solution of the crude product was thus used directly for further chlorination.

Hemisulfur mustard esters. (C) Thionyl chloride (slight excess) in a small amount of benzene was added dropwise to a solution of thiodiglycol monoester in benzene. After the initial reaction had subsided, the solution was refluxed until the evolution of hydrogen chloride ceased. Benzene was then removed and the product was obtained by distillation *in vacuo*.

(D) The acid chloride was added slowly into an equimolar quantity of hemisulfur mustard (I) with stirring and cooling to maintain the temperature below 30° (40° in some cases). After the heat of the reaction had subsided, the mixture was heated at 100–110° (120–130° in some cases) for 1–2 hours. The resulting liquid was distilled to obtain the ester. The stearic acid ester solidified on standing. It was then recrystallized from ethanol. The physical properties and analytical data are summarized in Table II.

Reaction of mercaptoethanol and p-toluenesulfonyl chloride. I. *With equimolar amounts of mercaptoethanol and p-toluenesulfonyl chloride.* *p*-Toluenesulfonyl chloride (190.5 g., 1 mole) was added in small portions to mercaptoethanol (78 g., 1 mole) in 200 ml. of pyridine at 30°. After stirring for an hour, the mixture was heated at 100° for another hour. On cooling, the reaction mixture was decomposed with ice-cold hydrochloric acid (1:1). After removal of the precipitate the solution was extracted alternately with ether and benzene, which were combined and dried. After removal of the solvents, the oily residue was distilled. 2(2′-Chloroethylthio)ethyl *p*-toluenesulfonate (II) (7 g., 2.4%) was obtained at b.p. 123–124° (3 mm.) as a pale yellow, heavy oil with n_D^{20} 1.5768. The infrared spectrum of this product has the characteristic C—S—C split band at 9.5 μ.

Anal. Calcd. for $C_{11}H_{15}S_2O_3Cl$: C, 44.81; H, 5.13; Cl, 12.03. Found: C, 44.71; H, 5.14; Cl, 12.15.

Further distillation yielded a yellow oil (V) (3 g.) at 159–175° (6–7 mm.) with some decomposition. It is a polysulfide compound.

Anal. Calcd. for $C_{24}H_{34}S_6O_6$: C, 47.18; H, 5.61; S, 31.49. Found: C, 47.39; H, 6.17; S, 32.60.

The residue after recrystallization from ether-petroleum ether (b.p. 30–60°) yielded 1.8 g. (3%) of white, shining flakes, m.p. 160–162° identified as *p*-tolyl sulfone (III). (Lit. m.p. 159°.)

Anal. Calcd. for $C_{14}H_{14}SO_2$: C, 68.26; H, 5.73; S, 13.02. Found: C, 67.85; H, 5.70; S, 13.29.

II. *With two moles of mercaptoethanol and one mole of p-toluenesulfonyl chloride.* *p*-Toluenesulfonyl chloride (95.3 g., 0.5 mole) was added in small portions to mercaptoethanol (78 g., 1 mole) in 100 ml. of pyridine. The mixture was refluxed for 3 hr. After decomposition of the reactants with hydrochloric acid (1:1), the reaction mass was extracted with ether, and the ethereal solution dried over sodium sulfate and potassium carbonate. After removal of the solvent, a yellow oil with white solid resulted.

The white solid was recrystallized from ether-petroleum ether (b.p. 30–60°) as shining flakes of the sulfone III, m.p. 157–159°, which did not depress the m.p. of an authentic sample.

The yellow oil was distilled. A colorless liquid was collected at 42–44° (3.9 mm.), which solidified on cooling into white flakes (IV), m.p. 44–45°. It did not depress the m.p. of authentic thiocresol (Eastman). Its addition product with *N*-(1-naphthyl)maleimide[21] was prepared from ethanol solution as white stout needles, m.p. 125–126°.

Anal. Calcd. for $C_{21}H_{17}NO_2S$: C, 72.60; H, 4.93; N, 4.03. Found: C, 72.31; H, 5.18; N, 4.18.

