9-(4-Dimethylaminobenzylidene)-	140–142[e,f]	35	1.4	1600	0/3	1600
2-Dimethylamino-9-(4-dimethylaminobenzylidene)-	188–191[g]	38	1.1	1600		
2-Dimethylamino-9-(4-dimethylaminobenzylidene)-	148–150[h]	32				

[a] All new compounds were analyzed for C and H; analytical results were within ±0.3% of the theoretical values. [b] Determined with Mel-Temp melting point apparatus. [c] We are grateful to Professor Sir Alexander Haddow, Mr. J. E. Everett, and Mr. C. V. Mitchley of the Chester Beatty Research Institute for data on toxicity and activity against the Walker 256 tumor in rats weighing 200–250 g. Each compound was administered as a single interperitoneal injection in arachis oil on the day following tumor implantation or on the first day of the toxicity observation. Tumor bearing animals were sacrificed approximately 8 days later and the average weights of tumors in treated and untreated hosts are reported as the ratio T/C. [d] Results of the standard *in vitro* KB tumor cell inhibition tests carried out under sponsorship of the Cancer Chemotherapy National Service Center at the University of Miami Cell Culture Laboratory. [e] Orange. [f] Reference 2. [g] Red. [h] Yellow.

Experimental Section

Our efforts to prepare these compounds by KOH-catalyzed condensation in EtOH were unsuccessful, but good results were obtained in DMSO. In a typical experiment a solution of 0.025 mole of 4-dimethylaminobenzaldehyde and 0.025 mole of 2-dimethylaminofluorene in 125 ml of DMSO at 90° was poured into a solution of 0.025 mole of KOH in 50 ml of DMSO at 163° and heated 30 min in a bath at 110°. The solvent and unreacted aldehyde were distilled off under vacuum and the product was extracted from the residue by isohexane,[4] recrystallized (EtOH), and purified further by use of a Florisil column. Two types of crystals were obtained, both of which had the correct analysis for the expected product and the same uv absorption spectrum in EtOH or AcOH. Ir spectra in Nujol were similar. The two solids may be *cis* and *trans* isomers.

(4) A mixture of isomeric branched chain hexanes.

Potential Antiradiation Agents. II.[1] Guanidinoalkanethiosulfuric Acids

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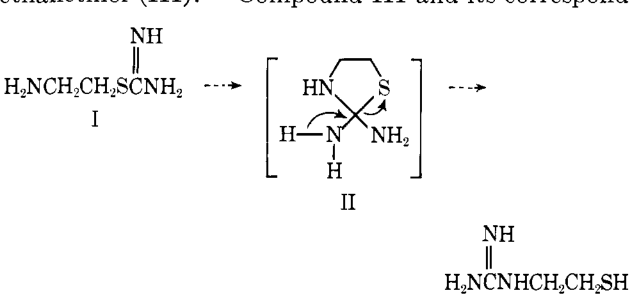
The mechanism by which 2-(2-aminoethyl)-2-thiopseudourea dihydrobromide (I) confers antiradiation properties is believed to involve an intratransguanylation to form an intermediate *gem*-diaminothiazolidine (II) which subsequently ring opens to give 2-guanidinoethanethiol (III).[2] Compound III and its correspond-



ing disulfide, bis(2-guanidinoethyl) disulfide (IV), have been studied extensively and have been shown to offer excellent protection against the effects of ionizing radiation.[3] A logical variation of III possessing the $H_2NC(=NH)NHCCS-$ moiety is the thiosulfate inner salt, 2-guanidinoethanethiosulfuric acid (**1**), first synthesized by Kaluszyner.[4] While he indicated that **1**

$$H_2NC(=NH)NHCH_2CH_2SSO_3H$$

1

was a good antiradiation agent, no further details of its actual effectiveness were provided. Compound **1** was reported by Mangina[5] to have radioprotective effect on the yeast, *Saccharomyces vini*. Westland, *et al.*,[6] found that several 2-(1-alkylguanidino)ethylthiosulfuric acids, prepared by the sulfitolysis of bis(2-guanidinoethyl) disulfides, had *in vitro* antibacterial action against *Streptococcus pyogenes* and *Staphylococcus aureus*. The phosphate analog of **1**, 2-guanidinoethanethiol S-phosphate (V), prepared by Åkerfeldt[7]

$$H_2NC(=NH)NH—CH_2CH_2SPO_3H_2$$

V

as the ammonium lithium salt, was reported by him to be a good radioprotector at doses lower than are required for 2-mercaptoethylamine (MEA).[8] Compound V, however, was found to be more toxic than MEA.

