Antiradiation Compounds. II. Dithiocarbamates[1]

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A number of substituted dithiocarbamic acid derivatives, some of which contain metal chelating functions additional to the dithiocarbamyl group, have been synthesized as potential protective agents against ionizing radiation. Some of the compounds showed significant protection of mice at a radiation level of 575–600 roentgens, but it appears that an additional nitrogen-containing chelating function contributes nothing to the radioprotective ability of the dithiocarbamates.

Dithiocarbamic acids and their salts have been stated to be effective radiation protective agents in animals, but no attempt has been reported to evaluate other dithiocarbamates than those immediately available. Bacq[2] found sodium diethyldithiocarbamate to be comparable to cysteamine in protecting mice against X-rays, in evaluating the protective effects of several chelating agents. Van Bekkum[3] tested a series of N-alkylated dithiocarbamates in mice and found that alkylation of the amine function with chains longer than propyl was generally unfavorable, since toxicity was increased and effective doses could not be administered. He also showed that dithiocarbamate esters were less effective than the salts. More recently, sodium diethyl dithiocarbamate[4] was described as having a good protective effect, but less than that of thiourea, which conflicts with the relative evaluations of Eldjarn and Pihl.[5] The preparation of β-aminoethyl esters of three substituted dithiocarbamic acids, the dimethyl, diethyl, and dimethylcarbazyl derivatives, has been reported[6] without antiradiation testing results. The dithiocarbamic acid of β-mercaptoethylamine (MEA) has also been synthesized[7]

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and found to have a radiation protective effect in mice approaching that of MEA itself and an intraperitoneal toxicity approximately one-half that of MEA. Cheymol, *et al.*[8] have tested several salts of dithiocarbamic acid derivatives including the N-phenyl, N-morpholinyl, and N-piperidinyl, without finding appreciable protective effects against a greater than lethal dose of X-irradiation (1025 r.).

Dithiocarbamates are well known as metal chelating agents, and metal ion chelation is frequently mentioned as a possible mechanism of radiation protection in the cells by organic compounds.[9-13] It is a logical deduction that metal ion chelation may be of importance in the antiradiation effects shown by dithiocarbamates, and with this in mind, a variety of dithiocarbamates has been prepared, some of which contain chelating groups additional to the dithiocarbamyl moiety. The possibility of metal ion chelation in antiradiation effects was first mentioned by Bacq,[2] and Albert[14] has pointed out that sequestration of iron and copper ions could halt or prevent the chain reaction of hydroxyl radical formation in the cells. Bonati[15] apparently considered metal ion chelation of importance in the protective action of mercaptoamines, since the toxicities of several of their metal chelates were reported. Brintzinger, *et al.*,[16] have also postulated that radiation damage in the cells is dependent upon metal ion equilibria and that protective agents affect these equilibria.

The dithiocarbamates were prepared either in aqueous media with carbon disulfide and ammonia or in absolute alcohol with carbon disulfide. In these reactions, analytical results indicated that the free dithiocarbamic acids were isolated where another amino group was present in the molecule, but the acids most likely exist as zwitterions. In the case of β-mercaptoethyldithiocarbamic acid,[7] however, a free dithiocarbamic acid apparently has been isolated, but the compound is quite unstable to air and moisture. Physical constants of the compounds prepared are recorded in Table I.

It is worthy of note that cystamine formed a mono-dithiocarbamate, whereas piperazine, also having two nitrogens of equal basicity, formed a bis-dithiocarbamate. The piperazine bisdithiocarbamic

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TABLE
PHYSICAL PROPERTIES OF

