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 $C_{23}H_{27}Cl_2NOS_2$: 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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(2) (a) S. McFarland, N. B. Granville, and W. Damershek, *Blood*, **14**, 503 (1959); (b) O. Creech, Jr., E. T. Kremontz, R. F. Ryan, and J. N. Winblad, *Ann. Surg.*, **148**, 616 (1958); (c) M. E. Conrad and W. H. Crosby, *Blood*, **16**, 1089 (1960); (d) D. G. Miller and W. Lawrence, *Proc. Am. Assoc. Cancer Res.*, **3**, 251 (1961); (e) C. T. Klopp, T. C. Alford, J. Bateman, G. N. Berrry, and T. Winship, *Ann. Surg.*, **132**, 811 (1950).

(3) C. E. Williamson, J. I. Miller, S. Sass, J. Casanova, S. P. Kramer, A. M. Seligman, and B. Witten, *J. Natl. Cancer Inst.*, **31**, 273 (1963).

(4) L. F. Fieser and M. Fieser, "Advanced Organic Chemistry," Reinhold Publishing Corp., New York, N. Y., 1962, p. 319.

mustards. This is consistent with the observations of Chapman and James[5a] and of Ross[5b] who reported that the bromo and iodo nitrogen mustards cyclize more rapidly than the corresponding chloro compounds.

Accordingly, a series of bifunctional bromo and iodo sulfur mustards was synthesized. The bromo and iodo mustards contained the same fundamental amide skeletal structure as N,N′-ethylenebis[(2-chloroethylthio)acetamide] (S-46) [—$CH_2CH_2S(CH_2)_nCONHCH_2CH_2NHCO(CH_2)_nSCH_2CH_2$—], which has shown good palliation of tumors in children.[6] Kinetic studies have been carried out to determine the suitability of these new amido mustards for use in the intraarterial infusion techniques for cancer chemotherapy.

Results and Discussion

The active species of sulfur mustards I is attributed to a highly reactive three-membered ethylene sulfonium ion II (eq. 1)[7] which reacts very rapidly with water as well as with other nucleophilic reagents, such as thiosulfate, amines, proteins, thiols, and nucleic acids.[8] The rates of these reactions are all essentially identical and depend only upon the rate of formation of the ethylene sulfonium ion II. Because of the extremely rapid reaction between II and nucleophilic substances, the identity of the nucleophile is unimportant from a kinetic viewpoint. The rate of ethylene sulfonium ion formation (the rate-controlling step) of a particular sulfur mustard thus defines its rate of reaction with all nucleophilic reagents. In accordance with eq. 1, these reactions exhibit first-order kinetics and the reaction rates are generally independent of pH over a wide range.[9a,b]

$$\underset{\text{I}}{RSCH_2CH_2Cl} \underset{\text{slow}}{\rightleftharpoons} \underset{\text{II}}{RS\overset{+}{\underset{CH_2}{\overset{CH_2}{|}}}} + Cl^- \begin{cases} \xrightarrow[\text{fast}]{H_2O} RSCH_2CH_2OH + HCl \\ \xrightarrow[\text{fast}]{Y^-} RSCH_2CH_2Y + Cl^- \end{cases} \quad (1)$$

Y^- = nucleophilic reagent

In Figure 1 are depicted typical curves showing the course of hydrolysis at 30° of the iodo mustard, N,N′-ethylenebis[(2-iodoethylthio)acetamide] (III), the bromo mustard, N,N′-ethylenebis[(2-bromoethylthio)-acetamide] (IV), and for comparison the analogous chloro sulfur mustard, N,N′-ethylenebis[(2-chloroethylthio)acetamide] (V). As in the bifunctional chloro series,[3] first-order kinetics are observed only during the initial phase of the hydrolysis. The extent of the reaction exhibiting first-order kinetics was usually greater than 60% in these studies. The rate of reaction for the latter phase of the hydrolysis was generally about two times slower than that observed for the initial phase.

