1-Alkyl-1-(2-mercaptoethyl)guanidines and the Corresponding Thioureas, Disulfides, and Thiosulfates as Antibacterial Agents[1]

ROGER D. WESTLAND, EVA R. KARGER, BILLIE GREEN, AND JOHN R. DICE

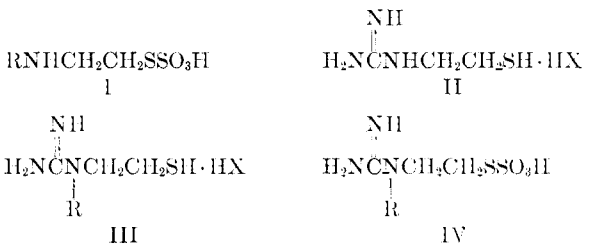
Research Laboratories, Parke, Davis and Company, Ann Arbor, Michigan 48106

Received May 29, 1967

A new class of antibacterial agent has been found, having good *in vitro* activity against *Streptococcus pyogenes* and *Staphylococcus aureus*. Certain compounds possess *in vivo* activity against *S. aureus* at nontoxic levels. The preparation and antibacterial activity of an interrelated series consisting of 2-[2-(alkylamino)ethyl]-2-thiourea dihydrobromides (V), 1-alkyl-1-(2-mercaptoethyl)guanidine salts (III), and S-2-(1-alkylguanidino)ethyl thiosulfates (IV) are reported.

Mercaptoethylamine has been variously substituted by many workers in attempts to develop a useful antiradiation agent.[2] In the present study some new derivatives of mercaptoethylamine have unexpectedly been found effective against the microorganisms *Streptococcus pyogenes and Staphylococcus aureus*.

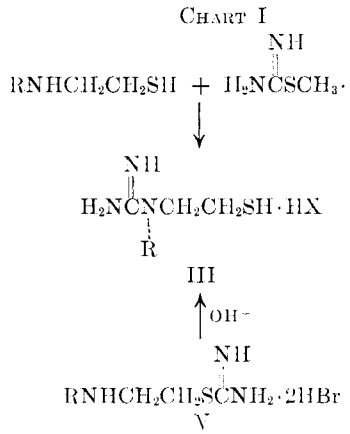
Structures which have stimulated much interest as antiradiation agents are S-2-(alkylamino)ethyl thiosulfates (I)[3] and analogs of 1-(2-mercaptoethyl)guanidine (II).[4] Long-chain alkyl derivatives of type III



(Table I) have not been reported, although compounds possessing R groups as large as isopropyl are known.[5] In the case of IV (Table II), only the unsubstituted, parent S-2-(1-guanidino)ethyl thiosulfate has been reported.[6]

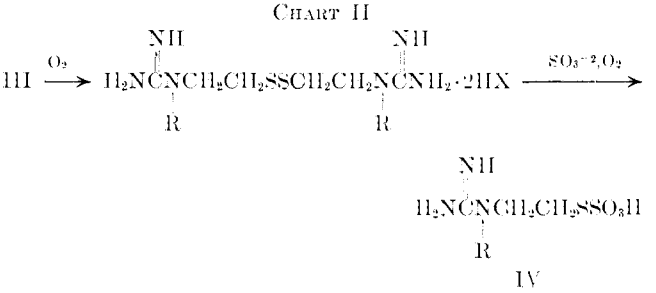
1-Alkyl derivatives III of 1-(2-mercaptoethyl)guanidine were prepared (Chart I) either by rearrangement[1] of isothiouronium salts (Table III) or by reaction[7] of 2-methyl-2-thiourea hemisulfate with suitable 2-alkylaminoethanethiols. The practical success of the method involving 2-methyl-2-thiourea appeared to depend on the degree of insolubility of III in the reaction medium. A pure product was readily isolated when a solid appeared immediately on mixing the two reactants in ethanol solution. When a solid did not immediately appear, oily mixtures were obtained which were difficult to purify. Oxidation and substitution of the thiol are side reactions which probably occurred.

