4. In the presence of small quantities (0.25 and 0.5%) of polysorbate 80, propylparaben showed the greatest inhibitory powers. However in greater concentrations of the surfactant (1, 2, and 5%) sorbic acid was more effective.

5. Methylparaben was the least effective of the compounds tested, alone or in the presence of polysorbate 80.

6. On the basis of these manometric results using baker's yeast as the test organism, further investigation is considered justified using this technique with other organisms, preservatives, and surfactants.

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Antiradiation Compounds I

Acylated Derivatives of β-Mercaptoethylamine

By WILLIAM O. FOYE, RONALD N. DUVALL, and JAMES MICKLES

A series of S-acyl derivatives of β-mercaptoethylamine (MEA) has been prepared in an attempt to provide compounds having protective effects in animal cells against ionizing radiation and which are less toxic than MEA itself. In general, toxicity to animals was reduced and good protective ability against X-radiation was retained in some of the esters. The dithiocarbamic acid of MEA was also prepared, but attempts to acylate this compound, which showed good antiradiation effects in close to toxic dose levels in mice, gave a variety of decomposition products.

Beta-Mercaptoethylamine (cysteamine, MEA) has been reported one of the most effective compounds discovered for protecting animal cells against the deleterious effects of ionizing radiation (1). This compound is somewhat toxic for human use (2), however, so an attempt has been made to mask the toxic effects by preparing derivatives subject to hydrolysis *in vivo* which would afford a liberation of MEA over a period of time. Several acylating agents were selected for this purpose.

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The hydrochloride of MEA was utilized on the assumption that acylation of the nitrogen would be prevented. Conventional blocking agents would be difficult to remove in the presence of the thioester formed, and nonhydrolyzable substituents on the nitrogen have generally resulted in diminution or loss of radio-protective action. Attempts to prepare the S-carbobenzoxy derivative of MEA, furthermore, resulted in the formation of S-benzylmercaptoethylamine. Also, MEA hydrochloride failed to react with ethyl chloroformate. However, the use of the hydrochloride of MEA was successful in preventing formation of N-acyl derivatives.

Some difficulty was experienced in finding a suitable solvent for both MEA·HCl and the selected acyl chlorides, but the reaction was found to take place in either dimethylformamide or an

TABLE I.—ESTERS OF 2-MERCAPTOETHYLAMINE HYDROCHLORIDE

$$R{-}C(=O){-}S{-}CH_2{-}CH_2{-}NH_2\cdot HCl$$

R	M.p., °C.	Purification Solvent	Reaction Time, hr.	Yield, %	Formula	Analyses Calcd.	Found
CH_3	130–132	Benzene-ether	1/2	84	$C_4H_{10}ClNOS$	S: 20.6	21.4
C_3H_7	86–88	Benzene-ether	1/2	55	$C_6H_{14}ClNOS$	S: 17.4	17.7
C_4H_9	98–100	Benzene-ether	1/2	74	$C_7H_{16}ClNOS$	S: 16.2	16.3
C_5H_{11}	108–110	Benzene-ether	1/2	75	$C_8H_{18}ClNOS$	S: 15.1	15.7
C_6H_{13}	106–108	Benzene-ether	1/2	86	$C_9H_{20}ClNOS$	S: 14.2	14.6
C_7H_{15}	111–113	Absolute ethanol	2	85	$C_{10}H_{22}ClNOS$	S: 13.4	13.9
$C_{13}H_{27}$	126–128	Water	2	75	$C_{16}H_{34}ClNOS$	C: 59.2	59.3
						H: 10.5	10.7
						S: 9.9	10.2
CH_2Cl	126–128	Benzene-ether	1/2	85	$C_4H_9Cl_2NOS$	C: 25.3	25.3
						H: 4.8	4.7
						S: 16.9	16.7
C_6H_5	170–172	Absolute ethanol	2	82	$C_9H_{12}ClNOS$	S: 14.7	15.1

TABLE II.—ANTIRADIATION PROPERTIES OF MEA DERIVATIVES IN MICE[a]

Compound	Drug Level, mg./Kg.[b]	Dose, r	30-Day Survival, % Treated	Controls
S-Acetyl-2-mercaptoethylamine	400	800	60	0
	400	575	80	20
S-Chloroacetyl-2-mercaptoethylamine	600	800	0	0
	600	575	85	70
S-Octanoyl-2-mercaptoethylamine	600	800	60	0
	600	575	90	50
S-Myristoyl-2-mercaptoethylamine	400	800	0	0
	400	575	65	35
S-Benzoyl-2-mercaptoethylamine	225	800	10	0
	225	575	85	75
2-Mercaptoethyldithiocarbamic acid	350	800	75	0
	350	575	95	40

[a] Determined at the Walter Reed Army Institute of Research by D. P. Jacobus. [b] Administered 15 minutes prior to X-irradiation.

excess of the acyl chloride. Physical properties and reaction data for the esters prepared are listed in Table I.

