Antiradiation Compounds VI

Metal Chelates and Complexes of 2-Mercaptoethylamine and 2-Mercaptoethylguanidine

By WILLIAM O. FOYE and JAMES MICKLES

To provide stable derivatives of 2-mercaptoethylamine (MEA) and 2-mercaptoethylguanidine (MEG) and to test one of the current hypotheses of the mechanism of radiation protection in animals (that metal binding is an important reaction in impeding cellular oxidations), metal complexes of MEA and MEG have been prepared. Reaction of the trithiocarbonate zwitterions of MEA and MEG with metal salts provided straight chain (2:1) metal complexes, and direct reaction of MEA and MEG with metal salts in acid solution provided cyclic (1:1) chelates. Structures of these compounds have been elaborated. One of the compounds, the Zn(II) complex of MEG has shown appreciable radiation protection in mice.

THE ABILITY to bind metal ions has been considered a possible mechanism for the protective action of the mercaptoamine and other chelating antiradiation agents in animals (1–4). Metal ion chelation or complexation may operate either for scavenging ions of copper or iron to prevent or interrupt cellular oxidations (5) initiated by radiation, for stabilization of the cuprous state of cuprous-containing enzymes (6), or for protection of the metal constituents of enzymes from free radical or ionic attack through transient chelation by the protective compound. In the event of such possibilities as these, administration of a preformed chelate to the animal would be expected to lower the radiation protective effect of the agent. Conversely, if the agent acts in the form of a metal chelate, or possibly is transported as a metal chelate, use of the preformed chelate should give equal or better protective ability than use of the agent alone.

Comparison of the radioprotective abilities of compounds capable of metal-ion chelation with those of the metal chelates themselves has been made in several instances. Alexander (7), for instance, showed that the copper chelates provided less protection to mice than the agents themselves, using diethyldithiocarbamate, dithiooxamide, and oxine. Rixon and Whitfield (8) found that disodium EDTA gave some protection to rats, whereas the copper complex of EDTA afforded little or no protection. An example where radiation protection was provided by metal complexes was reported by Smirnova (9) with the use of polyamine iron complexes.

Other evidence that metal-ion chelation may be of importance in radiation protection of animal cells also exists. Corradi (10) has claimed that such chelating agents as diethyldithiocarbamate, EDTA, *N*-nitroso-*N*-phenylhydroxylamine, and nitrilotriacetate are more active than MEA when given in equivalent fractions of the toxic dose. Protective action by metal ions themselves has been reported by Butler and Robins (11), who found that both ferrous and ferric ions protected trypsin against irradiation by electrons, possibly through complexation with the enzyme. A quantitative correlation between metal-binding ability and radiation protection has been observed by Jones (2), who found a constant ratio between the stability constant of the copper chelate and the protective effect of such agents as MEA, cysteine, histamine, EDTA, ethylenediamine, salicylic acid, and glycine. Knoblock and Purdy (12) have also shown a correlation between the instability constant of the copper complex and the radiation protective capacity in a series of mercaptoamines.

To compare the radioprotective abilities of 2-mercaptoethylamine (MEA) and 2-mercaptoethylguanidine (MEG) with the protective effects of their metal complexes, several reactions that might lead to metal complexes were investigated. Straight-chain metal sulfide complexes of MEA (I) and MEG (II) were obtained from the reaction of the trithiocarbonate zwitterions of MEA (13) and MEG (14) in dimethylformamide with aqueous solutions of divalent metal sulfates. The decomposition of trithiocarbonate-metal complexes to the metal alkyl sulfides appears to be a general reaction and takes place quantitatively (15). The metals employed were Zn (II), Cu (II), and Fe (II).

Cyclic metal complexes, or chelates, of MEA and MEG were obtained from the reactions of the ligands directly with metal salts. A 1:1

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$$^{+}NH_3CH_2CH_2S\overset{\overset{S}{\|}}{C}S^{-} + MSO_4 \rightarrow {}^{+}NH_2CH_2CH_2SMSCH_2CH_2NH_3{}^{+}SO_4{}^{-} + CS_2$$

I

$$NH_2CNHCH_2CH_2S\overset{\overset{S}{\|}}{C}S^{-} + MSO_4 \rightarrow NH_2\overset{\overset{NH_2^+}{\|}}{C}NHCH_2CH_2SMSCH_2CH_2NH\overset{\overset{NH_2^+}{\|}}{C}NH_2\ SO_4{}^{-} + CS_2$$

II

zinc (II) chelate of MEA was prepared using a procedure of Greth and Reese (16), which employs zinc oxide and carbon disulfide in aqueous alkali. Apparently an intermediate carbon disulfide complex decomposed to give the metal chelate. Copper (II) chelates (1:1) of MEA and MEG were obtained from aqueous solution by direct reaction of the sulfate with the ligand at pH 8.0 and allowing the pH to drop. In the case of MEG, it was necessary to keep the pH from dropping below 3.0 in order to obtain a crystalline product of good degree of purity. A similar reaction between ferrous sulfate and MEG, however, gave less drop in pH, and the product was identical with the open complex obtained from Fe (II) and the trithiocarbonate zwitterion of MEG. The chelates (III and IV) were distinguished from the complexes by elemental analyses, a much greater drop in pH during chelate formation, a difference in ratio between ligand and metal in the chelates (1:1) and the complexes (2:1). The complexes, in general, were more highly colored.