R	R′	M.p., °C.	Medium	Yield, %	Formula
C_6H_5NH	NH_4^+	92–93[a]	H_2O	75	$C_7H_{10}N_2S_2$
cyclo-$C_6H_{11}NH$	*cyclo*-$C_6H_{11}NH_3^+$	184–186	H_2O	42	$C_{13}H_{26}N_2S_2$
n-$C_8H_{17}NH$	*n*-$C_8H_{17}NH_3^+$	88–89	C_2H_5OH	74	$C_{17}H_{38}N_2S_2$
$(C_2H_5)_2NC_2H_4NH$	H	146–147	H_2O	73	$C_7H_{16}N_2S_2$
$(CH_3)_2NC_6H_6NH$	H	145–147	H_2O	85	$C_8H_{14}N_2S_2$
$HOC_2H_4NHC_2H_4NH$	H	121–123	C_2H_5OH	97	$C_5H_{12}N_2OS_2$
$C_2H_5N(C_2H_4)_2N$[b]	H	160–162(s)[c]	C_2H_5OH	84	$C_7H_{14}N_2S_2$
$N(C_2H_4)_2N$	$2HN(C_2H_4)_2NH_2^+$	190(s)	C_2H_5OH	51	$C_{14}H_{30}N_6S_4$
$HN(C_2H_4)_2NC_2H_4NH$	H	151(s)	C_2H_5OH	83	$C_7H_{15}N_3S_2$
NH_2NH	$NH_2NH_3^+$	119–120	C_2H_5OH	96	$CH_3N_4S_2$
$NH_2C_2H_4S_2C_2H_4NH$	H	87–90	H_2O	24	$C_5H_{12}N_2S_4$
NH_2	$C_2H_4NH_2.HBr$	199–200	C_2H_5OH	54	$C_3H_9BrN_2S_2$
$(C_2H_5)_2N$	$C_2H_4NH_2.HCl$	126–128[d]	H_2O	27	$C_7H_{17}ClN_2S_2$

[a] Lit.[18] m.p. 108°. [b] 1-Ethylpiperazine was prepared according to Moore, Boyle, and Thorn[19] by Dr. D. H. Kay. [c] Lit.[19] m.p. 160° (s). [d] Lit.[20] m.p. 122–124°.

acid also was isolated as the bis-piperazinium salt, whereas cystamine evidently underwent zwitterion formation. In the case of 1-(β-aminoethyl)-piperazine, the primary amino group must be a weaker base than the secondary piperazine nitrogen, and dithiocarbamation should therefore take place on the primary amino nitrogen and salt formation on the secondary.

In cases where more than one amino group was available for dithiocarbamation, the reactions were assumed to follow the generalities of Losanitsch,[17] who established that the less basic amine is attached to the carbon disulfide and the more basic amine appears as the cation of the salt. These assumptions were favored by the infrared absorption spectra of the dithiocarbamates where both primary and secondary amino groups were present in the amine reactant, the primary amine band generally disappearing after dithiocarbamation. For instance, in the case of the dithiocarbamate of 2-hydroxyethylaminoethylamine, the primary amine peak at 1620 cm.$^{-1}$ in the parent compound disappeared completely, and the absorption at 3450 cm.$^{-1}$ due to hydroxyl and amino groups was weakened in intensity. In the case of 1-(β-aminoethyl)-piperazine, the primary amino stretching vibration at 3460 cm.$^{-1}$ disappeared after dithiocarbamation. In addition, a weak band appeared at 3440 cm.$^{-1}$ in the dithiocarbamate, probably due to appearance of secondary amine which was concealed by primary amine absorption in the parent compound. The primary amine band at 1620 cm.$^{-1}$ also appeared in the dithio-

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DITHIOCARBAMIC ACIDS, RCS_2R'

Carbon, %		Hydrogen, %		Sulfur, %		Nitrogen, %	
Calcd.	Found	Calcd.	Found	Calcd.	Found	Calcd.	Found
56.93	55.80	9.49	9.10				
61.08	61.11	11.38	11.40	19.16	19.30		
43.75	43.90	8.33	8.70				
40.44	40.40	7.87	7.90				
33.33	33.80	6.67	7.00	35.56	35.00		
41.00	41.80	7.32	7.30	31.20	31.60		
40.97	40.70	7.32	7.60	31.20	31.30		
8.57	9.10	5.71	5.70				
26.31	26.53	5.26	5.58	56.14	55.17	12.28	12.51
16.59	16.59	4.15	4.31	29.49	29.54	12.90	12.81
36.76	35.98	7.44	7.64	28.01	27.43		

carbamate as a split weak band at 1590 and 1610 cm.$^{-1}$, which is attributed to secondary amine salt formation.