Further distillation gave a yellow oil (VI), b.p. 154–165° (4 mm.), n_D^{20} = 1.5988.

Anal. Calcd. for $C_{22}H_{34}S_5O_6$: C, 47.97; H, 5.49; S, 29.11. Found: C, 47.99; H, 6.18; S, 30.27.

Stability Study of I. (A) *Preparation of 4-(p-nitrobenzyl)-pyridine reagent.* 4-(*p*-Nitrobenzyl)pyridine (0.4 g.), phthalic anhydride (0.066 g.) and sodium perchlorate (5.6 g.) were dissolved in a solution of 10 ml. isopropyl alcohol, 8 ml. distilled water and 2 ml. 0.06*N* sodium hydroxide by stirring and warming on a water bath.

(B) *Procedure.* I was dissolved in methanol to predetermined concentration and added to 2 ml. of 4-(*p*-nitrobenzyl)-pyridine reagent in a volumetric flask. Immediately, it was stirred and heated in a boiling water bath for 10 min. Then the mixture was immediately cooled in ice water. After cooling, the content was diluted to 10 ml. with acetone. The dilution was immediately followed by the accurate addition of 1 ml. of piperidine, the solution stirred and its absorbance measured at 570 mμ in a Beckman DK-3. The calibration curve follows Beer's law and the slope 1.272 is used as an index of stability. Samples were taken over a period of 13 months and deviation of less than 5% was noticed throughout the period.

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[CONTRIBUTION FROM THE DAJAC LABORATORY OF THE BORDEN CHEMICAL CO., A DIVISION OF THE BORDEN CO.]

Synthesis of Possible Cancer Chemotherapeutic Compounds Based on Enzyme Approach. II. Mercaptosulfur Mustard and Its Esters[1]

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2-Chloro-2′-mercaptodiethyl sulfide (MSM) (I) was synthesized from ethanedithiol and vinyl chloride, and also from 1,2,3-oxadithiolane -2-oxide (III) with concentrated hydrochloric acid or 2-chloroethyl mercaptan. Its esters were obtained from acylation of I with acid chlorides. 2-Chloro-2′-thioacetoxy diethyl sulfide was also prepared from chlorination of 2-hydroxy-2′-thioacetoxy diethyl sulfide which in turn was obtained from the reaction of 2-chloroethyl thioacetate and sodiomercaptoethanol.

In the previous paper,[2] a number of esters of hemisulfur mustard, 2-chloro-2′-hydroxydiethyl sulfide were synthesized in order to take advantage of the selective activity of esterase in tumor. In view of the consensus that thioesters are probably

(1) This research was supported by a research grant (C-2530) from The National Cancer Institute, National Institutes of Health, Department of Health, Education and Welfare, Bethesda 14, Md.

(2) K. C. Tsou, Helen C. F. Su, C. Segebarth, and U. Mirarchi, Part I, *J. Org. Chem.*, 26, 4987 (1961).

$$HSCH_2CH_2SH + 2CH_2{=}CHCl \text{ (excess)} \longrightarrow ClCH_2CH_2SCH_2CH_2SCH_2CH_2Cl$$
[II]

$$2HSCH_2CH_2SH \text{ (excess)} + CH_2{=}CHCl \longrightarrow ClCH_2CH_2SCH_2CH_2SH$$
[I]

$$ClCH_2CH_2SCH_2CH_2S{-}\overset{\overset{O}{\|}}{C}{-}R \quad (\text{RCOCl})$$

Figure 1

R = Alkyl (C_1–C_5, C_{11}, C_{17}), *i*-C_3H_7, *i*-C_4H_9, *sec*-amyl, C_6H_5—, $C_6H_5CH_2$—

hydrolyzed by the same esterase[3] and followed essentially the same pattern, the preparation of mercaptosulfur mustard or 2-chloro-2′-mercaptodiethyl sulfide (I), therefore is desirable. Furthermore, as it has been assumed that alkylation reactivity of hemisulfur mustard type compounds involves the sulfhydryl group of the enzyme,[4] the 2-chloro-2′-mercaptodiethyl sulfide that is liberated from esterase hydrolysis of the mercaptosulfur mustard esters would be likely to inactivate the enzyme *in situ* by additional disulfide formation in addition to its alkylating ability.

