Heterocyclic Derivatives of 2-Chloroethyl Sulfide with Antitumor Activity[1]

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Several monofunctional nitrogen and sulfur mustards combined with a variety of heterocyclic nuclei through aminoalkyl side chains have been synthesized for comparisons of their antitumor activities. The presence of acridine, benz[*c*]acridine, and phenanthridine nuclei increased the antitumor effectiveness of both types of mustard moiety; the sulfur mustard derivatives displayed higher, but much broader, dosage ranges of activity. Since the sulfur mustards of 7-chloro- and 3,7-dichloroquinoline were considerably more effective against Ehrlich ascites tumors in the mouse than the corresponding nitrogen half-mustards, it is considered that studies utilizing sulfur mustard derivatives may be an improved procedure for the detection of potential antitumor activity in the carrier portion of the molecule.

Our previous determinations of the relationships between the chemical structure and biological activity of a variety of alkylating agents showed that almost all the bis(2-chloroethyl)aminoalkylamino derivatives of quinoline and acridine were highly effective against Ehrlich ascites tumors in the mouse, but that this inherent activity could be modified by the nature of the alkylamino side chain and of the heterocyclic group used as a carrier. In fact, certain carrier structures such as 6-methoxyquinoline and various acridines were found to potentiate the activity of the nitrogen mustard moiety.[2-4] This prompted the synthesis of a wide selection of monofunctional nitrogen mustards (half-mustards), some of which displayed exceptional antitumor activity.[5,6] For example, 2-methoxy-9-[3-(ethyl-2-chloroethyl)aminopropylamino]acridine dihydrochloride was found to be more active on a molar basis than nitrogen mustard [methylbis(2-chloroethyl)-amine hydrochloride].

Several other monofunctional nitrogen mustards containing the acridine and substituted acridine nuclei were highly effective at low molar dosage against ascites tumors, whereas the quinoline and quinazoline derivatives were either inactive or only moderately active at extremely high molar dosage. In our view the pronounced antitumor properties and mutagenic capabilities[7] of the acridine nitrogen half-mustards are due to the participation of the carrier portion of the molecule in cytotoxic activity in place of the function of the second alkylating center of the bis-mustards. Thus the half-mustards exhibit a modified form of bifunctionality. Supporting this view is the fact that acridines have been shown to have a remarkable affinity for deoxyribonucleic acid.[8]

These activating carriers permitted exploration of a series of sulfur mustards not otherwise possible, since the sulfur valence of two precludes attachment of a carrier to the bifunctional alkylating moiety. Consequently, several representative heterocyclic derivatives of 2-chloroethyl sulfide were synthesized and compared with their corresponding ethyl-2-chloroethylamines (nitrogen half-mustards) in studies made with mouse ascites tumors. The influence of different nuclei and side chains on the antitumor activity of the corresponding sulfur and nitrogen mustards are given in Table I along with the analytical information on these compounds. Data on the intermediates are presented in Table II. The intermediate hydroxy derivatives of the compounds with the alkyl side chains were made either (1) by direct condensation of the haloheterocyclic compound with the corresponding side chain or (2) by stepwise condensation with the appropriate amino alcohol followed by the replacement of the hydroxyl group by chlorine and then the use of the resultant compound for the alkylation of the alkylaminoethanol or sodium derivative of mercaptoethanol. The two sulfur mustards with the amide side chains were prepared as described in the Experimental Section.

Experimental Section

Melting points were taken in open capillary tubes in a Hershberg apparatus using total immersion thermometers and are reported as uncorrected values.

All of the 2-chloroethyl compounds in this paper were prepared by the action of excess $SOCl_2$ on their hydroxy precursors.[2] Where these precursors were obtained by direct condensation (method A in Table II) the reactants have all been previously described except in the case of 3-aminopropyl-2-hydroxyethyl sulfide; preparation of this intermediate is described below in the course of the preparation of compound D-2 from Table II. An experimental procedure utilizing method B is given below in the preparation of B-2 (Table II). Finally, the preparation of one of the compounds containing an amide side chain is given.

3-Aminopropyl-2-hydroxyethyl Sulfide and 7-Chloro-4-[3-(2-chloroethyl)mercaptopropylamino]quinoline Hydrochloride (D-2, Table II).—To a stirred mixture of 300 ml of ethanol containing 47 g of mercaptoethanol and 24 g of NaOH (0.6 mole each) and 300 ml of 1 *N* NaOH in methanol was added in portions a solution of 66 g (0.3 mole) of 3-bromopropylamine hydrobromide in 150 ml of absolute ethanol. The mixture was stirred and heated, and 400 ml of solvent was removed by distillation. After cooling, 100 ml of ether was added and the NaBr precipitate (84% yield) was removed by filtration. The filtrate was concentrated, 0.3 mole of concentrated HCl and 100 ml of ether were added, and the precipitated NaCl was removed. The filtrate was concentrated and the residue was distilled *in vacuo* in a modified von Braun flask, yielding 38 g (93%) of material, bp 81–98° (60–100 μ). Redistillation gave 34 g, bp 78–82° (70 μ), of slightly impure product.

