More importantly, a new activity against *N. gonorrhoeae* is found in these compounds.

Experimental Section

Melting points (corrected) were taken in a Thomas-Hoover capillary apparatus. Analyses indicated by symbols of the elements represent results within ±0.4% of the theoretical values. Most compounds were prepared by the methods of reference 3. Sample preparations are given for the compounds prepared by different methods.

Ethyl 4-[6-(1-Methyl-5-nitro-2-imidazolylthio)hexyloxy]benzoate (45). Ethyl 4-[6-(1-methyl-2-imidazolylthio)-hexyloxy]benzoate, 5.8 g, was dissolved in 15 mL of CF_3COOH with 2.5 mL of HNO_3 (concentrated). The solution was kept at room temperature for 18 h, heated to 90 °C for 0.5 h, and then poured into H_2O. The pH was adjusted to 6 with $NaHCO_3$ and the aqueous layer was decanted from an oil which was chromatographed on Woelm silica in a low-pressure system using 20% ethyl acetate–benzene. Fractions containing **45** (as indicated by thin-layer chromatography) were combined and evaporated to yield a solid which was triturated with ether to give 0.5 g (8%), mp 75–78 °C. Compound **19** was eluted next to yield 0.6 g (10%).

4-[6-(1-Methyl-5-nitro-2-imidazolylthio)hexyloxy]benzoic Acid (18). Compound **45**, 0.5 g, was dissolved in 4 mL of H_2SO_4 (concentrated). After 15 min the solution was diluted to 40 mL with H_2O. The solid which separated was recrystallized twice from acetone to give 0.15 g (30%) of **18**.

Sodium 4-[2-(1-Methyl-5-nitro-2-imidazolylthio)ethoxy]benzoate (12). Compound **10**, 1.8 g, was mixed with less than 1 equiv of NaOH solution and the mixture was stirred for several hours and then filtered. The filtrate was concentrated under vacuum. The residue was **12**, 0.9 g (47%).

Microbiology. Antimicrobial tests were conducted by tenfold serial dilution in suitable liquid or solid media, the highest concentration of compounds used being 100 μg/mL. Activities against the anaerobic bacteria, *C. perfringens* ATCC 13124 and *B. fragilis* ATCC 23745, were determined in Fluid Thioglycollate Broth (Bioquest, Cockeysville, Md.) while those against *N. gonorrhoeae* ATCC 19424, an aerobic bacterium, were determined on Chocolate Agar [GC Agar Base (Bioquest) supplemented with Bacto Hemoglobin and Bacto Supplement C (Difco Laboratories, Detroit, Mich.)]. Diamond medium[6] with 5% Dubos medium serum (Difco Laboratories) was used for antiprotozoal tests with *T. vaginalis* ATCC 30001, *T. foetus* 1002-96-27, and *P. hominis* ATCC 30000. The *C. perfringens* and *B. fragilis* test preparations were incubated for 24 h and the slower growing *N. gonorrhoeae* and the trichomonads were incubated for 48 h, all at 37 °C. After incubation the bacterial preparations were observed visually for the presence of growth and the trichomonad preparations were observed microscopically for the presence of motile cells. Minimal inhibitory concentrations (MIC values) are reported as the lowest concentrations of the compounds, in μg/mL, which prevented development of visible growth or resulted in the absence of motile organisms. Replicate tests were done (two to eight replicates) when the quantities of compounds available permitted, and in cases where variations in the results occurred the highest MIC values are reported.

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Chemoimmunotherapy of Cancer. 3.[1] Analytical Measurement of Chemical Half-Lives of Monofunctional Alkylators

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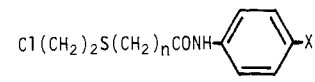
The objective of this study is the measurement of the rates of hydrolysis of a series of chloroethyl sulfide derivatives, under simulated physiological conditions. Interferences encountered with the conventional spectrophotometric method prompted the use of a rapid-response, chloride selective electrode. This probe was readily capable of monitoring the hydrolytic rate, which is identical with the rate of chloride ion formation. Since the desired subsecond half-lives were not achieved by any of the compounds, factors influencing the rates were investigated. The results suggest that the rate-controlling cyclization step may be inhibited, due to coordination of undissociated protonic functional groups on the aromatic portions of the structures with the lone-pair electrons on sulfur.