2-Chloro-2′-mercaptodiethyl sulfide was first isolated by Delepine and Eschenbrenner[5] as a by-product from the reaction of ethylene sulfide and hydrochloric acid. When ethanedithiol and twice its quantity of vinyl chloride[6] containing trace of diphenyl disulfide were irradiated with or without the use of solvent, 1,2-bis(2-chloroethylthio) ethane (II) was the product. But when a similar reaction was carried out with vinyl chloride and a large excess (at least 100%) of ethanedithiol in ether, 2-chloro-2′-mercaptodiethyl sulfide was isolated. It is an unstable compound with pungent mercaptan odor. On standing at room temperature for two to three days intramolecular cyclization took place with liberation of hydrogen chloride to yield *p*-dithiane (IV).

I also was synthesized from 1,2,3-oxadithiolane-2-oxide (III).[7] When III was heated in concentrated hydrochloric acid, sulfur dioxide evolved. *p*-Dithiane and I were obtained by distillation. The residue was treated with a hot concentrated sulfuric acid-nitric acid mixture; a soft spongy rubber-like substance resulted. If III was treated with 2-chloroethyl mercaptan (crude), the same products were isolated. Therefore, 2-chloroethyl mercaptan was the initial product for the reaction of III and hydrochloric acid. Similar to the previous work,[2] the esters of 2-chloro-2′-mercaptodiethyl

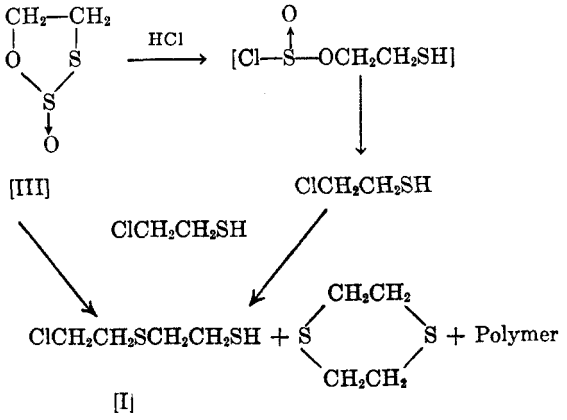

Figure 2

sulfide were conveniently prepared by the reaction of acid chlorides directly with 2-chloro-2′-mercaptodiethyl sulfide.

An alternate route to 2-chloro-2′-thioacetoxy diethyl sulfide was investigated. 2-Chloroethyl thioacetate was prepared by a modification of Dawson's method.[8] Condensation of 2-chloroethyl thioacetate with sodiomercapto ethanol gave 2-hydroxy-2′-thioacetoxy diethyl sulfide which was chlorinated with thionyl chloride to yield the desired ester. This method, however, gives only very low yield.

Toxicity studies of 2-chloro-2′-mercaptodiethyl sulfide and its esters were carried out by Dr. S. T. Kramer of Sinai Hospital of Baltimore. The esters are found to be more toxic than that of 2-chloro-2′-mercaptodiethyl sulfide on a molar basis. 2-Chloro-2′-mercaptodiethyl sulfide itself is about half (LD_{50} — 845 μM) as toxic as that of 2-chloro-2′-hydroxydiethyl sulfide. Screening data on these compounds against mouse tumor are generously provided by Cancer Chemotherapy National Service Center and will be reported elsewhere. Of the compounds studied, it might be of interest to mention that the α-ethyl-*n*-butyrate is active against CA-755.

EXPERIMENTAL[9,10]

Mercaptosulfur mustard (I). The procedure of Rueggeberg, *et al.*[6] was adapted, but large excess (at least 100%) of ethanedithiol was used in order to minimize the possible formation of the 1,2-bis(2-chloroethylthio)ethane.