Biological Activities.—Tests for the ability of some of these compounds to protect mice against X-irradiation have been carried out at the Walter Reed Army Institute of Research under the direction of Dr. D. P. Jacobus. These results are summarized in Table II, and the corresponding data for the dithiocarbamic acid of MEA are included for comparison. The dithiocarbamates reported here did not approach the MEA dithiocarbamic acid in protective ability, in general no protection being afforded at a radiation dosage level of 800 roentgens. Significant protection (35-40% increase in survival over that of the control animals) was afforded by a number of the dithiocarbamic acid derivatives when injected 30 minutes prior to a radiation dose of 575-600 r., however.

Although stability constants for the formation of metal chelates of a number of these dithiocarbamates are being determined at present, it does not appear from the antiradiation effects that inclusion of an additional chelating function (amino) in the dithiocarbamate molecule affects the radiation protective ability. For example, the phenyl derivative is completely inactive, and the cyclohexyl analog shows significant protection. However, both the β-diethylaminoethyl and β-hydroxyethylaminoethyl derivatives, as well as the piperazine derivatives, where the additional nitrogens might be expected to contribute to chelate stability, showed some protection. Furthermore, the dimethylaminopropyl derivative, where the additional nitrogen is too far removed from the dithio function to affect chelate stability, was without protective ability.

TABLE II

ANTIRADIATION PROPERTIES OF THE DITHIOCARBAMATES IN MICE

Compound	Drug level, mg./kg.	Dose, r.	30-Day survival, % Treated	Controls
2-Mercaptoethyldithiocarbamic acid	350[a]	800	75	0
	350[a]	575	95	40
Ammonium phenyldithiocarbamate	100	800	0	0
Cyclohexylammonium cyclohexyldithiocarbamate	50	575	65	25
2-Diethylaminoethyldithiocarbamic acid	25[a]	575	75	50
3-Dimethylaminopropyldithiocarbamic acid	15	575	75	70
2-Hydroxyethylaminoethyldithiocarbamic acid	600	575	60	20
1-Ethylpiperazine-4-carbodithioic acid	100	600	55	20
2-Piperazinoethyldithiocarbamic acid	400	600	65	30

[a] Administered 15 minutes prior to X-irradiation.

Radiation protection of *Escherichia coli* cells was also assayed using the dithiocarbamic acid derivatives. No protection was observed except with the β-hydroxyethylaminoethyl and β-aminoethylpiperazine derivatives.

Acute intraperitoneal toxicities in mice of the compounds tested for antiradiation effects are listed in Table III. Several of the compounds proved to be unexpectedly toxic, more so than MEA dithiocarbamic acid, for instance, which drastically limited the dose which could be given to the mice. No correlation between dosage size and protective ability is evident, however.

Metal ion chelation cannot be discarded as a possible mode of radioprotective action from these results, since practically all dithiocarbamic acids and salts are theoretically capable of complexing metal ions. All of the dithiocarbamates discussed here were found to chelate both cupric and ferric ions. However, inclusion of additional nitrogen-containing groups, to provide possible tridentate chelates, does not appear to increase their protective ability.

Experimental

Substituted Dithiocarbamic Acids.—The amine (0.1 mole) was added dropwise over a period of 20 min. to a stirred mixture of carbon disulfide (0.1 mole) and concentrated ammonia water (0.25 mole) cooled by an ice-salt bath. Where the nonaqueous procedure was used, the amine (0.1 mole) was dissolved in absolute ethanol, (80 ml. or more if required), cooled and treated dropwise with an equiva-

TABLE III

INTRAPERITONEAL TOXICITIES OF DITHIOCARBAMATES IN MICE[a]

