Antiradiation Compounds VII

Long-Chain Alkyl Derivatives of 2-Mercaptoethylguanidine

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Mono-*N*-alkyl and *N,N'*-dialkyl derivatives of 2-mercaptoethylguanidine (MEG) have been synthesized as potential radioprotective agents with the assumption that increase in liposolubility of MEG will improve its radioprotective ability. The compounds were obtained by reaction of bromoethylamine with the appropriate alkylated thiourea to give the corresponding aminoethylisothiouronium bromide hydrobromide, which was rearranged to the desired MEG derivative in alkaline solution. The alkyl MEG hydrobromides, unlike MEG·HBr itself, could be isolated from aqueous solution. Conversion to the trithiocarbonates generally gave products of better crystalline properties. The structure of the trithiocarbonate of MEG was supported by NMR analysis.

S-(2-AMINOETHYL)-ISOTHIOURONIUM bromide hydrobromide (AET) was first tested for antiradiation effects by Philpot (1) in 1951; but it seemed to give no better results than cysteamine (MEA) and was not studied further by his group. In 1955, Doherty (2) and coworkers reintroduced AET and claimed it was superior in radioprotective ability to other thiols, particularly after oral administration. In neutral or alkaline solution and under physiological conditions, the compound rearranges to 2-mercaptoethylguanidine (MEG) (3), which has not been isolated, but was first precipitated as the flavianate. It has been isolated subsequently as the sulfate (4). The authors also have found that MEG could be isolated from aqueous solution by reaction with carbon disulfide to give an adduct shown to be the trithiocarbonate (5). A series of long-chain *N*-alkyl and *N,N'*-dialkyl derivatives of MEG now has been prepared, with the assumption that increase in liposolubility will improve antiradiation effectiveness. These compounds could be isolated from aqueous solution, generally as the hydrobromide. Compounds of better crystalline properties were obtained by conversion of the MEG derivatives to the trithiocarbonates, however. Further evidence that these adducts are trithiocarbonates and exist as zwitterions also has been provided by proton nuclear magnetic resonance analysis.

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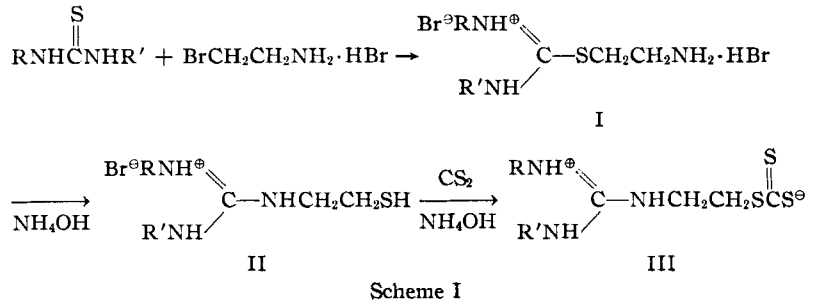
DISCUSSION

The *N*-alkyl and *N,N'*-dialkyl derivatives of MEG were prepared by reaction of the corresponding thiourea with bromoethylamine hydrobromide to give the substituted isothiouronium compound (I). The dialkyl thioureas were prepared by conversion of long-chain alkyl amines to the dithiocarbamates, followed by heating according to the method of Erickson (6), whereas the monoalkyl thioureas were obtained by conversion of the amines to isothiocyanates using thiophosgene. The isothiouronium compounds were rearranged to the corresponding MEG derivatives (II) in aqueous ammonia, and the mono-hydrobromides were generally isolated. In the case of the *N*-myristyl derivative, however, the free base precipitated from aqueous solution under the same conditions that precipitated the mono-hydrobromides of the other alkyl MEG derivatives isolated. The hydrobromides had wide melting point ranges, possibly because of cyclization by heating prior to melting. Paper chromatography of one of these compounds, the monomyristyl MEG derivative, showed only one spot. The general synthetic route followed is shown in Scheme I.

