acetamide IIg which was identical with that previously prepared by acetylation of IIa as indicated by infrared spectra and paper chromatographic analysis. The ethyl acetate insoluble material was crystallized from absolute methanol giving 130 mg. (43%) of diacetate IIc (identical with previously prepared material by infrared spectra and paper chromatographic analysis). This was subjected to n.m.r. spectral analysis to determine whether deuterium had become incorporated at position 1 by exchange. By the methods previously described, it was found that no deuterium exchange had taken place.

Reaction of Mitomycin C with Acetic Deuterioacid without Subsequent Acetylation (Test of Deuterium Incorporation into Methoxyamine IIa).—Mitomycin C (750 mg., 2.2 mmoles) was stirred in a sealed flask with 50 ml. of acetic deuterioacid for 5 hr. at room temperature. The acid was then removed *in vacuo* and a small sample of the residue was spotted on a thin layer plate. Analysis of this plate after development showed the residue to consist primarily of the acetamide IIb with smaller amounts of methoxyamine IIa and an unidentified substance (trace) which ran fast in the system used. The residue was dissolved in the minimum volume of hot acetone and 410 mg. (50.5%) of the acetamide IIb crystallized in three crops on cooling the solution. The filtrate was evaporated to dryness, and the residue was dissolved in the minimum amount of methanol and introduced onto a neutral alumina column (Brockman grade III). Elution with ethyl acetate and then 10% methanol in ethyl acetate removed the fast-running unidentified substance. Then elution with 30% methanol in ethyl acetate gave a red band which yielded an additional 30.5 mg., 5% of the acetamide IIb upon evaporation of the solvent. Finally elution with absolute methanol followed by 50% methanol–water gave an intense purple-red band which was shown to be methoxyamine IIa by thin layer chromatography. This was rechromatographed in a similar manner to yield 106.8 mg., 14.2%, of pure IIa as a residue. An n.m.r. spectrum of this compound showed all of the features of the previously obtained spectrum of IIa and integration of the δ 3.87–4.83 multiplet in the usual manner showed the presence of 4 protons (*i.e.*, no deuterium bound to carbon) as in the previous spectrum.

Preparation of Deuterated Methoxy Derivative IIa.—A solution 0.10 g. (0.3 mmole) of mitomycin C in 20 ml. of absolute methanol and 0.20 ml. of glacial acetic acid was refluxed gently for 5 hr. and left at room temperature for an additional 8 hr. Solvents were then evaporated *in vacuo*, toluene being added to help remove traces of acetic acid. The resulting red residue was dissolved in a small amount of methanol and passed onto an alumina column (Woelm, neutral, Brockman activity III). Elution with methanol produced two fractions, the first being purple and containing mostly unreacted mitomycin C. A second red fraction followed closely and yielded crystals after evaporation and addition of water. Recrystallization from water yielded 30–35 mg. (30–35%) of IIa, homogeneous on thin layer chromatography, R_f 0.08–0.10.

In a similar manner methanol-*d* and acetic acid-*d* were employed in converting mitomycin C to a deuterated methyl ether. All nitrogen-bound deuterium was re-exchanged in the work-up and recrystallization procedure, leaving only carbon-bound deuterium in the product. Analogy with diacetate IIc dictated that deuterium incorporation would be exclusively at position 1, and integration of the δ_{TMS} 3.87–4.83 multiplet in the n.m.r. spectrum showed it to be incorporated there to the extent of approximately 70%.

Potential Anticancer Agents. II. The Synthesis of Some Nitrogen Mustard Containing Sulfones and Thiosulfinates[1a]

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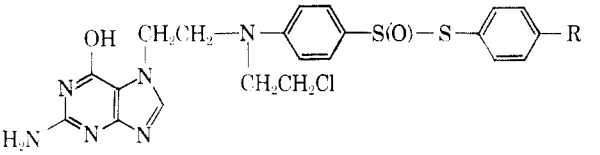
Three new thiosulfinates containing a nitrogen mustard moiety were prepared by treating *p*-[bis(2-chloroethyl)amino]benzenesulfinyl chloride with substituted thiophenols in the presence of pyridine. The sulfinyl chloride was synthesized from the corresponding sulfonyl chloride by reduction with lithium aluminum hydride followed by treatment with oxalyl chloride. In addition, several new sulfones containing a nitrogen mustard moiety were prepared by treating an alkyl halide with sodium *p*-[bis(2-chloroethyl)amino]benzenesulfinate. The antitumor activity of these compounds was studied in mice against the Ehrlich ascites carcinoma.