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(5) M. Delepine and S. Eschenbrenner, *Bull. soc. chim.*, (4) **33**, 703 (1923).

(6) W. H. C. Rueggeberg and W. A. Cook, U. S. Patent 2,810,687 (Oct. 22, 1957).

(7) Method of synthesis will be published in Part III of this series.

(8) T. P. Dawson, *J. Am. Chem. Soc.*, **69**, 1211 (1947).

TABLE I

MERCAPTOSULFUR MUSTARD AND ITS ESTERS

Esters	B.P. (M.P.)	Mm.	n_D^t	Yield, %	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found	Chlorine, % Calcd.	Chlorine, % Found
MSM	72–74	0.6	1.5582^{20}	22	30.65	30.59	5.79	5.40	22.63	22.65
Acetate	70–80	0.07	1.5440^{20}	60	36.26	35.97	5.58	5.71	17.84	17.98
n-Propionate	93	0.2	1.5395^{20}	68.5	39.52	39.64	6.17	5.89	16.67	16.54
n-Butyrate	99	0.1	1.5316^{20}	76	42.37	42.40	6.67	6.45	15.64	15.94
n-Valerate	104–106	0.1	1.5237^{20}	74	44.89	44.84	7.12	7.12	14.72	14.65
n-Caproate	141–144	0.45–0.50	1.5174^{20}	91	47.13	47.25	7.52	7.54	13.91	13.72
Laurate	140–150	0.9	1.4565^{24}	Low	56.69	57.32	9.22	9.48	10.46	9.96
Stearate	(44–45)	—	—	68	62.44	62.08	10.24	10.16	8.38	8.25
Isobutyrate	112	0.05	1.5253^{24}	92	42.37	42.47	6.67	6.74	15.64	15.73
Isovalerate	121–123	0.3	1.5196^{22}	65	44.89	44.84	7.12	7.05	14.72	14.76
α-Ethyl-*n*-butyrate	117–118	0.2	1.5186^{21}	84	47.13	47.13	7.52	7.51	13.91	14.00
Benzoate	170–174	0.3	1.6026^{25}	42	50.66	50.73	5.02	4.94	13.59	13.81
Phenylacetate	173–176	0.30–0.35	1.5798^{24}	75	52.44	52.26	5.50	5.55	12.90	13.15

Ethanedithiol (100 g., 1.06 moles) dissolved in 200 ml. of dry ether was cooled to −20°. About 0.1% of diamyl disulfide was then added, followed with the liquefied vinyl chloride (31.3 g., 0.5 moles). The mixture was then kept at room temperature for 5 days. The ether solution was filtered from some solid polymer and the solvent was removed gradually *in vacuo*. Most of the unchanged ethanedithiol (56 g.) was recovered by distillation at 60–70°/20 mm. The residue was further distilled to obtain 17 g. (22%) of a colorless oil at 74–75° (0.6 mm.) with n_D^{20} = 1.5582 and d_4^{20} = 1.230; λ_{max} 3.42; 3.95; 6.95; 7.75, 7.90 (split); 8.30; 8.75; 9.65; 11.65–11.75 (broad); 14.30–14.40 (broad) μ. This compound had a pungent mercaptan odor. Molar refraction = 41.01 (calcd., 41.1).

When mercaptosulfur mustard was left in a test tube at room temperature for 2 days, white crystals formed. After recrystallization from ether-petroleum ether (b.p. 30–60°) it melted at 111–112° and did not change the melting point of an authentic sample of *p*-dithiane (IV) when mixed.

Alternate mercaptosulfur mustard (I) *syntheses. A. Reaction of 1,2,3-oxadithiolane 2-oxide* (III) *with concentrated hydrochloric acid.* III (25 g.) and concentrated hydrochloric acid (sp. gr. 1.18; 17 ml.) were mixed and heated for 5 min. It was poured into ice water immediately. The heavy oil separated was collected, dried and distilled. First, the unchanged III (5–6 g.) was recovered. Then I (3 g., 19.2%) was obtained at b.p. 91–95° (4 mm.) with n_D^{20} = 1.5589.