CHART I



The substituted mercaptoethylguanidines III were partially oxidized by air to the corresponding disulfides (crude) (Chart II) and further oxidized by ammonium sulfite in the presence of air to thiosulfate esters IV.

CHART II



Biological Activity (Table IV).[8]—For the 1-alkyl derivatives of 1-(2-mercaptoethyl)guanidine (III), highest *in vitro* activity against *S. pyogenes* and *S. aureus* was found where the alkyl group was hexyl, heptyl, octyl, 2-ethylhexyl, and nonyl. The antibacterial activity was found to diminish with either longer or shorter alkyl groups. The most active compound in the series was the 2-ethylhexyl derivative. For the S-2-(1-alkylguanidino)ethyl thiosulfates (IV), optimum activity was found with heptyl, octyl, nonyl, and decyl as alkyl substituents.

Some of the compounds exhibited *in vitro* activity against other bacteria. Compound **9** (2-ethylhexyl derivative of III) had the broadest antibacterial spectrum, giving complete inhibition of the growth of *S. aureus, S. pyogenes, Salmonella typhimurium, Klebsiella pneumoniae, Escherichia coli, Diplococcus pneu-*

(1) This investigation was supported in part by the U. S. Army Medical Research and Development Command, Contract No. DA-49-193-MD-2306. We appreciate the interest and support of Drs. D. P. Jacobus and T. R. Sweeney of Walter Reed Army Institute of Research.

(2) For leading references see W. O. Foye in "Annual Reports in Medicinal Chemistry, 1965," C. K. Cain, Ed., Academic Press Inc., New York, N. Y., 1966, Chapter 30.

(3) D. L. Klayman and W. F. Gilmore, *J. Med. Chem.*, **7**, 823 (1964).

(4) J. X. Khym, D. G. Doherty, and R. Shapira, *J. Am. Chem. Soc.*, **80**, 3342 (1958). These agents generally are formulated as thiopseudoureas which readily rearrange under very slightly basic conditions to give mercaptoethylguanidines.

(5) R. Shapira, D. G. Doherty, and W. T. Burnett, *Radiation Res.*, **7**, 22 (1957).

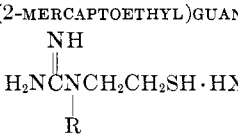
(6) A. Kaluszyner, *Bull. Research Council Israel*, **9A**, 35 (1960); *Chem. Abstr.*, **55**, 3427 (1961).

(7) Dr. T. R. Sweeney, personal communication, 1963.

(8) For the general *in vitro* and *in vivo* test procedures see M. W. Fisher, M. C. Manning, L. A. Gagliardi, M. R. Gaetz, and A. L. Erlandson in "Antibiotics Annual 1959–1960," Antibiotica, Inc., New York, N. Y., 1960, pp 293–303.

TABLE I

1-ALKYL-1-(2-MERCAPTOETHYL)GUANIDINES (III)