Weisberger and Pensky[2a,b] observed antitumor activity in several symmetrical thiosulfinates [–S(O)–S–], a moiety present in such naturally occurring plants as garlic.[3a,b] Kametani, *et al.*,[4a,b] synthesized additional symmetrical thiosulfinates which were effective against the Ehrlich ascites carcinoma.

The primary objective of the present research was to synthesize new thiosulfinates which contained a nitrogen mustard moiety. It was considered possible that these compounds might have antitumor activity for several reasons. (1) From the standpoint of reaction with essential sulfhydryl groups, both thiosulfinates[5a,b] and nitrogen mustards[6] could exhibit such reactivities; consequently, a combination of the two moieties in one compound could yield a type of dual antagonist.[7] (2) An interaction between one arm of the nitrogen mustard and the N-7 of guanine nucleotides could yield an unstable quaternary ammonium compound which upon hydrolysis would give com-



I, R = H, CH_3, $N(CH_2CH_2Cl)_2$

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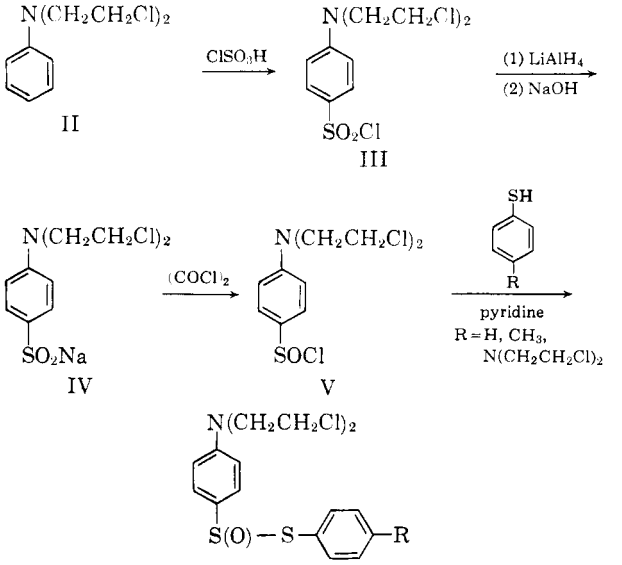
pound I.[8a-d] Such a compound could possibly exert an antimetabolite effect if the hydrolysis occurred before the second β-chloroethyl group reacted with another purine structure.[9] (3) Intermolecular cross linking of nucleic acids and proteins[10a,b] possibly through reaction of the nitrogen mustard with DNA and the thiosulfinate with protein sulfhydryl groups could occur.

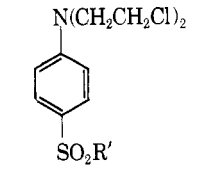
Two different routes were employed in synthesizing the thiosulfinates. Direct oxidation of a known disulfide with perbenzoic acid[11a,b] was used to prepare the symmetrical thiosulfinate (VIc), and a modification of the procedure of Backer and Kloosterziel[12] was employed in preparing both the symmetrical (VIc) and unsymmetrical (VIa and VIb) compounds. The steps involved in the latter synthesis are shown in Scheme I.

SCHEME I



VIa, R = H
b, R = CH_3
c, R = $N(CH_2CH_2Cl)_2$

The conversion of III to IV was based on the work of Field and Grunwald[13] in reducing sulfonyl halides to sulfinic acids by the "inverse" addition of the hydride to the halide at low temperature. Compound V was prepared by a modification of the work of Kurzer[14] who treated thionyl chloride with sodium *p*-toluenesulfinate. Compound IV was treated with alkyl halides to yield some new sulfones (VII–X).



VII, R = CH_3
VIII, R = CH_2COOH
IX, R = $(CH_2)_{15}CH_3$
X, R = $(CH_2)_3CN$

Proof of structure was afforded through the preparation of the symmetrical thiosulfinate by two different procedures to yield compounds which were found to be identical with regard to melting point, elemental analysis, and infrared spectra. Not only does this procedure prove the structure of the thiosulfinates, but it also verifies the structure of all of the intermediates, especially *p*-[bis(2-chloroethyl)amino]benzenesulfinyl chloride, which was not characterized.