We report here the synthesis and the antiradiation activity of **1** and six variants of its structure in which the carbon skeleton consists of 2, 3, or 4 CH_2 groups and in which some of the guanidino groups are alkyl substituted.

Chemistry.—Kaluszyner's method[4] for the preparation of **1** from 2-aminoethanethiosulfuric acid was slightly modified and adapted for the synthesis of the members of this series. It consists of heating a solution of an S-methyl derivative of a thiopseudourea with the Na salt of an aminoalkanethiosulfuric acid. When

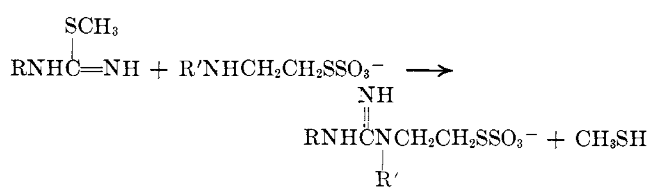


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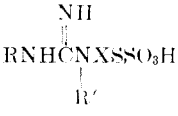
TABLE I
GUANIDINOALKANETHIOSULFURIC ACIDS



No.	R	R′	X[a]	Formula	Mp, °C	% yield	Recrystn solvent
1[c]	H	H	$(CH_2)_2$	$C_3H_9N_3O_3S_2$	181–184 dec[d]	51	H_2O
2[e]	CH_3	H	$(CH_2)_2$	$C_4H_{11}N_3O_3S_2$	148–149	17	MeOH
3[e]	C_2H_5	H	$(CH_2)_2$	$C_5H_{13}N_3O_3S_2$	138.5–139	18	H_2O
4[e]	CH_2=$CHCH_2$	H	$(CH_2)_2$	$C_6H_{13}N_3O_3S_2 \cdot 0.5H_2O$	133–134	52	H_2O
5[e]	H	CH_3	$(CH_2)_2$	$C_4H_{11}N_3O_3S_2$	198–200.5	57	H_2O
6[e]	H	C_2H_5	$(CH_2)_2$	$C_5H_{13}N_3O_3S_2$	200–203	33	H_2O
7[e]	H	*i*-C_3H_7	$(CH_2)_2$	$C_6H_{15}N_3O_3S_2$	187–190 dec	35	H_2O
8[f]	H	H	CH_3CHCH_2	$C_4H_{11}N_3O_3S_2$	180–182 dec	64	H_2O
9[g]	H	H	$C_2H_5CHCH_2$	$C_5H_{13}N_3O_3S_2$	204–206 dec	58	H_2O
10[h]	H	H	$(CH_2)_3$	$C_4H_{11}N_3O_3S_2$	154–156	37	H_2O
11[i]	H	H	$(CH_2)_4$	$C_5H_{13}N_3O_3S_2$	182–183	33	H_2O

[a] The thiosulfate group is on a terminal C in all cases. [b] All compounds were analyzed for C, H, N, S. [c] For starting aminoalkanethiosulfuric acid see H. Bretschneider, *Monatsh.*, **18**, 372 (1950); D. L. Klayman, W. F. Gilmore, and T. R. Sweeney, *Chem. Ind.* (London), 1632 (1965). [d] Kaluszyner[4] reported mp 181–183°. [e] For starting aminoalkanethiosulfuric acid see D. L. Klayman and W. F. Gilmore, *J. Med. Chem.*, **7**, 823 (1964). [f] For starting aminoalkanethiosulfuric acid see D. L. Klayman, J. W. Lown, and T. R. Sweeney, *J. Org. Chem.*, **30**, 2275 (1965). [g] See Experimental Section for starting aminoalkanethiosulfuric acid. [h] For starting aminoalkanethiosulfuric acid see A. Kaluszyner, P. Czerniak, and E. D. Bergmann, *Radiation Res.*, **14**, 23 (1961). [i] For starting aminoalkanethiosulfuric acid see ref 1.

methyl mercaptan, a by-product of the reaction, was no longer evolved, the reaction mixture was concentrated and cooled to give the guanidinoalkanethiosulfuric acid (Table I).

In the ir the guanidinoalkanethiosulfuric acids absorb in the region of 6.00–6.10 μ indicating the presence of a C=N group[9] while the peaks near 8.15, 8.40, and 9.80 μ are indicative of the thiosulfate group. The Sakaguchi test[10] for guanidines was positive only for those compounds in which the guanidino moiety was not further substituted by alkyl groups in the 1 or 3 position.

Biological Methods.—A detailed procedure for the preparation and administration of potential antiradiation agents and the irradiation of the mice has been given in an earlier report.[1] The minor deviations from this procedure which were followed in this study are given below.