Aqueous solubility of both the complexes and chelates was quite limited, with generally less than 0.01 Gm. of substance soluble in 100 ml. of water. Since the antiradiation tests discussed below are generally carried out using 1–3% solutions administered by i.p. injection, these compounds must have been given in suspension.

Analytical evidence for the complexes and chelates prepared is recorded in Table I. The agreement between found and theoretical values was, in general, good for compounds of this nature where exact duplication of the product on successive runs is often difficult. In the majority of cases, agreement of four or five elements with theoretical values was obtained. The compounds were analyzed at two different commercial laboratories, and values for carbon and hydrogen generally concurred. Reproducible values for nitrogen and sulfur were more difficult to obtain, and were not obtained for the complexes of MEG. In the case of the Fe (II) MEG complex prepared directly from MEG rather than from the MEG trithiocarbonate, however, acceptable values for nitrogen and sulfur were obtained (see *Experimental*).

For the Fe (II) complex of MEA, carbon, hydrogen, nitrogen, and metal analyses indicated the presence of four sulfur atoms instead of the three required by the general formula. Formulation of the product as either the bis-bisulfate of the Fe (II) complex or the sulfate of the Fe (III) complex provided theoretical values agreeing for these four elements but not for sulfur. Although the color of the product would suggest that it is the Fe (III) rather than the Fe (II) complex, no indication of Fe (III) was obtained with thiocyanate ion.

Antiradiation Properties.—Antiradiation testing of some of the compounds described was carried out at the Walter Reed Army Institute of Research under the direction of Dr. D. P. Jacobus. Tests were made in mice using lethal doses of X-radiation as previously mentioned (17), 30-day survivial being taken as evidence of protection. The MEG–zinc complex (II) was tested at a dosage level of 50–150 mg./

$$HCl\cdot NH_2CH_2CH_2SH + MSO_4 \rightarrow \begin{array}{l} CH_2\text{——}CH_2 \\ | \quad\quad | \\ NH_2 \quad S \\ \searrow \swarrow \\ M^{+}\ Cl^{-} \end{array}$$

III

$$HBr\cdot NH_2CH_2CH_2S\overset{\overset{NH_2^+Br^-}{\|}}{C}NH_2 \xrightarrow[NH_4OH]{} NH_2\overset{\overset{NH_2^+Br^-}{\|}}{C}NHCH_2CH_2SH + MSO_4 \rightarrow \begin{array}{l} CH_2\text{——}CH_2 \\ | \quad\quad\quad | \\ S \quad\quad NH \\ \quad\quad\quad \| \\ \quad\quad\quad C \\ \quad\quad\quad | \\ \quad\quad\quad NH_2 \\ \searrow \swarrow \\ M^{+}\ Br^{-} \end{array}$$

IV

TABLE I.—PHYSICAL PROPERTIES OF METAL COMPLEXES AND CHELATES

A. COMPLEXES OF MEA: $(^{+}NH_3CH_2CH_2S)_2M\ SO_4^{=}$

M	Yield, %	Color	Formula	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found	Nitrogen, % Calcd.	Nitrogen, % Found	Sulfur, % Calcd.	Sulfur, % Found	Metal, % Calcd.	Metal, % Found
Zn(II)	81	White	$C_4H_{14}N_2O_4S_2Zn$	15.22	15.22	4.47	4.67	8.87	9.11	30.46	30.07	20.70	19.65
Cu(II)	74	Brown	$C_4H_{14}N_2O_4S_2Cu$	15.31	15.21	4.50	4.05	8.92	8.46	30.65	30.62	20.24	20.24
Fe(II)	73	Tan	$C_4H_{18}N_2O_8S_4Fe$[a]	11.91	12.08	3.75	3.89	6.95	7.03	31.80	26.65	13.84	15.35