Screening Results.[15]—The compounds were tested *vs.* the Ehrlich ascites carcinoma in Swiss Webster white mice by procedures described previously.[16a,b] The results are recorded in Table I in which the compounds are designated by the Roman numerals used to identify the compounds in the text of this paper. The rapid increase in body weight of control mice is a measure of the accumulation of tumor cells and ascitic fluid (column 4). However, the total packed-cell volume of tumor cells (TPCV) (columns 5 and 6) determined on the sixth day after intraperitoneal transplantation of the tumor is the most reliable index of the multiplication of the tumor cells. The

TABLE I

SCREENING TESTS *vs.* THE EHRLICH ASCITES CARCINOMA[a]

Compd.	Dose,[b] mg./kg./day	Mortality of treated group	Av. wt. change T/C, g.	Av. TPCV T/C, ml.	Av. TPCV % of controls
IV	69*	0/8	5.3/4.9	2.1/1.6	>100
	100	2/8	4.2/5.3	1.8/2.3	78
VIa	124*	1/8	3.0/8.1	1.5/2.6	58
	100	0/8	4.1/6.2	1.4/2.9	48
	65	0/8	4.6/5.4	1.7/2.8	61
VIb	100*	1/8	3.1/6.2	0.6/2.4	25
	69*	1/8	3.6/4.9	1.1/2.2	50
	64	0/8	2.5/4.6	0.3/1.6	19
	54	0/8	3.4/4.4	0.2/0.9	22
	56	1/8	4.8/5.9	0.05/0.47	11
VIc	66*	0/8	4.8/4.9	2.1/2.2	96
	100	2/8	3.9/5.1	1.9/2.7	70
	60	1/8	4.6/4.9	1.5/1.6	94
	57	0/8	3.7/4.2	2.3/1.8	>100
VII	126*	5/8	6.2/8.1	2.5/2.6	96
	63*	1/8	6.3/6.9	1.6/1.9	84
	60	2/8	5.1/6.2	1.5/2.0	75
VIII	95*	2/8	5.6/8.1	2.2/2.6	85
	66	0/8	6.9/6.9	2.1/1.9	>100
IX	125*	6/8	6.8/8.1	2.0/2.6	77
	56*	1/8	5.6/6.4	2.3/2.2	>100
	69	0/8	3.0/3.5	1.6/1.7	9
X	66*	4/8	4.1/4.9	3.1/2.9	>100
	70	3/8	5.1/5.7	2.0/2.4	83
XI	70*	0/8	4.8/4.6	1.6/1.6	100
	66	1/8	4.5/4.9	2.2/2.4	92

[a] T = treated, C = control, TPCV = total packed-cell volume. Average mortality of control groups to day of assay = 33%. [b] The asterisks indicate that the compound was suspended in 0.9% NaCl; all others were suspended in olive oil.

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dosages recorded in column 2 were divided into two intraperitoneal injections/day commencing 24 hr. after transplantation of the tumor and continuing for 4.5 days.

On the basis of significantly lower TPCV in treated mice in comparison with controls, two thiosulfinate nitrogen mustards (VIa and VIb) showed sufficient activity to warrant further study. The low solubilities of the various compounds could have contributed to the absence of significant antitumor activity.

Experimental[17]

S-{*p*-[Bis(2-chloroethyl)amino]phenyl} *p*-[Bis(2-chloroethyl)amino]thiobenzenesulfinate (VIc) by Perbenzoic Acid Oxidation.—To 3.00 g. (0.00602 mole) of 4,4′-dithiobis[*N*,*N*-bis(2-chloroethyl)aniline][18] in 50 ml. of reagent chloroform, cooled to 0°, was added dropwise 0.831 g. (0.00602 mole) of perbenzoic acid contained in 7.5 ml. of stock solution.[19] The solution was stirred at room temperature for 50 min., cooled to 10°, and washed with 5 ml. of cold 5% $NaHCO_3$ solution, 10 ml. of 2% $NaHCO_3$ solution, and 10 ml. of cold water. The combined aqueous extracts were washed with two 10-ml. portions of chloroform, and this was combined with the original chloroform solution which was subsequently dried ($MgSO_4$). The chloroform solution was cooled to −10° and 320 ml. of cold ligroin (66–75°) was added. The mixture was kept at −15° overnight, and the precipitate was removed by filtration and air-dried; yield, 51%; m.p. 108–109°.