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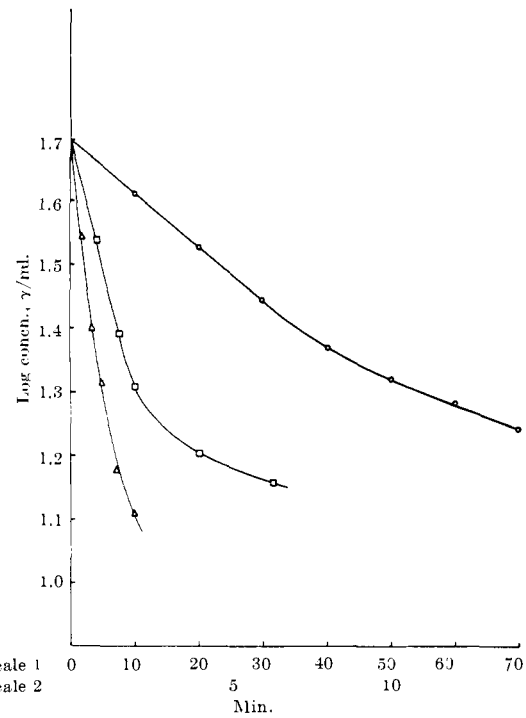

Figure 1.—Hydrolysis of iodo (III, □, scale 2), bromo (IV, Δ, scale 2), and chloro mustards (V, ○, scale 1) at 30°, pH 7.4, 0.04 *M* aqueous barbital buffer, 2% DMA; mustard concentration, 50 γ/ml.

Of significance is the rapid reaction rate of the bromo derivative IV (Figure 1). It is noted that neither the quality of the bromine as a leaving group nor its electron-withdrawing power can explain the greater reactivity of IV as compared with that of the iodo derivative III. However, this anomaly is not completely unprecedented. It has been shown by Ross[5b] that bromoethyl nitrogen mustards cyclize more rapidly than the corresponding iodoethyl nitrogen mustards.

Table I contains the first-order reaction constants of the bromo and iodo mustards at 3.5°, a convenient temperature for comparison. These rate constants

TABLE I

HYDROLYSIS DATA FOR A SERIES OF BIFUNCTIONAL CHLORO, BROMO, AND IODO SULFUR MUSTARDS AT pH 7.4

$XCH_2CH_2S(CH_2)_nCONHCH_2CH_2NHCO(CH_2)_nSCH_2CH_2X$

			3.5°		37°[a]		
				$t_{1/2}$,		$t_{1/2}$,	
No.	X	n	log k	min.	log k	min.	sec.
V	Cl	1			−1.393	17.1	
VIII	Cl	2			−0.242	1.21	72.6
XI	Cl	3			0.0762	0.58	34.6
XIII	Cl	4			0.482	0.231	13.7
IV	Br	1	−1.567	25.55	0.399	0.28	16.6
VII	Br	2	−0.241	1.20	1.726	0.013	0.78
X	Br	3	0.131	0.51	2.097	0.006	0.33
XII	Br	4	0.393	0.28	2.359	0.003	0.18
III	I	1	−2.028	73.8	−0.0241	0.73	43.9
VI	I	2	−0.826	4.63	1.178	0.046	2.75
IX	I	3	−0.380	1.66	1.624	0.018	0.99

[a] Values for bromo and iodo sulfur mustards extrapolated from rates at 3.5° (see text).

are derived from the initial slopes of the curves illustrated in Figure 1. It will be noted from Table I that interposing methylene groups between the sulfur atom and the electron-withdrawing amide group results in