The animals used were female inbred Charles River mice, 8–9 weeks old, weighing 25–30 g. Groups of 15 mice were injected with the maximum tolerated doses of drugs, freshly prepared as neutral solutions or suspensions and administered intraperitoneally at 15–30 min before exposure to lethal whole-body irradiation.

Mice were given a dose of 1000 R in a specially designed ^{60}Co irradiator whose dose rate was 45–48 R/min. Equal numbers of control mice were irradiated simultaneously with the treated mice and thereafter they were housed jointly.

Mortality was recorded daily over a 30-day observation period. The mice were given food and water *ad libitum* containing 10–15 ppm chlorine to suppress the growth of *Pseudomonas aeruginosa*.[11] The principles of laboratory animal care as promulgated by the National Society for Medical Research were observed throughout.

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(10) R. A. B. Bannard, A. A. Casselman, W. F. Cockburn, and G. M. Brown, *Can. J. Chem.*, **36**, 1541 (1958).
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Results and Discussion

Of the guanidinoalkanethiosulfuric acids studied, only **1** showed radioprotective activity comparable to that afforded by MEA (see Table II). Substitution

TABLE II
PROTECTION OF MICE AGAINST γ RADIATION BY GUANIDINOALKANETHIOSULFURIC ACIDS

Compd	LD_{50}, mg/kg[a]	Drug dose, mg/kg[a]	Time interval, min[b]	% survival (30 day)
1	275	100	15	80
2	250	150	30	20
3	200	100	15	0
4	135	90	30	20
5	175	100	15	27
6	250	100	15	53
7	130	45	15	0
8	350	200	15	53
9	250	150	15	40
10	350	125	15	67
11	450	200	30	0

[a] Intraperitoneal administration. [b] Administration prior to 1000 R of ^{60}Co γ irradiation (dose rate 45–48 R/min).

of an alkyl group at the 3 position of the guanidino moiety (**2–4**) gave products possessing little or no activity. Substitution of a CH_3 at the 1 position of the guanidino function produced a compound (**5**) with slight activity, while Et substitution in that position gave **6** with moderate activity. Isopropyl substitution in the 1 position gave **7** which was devoid of antiradiation activity. This may be due, in part, to its increased toxicity.

Branching of the carbon atom bearing the guanidino group by either a Me (**8**) or an Et (**9**) group gave compounds possessing moderate activity. Extension of the carbon chain separating the guanidino and thiosulfate functions to three CH_2 groups gave **10** which had moderate effectiveness. The analogous phosphorothioate, 3-guanidinopropanethiol S-phosphate (NH_4–Li

salt), was reported by Åkerfeldt[8] to provide 80% protection at the maximum tolerated dose. Compound **11**, having a four-carbon backbone, lacked effectiveness.

A comparison of the radioprotective activities of aminoalkanethiosulfuric acids with the corresponding substituted or unsubstituted guanidinoalkanethiosulfuric acids revealed that superior activity is obtained if the guanidino group is unsubstituted. Diminished activity results, however, if the guanidino moieties are alkyl-substituted. As was the case with aminoalkanethiosulfuric acids,[1] optimal effectiveness is found in guanidinoalkanethiosulfuric acids when the N and S functions are separated by two carbon atoms.

Experimental Section[12]

2-Methyl-2-thiopseudourea Iodides.—An EtOH solution of a thiourea was heated under reflux with 1.1 equiv of MeI for *ca.* 0.5 hr. Evaporation of the solvent under reduced pressure gave the 2-methyl-2-thiopseudourea iodide in high yield. Purification was usually achieved by recrystallization of the product from EtOH–Et_2O.

Guanidinoalkanethiosulfuric Acids.—This general procedure is a modification of one described by Kaluszyner.[4] To an aqueous solution containing 1 equiv of a 2-methyl-2-thiopseudourea iodide (or sulfate) was slowly added with stirring an aqueous solution of 1 equiv of an aminoalkanethiosulfuric acid and 1 equiv of NaOH. When addition was complete, the resultant solution was heated on a steam bath until the evolution of MeSH virtually ceased. The latter was detected as a yellow lead mercaptide by holding a piece of filter paper spotted with $Pb(OAc)_2$ near the mouth of the flask. The solution was concentrated at reduced pressure and cooled causing the separation of the product. The guanidinoalkanethiosulfuric acid was recrystallized until free of I^- (or SO_4^{2-}).