Anal. Calcd. for $C_{20}H_{24}Cl_4N_2OS_2$: C, 46.70; H, 4.70; Cl, 27.57; N, 5.45; S, 12.47. Found: C, 46.26; H, 4.66; Cl, 27.62; N, 5.36; S, 12.65.

N,N-Bis(2-chloroethyl)aniline (II).—Either the procedure of Robinson and Watt[20] or that of Elderfield and co-workers[21] was followed, but in both cases the final product was vacuum distilled and then immediately recrystallized from anhydrous methanol.

N,N-Bis(2-chloroethyl)sulfanilyl Chloride (III).—To 98 g. (0.45 mole) of *N*,*N*-bis(2-chloroethyl)aniline in 250 ml. of reagent chloroform was slowly added a solution of 148 ml. (2.25 moles) of chlorosulfonic acid in 250 ml. of reagent chloroform maintaining the temperature of the reaction between 15–20°. The addition took approximately 1 hr. The solution was refluxed for approximately 70 hr. with stirring, cooled, and slowly poured into a 4-l. beaker containing a rapidly stirred ice–water mixture. The chloroform and water layers were separated, the aqueous phase washed with one 250-ml. portion and one 100-ml. portion of chloroform. The chloroform extracts were combined and washed with two 150-ml. portions of cold water. The chloroform solution was then slowly poured into 3 l. of cold petroleum ether (30–60°) and the green-gray solid was collected and dried immediately over phosphorus pentoxide. Additional product was obtained by adding to the filtrate an equal volume of petroleum ether and storing overnight at −15°. The light green-gray solid was isolated in 61% yield, m.p. 99.5–101°.

The infrared spectrum showed the desired symmetrical and asymmetrical $-SO_2-$ absorption peaks at 1165–1175 and 1360 cm.$^{-1}$, respectively.[22] In addition, the absorption due to 1:4 aromatic substitution was found at 1090 and 815 cm.$^{-1}$.[23a,b]

Anal. Calcd. for $C_{10}H_{12}Cl_3NO_2S$: C, 37.93; H, 3.82; Cl, 33.59; N, 4.42; S, 10.13. Found: C, 38.07; H, 4.01; Cl, 33.50; N, 4.34; S, 10.02.

Sodium *p*-[Bis(2-chloroethyl)amino]benzenesulfinate (IV). To a suspension of 60 g. (0.17 mole) of *N*,*N*-bis(2-chloroethyl)-sulfanilyl chloride in 1000 ml. of dry ether there was slowly added a mixture of 100 ml. of a 1 *M* lithium aluminum hydride solution[24] and 100 ml. of dry ether, maintaining the temperature between 0–5° during the addition. About 50 min. was required for the addition, after which the solution was allowed to come to room temperature, refluxed gently for about 65 min., and stirred for an additional 90 min. at room temperature. The solution was cooled, 200 ml. of cold water slowly was added, followed by the addition of 200 ml. of 10% sulfuric acid, resulting in two layers and a brown gummy mass. The layers were separated and the aqueous layer was recombined with the brown mass and vigorously shaken with three 100-ml. portions of ether until the mass was completely in solution. The ether layers were combined and washed with several 25-ml. portions of 15% NaOH solution until the washings were almost colorless. The compound was crystallized by cooling to −15° for 1 hr., storing at 5° for 4 hr., and filtering in the cold. The solid was immediately dried over phosphorus pentoxide. Additional compound was obtained by storing the filtrate at −15° for 1 hr. and then at 5° for several hours; yield, 58%, based on the monohydrate; m.p. 153–158°.

This compound had three strong absorbing peaks at 3500, 1005, and 960 cm.$^{-1}$. The first peak was due to the absorption of the mole of water in the molecule and the two latter peaks were due to the absorption of the sodium sulfinate group.[25]

Anal. Calcd. for $C_{10}H_{12}Cl_2NNaSO_2 \cdot H_2O$: C, 37.29; H, 4.38; Cl, 22.00; N, 4.35; S, 9.95. Found: C, 37.20; H, 4.48; Cl, 21.95; N, 4.20; S, 10.07.

Recrystallization can be achieved, if desired, by dissolving 14 g. of sodium sulfinate in 37 ml. of water and allowing it to crystallize at 5°.

