0.00. Found: C, 62.65, 62.68; H, 7.37, 7.41; Si, 19.23, 19.30; N, 1.33, 1.41.

The second fraction, 4.3 g. (19%), b.p. 94–98° at 0.28 mm., n_D^{25} 1.5470, was identified by its infrared spectrum and elemental analysis as crude 2-phenyldimethylsilyl-3-methylpyrazine containing some 1,3-diphenyltetramethyldisiloxane.

Anal. Calcd. for $C_{13}H_{16}N_2Si$: C, 68.37; H, 7.06; N, 12.27; Si, 12.30. Found: C, 66.85, 67.04; H, 7.05, 7.13; N, 9.14, 9.23; Si, 14.31, 14.42.

(b) The above reaction was repeated on the same scale using identical conditions except that only a 1-hr. rather than 2-hr. reaction time was used in the present experiment. In this reaction the tetrahydrofuran was freshly distilled from lithium aluminum hydride before use. The work-up procedure was modified to exclude water in an effort to prevent the formation of the 1,3-diphenyltetramethyldisiloxane side product. The reaction mixture was treated with 200 ml. of benzene to precipitate magnesium chloride and filtered. The clear dark red filtrate was distilled at reduced pressure to give 6.0 g. (46.5%) of recovered crude 2-chloro-3-methylpyrazine, b.p. 28–35° at 1.6–1.0 mm., and 6.4 g. (29%) of crude 2-phenyldimethylsilyl-3-methylpyrazine, b.p. 102–108° at 0.5–0.7 mm. The crude desired product was redistilled to give 6.0 g. (27%) of pure 2-phenyldimethylsilyl-3-methylpyrazine, b.p. 81° at 0.030 to 0.025 mm., n_D^{25} 1.5486.

Anal. Calcd. for $C_{13}H_{16}N_2Si$: C, 68.37; H, 7.06; N, 12.27; Si, 12.30. Found: C, 68.11, 68.42; H, 7.11, 7.16; N, 12.32, 12.40; Si, 12.22, 12.29.

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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. I. Hemisulfur Mustard and Its Esters[1]

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Hemisulfur mustard, 2-chloro-2′-hydroxydiethyl sulfide (I), has been prepared from sodiomercaptoethanol and ethylene chloride. The stability of I is measured by the use of 4-(*p*-nitrobenzyl)pyridine (NBP). Direct acylation of I with acid chlorides yields its corresponding esters. Alternatively, these esters may be prepared by first addition of mercaptoethanol to vinyl esters or direct monoacylation of thiodiglycol, followed by chlorination of the thiodiglycol monoesters thus produced. The synthesis of these new esters generates interest in the preparation of possible chemotherapeutic compounds based on enzymatic approach.

The reaction of *p*-toluenesulfonyl chloride and mercaptoethanol has been found to yield 2-chloro-2′-*p*-toluenesulfonyldiethyl sulfide (II), ditolyl sulfone (III), *p*-thiocresol (IV), and other polysulfide compounds. The anomaly in this reaction can be accounted for in part by the strong reducing power of mercaptoethanol.

In the design and synthesis of possible chemotherapeutic agents for cancer, Seligman and his associates have proposed[2] an interesting rationale based on the difference in the distribution of enzymes in normal and neo-plastic tissue. Thus, the tumor rich in a specific hydrolytic enzyme might liberate the toxic moiety that had been incorporated in a less toxic compound after hydrolysis. Conversely, if a tumor is known to be deficient in a specific enzyme, toxic compounds that could easily be detoxified by such enzymatic hydrolysis in normal cells should then be synthesized. The present series of synthetic work has been initiated to take advantage of the observation that carcinomas are particularly deficient in esterase.[3,4]

Hemisulfur mustard, or 2-chloro-2′-hydroxydiethyl sulfide (I), has been of interest to cancer chemotherapy not only because of its known beneficial effect[5–7] to ascites tumor, but also because of the absence of bone marrow effect, in contrast to the well-known alkylating agent nitrogen mustard. The synthesis of the esters of I was therefore undertaken with the expectation that the beneficial hematopoietic effect would be retained and that the difference in toxicity of the esters and the parent compound might provide a basis for taking advantage of the above mentioned rationale.