Formation of the trithiocarbonates (III) of the mercaptoethylguanidines took place in the usual manner (5) by treatment of either the AET or the MEG derivative with carbon disulfide in aqueous ammonia. The trithiocarbonates were crystalline products of good stability and better melting points.

Nuclear magnetic resonance analysis indicated that the MEG-carbon disulfide adduct has the trithiocarbonate structure and thus that here, as with similar derivatives of 2-mercaptoethylamine (MEA) and its *N,N*-diethyl analog (7, 8), carbon disulfide becomes attached to sulfur rather than to nitrogen. This conclusion is derived as follows. The *N,N*-diethylaminoethanthiol derivative can have only this structure (8). In trifluoroacetic acid solution, the chemical shifts of both methylene groups in its NCH_2CH_2S unit are very near 6.3 τ, giving rise to a complex multiplet as a consequence of their mutual spin-coupling and possibly also due to further coupling of the NCH_2 to the NH, nitrogen being protonated in this solvent.[1] The *N*-methylene

[1] Proton magnetic resonance spectra were determined at approximately 30° using a Varian A-60 spectrometer with tetramethylsilane as internal reference.



Scheme I

resonance is shifted downfield to this position by the adjacent quaternary nitrogen and also by the sulfur function β to it; the *N*-methylene protons of the ethyl groups, which are more remote from the latter influence, are shifted only to near 6.53 τ.[2] [By comparison, the methylene proton resonance of tetraethylammonium iodide falls at 6.60 τ (9).] The *S*-methylene chemical shift clearly shows the influence of the adjacent trithiocarbonate grouping. If these protons were α only to a sulfhydryl group, their resonance would be expected near 7.6 τ [ethyl mercaptan, 7.56 τ (9)], and the β-quaternary nitrogen would be expected to produce an additional downfield shift of approximately 0.5 p.p.m., as it does in the methyl resonance of tetraethylammonium iodide. For example, the *S*-methylene resonance of MEA itself (in trifluoroacetic acid) is near 7.0 τ, while its *N*-methylene resonance falls near 6.5 τ. Furthermore, it is probably the *S*-methylene resonance of *N*-myristyl MEG which appears as a multiplet near 7.16 τ. The additional deshielding (to 6.3 τ) experienced by the *S*-methylene in the diethyl MEA trithiocarbonate is due undoubtedly in large measure to the magnetic anisotropy of the thiocarbonyl group and thus is qualitatively analogous to the effect produced by the carbonyl group when an alcohol is converted to its acetate (10).

The methylene proton resonance of the MEA adduct is similar to that of the diethylamino derivative, *viz.*, a complex multiplet in which both the *N*-methylene and the *S*-methylene chemical shifts are near 6.25 τ. If this compound had possessed the alternative β-mercaptoethyldithiocarbamate structure, such similarity would not have been anticipated. Among other things the *S*-methylene should not have been nearly so deshielded by the thiocarbonyl, and its resonance would have occurred at substantially higher field. Thus, these data give added support to the structure assigned to the MEA adduct on the basis of infrared and ultraviolet (8) and other NMR data (7).

Both methylene chemical shifts in the MEG-carbon disulfide adduct are near 6.42 τ, the multiplet resembling those from the MEA adduct and its diethyl derivative. The positive guanidinium ion and the ammonium ion have about the same net effect on the chemical shift of protons α to them. Although the charge on the former is delocalized and hence probably not so effectively deshielding as that of the latter, the trigonally hybridized guanidinium ion is probably more deshielding than the ammonium ion as a consequence of its greater anisotropy. That these opposing effects nearly cancel can be seen from spectra of methylguanidinium sulfate and methylamine hydrochloride, in which the methyl chemical shifts are 7.03 and 7.12 τ, respectively. Also the *N*-methylene chemical shifts of *N*-myristyl-MEG fall in the 6.55–6.73 τ range, reasonably close to the 6.5 τ observed in the MEA spectrum. Thus, the chemical shifts observed for the MEG adduct are completely consistent with the trithiocarbonate structure. If this product had contained a free CH_2SH group rather than the trithiocarbonate, the *S*-methylene resonance would have been at higher field.