When a small portion of the alkaline solution was carefully acidified with 1 *N* HCl to pH 6, no solid precipitated. By adding more acid, however, an immediate precipitate was produced which was presumably the free sulfinic acid. If any *p*-[bis(2-chloroethyl)amino]benzenethiol had been formed during the reaction, this should have precipitated out at pH 6.

N,N-Bis(2-chloroethyl)-*p*-(methylsulfonyl)aniline (VII). To 2 g. (0.0066 mole) of sodium *p*-[bis(2-chloroethyl)amino]-benzenesulfinate in 75 ml. of absolute ethanol there was added slowly a solution of 15 ml. of methyl iodide in 50 ml. of absolute ethanol. The mixture was refluxed for 22 hr., the solvent was removed under reduced pressure, and the residual red oil was added to 30 ml. of chloroform. A white solid precipitated, and the solution was washed with two 5-ml. portions of water. The chloroform solution was dried ($MgSO_4$), filtered, and sufficient ligroin was added until the solution turned cloudy. It was then placed in the deep freeze for several hours and yielded a yellow precipitate which contained some black tar. This was filtered and washed with water. The solid was again dissolved in a small amount of chloroform and enough ligroin was added to bring out the black tar. The solution was decanted and sufficient ligroin was added to cause crystallization; yield, 51%; m.p. 88–89°.

This compound absorbed strongly at 1300 and 1140–1150 cm.$^{-1}$. These bands were in agreement with the findings of Barnard and co-workers[26] who compared the spectra of seven sulfones in the solid and solution states, and found the compounds in the solid state to absorb in the range of 1160–1120 and 1350–1300 cm.$^{-1}$.

Anal. Calcd. for $C_{11}H_{15}Cl_2NO_2S$: C, 44.60; H, 5.10; Cl, 23.94; N, 4.73; S, 10.83. Found: C, 44.87; H, 5.07; Cl, 23.63; N, 4.93; S, 10.98.

N,N-Bis(2-chloroethyl)sulfanilylacetic Acid (VIII).—To 3.0 g. (0.0093 mole) of sodium *p*-[bis(2-chloroethyl)amino]benzenesulfinate in 250 ml. of absolute ethanol there was added a solution of 1.9 g. (0.010 mole) of iodoacetic acid in 25 ml. of absolute ethanol. The mixture was refluxed for 6 hr. The ethanol was removed under reduced pressure, and the resulting residue was dissolved in a 10% aqueous sodium carbonate solution containing a small crystal of sodium thiosulfate. This was then treated with 2 *N* HCl, and, upon standing, long white needles were obtained in 40% yield, m.p. 141–143°.

This compound showed the typical peaks of a carboxylic acid dimer at 3000–2500, 1700, 1435, and 940 cm.$^{-1}$. It also

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had strong absorption at 1310 and 1140 cm.$^{-1}$ due to $-SO_2-$ stretching. The asymmetrical $-SO_2-$ stretching peak (1310 cm.$^{-1}$) occurred at a slightly higher frequency than the methyl sulfone. This was due to the electron-attracting ability of the acidic group which conveyed a stronger double bond character to the sulfur–oxygen bond.[27]

Anal. Calcd. for $C_{12}H_{15}Cl_2NSO_4$: C, 42.36; H, 4.44; Cl, 20.84; N, 4.11; S, 9.42. Found: C, 41.63; H, 4.59; Cl, 20.81; N, 4.44; S, 9.90.

N,N-Bis(2-chloroethyl)-*p*-(hexadecylsulfonyl)aniline (IX).—To 1 g. (0.0033 mole) of sodium *p*-[bis(2-chloroethyl)amino]-benzenesulfinate in 25 ml. of absolute ethanol there was added 5 ml. of 1-bromohexadecane. This was refluxed for about 7–8 hr., the solid was filtered, washed with water and absolute ethanol, and air-dried; yield, 60%; m.p. 50–52°.

The infrared absorption was similar to that of VIII at 1300 and 1140–1150 cm.$^{-1}$. In addition, the long-chain sulfone had a strong band at 2930 cm.$^{-1}$ and a medium band at 1460 cm.$^{-1}$, both indicative of C–H stretching in the hexadecyl group.[28]

Anal. Calcd. for $C_{26}H_{43}Cl_2NO_2S$: C, 61.60; H, 8.95; Cl, 13.99; N, 2.76; S, 6.32. Found: C, 61.55; H, 9.09; Cl, 13.59; N, 2.45; S, 6.20.