Compound	Dose, mg./kg.	Mortality (dead/total) Acute	10 Days
2-Mercaptoethyldithiocarbamic acid	500	0/5	1/5
	600	4/5	5/5
Ammonium phenyldithiocarbamate	300	0/5	2/5
	400	0/5	5/5
Cyclohexylammonium cyclohexyldithiocarbamate	150	0/5	2/5
	200	0/5	5/5
2-Diethylaminoethyldithiocarbamic acid	50	2/5	2/5
	100	2/5	2/5
3-Diethylaminopropyldithiocarbamic acid	20	3/5	3/5
	25	2/5	2/5
2-Hydroxyethylaminoethyldithiocarbamic acid	700	0/5	0/5
1-Ethylpiperazine-4-carbodithioic acid	150	2/5	2/5
	200	5/5	5/5
2-Piperazinoethyldithiocarbamic acid	500	1/5	1/5
	600	1/4	1/4

[a] Determined at the Walter Reed Army Institute of Research by Dr. D. P. Jacobus and staff.

lent of carbon disulfide with constant stirring. The products generally precipitated without concentration of the solvent, were washed well with water or absolute ethanol, and dried for a short time on blotting paper before storage in a desiccator. Attempts at recrystallization frequently resulted in partial decomposition. Where gummy products were isolated, resuspension of the product in absolute ethanol and carbon disulfide generally gave a crystalline compound of good purity.

Aminoethyl Esters of Dithiocarbamic Acids.—A mixture of 5.5 g. (0.05 mole) of ammonium dithiocarbamate,[18] 10.3 g. (0.05 mole) of 2-bromoethylamine hydrobromide (Eastman Organic Chemicals) and 40 ml. of ethanol was refluxed for 40 min. The white, crystalline product was filtered after being cooled, washed with chilled ethanol, and recrystallized from ethanol. In the case of the N,N-diethyldithiocarbamate ester, the reaction was carried out at 30–40° in aqueous alkaline solution with 2-bromoethylamine hydrobromide and sodium diethyldithiocarbamate, prepared *in situ* from diethylamine (0.35 mole in 50 ml. of water), carbon disulfide (0.25 mole), and sodium hydroxide (20 g. in 90 ml. of water). The product was converted to the hydrochloride after extraction from the reaction medium with ether and drying over anhydrous sodium sulfate.

Chelation of Dithiocarbamates with Cupric and Ferric Ions.—In a representative experiment, 50 ml. of a solution containing 4.6 g. (0.017 mole) of $FeCl_3 \cdot 6H_2O$ or 6.25 g. (0.025 mole) of $CuSO_4 \cdot 5H_2O$ was added dropwise to a solution containing 8.75 g. (0.05 mole) of a sodium dithiocarbamate. A solution of 0.1 *N* sodium hydroxide was added simultaneously to maintain the pH above 8.0. The precipitate which appeared was allowed to settle overnight, isolated, and washed with water. The product was washed with ether and dried under warm air. Evidence

of chelation was given by ashing the products at 1000° and obtaining metal contents that indicated a chelate ratio of 1:1 or 2:1 of organic ligand to metal. Analytically pure products were not obtained.

Synthesis of Chromogenic Substrates for the Assay of Aminopeptidase Activity[1]

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The 2-naphthylamides of six amino acids were prepared as substrates in order to determine if the enzymatic splitting of the amide link is due to a non-specific aminopeptidase or an aminopeptidase specific for each amino acid.

Although the enzymes aminopolypeptidase, aminodipeptidase, and leucyl aminopeptidase have been described[2,3,4] it is not known whether there exists one non-specific aminopeptidase or many aminopeptidases specific for each amino acid. Since a biochemical and histochemical method for leucyl aminopeptidase has been described utilizing L-leucyl-2-naphthylamide as the substrate,[5,6] a series of naphthylamides of other amino acids was prepared and their enzymatic cleavage compared to the leucyl compound. This paper describes the synthesis of the substrates.

The results of the biochemical study[7] may be summarized as follows. The leucyl, methionyl, alanyl, and arginyl amides were assayed at 1×10^{-3} *M;* the glycyl amide at 1×10^{-2} *M;* the phenylalanyl sub-

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