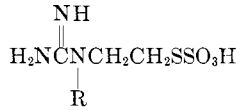


No.	R	Method[a]	Yield, %	Mp, °C	Formula	Calcd, % C	H	N	SH	Found, % C	H	N	SH[b]
4	$CH_3(CH_2)_3$	A[c]	43	213–215	$C_7H_{17}N_3S\cdot0.5H_2SO_4$	37.46	8.09	18.74	14.75	37.19	7.76	18.90	15.15
5	$CH_3(CH_2)_5$	A[d]	45	208–210	$C_9H_{21}N_3S\cdot0.5H_2SO_4$	42.99	8.42	16.72	13.16	42.85	8.69	16.51	12.43
6	$CH_3(CH_2)_6$	A[d]	92	204–207	$C_{10}H_{23}N_3S\cdot0.5H_2SO_4$	45.08	9.08	15.77	12.41	44.92	9.01	15.71	12.11
7	$CH_3(CH_2)_7$	A[c]	56	195	$C_{11}H_{25}N_3S\cdot0.5H_2SO_4$	47.11	9.34	14.98	11.81	47.40	9.21	14.99	11.90
8	$CH_3(CH_2)_7$	B	77	102–104	$C_{11}H_{25}N_3S\cdot HBr$	42.30	8.39	13.46	10.60	42.00	8.18	13.41	10.09
9	$CH_3(CH_2)_3CH(C_2H_5)CH_2$	B	31	106–109	$C_{11}H_{25}N_3S\cdot HBr$	42.30	8.39	13.46	10.60	42.44	8.26	13.40	10.04
10	$CH_3(CH_2)_8$	A[d]	23	198–201	$C_{12}H_{27}N_3S\cdot0.5H_2SO_4$	48.93	9.59	14.27	11.23	49.26	9.60	14.15	10.19
11	$CH_3(CH_2)_9$	A[c]	60	202–204	$C_{13}H_{29}N_3S\cdot0.5H_2SO_4$	50.61	9.81	13.61	10.73	50.75	9.76	13.43	9.78
12	$CH_3(CH_2)_{11}$	B	54	119–123	$C_{15}H_{33}N_3S\cdot HBr$	48.89	9.30	11.41	8.70	48.65	9.03	11.35	8.96

[a] A, $RNHCH_2CH_2SH + H_2NC(=NH)SCH_3\cdot0.5H_2SO_4$; B, $RNHCH_2CH_2SC(=NH)NH_2\cdot2HBr + NaOH$. [b] Iodine titration; for a similar assay see J. W. Kimball, R. L. Kramer, and E. E. Reid, *J. Am. Chem. Soc.*, **43**, 1199 (1921). It is apparent that the corresponding disulfide was present in some samples as a minor component. Occasionally repeated recrystallization served only to increase the disulfide content. [c] For the 2-(alkylamino)ethanethiol, see D. D. Reynolds, D. L. Fields, and D. L. Johnson, *J. Org. Chem.*, **26**, 5125 (1961). [d] For the 2-(alkylamino)ethanethiol, see ref 13.

TABLE II

S-2-(1-ALKYLGUANIDINO)ETHYL THIOSULFATES (IV)



No.	R	Yield, %	Mp, °C	Formula	Calcd, % C	H	N	S	Found, % C	H	N	S
13	$CH_3(CH_2)_3$	20	197–200	$C_7H_{17}N_3O_3S_2$	32.92	6.71	16.46	25.11	33.20	6.60	16.59	24.92
14	$CH_3(CH_2)_5$	53	202–205	$C_9H_{21}N_3O_3S_2$	38.13	7.47	14.83	22.63	38.37	7.42	14.82	22.38
15	$CH_3(CH_2)_6$	20	175–178	$C_{10}H_{23}N_3O_3S_2$	40.38	7.79	14.13	21.56	40.65	7.62	14.16	21.31
16	$CH_3(CH_2)_7$	69	128–130	$C_{11}H_{25}N_3O_3S_2$	42.41	8.09	13.49	20.59	42.65	7.89	13.20	20.48
17	$CH_3(CH_2)_8$	72	149–152	$C_{12}H_{27}N_3O_3S_2$	44.31	8.36	12.91	19.10	44.59	8.31	13.08	19.37
18	$CH_3(CH_2)_9$	75	152–154	$C_{13}H_{29}N_3O_3S_2$	45.99	8.61	12.38	18.89	46.05	8.50	12.12	18.60
19	$CH_3(CH_2)_{11}$	69	123–126	$C_{15}H_{33}N_3O_3S_2$	49.01	9.05	11.43	17.45	49.32	9.05	11.55	17.20

TABLE III

2-[2-(ALKYLAMINO)ETHYL]-2-THIOUREA DIHYDROBROMIDES (V)