Since dithiocarbamates have also shown relatively good protective effects against ionizing radiation, the dithiocarbamic acid of MEA was prepared, which may be considered an acylation with carbon disulfide. A two-phase system of carbon disulfide and ammonia water was applicable for this preparation; apparently the ammonium salt of the dithiocarbamic acid of MEA, which would be expected, was hydrolyzed under the reaction conditions, and the free acid was obtained.

Relatively good antiradiation properties in mice were found with the dithiocarbamic acid of MEA (see Table II), but the compound was found to be unstable in air, and close to toxic dosage levels were necessary for protection against lethal doses of X-radiation. Accordingly, attempts were made to acylate the β-mercapto group of this compound with the intention of increasing stability and reducing toxicity. Reaction of MEA dithiocarbamic acid with acetyl chloride at various temperatures, however, resulted in decomposition with the loss of hydrogen sulfide or carbon disulfide. Three products were identified from the attempted acetylation: 2-mercaptothiazoline, S-acetyl-β-mercaptoethylamine hydrochloride, and β-mercaptoethylamine disulfide dihydrochloride. See I. Attempted acylation in buffered aqueous solution also resulted in decomposition of the dithiocarbamic acid. The high degree of insolubility of this compound in organic media proved to be a major handicap.

The reverse procedure of dithiocarbamation of an acyl derivative of MEA was also investigated. The reaction of S-benzoyl-β-mercaptoethylamine hydrochloride with carbon disulfide and ammonia water was accordingly carried out, but the major product proved to be N-benzoyl-β-mercaptoethylamine. The migration of the benzoyl group appeared to be nearly instantaneous, as shown by immediate ether extraction of a solution of S-benzoyl-β-mercaptoethylamine hydrochloride when made basic with ammonia water. Similar results were obtained when the S-acetyl, S-octanoyl, and S-myristoyl esters of MEA were made alkaline in ammonia water; no evidence of dithiocarbamation was found. See II. The desired reaction was therefore found to be im-

possible in either neutral, acid, or basic aqueous media. Similar acyl migrations from sulfur to nitrogen have been observed by others (3).

Reaction of the dithiocarbamic acid of MEA with acid chlorides in ether also gave products that decomposed either on storage in a desiccator or on suspension in water. The N-acyl derivatives were isolated from this reaction, however. Use of pyridine and acetic anhydride likewise resulted in the formation of N-acetyl-2-mercaptoethylamine, a known compound.

Antiradiation Properties.—Tests for the ability of these compounds to protect mice against X-radiation were carried out at the Walter Reed Army Institute of Research under the direction of Dr. D. P. Jacobus. The S-acetyl derivative of MEA was found to provide 60% protection over a 30-day survival period when the mice were irradiated at a dose of 800 r. An 80% survival was observed after a dose of 575 r, nontoxic doses of compound being injected in each case 15 minutes prior to exposure. The S-octanoyl ester gave similar results. The S-myristoyl ester provided 65% protection against a dose of 575 r, but was ineffective against 800 r. The dithiocarbamic acid gave 75% protection against 800 r, and 95% protection against 575 r, making this compound almost the equal of MEA itself. These results are summarized in Table II.

The benzoyl ester of MEA was not appreciably effective, affording only 10% protection from 800 r. The chloroacetyl ester, however, which would be expected to hydrolyze in water at a somewhat more rapid rate than the other esters tested (4), surprisingly gave no protection at all. It is possible that the compound underwent polymerization or cyclization before reaching the cells, when neutralized prior to injection.

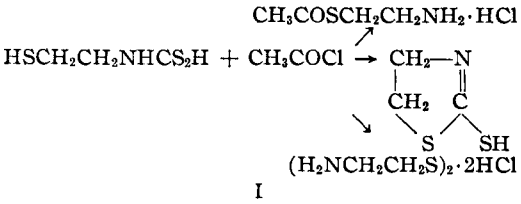
The acute intraperitoneal toxicities in mice of several of the esters are recorded in Table III, where they may be compared to the toxicity of 2-mercaptoethylamine. It is apparent that a lowering of toxicity has resulted on ester formation. Although hydrochlorides of the esters were used, a valid comparison may be made with 2-mercaptoethylamine itself, since neutralization would be expected to occur fairly rapidly *in vivo*.