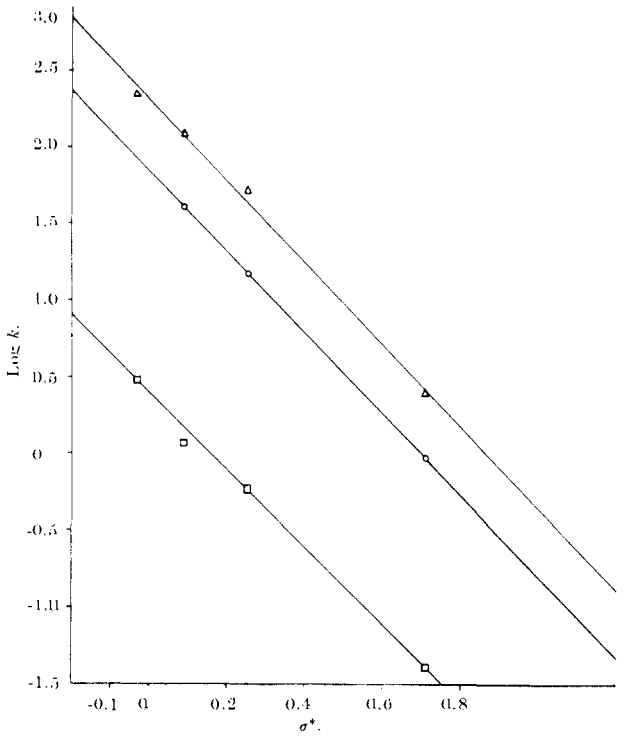

Figure 2.—Hydrolysis of iodo (○), bromo (△), and chloro (□) mustards at 37°, pH 7.4. Effect of polar substituents on reaction rates.

rate acceleration. This influence reflects the increased relative nucleophilicity of the sulfur atom. In these respects, the bromo and iodo sulfur mustards parallel the effects observed in the chloro mustards where the rate of reaction was shown to depend directly upon the relative nucleophilicity of the sulfur atom.[3]

Reaction constants for III and IV were obtained at five temperatures and the energies of activation were calculated. The data used for these calculations are shown in Table II. The energies of activation, 23.9 kcal./mole for III and 23.3 kcal./mole for IV, were assumed to be the same for the other highly reactive members of the respective iodo and bromo series. This assumption appears reasonable in view of the fairly constant activation energy observed for a large series of sulfur mustards containing widely varied substituents.[3] The analytical procedure utilized did not lend itself to measurement of the reaction rates of the very reactive compounds at temperatures higher than 3.5°. Accordingly, reaction constants and half-lives at 37° were calculated for the bromo and iodo mustards, extrapolating by means of the Arrhenius equation from the data at 3.5° (Table I). For comparison, the hydrolysis data for the corresponding chloro series are included in Table I.

TABLE II

HYDROLYSIS DATA FOR IODO (III) AND BROMO (IV) SULFUR MUSTARDS USED AS THE BASIS FOR CALCULATION OF E_a[a]

	III		IV	
Temp., °C.	k_{av}, min.$^{-1}$	$t_{1/2}$, min.	k_{av}, min.$^{-1}$	$t_{1/2}$, min.
10	0.031	22.32	0.085	8.14
15	0.066	10.48	0.174	3.98
20	0.136	5.09	0.334	2.07
25	0.272	2.54	0.709	0.98
30	0.511	1.35	1.275	0.54

[a] These results are the average of at least five determinations at each temperature. All data were within 8% of the mean. Hydrolysis was conducted at pH 7.4.

The calculated hydrolysis data indicate that at 37°, some of the bromo and iodo mustards possess half-lives measured in fractions of 1 sec. Under comparable conditions, the *bromo* mustards hydrolyze about three times more rapidly than the corresponding *iodo* mustards and about 100 times more rapidly than the corresponding *chloro* mustards.

A re-examination of the hydrolysis data for the amides previously reported[3] indicates that the assignment of a σ^* value of 2.0 for the substituent $-CONHR$ (where R is H or an alkyl group) agrees with the data better than the previously assigned value (1.74). It is noteworthy that the revised value is identical with the σ^* value reported for the ester function, $-COOCH_3$.[10] The σ^* values for the homologous amides, $(CH_2)_n$-$CONHR$ are calculated as $\sigma^* = 2.8^n$ for $n = 1$, 2, and 3. As previously reported,[3] this method is not applicable when $n = 4$ or higher.

In Figure 2, log k is plotted *vs.* σ^* for each series. The dependence of the hydrolysis rates upon polar effects is illustrated. The linearity exhibited suggests that steric factors are unimportant for the compounds given an observation that has been confirmed for the chloro mustards.[3] Slopes obtained from Figure 2 are recorded as the ρ^* values in Table III.

TABLE III

CONSTANTS DERIVED FROM FIGURE 2

Series	ρ^*	log k_0
Chloro	−2.51	0.404
Bromo	−2.66	2.321
Iodo	−2.81	1.962

The nature of the halogen atom in the sulfur mustards does not markedly influence the ρ^* value, which varies between −2.51 and −2.81. Insufficient data are available to determine whether this difference is significant.