2-Aminobutane-1-thiosulfuric Acid.—A solution of 23.3 g (0.1 mole) of 2-amino-1-bromobutane hydrobromide, prepared by the reaction of 48% HBr with 2-aminobutanol according to the method of Cortese,[13] and 24.8 g (0.1 mole) of sodium thiosulfate pentahydrate in 30 ml of H_2O was heated on a steam bath *ca.* 0.5 hr. The product, which was separated from the cooled solution, was recrystallized from H_2O and washed with hot MeOH until halide free to give 11.5 g (62%) of white crystals, mp 208–209° dec (lit.[14] mp 206° dec). *Anal.* ($C_4H_{11}NO_3S_2$) C, H, N, S.

Acknowledgment.—We thank Dr. T. R. Sweeney and Col. W. E. Rothe for helpful suggestions and Mr. L. Hafner and Dr. S. Abdou-Sabet for technical assistance.

(12) Melting points were determined on a Fisher-Johns melting point apparatus and are uncorrected. Microanalyses were performed by Mr. Joseph F. Alicino, Metuchen, N. J. 08840, and Schwarzkopf Microanalytical Laboratory, Woodside, N. Y. 11377. Infrared spectra were determined as KBr pellets on a Beckman IR-5 spectrophotometer.

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Terpene Compounds as Drugs. VI. Acyclic Mono- and Sesquiterpene Thiocyanates and Isothiocyanates

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Many aliphatic and aromatic thiocyanates and isothiocyanates, especially the allyl and benzyl derivatives, are endowed with interesting antimicrobial properties.[1] This, together with our interest in the terpene field, has led us to synthesize esters of thiocyanic and isothiocyanic acid with terpene alcohols such as citronellol, geraniol, and farnesol.

Citronellyl (II), geranyl (IV), and farnesyl isothiocyanate (VI) were obtained from the proper amines by reaction with CS_2 and $ClCOOC_2H_5$. Thiocyanates were obtained by treating terpenyl bromides with NaSCN. Citronellyl bromide gave the related thiocyanate (I); geranyl bromide yielded linalyl isothiocyanate together with geranyl thiocyanate (III); farnesyl bromide yielded nerolidyl isothiocyanate together with farnesyl thiocyanate (V).

The structure of all the compounds and the composition of III and V were determined by pmr. Our values were in good agreement with those reported for similar compounds.[2] Integration of the linalyl olefinic protons and CH_2SCN methylene protons gave 80.56% of linalyl isothiocyanate and 19.44% of geranyl thiocyanate for III. A similar assay gave 83.34% of nerolidyl isothiocyanate and 16.66% of farnesyl thiocyanate for V. Since these data were taken in CCl_4 solution at 36° and an equilibrium is involved, it may be expected that changes in the experimental conditions give different percentage compositions of III and V.

Table I reports the new products, along with yields and analytical data.

TABLE I

PHYSICOCHEMICAL PROPERTIES OF ACYCLIC TERPENE THIOCYANATES AND ISOTHIOCYANATES

Compd	Yield, %	Bp, °C (mm)	n_D (25°)	R_f (tlc)	Formula[a]
I	44	79–80 (0.15)	1.4832	0.48	$C_{11}H_{19}NS$
II	45	81–82 (0.2)	1.5021	0.74	$C_{11}H_{19}NS$
III	57	62–64 (0.1)	1.5061	0.47, 0.81	$C_{11}H_{17}NS$
IV	43	81–82 (0.15)	1.5221	0.76	$C_{11}H_{17}NS$
V	45	115–116 (0.22)	1.4893	0.48, 0.83	$C_{16}H_{25}NS$
VI	53	125–127 (0.2)	1.5206	0.77	$C_{16}H_{25}NS$

[a] All compounds were analyzed for C, H, N, S; the analytical values were within ±0.4% of the theoretical values.

Biological Results.—*Micrococcus pyogenes var. aureus* (ATCC 6538 P), *Bacillus subtilis* (ATCC 6633), *Escherichia coli* Mc. Leod (ATCC 10536), *Salmonella typhi* (T 30 Roma), *Candida albicans* (DM), *Trichomonas foetus*, and *Entamoeba histolytica* (F 22) were used as test organisms. Minimum inhibitory concentrations required to prevent growth of bacteria for 24 hr at 37° were determined by serial dilution in tryptose phosphate broth (Difco). The *in vitro* activity against *T. foetus* was determined by serial dilution in fluid thioglycolate medium (Difco) with 10% horse serum added; test tubes containing 5 ml of the liquid media were inoculated with 40,000–70,000 parasites/ml; minimum inhibitory concentrations were determined microscopically after 48 hr of standing at 37°. The antiamebic activity *in vitro* was determined according to De Carneri.[3]

In none of the assays did the compounds exert any observable inhibitory activity even at a concentration level of 400 μg/ml. In contrast, the standard allyl and benzyl isothiocyanate confirmed their good activity.

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