On the basis of the mixed disulfide hypothesis (5) of radiation protection by mercaptans, the S-acyl derivatives of MEA would be expected to suffer hydrolysis *in vivo*, thus liberating MEA to form mixed disulfides with vital thiol groups in the cells. However, rearrangement to the N-acyl derivatives may take place, which is shown here to occur quite readily at pH's above 7. This, too, would provide a free thiol group for mixed disulfide formation, but the basicity of the nitrogen, usually present in compounds possessing antiradiation ability, would be lost. Antiradiation tests on the N-acyl derivatives should help resolve the question of the necessity of a basic nitrogen in compounds of this type.

TABLE III.—INTRAPERITONEAL TOXICITIES OF MEA DERIVATIVES IN MICE[a]

Compound	Dose in Mice, mg./Kg.	Mortality, Acute	Dead/Total 10 Days
2-Mercaptoethylamine	230[b]	..	..
	350[c]	..	..
S-Acetyl-2-mercaptoethylamine·HCl	400	0/5	0/5
	500	2/5	4/5
S-Chloroacetyl-2-mercaptoethylamine·HCl	800	0/5	0/5
	1000	0/5	4/5
S-Octanoyl-2-mercaptoethylamine·HCl	650	0/5	0/5
	800	1/5	2/5
S-Myristoyl-2-mercaptoethylamine·HCl	400	0/5	0/5
	800	0/5	0/5
S-Benzoyl-2-mercaptoethylamine·HCl	250	0/5	0/5
	350	2/3	4/5
2-Mercaptoethyldithiocarbamic acid	400	0/5	0/5
	500	0/5	1/5
	600	4/5	5/5

[a] Determined at the Walter Reed Army Institute of Research by D. P. Jacobus. [b] LD_{50} value in rats obtained by Bonati, F., *Arch. ital. sci. farmacol.*, **9**, 125(1959). [c] LD_{50} value in mice obtained by Bacq, Z. M., and Herve, A., *Schweiz. med. Wochschr.*, **82**, 1018(1952).



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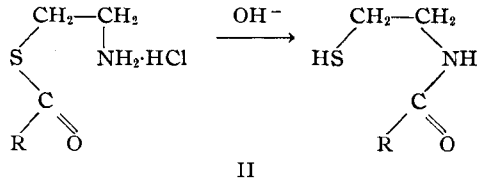

II

EXPERIMENTAL

Melting points were taken on a Fisher-Johns block and are uncorrected. Carbon-hydrogen analyses were done by Weiler and Strauss, Oxford, England, or by Carol K. Fitz, Needham, Mass.

2-Mercaptoethylamine Hydrochloride.—A modification of the procedure of Mills and Bogert (6) was used. Ethyleneimine (27.5 ml., 0.52 mole) dissolved in 205 ml. of absolute ethanol was added dropwise, with stirring, during 1 hour to 200 ml. of absolute ethanol. A stream of dry hydrogen sulfide was passed into the ice-cooled solution simultaneously. The resulting solution was partially dis-

tilled under nitrogen and reduced pressure, and dry hydrogen chloride was then bubbled through the remaining solution until it was acidic. The solvent was distilled under nitrogen and reduced pressure, and the resulting oil was allowed to crystallize by being chilled in tightly stoppered bottles. A 72% yield was obtained of material melting at 70°, which agrees with the reported value (7).

Esters of 2-Mercaptoethylamine Hydrochloride.—The following procedure is representative. A mixture of 2-mercaptoethylamine hydrochloride (4.5 Gm., 0.04 mole) and myristoyl chloride (15 ml., Eastman Organic Chemicals) was refluxed on a water bath for 2 hours or until the mixture solidified (the lower esters, with the exception of the benzoyl, gave solutions). The solid mass (which generally resulted on cooling) was pulverized, washed with benzene and ether, and recrystallized from absolute ethanol. Washing with water was possible in the case of the myristoyl ester. A yield of 75% was obtained of material melting at 126–128°.

Anal.—Calcd. for $C_{16}H_{34}ClNOS$: C, 59.2; H, 10.5; S, 9.9. Found: C, 59.3; H, 10.7; S, 10.2.

Reaction of 2-Mercaptoethylamine with Benzyloxycarbonyl Chloride.—2-Mercaptoethylamine hydrochloride (10.0 Gm., 0.09 mole) was refluxed with 25 ml. of benzyloxycarbonyl chloride for 20 hours on a water bath or 4 hours at 150°, when a clear solution resulted. Benzene was added, and an oil separated which soon crystallized. The product was recrystallized from absolute alcohol with the addition of a large amount of ether. The product, 2-benzylthioethylamine hydrochloride, which did not give a mercapto test with iodine, melted at 112–114° [Walton (8) reported 120–136°].