(1) Supported by research Grants CA 02975 and CA 06927 from the National Cancer Institute, National Institutes of Health, U. S. Public Health Service.

(2) R. M. Peck, R. K. Preston, and H. J. Creech, *J. Am. Chem. Soc.*, **81**, 3984 (1959).

(3) H. J. Creech, E. Breuninger, R. F. Hankwitz, Jr., G. Polsky, and M. L. Wilson, *Cancer Res.*, **20**, 471 (1960).

(4) R. M. Peck, R. K. Preston, and H. J. Creech, *J. Org. Chem.*, **26**, 3409 (1961).

(5) R. K. Preston, R. M. Peck, E. R. Breuninger, A. J. Miller, and H. J. Creech, *J. Med. Chem.*, **7**, 471 (1964).

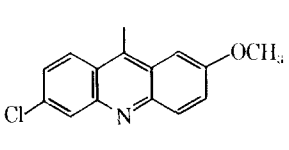
(6) R. M. Peck, E. R. Breuninger, A. J. Miller, and H. J. Creech, *ibid.*, **7**, 480 (1964).

(7) E. A. Carlson and I. I. Oster, *Genetics*, **47**, 561 (1962).

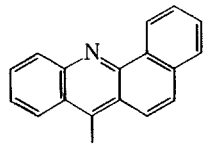
(8) L. S. Lerman, *J. Mol. Biol.*, **3**, 18 (1961).

Table I

Antitumor Activity and Analytical Information



A



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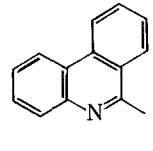
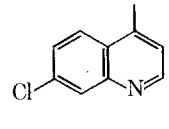
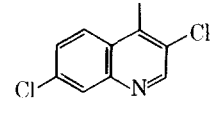


C



D



E

Compd[a]	Side chain	Salt	Antitumor activity[b] Range, μmoles/kg	Degree	Yield, %	Mp, °C	Calcd, %[c] C	H	Cl	N (S)[c]	Found, %[d] C	H	Cl	N (S)
A-1[e]	$NHCH_2CH_2N<^{CH_2CH_2Cl}_{C_2H_5}$	2HCl	4–16	2.3										
A-2	$NHCH_2CH_2SCH_2CH_2Cl$	HCl	12–40	2.4	74	221–223 dec	51.72	4.59	25.49	7.68	51.71	4.66	25.74	7.79
A-3[e]	$NH(CH_2)_3N<^{CH_2CH_2Cl}_{C_2H_5}$	2HCl	1.5–4	2.5										
A-4	$NH(CH_2)_3SCH_2CH_2Cl$	HCl	2.5–20	2.3	93	183–187 dec	51.77	5.03	24.11	7.28	52.08	5.40	23.07	7.48
A-5[e]	$NHCH_2CH_2NHCOCH_2N<^{CH_2CH_2Cl}_{C_2H_5}$	2HCl	3–10	2.2										
A-6	$NHCH_2CH_2NHCOCH_2SCH_2CH_2Cl$	HCl	2.5–15	2.3	60	221–222 dec	50.65	4.67	22.40	6.76	50.56	4.76	21.82	6.83
B-1	$NHCH_2CH_2N<^{CH_2CH_2Cl}_{C_2H_5}$	$2HCl\cdot1.5H_2O$	2–8	2.0	90	233–235 dec	57.88	6.12	22.20	8.80	57.60	6.60	21.87	9.12
B-2	$NHCH_2CH_2SCH_2CH_2Cl$	HCl	5–50	2.2	34	238–239 dec	62.53	5.00	17.57	7.96	63.07	5.12	17.06	7.62
B-3	$NH(CH_2)_3N<^{CH_2CH_2Cl}_{C_2H_5}$	$2HCl\cdot H_2O$	1.5–5	2.2	84	181–186	59.70	6.27	22.04	8.71	59.95	6.19	22.03	9.14
B-4	$NH(CH_2)_3SCH_2CH_2Cl$	HCl	2.5–15	2.2	56	180–181.5 dec	63.32	5.31	16.98	7.69	63.38	5.44	16.73	7.66
C-1	$NH(CH_2)_3N<^{CH_2CH_2Cl}_{(CH_2)_2CH_3}$	2HCl	6–12	2.3	51	232–234.5 dec	58.61	6.58	24.81	9.80	58.31	6.64	24.78	9.71
C-2	$NH(CH_2)_3SCH_2CH_2Cl$	HCl	15–60	2.2	49	111–116 dec	56.12	5.76	18.40	8.34	55.41	5.80	18.43	8.62
D-1[e]	$NH(CH_2)_3N<^{CH_2CH_2Cl}_{C_2H_5}$	2HCl	1.5–9	1.0										
D-2	$NH(CH_2)_3SCH_2CH_2Cl$	HCl	40–60	2.2	55	150–151.5	47.82	4.88	30.25	9.12	48.23	5.37	28.74	8.88