These spectra all were obtained from trifluoroacetic acid solutions, both for reasons of solubility and to ensure that the amino and guanido groups were in the protonated form in each case, so that chemical shift comparisons could be made. In none of the trithiocarbonate spectra could reasonance due to SH protons be observed. This could be because the compounds are present as zwitterions in which the trithiocarbonate is not protonated [as is the case with the MEA adduct in neutral solvents (7, 8)], but it might be equally because exchange of the SH proton with solvent is rapid in trifluoroacetic acid so that the SH resonance simply is merged with solvent resonance.

Antiradiation Properties.—Tests for the ability of a couple of the MEG derivatives to protect mice against a lethal dose of X-radiation have been carried out at the Walter Reed Army Institute of Research under the direction of Dr. D. P. Jacobus. Tests have been carried out, as previously indicated (11), on *N*-myristyl-2-aminoethylisothiouronium bromide hydrobromide and *N*-myristyl-2-mercaptoethylguanidine trithiocarbonate in doses of 50 mg./Kg. or less. At this dose level, neither compound was protective.

EXPERIMENTAL

Melting points were taken on a Mel-Temp block and are uncorrected. Analyses for carbon, hydrogen, and nitrogen were done by Weiler and Strauss, Oxford, England. The following procedures are representative. See Table I for properties of all the prepared compounds.

N,N'-Dioctylthiourea.—To a solution of 25.8 Gm. (0.2 mole) of *n*-octylamine (Matheson, Coleman and Bell) in 125 ml. of toluene, cooled by an ice bath, was added dropwise and with stirring 7 ml. (0.1 mole) of carbon disulfide. A snow-white precipitate appeared. The reaction mixture was re-

[2] The protons of these methylenes are nonequivalent owing to their location in the asymmetric environment provided by the tetrahedral (protonated) nitrogen. [*cf.* Nair, P. M., and Roberts, J. D., *J. Am. Chem. Soc.*, **79**, 4565(1957).] Thus, they give rise to the AB part of an ABC_3 spectrum.