No.	R	Yield, %	Mp, °C	Formula	Calcd, % C	H	N	S	Found, % C	H	N	S
1	$CH_3(CH_2)_7$	80	108–110	$C_{11}H_{25}N_3S\cdot2HBr$	33.60	6.93	10.69	8.16	33.65	6.64	10.68	8.18
2	$CH_3(CH_2)_3CH(C_2H_5)CH_2$	81	165–168	$C_{11}H_{25}N_3S\cdot2HBr$	33.60	6.93	10.69	8.16	33.89	6.87	10.56	8.33
3	$CH_3(CH_2)_{11}$	75	250–252	$C_{15}H_{33}N_3S\cdot2HBr$	40.00	7.83	9.33	7.12	40.00	7.40	9.45	7.29

[a] $RNHCH_2CH_2Br\cdot HBr + H_2NC(=NH)NH_2$.

moniae, and *Shigella sonni* and partial inhibition of *Pseudomonas aeruginosa* and *Mycobacterium tuberculosis*.

Compounds were tested against *S. aureus* in mice by oral and by subcutaneous administration. General *in vivo* activity was demonstrated, but dose levels required were very close to toxic limits. A minimum-protective dose of 12.5 mg/kg given as a single subcutaneous injection was found for **1**, **5**, **6**, **17**, and **18**. Similarly, the minimum protective dose was 10 mg/kg for **10** and 50 mg/kg for **15**. Compound **7** was tested in mice (subcutaneous injection) against *S. aureus*. It exhibited a protective effect at 3.1 mg/kg.

Experimental Section[9]

2-(Octylamino)ethyl Bromide Hydrobromide.—To 900 g of 48% HBr was added 232 g (1.34 moles) of 2-(octylamino)-ethanol,[10] and the resulting solution was heated under reflux for 2 hr. Approximately 250 ml of solvent was removed by distillation, and the remaining solution was again heated under reflux for 2 hr. The distillation of 250 ml of solvent and heating under reflux for 2 hr were repeated once more, and the solution was cooled and diluted with about 200 ml of Et_2O. The solid 2-(octylamino)ethyl bromide hydrobromide was isolated and recrystallized from MeOH–Et_2O to give 180 g (42%) of product, mp 212–215°.

Anal. Calcd for $C_{10}H_{22}BrN\cdot HBr$: C, 37.88; H, 7.31; N, 4.42. Found: C, 37.83; H, 7.23; N, 4.55.

2-(2-Ethylhexylamino)ethyl Bromide Hydrobromide.—From 82 g (0.47 mole) of 2-(2-ethylhexylamino)ethanol[10] and 450 g of 48% HBr was obtained 12 g (8%) of pure product, mp 167–168° (in addition to crops of less pure material) in a manner identical with that used for 2-(octylamino)ethyl bromide hydrobromide above.

(9) Melting points, determined using a Thomas–Hoover melting point apparatus, are uncorrected.

(10) J. R. Reasenberg and S. D. Goldberg, *J. Am. Chem. Soc.*, **67**, 933 (1945).

TABLE IV

In Vitro ANTIBACTERIAL ACTIVITY[a]

No.	Min inhib concn, μg/ml — *S. pyogenes*	*S. aureus*
2-[2-(Alkylamino)ethyl]-2-thiourea Dihydrobromides		
1	2.5	5
3	*b*	*b*
1-Alkyl-1-(2-merraptoethyl)guanidines, Hemisulfates, or Hydrobromides		
4	20	*b*
5	5	10
6	2.5	2.5
7	0.3	2.5
9	<0.08	0.3
10	2.5	5
11	10	20
12	*b*	*b*
S-2-(1-Alkylguanidino)ethyl Thiosulfates		
13	*b*	*b*
14	*b*	*b*
15	5	20
16	5	20
17	5	20
18	20	*b*

[a] See ref 8 for test procedures. [b] No inhibition at highest concentration used (20 μg/ml).

Anal. Calcd for $C_{10}H_{22}BrN \cdot HBr$: C, 37.88; H, 7.31; N, 4.42. Found: C, 38.17; H, 7.24; N, 4.41.