Further distillation yielded a white solid. After recrystallization from ether-petroleum ether, white flakes (0.8 g.) melted at 110–111°. It did not depress the melting point of authentic IV.

The residual dark viscous paste was treated with hot concentrated sulfuric acid-nitric acid mixture. A dark, spongy, rubbery polymer was obtained.

B. Reaction of III with 2-chloroethyl mercaptan. III (6.2 g.) and 2-chloroethyl mercaptan (crude, 4.8 g.) were mixed and heated to 110°. On addition of 10 mg. of potassium carbonate, the evolution of sulfur dioxide occurred immediately. When this has subsided, the reaction mixture was distilled. The pale yellow oil of I (1 g.) was collected at b.p. 122–128° (15 mm.), together with white solid of *p*-dithiane (IV).

Mercaptosulfur mustard esters. Acid chloride (0.1 mole) was added slowly to I (0.1 mole) with stirring. After complete addition and the initial reaction has subsided, the mixture was heated gradually to 90–100° for 3 hr. The heavy liquid was left standing overnight and then distilled *in vacuo* to obtain the ester.

When stearoyl chloride was used and the reaction mixture heated for 3 hr., the product solidified on standing. The solid was recrystallized from ether. The physical constants are summarized in Table I.

2-Chloroethyl thioacetate.[8] 2-Chloroethyl thioacetate was prepared by a modification of the method given by Dawson.[8] 2-Chloroethylmercaptan was prepared from mercaptoethanol and concentrated hydrochlorid acid. Both reactants were preheated to approximately 100° and mixed. On standing the milky dispersion separated into two layers. The lower oily layer was separated quickly, and dried over sodium sulfate. From 112 g. of mercaptoethanol there was obtained 88 g. of crude product. This product contains unchanged mercaptoethanol and makes purification difficult. It can, however, be refluxed over 80 g. of acetyl chloride and then distilled first at atmospheric pressure to remove acetyl chloride and then under reduced pressure to give 62 g. of the product, b.p. 70–72°/12 mm. (yield: 49%), n_D^{22} = 1.4455 (lit.[8] b.p. 76°/16 mm.).

2-Hydroxy-2'-thioacetoxy diethyl sulfide.[8] One mole of 2-chloroethyl thioacetate was mixed with equimolar quantity of sodiomercaptoethanol at 0° and allowed to stand overnight at room temperature. The white polymeric precipitate formed was filtered and the filtrate was distilled to give three fractions: fraction 1, b.p. 64°/20 mm., n_D^{21} = 1.4680; fraction 2, b.p. 125–149°/20 mm. and 128°/1.5 mm., n_D^{21} = 1.5292; fraction 3, b.p. 185–191°/1.5 mm., n_D^{21} = 1.5523. No attempts were made to identify fraction 1. Fraction 3 was extracted into hexane and precipitated with benzene to give long needles of *p*-dithiane, m.p. 111–112°. Fraction 2 was redistilled to give pure 2-hydroxy-2'-thioacetoxy diethyl sulfide b.p. 80–83°/0.1 mm., n_D^{20} = 1.5309.

2-Chloro-2'-thioacetoxy diethyl sulfide. A 1.8-g. sample of the above hydroxy derivative was chlorinated with 1.2 g. of thionyl chloride in ether at 0°. After standing at room temperature for 15 min., the ether was removed by evaporation. The residue was then distilled at 0.2–0.1 mm., and only 0.7 g. of the product was collected at 100–110°, n_D^{20} = 1.5460. Its infrared spectra was identical to that of the mercaptosulfur mustard acetate in Table I. Because of the relative low yield from this route, this method was not used when mercaptosulfur mustard became available to us in larger quantity.

Acknowledgment. Acknowledgment is due to Mr. U. Mirarchi for helpful assistance in this work.

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(9) All melting points and boiling points are corrected.

(10) All analyses were done by Dr. Stephen Nagy, Microchemical Laboratory, Massachusetts Institute of Technology.