After three decades of clinical experience with cytotoxic alkylating agents in the treatment of cancer, myelosuppressive side effects remain a major pitfall of chemotherapy.[2] Approaches to this problem have included a number of techniques intended to permit the shunting of anticancer agents through the diseased region, while bypassing sensitive hemopoietic tissues.[3-7] The goal of developing such methods had prompted Seligman and his associates to synthesize a number of alkylators which may be rapidly deactivated by hydrolysis, following a single passage through a tumor's capillary bed. Kinetic studies of these agents showed that the desired subsecond half-lives were achieved by a number of compounds in the chloroethyl sulfide series.[8]

Our recent efforts have sought to incorporate this capability of rapid deactivation into the design of alkylators suitable for chemoimmunotherapy of cancer.[1] In this report, kinetic studies on the hydrolysis of such alkylating immunogens are presented. The structures are shown in Figure 1.

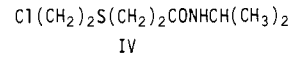
Previously, kinetic studies of alkylating agents have utilized the Epstein reagent, 4-(4′-nitrobenzyl)pyridine, to measure the unhydrolyzed mustard remaining after suitable time intervals.[8,9] Our initial studies showed this



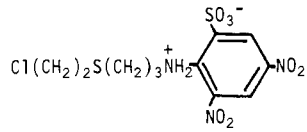
X = PO_3H_2 AsO_3H_2 OH

n = 2 (Ia) 2 (IIa) 3 (III)

3 (Ib) 3 (IIb)



IV



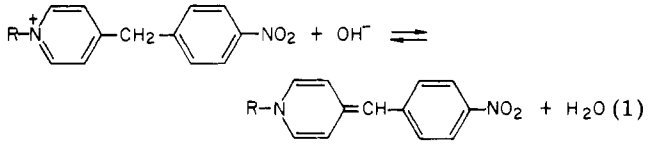
V

Figure 1. Structures of the monofunctional sulfur mustards.

Table I. Initial Concentrations, c (M), First-Order Rate Coefficients, k (s^{-1}), Half-Lives, $t_{1/2}$ (s), and Linear Regression Constants, r, for the Mustard Hydrolyses

Compd no.	c, M	k, s^{-1}	$t_{1/2}$, s	r
Ia	1.0×10^{-2}	4.8×10^{-3}	140	0.972
Ib	6.55×10^{-3}	8.9×10^{-3}	78	0.977
	1.00×10^{-2}	6.8×10^{-3}	100	0.998
	1.33×10^{-2}	5.6×10^{-3}	120	0.987
IIa	6.57×10^{-3}	9.5×10^{-3}	73	0.987
	9.03×10^{-3}	7.6×10^{-3}	90	0.992
	1.03×10^{-2}	7.0×10^{-3}	99	0.992
	1.31×10^{-2}	5.6×10^{-3}	120	0.995
IIb	6.56×10^{-3}	2.0×10^{-2}	34	0.969
	1.31×10^{-2}	7.8×10^{-3}	88	0.988
III	6.21×10^{-3}	1.0×10^{-2}	69	0.988
	1.20×10^{-2}	9.4×10^{-3}	74	0.984
IV	3.3×10^{-4}	5.6×10^{-3}	120	0.995
V	3.4×10^{-3}	2.0×10^{-3}	350	0.979

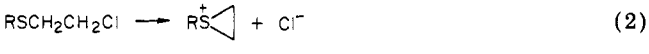
method to be unsatisfactory for use with many of the chemoimmunotherapeutic agents. Normally, the alkylated dye is developed by deprotonation of the pyridinium complex with base, as shown in eq 1. Using compounds



(1)

I, II, or V, however, the initially vivid purple dye was seen to fade within 5 s, obviating the usefulness of the method for our purposes. The exact reason for this phenomenon is unknown.

Recent measurements of nitrogen mustard hydrolysis rates have utilized chloride ion selective electrodes.[10] This approach is equally useful for monofunctional sulfur mustards, since their hydrolyses, in general, depend upon a related cyclization step, as shown in eq 2.[8]



(2)

The rate-controlling step may be followed directly, through increases in the measured potential which varies directly with the log of chloride ion concentration.