In Table IV, the values of log (k/k_0) *vs.* $\sigma^*\rho^*$ are compared. The agreement between the experimentally determined values and those calculated theoretically confirms that the Taft equation in its simplified form, log $(k/k_0) = \sigma^*\rho^*$, can be used to predict the hydrolysis rates of bifunctional sulfur mustards containing either

TABLE IV

COMPARISON OF LOG (k/k_0) *vs.* $\sigma^*\rho^*$[a]

$XCH_2CH_2S(CH_2)_nCONHCH_2CH_2NHCO(CH_2)_nSCH_2CH_2X$

No.	n	X	σ^*	log (k/k_0)	$\sigma^*\rho^*$
V	1	Cl	0.71	−1.797	−1.782
IV	1	Br	0.71	−1.913	−1.889
III	1	I	0.71	−1.986	−1.995
VIII	2	Cl	0.254	−0.646	−0.638
VII	2	Br	0.254	−0.600	−0.676
VI	2	I	0.254	−0.749	−0.714
XI	3	Cl	0.091	−0.325	−0.228
X	3	Br	0.091	−0.253	−0.242
IX	3	I	0.091	−0.231	−0.256
XIII	4	Cl	−0.031[b]	0.078	0.078
XII	4	Br	−0.031[b]	0.045	0.082

[a] At 37°, pH 7.4. [b] Value obtained from ref. 3.

(10) R. W. Taft, Jr., in "Steric Effects in Organic Chemistry," M. S. Newman, Ed., John Wiley and Sons, Inc., New York, N. Y., 1956, pp. 556–675.

TABLE V

SYNTHESIS OF BIFUNCTIONAL 2-BROMOETHYL AND 2-IODOETHYL SULFUR MUSTARDS

$XCH_2CH_2S(CH_2)_nCONHCH_2CH_2NHCO(CH_2)_nSCH_2CH_2X$

No.	n	X	Yield, %	M.p.,[a] °C.	Formula	Calcd., % C	Calcd., % H	Calcd., % X[b]	Found, % C	Found, % H	Found, % X
IV	1	Br	56	134–135	$C_{10}H_{18}Br_2N_2O_2S_2$	28.4	4.3	37.9	28.7	4.4	37.8
VII	2	Br	63	155–156	$C_{12}H_{22}Br_2N_2O_2S_2$	32.0	4.9		32.4	4.9	
X	3	Br	37	128	$C_{14}H_{26}Br_2N_2O_2S_2$	35.2	5.5	33.4	35.5	5.6	33.7
XII	4	Br	20	123–124	$C_{16}H_{30}Br_2N_2O_2S_2$	38.0	6.0	31.5	38.1	5.8	31.0
III	1	I	78	121–124	$C_{10}H_{18}I_2N_2O_2S_2$	23.3	3.5	49.2	24.0	3.3	47.5
VI	2	I	50	140–141	$C_{12}H_{22}I_2N_2O_2S_2$	26.5	4.1		27.1	4.3	
IX	3	I	62	124–125	$C_{14}H_{26}I_2N_2O_2S_2$	29.4	4.6		28.4	4.2	

[a] All melting points were determined on a Fisher-Johns hot stage melting point apparatus and are corrected. [b] X is halogen atom.

chlorine, bromine, or iodine atoms, provided resonance factors are minimal.

The factors which influence the hydrolysis rates, therefore, are (1) the electron-withdrawing effect of the substituent adjacent to the sulfur atom, and (2) the type of halogen atom on the carbon β to the sulfur atom.

Clinical studies are currently in progress with XII (half-life = 0.2 sec. at pH 7.4, 37°) utilizing regional intraarterial infusion techniques. It is anticipated that these studies will give an insight to the desired hydrolysis rate for optimum anticancer activity without the concomitant bone marrow depression.

Experimental

Bromo Mustards.—The bifunctional 2-bromoethyl sulfur mustards described herein were prepared by treatment of their corresponding glycols[11] with phosphorus tribromide.

The bromo mustards in this series were well-defined white crystalline solids. Although readily prepared in a dry atmosphere, much difficulty was encountered in handling these compounds in a humid atmosphere. The yields, elemental analyses, and physical properties obtained in the synthesis of these compounds are recorded in Table V.