B. COMPLEXES OF MEG: $(NH_2{}^{+}CNHCH_2CH_2S)_2\ M\ SO_4^{=} \cdot n\ H_2O$

M	Yield, %	Color	n	Formula	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found	Metal, % Calcd.	Metal, % Found	Water, %[b] Calcd.	Water, %[b] Found
Zn(II)	71	White	1	$C_6H_{20}N_4O_6S_2Zn$	17.25	17.01	4.82	4.79	15.65	14.43	4.31	4.05
Cu(II)	50	Red-brown	1	$C_6H_{20}N_4O_6S_2Cu$	17.32	18.92	4.85	4.75	15.27	14.66	4.33	4.52
Fe(II)	84	Green	2	$C_6H_{22}N_4O_8S_2Fe$	16.90	16.77	5.17	4.85	13.10	13.94	8.45	6.69

C. CHELATES OF MEA AND MEG

Chelate	Yield, %	Color	Formula	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found	Nitrogen, % Calcd.	Nitrogen, % Found	Sulfur, % Calcd.	Sulfur, % Found	Metal, % Calcd.	Metal, % Found
Zn(II)MEA	71	White	C_2H_6NOSZn[c]	15.25	14.47	3.84	3.63	8.89	10.30	20.36	22.97	41.50	45.75
Cu(II)MEA	92	Yellow	$C_2H_6NSClCu$	13.72	13.44	3.45	4.53	8.00	8.79	18.31	19.66	36.28	36.24
Cu(II)MEG	59	Yellow	$C_3H_9N_3SBrCu$	13.77	13.57	3.08	3.81	16.06	14.80	12.25	12.87	24.28	24.79

[a] Formulated as bis-bisulfate. [b] Determined by weight loss on vacuum drying at 100°. [c] The anion is hydroxyl ion.

Kg. and was described as giving good protection, but somewhat less than that afforded by MEG itself. The MEG–copper complex (II) gave only slight protection, as did the zinc chelate of MEA (III). The latter two compounds were appreciably more toxic than the MEG–zinc complex, however, and could be tested only at dosage levels under 50 mg./Kg.

EXPERIMENTAL

Analyses for carbon, hydrogen, nitrogen, and sulfur were determined by both Weiler and Strauss, Oxford, England, and Schwarzkopf Microanalytical Laboratory, Woodside, N. Y. Metal analyses were done by ashing at 900°. Melting points were taken on a Fisher-Johns block.

Complexes of 2-Mercaptoethylamine.—The following procedure is representative. An aqueous solution (20 ml.) of 4.32 Gm. (0.015 mole) of zinc sulfate heptahydrate or 3.74 Gm. (0.015 mole) of cupric sulfate pentahydrate or 4.18 Gm. (0.015 mole) of ferrous sulfate heptahydrate was added dropwise with stirring to a solution of 4.39 Gm. (0.03 mole) of the zwitterion of 2-aminoethyltrithiocarbonic acid (13) in 150 ml. of dimethylformamide. A precipitate immediately formed. Stirring was continued for an hour after the addition was complete, and the product was isolated and washed with benzene followed by ether. The product was placed under vacuum to remove the excess solvent and was then air dried. The complexes decomposed on heating above 200°.

Complexes of 2-Mercaptoethylguanidine.—The following procedure is representative. An aqueous solution (15 ml.) of 2.16 Gm. (0.0075 mole) of zinc sulfate heptahydrate or 1.87 Gm. (0.0075 mole) of cupric sulfate pentahydrate or 2.09 Gm. (0.0075 mole) of ferrous sulfate heptahydrate was added dropwise with stirring to a solution of 2.93 Gm. (0.015 mole) of the zwitterion of 2-guanidoethyltrithiocarbonic acid (14) in 100 ml. of dimethylformamide. A precipitate formed immediately. Stirring was continued for an hour after the addition was complete, and the product was isolated and washed with benzene followed by ether. The product was placed under vacuum to remove the excess solvent and was then air dried. The complexes decomposed above 200°.

Zinc Chelate of 2-Mercaptoethylamine.—2-Mercaptoethylamine hydrochloride (11.3 Gm., 0.1 mole) (Chemicals Procurement Labs.) was dissolved in 65 ml. of water and neutralized by the addition of an aqueous solution of sodium hydroxide (4 Gm., 0.1 mole). To this solution was added 8.1 Gm. (0.1 mole) of zinc oxide, and the resulting suspension was stirred while the temperature was maintained at 40°. Carbon disulfide (10 ml., 0.15 mole) was added dropwise with stirring. The temperature was maintained at 40°, and stirring was continued for 3 hours. During this period the color of the suspension turned from yellow to white. A white solid was isolated and washed with water followed by ethanol, and the product was dried in a vacuum desiccator. The yield was 11.1 Gm. (71.4%) of a white product which decomposed above 210°.