Hydrolysis rates and half-lives of the mustards obtained under simulated physiological conditions (37 °C, pH 7.4) were determined at various mustard concentrations.[11] The results are listed in Table I. None of the mustards achieved the desired subsecond half-life. Rates were seen to decrease with increasing initial concentration of mustard, implying autoinhibition of the hydrolysis.

Table II. Dependence of Hydrolysis Rate of Compound IIb, Initially 6.56×10^{-3} M, upon pH of the Medium. Values: pH; First-Order Rate Coefficient, k (s^{-1}); Half-Life, $t_{1/2}$ (s); and Linear Regression Constant, r

pH	k, s^{-1}	$t_{1/2}$, s	r
7.4	2.0×10^{-2}	34	0.969
5.4	9.4×10^{-3}	73	0.987
2.6	6.6×10^{-3}	100	0.980

Table III. Temperature Dependence of Hydrolysis Rate of Compound IIb, 6.56×10^{-3} M. Values: Temperature, T (K), T^{-1} (K^{-1}); First-Order Rate Coefficient, k (s^{-1}); and Ln k[a]

T, K	T^{-1}, K^{-1}	k, s^{-1}	Ln k
296.7	3.37×10^{-3}	3.48×10^{-3}	−5.66
302.2	3.31×10^{-3}	7.75×10^{-3}	−4.86
310.2	3.22×10^{-3}	2.02×10^{-2}	−3.90

[a] For plot of T^{-1} vs. ln k, see Figure 2.

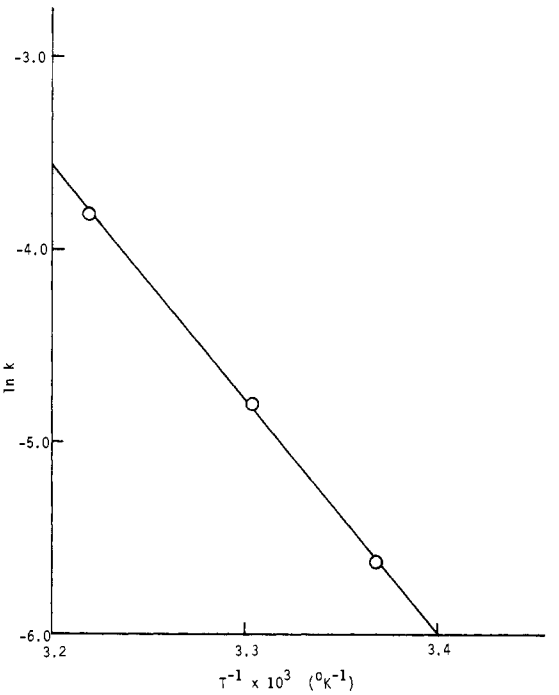


Figure 2. Plot of inverse temperature vs. natural logarithm of the first-order rate coefficient for hydrolysis of compound IIb, 6.56×10^{-3} M. Activation energy, $E_a = R(\mathrm{d} \ln k/\mathrm{d}T^{-1}) = 23.3$ kcal mol^{-1}.

Since this effect was most pronounced in the case of the dibasic acids and least with the neutral species, we hypothesize that coordination of the lone pairs of electrons on sulfur with undissociated protonic functional groups on the mustard may cause the observed retardation. This is reasonable since such an interaction would be expected to reduce the anchimeric potential. With this in mind, a study of the pH dependence of rate was undertaken using compound IIb. The results, shown in Table II, support the hypothesis.

Estimation of the activation energy, E_a, for the hydrolysis of compound IIb was accomplished by means of three different kinetic determinations at 23.5, 29.0, and 37.0 °C. The data are shown in Table III and Figure 2. The observed value, $E_a = 23.3$ kcal mol^{-1}, is consistent with the formulation of the rate-controlling step as reported previously.[8]

Experimental Section

Apparatus. Chloride concentrations were measured using an Orion Model 801 inolyzer equipped with an Orion Model 94-17A

chloride selective electrode and an Orion Model 90-02-00 double junction reference electrode. A Broadley-James Model 9015 combination pH/reference electrode was used for the pH measurements. Temperature was regulated (±0.1 °C) by means of an isothermal water bath equipped with a Precision Scientific Porta-temp heater–mixer for studies at and above ambient temperature and a Forma Scientific cold-finger immersion cooler for below ambient temperature.