Anal.—Calcd. for $C_9H_{14}ClNS$: C, 53.1; H, 6.9; S, 15.7. Found: C, 52.6; H, 6.7; S, 15.7.

2-Mercaptoethyldithiocarbamic Acid.—A solution of 2-mercaptoethylamine hydrochloride (11.3 Gm., 0.1 mole) in 20 ml. of water was added dropwise, with stirring, to an ice-cooled mixture of 7 ml. of carbon disulfide (0.1 mole) and 22 ml. of ammonia water (0.32 mole). Stirring was continued for an hour after the addition was complete, and the yellow product was isolated and dried rapidly on blotting paper at room temperature. The product was stored in a desiccator under nitrogen at refrigerator temperature. The yield was 10 Gm. (65%) of material melting at 76–78°.

Anal.—Calcd. for $C_3H_7NS_2$: C, 23.5; H, 4.6; S, 62.7. Found: C, 23.7; H, 4.9; S, 61.9.

Acetylation of 2-Mercaptoethyldithiocarbamic Acid.—2-Mercaptoethyldithiocarbamic acid (2 Gm., 0.013 mole) was treated with excess acetyl chloride both at room temperature and at 0–5° and allowed to stand for a half-hour. Hydrogen sulfide was liberated and an oil was formed which was washed thoroughly with benzene and ether. The oil was dissolved in alcohol and pale yellow crystals appeared. The material melted at 127–130° and was identified as S-acetyl-2-mercaptoethylamine hydrochloride [lit. (9) m.p. 137°].

Anal.—Calcd. for $C_4H_{10}ClNOS$: S, 20.6. Found: S, 21.4.

A water-insoluble product that appeared in the original reaction melted at 101–103° and was identified as 2-mercaptothiazoline [lit. (10) m.p. 101–102°].

A small amount of material melting at 210° was also isolated from the alcohol extract of the oil. This proved to be 2-mercaptoethylamine disulfide dihydrochloride [lit. (6) m.p. 212–212.5°].

Acylation of 2-Mercaptoethyldithiocarbamic Acid in Ether.—Freshly prepared 2-mercaptoethyldithiocarbamic acid (2.0 Gm., 0.013 mole) was suspended in ether (100 ml.) surrounded by an ice pack. An excess of octanoyl chloride was added dropwise, and the yellow material turned to an orange color in a few minutes. The orange product was filtered and washed thoroughly with ether. As soon as the ether evaporated, however, the crystals turned yellow and became oily, which took place even on storage in a desiccator. After several days in a desiccator, the yellow product was dry and proved to be a mixture of N-octanoyl-2-mercaptoethylamine, m.p. 46–48°, and bis(2-aminoethyl) disulfide dihydrochloride, m.p. 209–210°.

N-Octanoyl-2-mercaptoethylamine.—S-Octanoyl-2-mercaptoethylamine hydrochloride (5.0 Gm., 0.02 mole) was dissolved in 50 ml. of water. The solution was made alkaline with ammonia water and an oily precipitate formed immediately. The mixture was extracted with two 100-ml. portions of ether, the extract was dried over sodium sulfate, and the ether was removed under a current of nitrogen. The gray-white product was recrystallized from 25% aqueous ethanol and 3.5 Gm. (82%) of material was obtained which melted at 46–48°. It decolorized iodine solution rapidly.

Anal.—Calcd. for $C_{10}H_{21}NOS$: C, 59.1; H, 10.3; S, 15.7. Found: C, 59.7; H, 9.9; S, 15.1.

N-Myristoyl-2-mercaptoethylamine.—This compound was prepared in the same manner as the N-octanoyl derivative.

Anal.—Calcd. for $C_{16}H_{33}NOS$: C, 66.9; H, 11.5. Found: C, 67.5; H, 11.4.

Attempted Dithiocarbamation of S-Acyl Esters of 2-Mercaptoethylamine Hydrochloride.—An ice-cooled aqueous solution of S-benzoyl-2-mercaptoethylamine hydrochloride (2.0 Gm.) was made basic with concentrated ammonia water and an equivalent of carbon disulfide was added dropwise with stirring. The yellow solution was made very slightly acidic with hydrochloric acid and extracted with ether. The ether extract was evaporated under a current of nitrogen and a colorless product crystallized, m.p. 65–67°. The product reduced iodine solution rapidly and was shown to be N-benzoyl-2-mercaptoethylamine [lit. (11) m.p. 69–71°].

Anal.—Calcd. for $C_9H_{11}NOS$: S, 17.7. Found: S, 17.8.

An identical product was obtained when an aqueous solution of S-benzoyl-2-mercaptoethylamine hydrochloride was made basic with ammonia water and extracted with ether.

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