TABLE I.—PHYSICAL PROPERTIES OF AET AND MEG DERIVATIVES

Compd.	M. p., °C.	Yield, %	Formula	Anal., % Calcd.	Found
A. 2-Aminoethylisothiouronium Bromide Hydrobromides (I)					
N-Octyl AET·2HBr[a,b]	...	71	$C_{11}H_{27}Br_2N_3S$	C, 33.58	31.25
				H, 6.87	7.27
				N, 10.69	10.19
				S, 8.14	7.85
N,N'-Dioctyl AET·2HBr	178–180	41	$C_{19}H_{43}Br_2N_3S$	C, 45.13	45.28
				H, 8.51	8.48
				N, 8.31	8.32
				S, 6.33	6.29
N,N'-Didecyl AET·2HBr	179–182	0.01	$C_{23}H_{51}Br_2N_3S$	C, 49.20	49.10
				H, 9.09	9.18
				N, 7.49	7.88
N-Dodecyl AET·2HBr[a]	...	75	$C_{15}H_{35}Br_2N_3S$	C, 40.09	39.63
				H, 7.79	8.01
				N, 9.35	9.22
				S, 7.13	7.11
N-Myristyl AET·2HBr	78–248	73	$C_{17}H_{39}Br_2N_3S$	C, 42.76	41.38
				H, 8.23	8.34
				N, 8.80	8.68
				S, 6.71	6.44
N,N'-Dimyristyl AET·2HBr[c]	165–177	62	$C_{31}H_{67}Br_2N_3S$	C, 55.28	56.17
				H, 9.96	9.98
				N, 6.24	6.21
				S, 4.76	4.76
B. 2-Mercaptoethylguanidine Hydrobromides (II)					
N-Octyl MEG·HBr	142–155	16	$C_{11}H_{26}BrN_3S$	C, 42.31	42.33
				H, 8.33	7.97
				N, 13.46	13.41
N,N'-Dioctyl MEG·HBr	60–125	37	$C_{19}H_{42}BrN_3S$	C, 53.75	52.53
				H, 9.97	9.50
				N, 9.90	9.16
				S, 7.55	7.73
N-Dodecyl MEG·HBr	94–119	82	$C_{15}H_{34}BrN_3S$	C, 48.91	51.77
				H, 9.24	9.40
				N, 11.41	11.81
				S, 8.70	8.35
N-Myristyl MEG	115–140	76	$C_{17}H_{37}N_3S$	C, 64.72	64.73
				H, 11.82	11.80
				N, 13.32	13.38
				S, 10.16	10.33
N,N'-Dimyristyl MEG·HBr	53–97	46	$C_{31}H_{66}BrN_3S$	C, 62.80	62.82
				H, 11.22	11.02
				N, 7.09	6.95
				S, 5.41	5.56
C. 2-Mercaptoethylguanidine Trithiocarbonates (III)					
N-Octyl MEG·CS_2	65–80	48	$C_{12}H_{25}N_3S_3$	C, 46.90	47.04
				H, 8.11	8.24
				N, 13.68	13.62
N,N'-Dioctyl MEG·CS_2	123–125	51	$C_{20}H_{41}N_3S_3$	C, 57.28	57.07
				H, 9.78	10.33
				N, 10.02	10.19
				S, 22.91	22.85
N-Dodecyl MEG·CS_2	104–115	61	$C_{16}H_{33}N_3S_3$	C, 52.89	53.32
				H, 9.09	9.43
				N, 11.57	12.08
N-Myristyl MEG·CS_2	93–122	40	$C_{18}H_{37}N_3S_3$	C, 55.24	55.41
				H, 9.46	9.45
				N, 10.74	11.00
				S, 24.55	24.11
N,N'-Dimyristyl MEG·CS_2	80–92	45	$C_{32}H_{66}N_3S_3$	C, 65.42	65.23
				H, 11.07	11.08
				N, 7.16	7.36
				S, 16.35	15.96

[a] Isolated as a glass. [b] Ethyl acetate was used as solvent. [c] Isopropyl alcohol was used as solvent.

fluxed on a steam bath until hydrogen sulfide was no longer evolved; this required about 10 hr. The resulting solution was concentrated to about one-half its volume; after the residue was cooled in an ice bath, a white crystalline product formed. It was collected, washed with petroleum ether, and air-dried; a yield of 27.0 Gm. (90%) was obtained, m.p. 53–55°. [Lit. m.p. 53° (12).]

N,N'-Didecylthiourea was not found in the literature and therefore submitted to analysis.

Anal.—Calcd. for $C_{21}H_{44}N_2S$: C, 70.80; H, 12.33; N, 7.87; S, 8.99. Found: C, 71.28; H, 12.68; N, 7.71; S, 8.99.

S - (2 - Aminoethyl) - N,N' - dioctylisothiouronium Bromide Hydrobromide.—A solution of 10 Gm. (0.033 mole) of dioctylthiourea and 10 Gm. (0.05 mole) of bromoethylamine hydrobromide (K and K Laboratories, Inc.) in 50 ml. of hot absolute ethanol was refluxed for 4 hr. The solution was cooled to room temperature, diluted with 200 ml. of water, and refrigerated overnight. A white gelatinous precipitate was collected, washed with water, and dried in a desiccator; and 6.9 Gm. (41% yield) was obtained, m.p. 178–180°.