Reagents. In order to simulate in vitro the ionic composition and buffering capacity of human arterial blood, Dulbecco TC salt solution (Difco Laboratories, Ann Arbor, Mich.) was adjusted to 0.103 M in NaCl at pH 7.4 for determinations at 37 °C.[11] For determinations at lower pH values, appropriate additions of 5 M sulfuric acid were made to achieve the required levels. For calibration of electrode response, the corresponding buffer was reconstituted with NaCl to a final concentration of 0.309 N.

The mustards were prepared as previously reported[1b] and were dissolved in methanol immediately prior to use.

Electrode Calibration. The Nernst expression relates the potential difference measured between the indicator and reference electrodes to chloride ion activity at constant temperature

$$E_{ind} - E_{ref} = RT/nF \ln a$$

where $E_{ind} - E_{ref}$ is the measured potential in volts between the indicator and reference electrodes; R = 1.987 cal mol^{-1} K^{-1}, the gas constant; T is the Kelvin temperature; n = 1, the number of electrons transferred per reacting molecule; F = 23 000 cal V^{-1} equiv^{-1}; a is the molar activity of chloride ions in the solution. In practice, values of $Q = 2.303\ RT/nF$ were determined empirically prior to each kinetic run, as follows.[10] The electrodes were immersed in 25 mL of the appropriate buffer and the temperature was allowed to equilibrate at the desired value. A stock solution of buffer reconstituted to three times the original chloride concentration was also maintained at the experimental temperature. From this stock, successive aliquots of 5 mL each were added to the cell. Potentials stabilized within 10 s and were recorded after each addition. Values of $\Delta E = E_{ind} - E_{ref}$ were plotted against ln [Cl$^-$] (molar) and linear regression of these points was used to calculate an empirical value of Q for each run. Regression coefficients for the calibration always exceeded 0.999 for a minimum of five data points.

Blank Determinations. Prior to each kinetic run, a blank consisting of pure methanol was monitored to correct for voltage drift due to the solvent. Electrode potentials were recorded at 37 °C in 24.5 mL of buffer at equilibrium and at 10-s intervals following addition of 0.5 mL of methanol, over a 200-s period, and then every 20 s for an additional 400-s period. Blank potentials were <1 mV while kinetic runs typically spanned 6 mV. These values were substracted from the potentials obtained at corresponding intervals during the kinetic runs.

Kinetic Runs. Weighed samples of mustard were dissolved in 0.6 mL of methanol such that a 0.5-mL aliquot of the solution diluted with 24.5 mL of buffer would yield the desired concentration within the 3×10^{-4}–2×10^{-2} M range. Timing was begun when the addition of mustard solution by pipet was half completed. Rapid mixing of the buffer and mustard solution allowed data collection to begin within 10 s following the addition. Data were recorded at intervals corresponding to those for the blank runs. Stability of the methanolic solutions of mustards over 3 days was shown by NMR and TLC[1b] of recovered solutes and by means of the Epstein test.[8]

Initial concentrations of each mustard were varied over consecutive determinations in order to evaluate the concentration dependence of the rate coefficient. Similarly, kinetics for compound IIb were determined at two additional values below pH 7.4 and at two additional temperatures below 37 °C in order to estimate the activation energy.[12,13]

Rate Coefficient Calculations. First-order rate coefficients were calculated by the Guggenheim method[12]

$$-kt = 2.303 \log (10^{E/Q} - 10^{E'/Q}) + C$$

where E and E' are potentials (volts) at times t and $2.5t_{1/2}$ (s), respectively. Q is the Nernst factor obtained previously and k the first-order rate coefficient in s^{-1}. Plotting log $(10^{E/Q} - 10^{E'/Q})$ vs. time gave a slope of $-k/2.303$ in s^{-1}. Numerical values were obtained by linear regression of 15 or more pairs of data points. Regression coefficients exceeding 0.96 confirmed the validity of the first-order fit.

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