N,N-Bis(2-chloroethyl)sulfanilylbutyronitrile (X).—To a solution of 3.0 g. (0.01 mole) of IV in 125 ml. of absolute ethanol was added 2.09 g. (0.01 mole) of γ-iodobutyronitrile. The resulting mixture was refluxed for 3–4 hr. After cooling, the solvent was evaporated and the residue was triturated with cold water and ether. The product was recrystallized from absolute ethanol to give 1.39 g. (40%) of a crystalline product, m.p. 74–75°. This compound absorbed strongly at 1300 and 1140–1150 cm.$^{-1}$, similar to VII and IX. In addition, it possessed the characteristic peak at 2280 cm.$^{-1}$ due to the cyano group.

Anal. Calcd. for $C_{14}H_{18}Cl_2N_2O_2S$: C, 48.14; H, 5.19; Cl, 20.30; N, 8.02; S, 9.18. Found: C, 48.01; H, 5.12; Cl, 20.23; N, 7.95; S, 9.09.

***p*-[Bis(2-chloroethyl)amino]benzenesulfinyl Chloride (VI).**—To a solution of 27 ml. of oxalyl chloride in 180 ml. of chloroform, immersed in an ice–salt bath was added 18 g. (0.056 mole) of sodium *p*-[bis(2-chloroethyl)amino]benzenesulfinate and the mixture was allowed to stand at 5° for several hours until there was no visible bubbling. It was then filtered into 500 ml. of cold ligroin and stirred until an orange precipitate came out. This was filtered immediately, washed with several portions of cold ligroin, and dried between several sheets of filter paper. This product was used immediately in synthesizing the thiosulfinates. The filtrate was refrigerated (−15°) with an additional 100 ml. of ligroin and more compound was isolated. The approximate yield of the orange solid was 83%. No accurate melting point nor analysis could be obtained for this compound due to its instability. If the orange solid were allowed to stand in the air for a few minutes, it was found to decompose and turn into a gummy mass. When a drop of phenetole was added, this gummy solid, dissolved in cold sulfuric acid, turned blue indicating the presence of a sulfinic acid (Smiles' test).[29] Since the ease of decomposition of the sulfinyl chloride depended on the humidity, this reaction must be carried out under very anhydrous conditions. Thionyl chloride was also found to be a satisfactory chlorinating agent.

Preparation of the Thiosulfinates (VIa–c).—The preparation of S-(*p*-tolyl) *p*-[bis(2-chloroethyl)amino]thiobenzenesulfinate (VIb) is presented as an example of the procedure followed in preparing all three thiosulfinates.

To a mixture of 1.2 g. (0.0093 mole) of *p*-toluenethiol, 1.0 ml. of dry pyridine, and 15 ml. of dry chloroform in an ice bath, there was slowly added a solution of 2.8 g. (0.0093 mole) of *p*-[bis(2-chloroethyl)amino]benzenesulfinyl chloride in 15 ml. of dry chloroform. The solution was warmed between 40–50° for 10 min., cooled, and poured slowly into a cold solution of 8 ml. of 1 *N* sulfuric acid and 30 ml. of water. The layers were separated, the chloroform was washed with two 15-ml. portions of cold water, dried ($MgSO_4$), filtered, and cooled to −10°. To this was slowly added, with stirring, 380 ml. of cold ligroin. The flask was then scratched until a small quantity of solid precipitated, and the solution was refrigerated (−10°) for several hours during which time a voluminous solid formed. This was filtered and recrystallized from cold chloroform and ligroin. A second crop was obtained by returning the filtrate to the refrigerator (−10°) for several days with an additional quantity of ligroin. This afforded the compound as a light yellow solid in 56% yield, m.p. 91–92°.

Anal. Calcd. for $C_{17}H_{19}Cl_2NOS_2$: C, 52.57; H, 4.93; Cl, 18.26; N, 3.61; S, 16.51. Found: C, 52.34; H, 5.07; Cl, 18.34; N, 3.75; S, 16.45.

S-Phenyl *p*-[bis(2-chloroethyl)amino]thiobenzenesulfinate (VIa) was prepared in an analogous manner except that two different fractions were obtained during the isolation. The first fraction was a yellow solid, m.p. 132–138°, and its infrared spectrum was identical with that of S-{*p*-[bis(2-chloroethyl)-amino]phenyl} *p*-[bis(2-chloroethyl)amino]thiobenzenesulfonate. The second fraction was obtained by adding an additional 240 ml. of cold ligroin to the cold chloroform solution and allowing it to stand for several days at −10°. More product was obtained by adding additional ligroin to the filtrate. This gave the desired product as a light yellow solid in 19% yield, m.p. 69.5–71.5°.