2-(Dodecylamino)ethyl bromide hydrobromide was prepared in 27% yield from 2-(dodecylamino)ethanol[11] in a manner identical with that used for 2-(octylamino)ethyl bromide hydrobromide; mp 232–235°.

Anal. Calcd for $C_{14}H_{30}BrN \cdot HBr$: C, 45.05; H, 8.38; N, 3.76. Found: C, 45.35; H, 8.13; N, 3.80.

2-[2-(Octylamino)ethyl]-2-thiourea Dihydrobromide[12] (1).—To 24 g (0.31 mole) of thiourea in 125 ml of *i*-PrOH heated to 50° was added 100 g (0.31 mole) of 2-(octylamino)ethyl bromide hydrobromide, and the resulting mixture was heated under reflux for 40 min. The mixture was cooled, and the solid that precipitated was isolated, washed successively with *i*-PrOH and EtOAc, and dried to give 100 g (80%) of **1**, mp 108–110°.

1-(2-Mercaptoethyl)-1-octylguanidine Hydrobromide[12] (8). A solution of 100 g (0.25 mole) of **1** in 1.3 l. of H_2O was treated with 1 *N* NaOH until the pH was adjusted to 7.5. The white solid that precipitated was isolated, washed with H_2O, dried, and recrystallized from MeOH–Et_2O to give 61 g (77%) of product, mp 102–104°, infrared absorption (KBr) at 2520 cm^{-1} (SH).

1-Hexyl-1-(2-mercaptoethyl)guanidine Hemisulfate[12] (5).—To a stirred solution of 8.6 g (0.062 mole) of 2-methyl-2-thiourea hemisulfate in 60 ml of H_2O at room temperature was added dropwise a solution of 10 g (0.062 mole) of 2-(hexylamino)-ethanethiol[13] in 125 ml of MeOH. The resulting mixture was kept at room temperature for 18 hr, and the solid that precipitated was isolated and recrystallized from MeOH to give 7 g (45%) of product, mp 208–210°, infrared absorption (KBr) at 2520 cm^{-1} (SH).

S-2-(1-Octylguanidino)ethyl Thiosulfate[12] (16).—Air was bubbled for 48 hr through a solution of 15 g (0.048 mole) of **8** in 2 l. of MeOH. The MeOH was removed at reduced pressure, and the viscous oil was solidified by the addition of Et_2O. The solid was recrystallized from MeOH–Et_2O to give 15 g of crude disulfide dihydrobromide, mp 126–128°.

To a solution of 13 g (0.024 mole) of the above crude disulfide in 100 ml of MeOH was added 350 ml of 10% aqueous $(NH_4)_2SO_3$ (freshly prepared by bubbling SO_2 into H_2O and neutralizing the resulting solution with concentrated NH_4OH[14]), and air was passed through the solution at room temperature for 16 hr. The mixture was evaporated to dryness under reduced pressure, and the residue was extracted with boiling MeOH. The hot extract was filtered, and the filtrate was evaporated to dryness under reduced pressure. The solid residue was washed well with H_2O and recrystallized from MeOH to give 9 g (69%) of product, mp 128–130°.

Acknowledgment.—We wish to express appreciation to Mr. C. E. Childs and his associates for the microanalytical data; to Dr. J. M. Vandenbelt, Mrs. Carola Spurlock, and Mr. E. J. Schoeb for ultraviolet and infrared analyses; to Dr. M. W. Fisher and his associates of the Department of Microbiology of Parke-Davis for the biological data; and to Mr. Leonard Doub for encouraging us to pursue the antibacterial lead.

(11) J. S. Pierce, J. M. Salsbury, and J. M. Fredericksen, *J. Am. Chem. Soc.*, **64**, 1691 (1942).

(12) The general procedure is illustrated by this preparation.

(13) R. J. Wineman, M. H. Gollis, J. C. James, and N. M. Pompeni, *J. Org. Chem.*, **27**, 4222 (1962).

(14) D. H. Ball, J. M. Williams, and L. Long, Jr., *ibid.*, **28**, 1521 (1963).