Even though it is known that hemisulfur mus-

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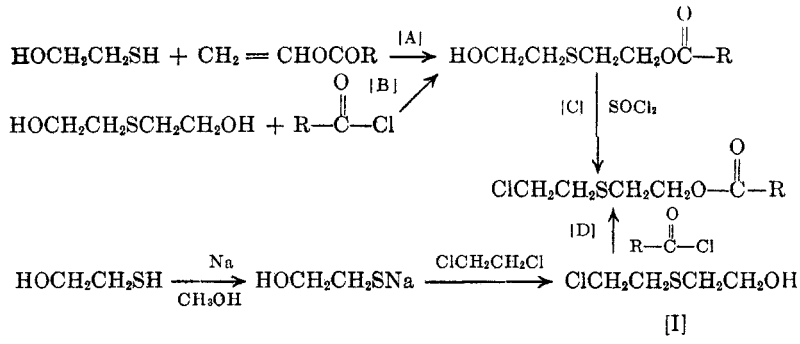

Figure 1
R = Alkyl [C_1 – C_5, C_{11}, C_{17}]; *i*-Pr; *i*-Bu; *sec*-Am; phenyl; benzyl

tard can be prepared by direct photochemical addition of mercaptoethanol to vinyl chloride[8,9] or partial chlorination of thiodiglycol with thionyl chloride,[10,11] the latter method gives a product contaminated with 3–4% of sulfur mustard and is therefore undesirable for clinical study. The best and most convenient method to obtain pure I is from the reaction of sodium β-hydroxyethylmercaptide and excess of ethylene chloride.[5]

In view of the known instability of I, it was necessary to devise a method for the determination of its concentration. This was done by the use of 4-(*p*-nitrobenzyl)pyridine based on the early work of Grant and Kinsey.[10]

This method is a direct measurement of the alkylating ability of hemisulfur mustard when complexed with 4-(*p*-nitrobenzyl)pyridine and the color density at 570 mμ measured as an index of its stability. Calibration of color density versus concentration indicates that the relationship follows Beer's Law and that this method is useful for determination of the stability of hemisulfur mustard and its derivatives.[12]

It was found that purified solid I remained unchanged for several months when kept below −10°, but decomposed completely at room temperature within a few days. A 10% dry ether solution kept at −15 to −20° remained unchanged after 18 months.

The direct addition of mercaptoethanol to vinyl acetate (method A[13]) yielded the thiodiglycol monoester which was chlorinated with thionyl chloride to give the corresponding chloro compound. However, when this method was applied to vinyl stearate, only the distearyl ester was obtained. This might be attributed to the shielding effect of the long alkyl chain to the hydroxyl group.

Direct acylation of thiodiglycol with equimolar acid chlorides also gave the mono esters (method B) which were then readily converted to the corresponding chloro compound with thionyl chloride.

The esters of the higher fatty acids could not be synthesized by the two methods already described, but were made in good yield by the direct acylation of I (method D). Both the monoesters and the chloro derivatives are listed in Tables I and II.

While 2-(2′-chloroethylthio)ethyl *p*-toluenesulfonate (II) could not be prepared by method B and C as described above, it was obtained when equimolar amounts of mercaptoethanol and *p*-toluenesulfonyl chloride were heated above 100°. *p,p′*-Ditolyl sulfone (III) and a yellow oil identified as a polyethylene sulfide (V) were isolated as by-products. When the reaction was carried out with two moles of mercaptoethanol to one mole of *p*-toluenesulfonyl chloride, only III was isolated together with *p*-thiocresol (IV) and a yellow polymeric oil identified as VI.

The anomaly in these reactions can best be explained by the strong reducing power of mercaptoethanol. As it has been reported that aryl sulfonyl chlorides are reduced[14] to thiophenols, the reduction of *p*-toluenesulfonyl chloride by mercaptoethanol in pyridine resulted in IV. The reaction of mercaptoethanol and *p*-toluenesulfonyl chloride[15] could lead to the formation of thiodiglycol *p*-toluene sulfonate which was then converted to II in one step since *p*-toluenesulfonyl chloride is also a chlorinating agent.[16] It might be necessary to mention here that II was found to be a potent vessicant.

The formation of *p,p′*-ditolyl sulfone (III) in the above reaction was not expected. Even though

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TABLE I

THIODIGLYCOL MONOESTERS

Esters	Method of Preparation	B.P. °C	B.P. Mm.	n_D	Yield, %	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found
Acetate	A	121	4	1.4901^{22}	43	—	—	—	—
n-Propionate	A	95–98	0.02	$1.4858^{19.5}$	61	47.20	46.46	7.91	7.64
n-Butyrate	A	120	1.2	1.4858^{20}	68	49.97	50.05	8.39	8.44
n-Valerate	A	121	4	1.4901^{22}	43	52.40	51.30	8.79	8.66
n-Caproate[a]	B	112–118	0.15	1.4692^{22}	—	—	—	—	—

[a] The distilled sample was used immediately for the subsequent step.