Preparation of Representative Compound, N,N′-Ethylenebis[4-(2-bromoethylthio)butyramide](X).—N,N′-Ethylenebis[4-(2-hydroxyethylthio)butyramide] (3.0 g., 0.0085 mole) was placed in a test tube and an equal volume of phosphorus tribromide (precooled to −40°) was added in one portion. The suspension was stirred with a thermometer and allowed to warm slowly to room temperature. After standing at room temperature for 1 hr., the suspension was heated to 75°, with stirring, for 10 min. and then allowed to cool to room temperature. After anhydrous ether was added, the mixture was stirred well and the solvent was decanted. This process was repeated until the crude solid X was suspended in almost pure ether. The solid was collected on a filter and then dissolved in a minimum quantity of N,N-dimethylformamide with mild heating. Acetonitrile was added until the solution became faintly turbid. Upon cooling, the desired compound crystallized as a fluffy white solid. Recrystallization from 2-butanone yielded 1.5 g. (37%) of pure X, m.p. 128°.

Iodo Mustards.—The synthesis of the chloro sulfur mustards has been reported previously.[3,11] These were converted to the corresponding iodo compounds by treatment with sodium iodide in 2-butanone.

The iodo mustards of this series are all crystalline solids obtained as white needles that melt with decomposition. Although the chloro sulfur mustards reacted with sodium iodide as expected, considerable difficulty was encountered in the purification of the products. Even after repeated recrystallization from various solvents, there was evidence of impurities remaining in the solid. Exposure to the atmosphere at room temperature results in rapid deterioration of these compounds. They can, however, be stored successfully in a desiccator at 0°.

The yields, elemental analyses, and physical properties obtained in the synthesis of these compounds are recorded in Table V.

Preparation of N,N′-Ethylenebis[2-iodoethylthio)acetamide].—N,N′-Ethylenebis[(2-chloroethylthio)acetamide] (V, 25 g., 0.075 mole) was dissolved in 100 ml. of 2-butanone contained in a 1-l. reaction flask equipped with a mechanical stirrer, reflux condenser, and thermometer. Sodium iodide (27.3 g., 0.150 mole) in 700 ml. of 2-butanone was added, and the reaction mixture was stirred at reflux for 10 hr. The mixture was filtered while hot and, upon cooling, a solid material separated from the filtrate. The crude product (30 g., 78%) was recrystallized repeatedly from ethyl acetate and then benzene. The final product was obtained as tiny white needles, m.p. 121–124°.

Kinetic Studies.—The 4-(4-nitrobenzyl)pyridine (NBP) method for the determination of the hydrolytic stability of the carbon to chlorine bonds of sulfur mustards as a measure of alkylating agent remaining, has been described in previous papers of this series.[3,11] The rates of hydrolysis of the carbon to bromine and carbon to iodine bonds in 0.04 M, pH 7.4 aqueous barbital buffer were determined by this method. As a solvent for the preparation of stock solutions, N,N-dimethylacetamide (DMA) was selected instead of Methyl Cellosolve because it contained no reactive hydroxyl groups and because it was the solvent of choice in clinical studies of these mustards.[6] In the aqueous reaction medium, DMA was present to a maximum of 2% by volume, although an increase in DMA to 5% did not alter the rates. The concentration of mustard was varied from as high as 70 γ/ml. to as low as 25 γ/ml. with no change in reaction rate. Most of the rates were determined at 50 γ/ml.

Due to the extremely rapid reaction at 37° in most instances, reaction constants could be obtained only at much lower temperatures. For low-temperature studies, aliquots were removed from the reaction medium using previously chilled pipets and added immediately to the NBP reagent. With N,N′-ethylenebis[(2-bromoethylthio)acetamide] (IV) and N,N′-ethylenebis[(2-iodoethylthio)acetamide] (III), the half-lives were sufficiently long that reaction constants could be obtained at several temperatures. The experimental energy of activation E_a was calculated by use of the Arrhenius equation. The extent of error in these studies was less than 8%.[12]

Acknowledgment.—Acknowledgment is due Walter J. Culbreth, Alexander Janowski, Charles E. McCarroll, Charles Burns, John Dopp, Daniel Rafferty, and G. Robert Salvi for technical assistance.

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(12) Maximum deviation from the mean in the experimental determination of reaction rates.