Anal. Calcd. for $C_{16}H_{17}Cl_2NOS_2$: C, 51.33; H, 4.57; Cl, 18.94; N, 3.76; S, 17.13. Found: C, 51.39; H, 4.62; Cl, 18.85; N, 3.88; S, 17.25.

S-{*p*-[bis(2-chloroethyl)amino]phenyl} *p*-[bis(2-chloroethyl)-amino]thiobenzenesulfinate (VIc) was prepared in an analogous manner, only one fraction being obtained. Recrystallization from cold chloroform and ligroin afforded a yellow solid in 76% yield, m.p. 109°.

Anal. Calcd. for $C_{20}H_{24}Cl_4N_2OS_2$: C, 46.70; H, 4.70; Cl, 27.57; N, 5.45; S, 12.47. Found: C, 46.34; H, 4.58; Cl, 27.59; N, 5.31; S, 12.58.

Bredereck and co-workers[30] found that the absorption bands of a disulfide were present, neglecting small differences in wave length and relative intensities, in all the spectra of its oxidation products. The oxidized compounds, however, possessed additional bands characteristic of the particular oxidation state. The spectrum of S-*p*-tolyl *p*-toluenethiosulfinate contained an additional band at 1094 cm.$^{-1}$ which was assigned to the S=O linkage. This additional peak was between the S=O absorption of a sulfoxide (1040–1060 cm.$^{-1}$) and the S=O absorption of a sulfinic acid ester (1126–1136 cm.$^{-1}$) or a sulfinic acid chloride (1150 cm.$^{-1}$).[25]

Carson and Wong[31] also reported strong absorption at 1093 cm.$^{-1}$ for *p*-tolyl *p*-toluenethiosulfinate. They stated that the compound did not absorb between 1163 and 1110 cm.$^{-1}$, thus showing the absence of a $-SO_2-$ group in the thiosulfonates. Protogen-B (β-lipoic acid) had the S=O absorption at 1040 cm.$^{-1}$.[32]

The spectra of S-{*p*-[bis(2-chloroethyl)amino]phenyl} *p*-[bis-(2-chloroethyl)amino]thiobenzenesulfinate (VIc), made *via* the perbenzoic acid or the sulfinyl chloride method, were the same, This established that the chlorosulfonation of bis(2-chloroethyl)-aniline occurred in the *para* position. In comparing the spectrum of this thiosulfinate to the corresponding disulfide it was seen that the former spectrum had a distinct peak at 1070 cm.$^{-1}$ due to S=O absorption. Also the thiosulfinate showed no additional absorption between 1110 and 1163 cm.$^{-1}$. The spectra of the two unsymmetrical thiosulfinates had the same S=O absorption peak as the symmetrical compound. The S=O absorption in these three thiosulfinates was at a lower frequency than reported by Bredereck, unless we assume that the peak at 1100 cm.$^{-1}$ in the disulfide had shifted to the lower frequency of 1060–1070 cm.$^{-1}$ in the thiosulfinates. Then the peak at 1090–1100 cm.$^{-1}$ in the latter spectra was due to the S=O absorption.

S-{*p*-[Bis(2-chloroethyl)amino]phenyl} *p*-[Bis(2-chloroethyl)amino]thiobenzenesulfonate (XI).—This was prepared by the same procedure as presented for the thiosulfinates, only the mode of isolation was different. The thiosulfinates were found to disproportionate into a thiosulfonate and a disulfide if they were isolated by pouring the chloroform solution into ligroin at room temperature and then placing the solution into the refrigerator (−10°). Various mercaptans such as dodecyl mercaptan,

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thiophenol, and thiotoluene were used in making the thiosulfinates and in each case the same symmetrical thiosulfonate was obtained. The yellow solid was recrystallized from absolute ethanol and had a m.p. of 136–139°. The disulfide was not recovered.

In comparing the spectra of this thiosulfonate with its corresponding disulfide there were found two large peaks at 1135 and 1300 cm.$^{-1}$ due to asymmetrical and symmetrical $-SO_2-$ absorption.[30,33] In addition, the peak at 1100 cm.$^{-1}$ in the disulfide has shifted to 1075 cm.$^{-1}$ in the thiosulfonate, which is in agreement with the assumption made concerning the S=O peak in the thiosulfinate.