TABLE II

HEMISULFUR MUSTARD AND ITS ESTERS

Esters	Method of Preparation	B.P. °C	(M.P.) Mm.	n_D	Yield, %	Carbon, % Calcd.	Carbon, % Found	Hydrogen % Calcd.	Hydrogen % Found	Chlorine, % Calcd.	Chlorine, % Found
I	—	82–83	0.25–0.30	1.5195^{28}	24	—	—	—	—	—	—
Acetate	C	80	1.6	1.4723^{24}	38	39.45	39.74	6.07	6.22	—	—
n-Propionate	C	75–76	0.25	1.4879^{20}	66	42.75	42.04	6.66	6.61	—	—
n-Butyrate	C	79–83	0.15–0.20	1.4859^{22}	37	45.61	45.33	7.17	7.19	16.83	16.13
n-Valerate	C	82–90	0.15–0.20	1.4844^{23}	36	—	—	—	—	15.78	15.98
n-Caproate	C	105–106	0.35	1.4820^{24}	29[a]	50.30	49.84	8.02	8.05	—	—
Laurate	D	185	1.0	1.4762^{21}	27	59.51	59.24	9.68	9.56	10.98	10.87
Stearate	D	(42–43)	—	—	70	64.91	65.22	10.65	10.44	8.71	8.58
i-Butyrate	D	89–90	0.35	1.4811^{20}	52	45.61	45.25	7.17	7.22	16.83	16.98
i-Valerate	D	119–124	1.1	1.4785^{20}	56	48.10	47.70	7.62	7.49	15.78	15.63
α-Ethyl-*n*-Butyrate	D	110–123	1.35	1.4799^{21}	62	—	—	—	—	14.85	14.65
Benzoate	C	130–131	0.1	1.5531^{29}	18[a]	53.98	53.68	5.35	5.47	14.49	14.61
Phenylacetate	D	164–167	0.9–1.0	1.5437^{20}	40	—	—	—	—	13.70	13.30

[a] Over-all yield calculated thiodiglycol used.

p-thiocresol that was formed might react with *p*-toluenesulfonyl chloride to yield either a sulfinate or a disulfide or a thiosulfonate[16] as possible intermediates for III, none of these compounds were isolated. When an authentic sample of *p*-tolyl *p*-toluenethiosulfonate (m.p. 76–77°) was prepared[17] and refluxed in pyridine with or without mercaptoethanol, in the absence or presence of *p*-toluenesulfonyl chloride, or *p*-thiocresol, no rearrangement took place and the starting material was recovered unchanged. Prolonged heating at its melting point resulted in extensive decomposition to a tarry mass, yet no sulfone was isolated. Therefore, it appears that the thiosulfonate is not likely to be the intermediate for sulfone formation, and the mechanism of the formation of the sulfone (III) remains to be elucidated.

While toxicity studies[18] will be reported elsewhere, it might be of interest to report here in a preliminary way that all esters are more toxic than hemisulfur mustard itself on a molar basis even though the difference is only two- to threefold. Such difference therefore provides a basis for our further work in these and related compounds. These compounds have also been screened against mouse tumor S-180, CA-755 and L-1210. They are found to be active against CA-755. Details of such data will appear elsewhere.

EXPERIMENTAL[19,20]

Hemisulfur mustard (I). Metallic sodium (11.5 g., 0.5 g.-atom) was added in small pieces to freshly distilled mercaptoethanol (40 g., 0.5 mole) and 300 ml. of anhydrous methanol with cooling. After the sodium had reacted, ethylene chloride (300 ml.) was added. After standing at room temperature overnight, the precipitated salt was removed. The solution was concentrated *in vacuo* and distilled at 82–83° (0.25-0.3 mm.) to a viscous, colorless oil with n_D^{28} 1.5195 (Lit.[9] n_D^{20} 1.5188). Yield, 17 g. (24.3%). It solidified into white glassy solid below −10°; λ_{max} (infrared) 3.00, 3.45, 6.95, 7.05, 7.14 (spl.), 7.75, 7.90 (spl.), 8.28, 9.45, 9.60 (spl.), 14.38–14.45, 15.42 μ.

Thiodiglycol monoesters. (A) Equimolar amounts of mercaptoethanol and the vinyl ester were mixed with slight warming. After the reaction had subsided, benzene was added and the mixture was refluxed for 1–2 hr. Benzene was removed and the residual liquid was distilled *in vacuo*. A small amount of vinyl ester was first recovered. The pure thiodiglycol monoesters were obtained with good yields.

(B) The acid chloride was added slowly to an equimolar quantity of thioglycol in benzene with or without triethylamine. The mixture was refluxed for two hr. The precipitated triethylamine hydrochloride (if any) was removed by filtration and the benzene solution was evaporated *in vacuo*. The residual syrup was distilled. For fatty acid esters, it was difficult to purify the products by distillation. The benzene

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

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

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.

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