Cupric Chelate of 2-Mercaptoethylamine.—2-Mercaptoethylamine hydrochloride (3.42 Gm., 0.03 mole) was dissolved in 35 ml. of water and the pH of the resulting solution was adjusted to 8.0 with dilute ammonium hydroxide. Ten milliliters of a solution containing 3.74 Gm. (0.015 mole) of cupric sulfate pentahydrate was added dropwise with stirring, and at a pH of 4.5, a yellow precipitate appeared. After the addition was complete, the pH had decreased to 2.3. Stirring was continued for an hour after the addition was complete, and the yellow product was isolated and washed with water followed by ethanol. The yield was 2.4 Gm. (92.2%), and the product decomposed above 200°.

Cupric Chelate of 2-Mercaptoethylguanidine.—*S*-β-Aminoethylisothiouronium bromide hydrobromide (8.52 Gm., 0.03 mole) (Matheson, Coleman and Bell) was dissolved in 35 ml. of water and the pH of the resulting solution was adjusted to 8.0 with dilute ammonium hydroxide. Ten milliliters of a solution containing 3.74 Gm. (0.015 mole) of cupric sulfate pentahydrate was added dropwise with stirring, and at a pH of 6.5, a yellow precipitation appeared. The pH was allowed to decrease to 3.0 and was maintained at this value by the alternate dropwise addition of dilute ammonium hydroxide and the cupric sulfate solution. Stirring was continued for 1

hour after the addition was complete, and the yellow product was isolated and washed with water followed by ethanol. The yield was 2.3 Gm. (58.7%), and the product decomposed above 110°.

Ferrous Complex of 2-Mercaptoethylguanidine.—*S*-β-Aminoethylisothiouronium bromide hydrobromide (8.52 Gm., 0.03 mole) was dissolved in 35 ml. of water and the pH was adjusted to 8.0 using dilute ammonium hydroxide. A solution of 4.18 Gm. (0.015 mole) of ferrous sulfate heptahydrate in 15 ml. of water was added dropwise with stirring and a dark green precipitate formed immediately. After the addition was complete, the stirring was continued for 1 hour and the pH of the solution had decreased to a value of 6.6. The green product was isolated and washed with water followed by ethanol. The yield was 1.1 Gm. (17.3%) of a dark green product which started to decompose at 225°.

Anal.—Calcd. for $C_6H_{22}FeO_6S_3$: C, 16.90; H, 5.17; Fe, 13.10; N, 19.71; S, 22.56. Found: C, 17.06; H, 5.16; Fe, 11.99; N, 19.00; S, 22.24.

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Investigation of Egyptian Basil Essential Oils by Simple Chromatographic Method

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Two kinds of basil oil are produced in Egypt under the so-called white and red basil oils. The present study reveals the occurrence of terpineol, linalool, cineole, citral, eugenol, esterified terpineol, geraniol, linalool, and citronellol with acetic and formic acids (geranyl and/or citronellyl acetate, linalyl and/or terpinyl acetate and citronellyl formate) a sesquiterpene alcohol (nerolidol?) and unidentified terpenes in both types of oil. The red type contains in addition methyl chavicol and cinnamic acid ester. The white type contains methyl cinnamate and (safrol ?). A simple economic method for the oil investigation is described.

THE GENUS *Ocimum* includes, according to Hegi (1), 50–60 species in Africa, Asia, and America. Guenther (2) and Gildemeister and Hoffmann (3) state that there is a great possibility of cross pollination in the species of *O. basilicum* L., hence the occurrence of a larger number of varieties and physiological forms is possible. They claim that it seems appropriate to classify the basil oil types according to their chemical composition and geographical sources rather than ascribing them to definite plant varieties. They mention main types of *Ocimum* plants according to the chemical compound dominating in the oil.

Studies reported on the chemical composition of *O. basilicum* oils as reviewed by these authors, indicate that they contain methyl chavicol, anethole, methyl cinnamate, linalool, eugenol, cineole, camphor, terpenes, and sesquiterpenes.

Tackholm (4) describes only two indigenous *Ocimum* plants in the Egyptian flora under the names of *O. menthaefolium* Hoechst and *O. menthaefolium* var. *staminosum* Sims. Ascherson (5) mentions *O. basilicum* L. in Egypt under the cultivated plants. Basil is named "rihan" or "saatar hindy" by the natives of Egypt.

Since no previous work has been reported on the analysis of the Egyptian basil volatile oils, this study was undertaken to reveal their constituents. This study is also made to verify, as far as possible with future studies, the appropriate chemotaxonomical order of the Egyptian basil plants. Further aim of the study is to find out a simple economic method for the oil investigation which could be applied easily in agricultural experimental stations occupied with the production of essential oils, where expensive and delicate apparatus cannot always be afforded easily.

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