Anal. Calcd. for $C_{22}H_{37}Cl_2NOS_2$: C, 45.29; H, 4.56; Cl, 26.74; N, 5.28; S, 12.10. Found: C, 45.16; H, 4.71; Cl, 26.20; N, 5.35; S, 11.82.

Reaction of S-(*p*-Tolyl) *p*-[Bis(2-chloroethyl)amino]thiobenzenesulfinate (VIb) with Triphenylphosphine.—The procedure employed by Carson and Wong[31] was followed. In a test tube was placed 0.6 g. (0.0023 mole) of triphenylphosphine and 0.882 g. (0.0023 mole) of S-(*p*-tolyl) *p*-[bis(2-chloroethyl)-amino]thiobenzenesulfinate. They were mixed and gently shaken together. After 15 min., the dry mixture had not liquefied to a yellow melt as reported by Carson and Wong with their thiosulfinates. The tube was allowed to stand overnight, then it was warmed in a water bath for 30 min. The solid melted when warmed and re-formed as a yellow solid at room temperature. This was dissolved in 4 ml. of benzene, and 20 ml. of petroleum ether was added. The mixture was refrigerated for several hours and filtered, and the solid was washed with several portions of petroleum ether. It was redissolved in a small amount of benzene, and petroleum ether again was added. The resulting white solid had a m.p. of 154–157°. This confirmed the presence of a reactive oxygen on the sulfur atom. An unsuccessful attempt was made to isolate the disulfide from the chloroform filtrate.

Since the eutectic mixture reported by Carson and Wong did not form of its own accord, a blank was run to verify that the oxidation of the triphenylphosphine was truly caused by the thiosulfinate. The only product that was isolated from the blank was a white solid melting at 70–75°. Therefore, it can be assumed that the oxidation was caused by the thiosulfinate.

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Enzyme-Alterable Alkylating Agents. VII. The Design of Short-Lived Mustards[1]

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In a search for short-lived alkylating agents suitable for intraarterial injection in cancer chemotherapy, a series of 2-bromo- and 2-iodoethyl sulfur mustards has been synthesized. The mustards have the general structure, $XCH_2CH_2S(CH_2)_nCONHCH_2CH_2NHCO(CH_2)_nSCH_2CH_2X$, where X is a halogen atom and n is an integer from 1 to 4. The hydrolytic stability decreased in the following order: (1) as a function of X, Cl > I > Br, and (2) as a function of n, 1 > 2 > 3 > 4. One bromo mustard ($n = 4$) was synthesized which had a half-life of approximately 0.2 sec. at 37°, extrapolated from rate data at a lower temperature. These data indicate that sulfur mustards can be designed with extremely short half-lives utilizing two regulatory factors: (1) the relative nucleophilicity of the sulfur atom as controlled by the integer n, and (2) the type of halogen on the carbon β to the sulfur atom.

The therapeutic effectiveness of the alkylating agents in cancer chemotherapy is limited by the undesirable side effects normally associated with these drugs when they are administered in sufficient dose to cause tumor regression. Because of the great sensitivity of one or more elements of the bone marrow, many attempts to by-pass this area have been proposed.[2] The current intraarterial infusion technique depends for its success on the rapid and complete decomposition of the agent after it has passed through the tumor and prior to its contact with bone marrow. In a previous search for short biological life agents suitable for intraarterial infusion, a large number of sulfur mustards were synthesized and evaluated. Among these were bifunctional sulfur mustards with half-lives as short as 14 sec. The reaction mechanism for these mustards was studied in detail and the parameters controlling reactivity were reported.[3]

In view of the short circulation time in man, however, further reduction in the half-lives of these agents was considered necessary to prevent significant quantities of the agent from reaching sensitive, nontumor areas after intraarterial injection to the tumor site. An analysis of the quantitative data for the first-order reaction rates of chloro sulfur mustards[3] indicates a low probability of selecting substituents that would further shorten the half-lives of these agents. Therefore, an alternate approach was sought.

Because the bromine and iodine atoms possess better leaving properties than the chlorine atom,[4] the former would be expected to yield sulfur mustards that cyclize (and react) more rapidly than the corresponding chloro

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