N,N' - Dioctyl - N'' - (2 - mercaptoethyl)guanidine Hydrobromide.—A solution of 3.0 Gm. (0.006 mole) of the above product in methanol was made alkaline with 2 ml. of concentrated ammonium hydroxide, and the mixture was refrigerated for 1 week. It was then separated by decantation, and the residue was dissolved in ethanol. The ethanol solution was filtered and allowed to evaporate. The gummy residue was rubbed vigorously with a glass rod until solidification took place. The solid was air-dried and powdered; 0.94 Gm. (37% yield) was obtained, m.p. 60–125°.

The presence of a mercaptan group was verified by reduction of iodine solution and a positive nitroprusside test.

N,N' - Dioctyl - N'' - (2 - mercaptoethyl)guanidinium Trithiocarbonate.—To a solution of 5 Gm. (0.01 mole) of 2-(2-aminoethyl)-1,3-dioctylisothiouronium bromide hydrobromide and 4.4 ml. (0.06 mole) of concentrated ammonium hydroxide in 25 ml. of ethanol, cooled by an ice bath, was added dropwise and with stirring 0.7 ml. (0.01 mole) of carbon disulfide. A yellow oil separated and became semisolid after being stirred 1 additional hr. The mixture was refrigerated overnight, and the hard mass isolated was powdered and triturated with absolute ethanol. A yield of 2.1 Gm. (51%) of yellow powder was obtained after drying in a vacuum desiccator, m.p. 123–125°. Infrared absorption showed the presence of a trithiocarbonate peak (13) at 1030 cm.$^{-1}$.

Myristylthiourea.—A solution of 10 ml. (0.13 mole) of thiophosgene (K and K Laboratories, Inc.) in 100 ml. of toluene was cooled by an ice bath, and a solution of 21.3 Gm. (0.1 mole) of myristylamine (K and K Laboratories, Inc.) in toluene and a concentrated aqueous solution of sodium carbonate were added slowly simultaneously with stirring. Stirring was continued for 0.5 hr., and the mixture was warmed by a water bath until effervescence ceased. The mixture was cooled, and the toluene layer was collected, washed with distilled water and clarified with a small amount of ether. The solution was dried over calcium chloride, then evaporated; the residue was dissolved in warm methanol. Excess ammonium hydroxide was added, and the mixture was refluxed for 15 min. and refrigerated. The solid was collected and recrystallized from ethanol. A yield of 15 Gm. (15%) of white crystalline product was obtained, m.p. 110–113°. [Lit. m.p. 107° (14).]

Anal.—Calcd. for $C_{15}H_{32}N_2S$: S, 11.76. Found: S, 11.57.

S - (2 - Aminoethyl) - N - myristylisothiouronium Bromide Hydrobromide.—To a hot solution of 11.0 Gm. (0.04 mole) of myristylthiourea in 100 ml. of methanol and 20 ml. of absolute ethanol was added 8.4 Gm. (0.04 mole) of bromoethylamine hydrobromide (K and K Laboratories, Inc.). The solution was refluxed until no crystallization occurred on cooling to room temperature. The solution was refrigerated, and the resultant precipitate was collected, washed with cold ethanol, and air-dried. A yield of 11.4 Gm. (73%) of white solid was obtained, m.p. 78–248°.

Anal.—Calcd. for $C_{17}H_{39}Br_2N_3S$: Br, 33.54. Found: Br, 34.11.

N - Myristyl - N' - (2 - mercaptoethyl)guanidine.—To a solution of 4.8 Gm. (0.01 mole) of the preceding product in 20 ml. of ethanol were added 5 ml. of ammonium hydroxide and 15 ml. of distilled water. After refrigeration overnight, the mixture was filtered, and the product was air-dried. The product was triturated with cold absolute ethanol, filtered, and air-dried. A white powder was obtained which was recrystallized from benzene–ethanol; 2.5 Gm. (76% yield) was obtained, m.p. 115–140°.

The presence of a mercapto group was indicated by the ability to reduce iodine solution and a positive nitroprusside test. Paper chromatography of the product, using butanol saturated with ammonium hydroxide, showed only one spot after spraying with either ferrous sulfate or alizarin red S solution.

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