D-3[e]	$NHCH_2CH_2NHCOCH_2N<^{CH_2CH_2Cl}_{C_2H_5}$	2HCl	36–54	2.0										
D-4	$NHCH_2CH_2NHCOCH_2SCH_2CH_2Cl$	$HCl\cdot 0.5H_2O$	35–75	2.3	25	162–163.5 dec	44.60	4.75	26.35	7.95	44.57	4.77	26.24	7.59
E-1	$NH(CH_2)_3N<^{CH_2CH_2Cl}_{C_2H_5}$	2HCl	60–90	1.9	48	202–204	44.34	5.10	40.88	9.69	44.53	5.56	40.47	9.77
E-2	$NH(CH_2)_3SCH_2CH_2Cl$	HCl	75–325	2.4	51	160–162 dec	43.56	4.18	36.70	8.31	43.78	4.31	35.91	8.54

[a] Letters A to E represent the heterocyclic group attached to the side chains. The structures are shown above. [b] See text. [c] N (S). The analytical values refer to S when this element is present and to N when S is absent. [d] Values are either single analyses or averages of checks. [e] Physical and analytical data previously published.[4,6]

TABLE II

PRECURSORS TO COMPOUNDS IN TABLE I

Precursors of compd	Side chain	Prepn[a]	Yield, %	Mp, °C	Salt	Calcd, %				Found, %[b]			
						C	H	Cl	N (S)	C	H	Cl	N (S)
A-2	$NHCH_2CH_2SCH_2CH_2OH$[c]	B	37	145-147	...	59.60	5.28		8.84	59.83	5.42		8.79
A-4	$NH(CH_2)_3SCH_2CH_2OH$	B	84	140.5–142	...	60.57	5.62		8.51	60.24	5.72		8.62
	$NH(CH_2)_3Cl$		77	106.5–108	...	60.95	4.82	21.15	8.35	61.62	5.16	20.78	7.83
A-6	$NHCH_2CH_2NHCOCH_2SCH_2CH_2OH$		17	200–205 dec	HCl	52.63	5.08		7.03	51.89	5.12		6 99
B-1	$NHCH_2CH_2N<^{C_2H_5}_{CH_2CH_2OH}$	A	72	250–251 dec	$2HCl\cdot 1.5H_2O$	60.18	6.59	15.44	9.15	60.51	7.25	15.63	9.17
B-2	$NHCH_2CH_2SCH_2CH_2OH$[d]	B	94	200–202	HCl	65.54	5.50		8.34	64.74	5.85		8.19
B-3	$NH(CH_2)_3N<^{C_2H_5}_{CH_2CH_2OH}$[d]	B	57	97–98.5	...	77.23	7.29		11.25	76.93	7.46		11.97
B-4	$NH(CH_2)_3SCH_2CH_2OH$[d]	B	77	174.5–175.5	HCl	66.25	5.81		8.04	66.10	6.00		7.78
C-1	$NH(CH_2)_3N<^{C_3H_7}_{CH_2CH_2OH}$[e]	A	73	162–164	$2HCl\cdot H_2O$	58.92	7.30	16.55	9.81	59.05	7.46	16.82	10.09
C-2	$NH(CH_2)_3SCH_2CH_2OH$	A	71	96–98	...	69.20	6.45		10.26	68.94	6.64		10.67
D-2	$NH(CH_2)_3SCH_2CH_2OH$	A	73	148–149.3	...	56.67	5.78		10.81	56.75	5.75		10.48
D-4	$NHCH_2CH_2NHCOCH_2SCH_2CH_2OH$		53	165.5–168	...	53.01	5.34		9.44	53.14	5.67		10.54
E-1	$NH(CH_2)_3N<^{C_2H_5}_{CH_2CH_2OH}$	A	72	91–92.6	...	56.15	6.21	20.70	12.28	56.72	6.63	20.82	12.04
E-2	$NH(CH_2)_3SCH_2CH_2OH$	A	54	98–100	...	50.80	4.88	21.38	9.68	50.31	5.07	22.68	9.27

[a] Method A consists of direct condensation of the appropriate haloheterocyclic compound with the primary amine corresponding to the side chain. Method B consists of alkylation of either mercaptoethanol anion or of N-ethylethanolamine with the appropriate arylaminoalkyl halide. [b] Values are either single analyses or averages of checks. [c] The appropriate halide ($ANHCH_2CH_2Cl\cdot HCl$) is described in J. H. Burckhalter, F. W. Short, E. F. Elslager, A. M. Moore, M. J. Sullivan, and F. W. Tendick, *J. Am. Chem. Soc.*, **65**, 2012 (1943); here the reactant was the previously unreported free base, mp 120–121.5°. [d] The appropriate halide is described by F. W. Short, J. H. Burckhalter, E. M. Jones, W. F. Holcomb, and L. A. Sweet, *ibid.*, **80**, 223 (1958). [e] The necessary side chain[4] and nucleus, G. M. Badger, J. H. Seidler, and B. Thomson, *J. Chem. Soc.*, 3207 (1951), are both previously reported.

Anal. Calcd for $C_5H_{13}NOS$: C, 44.31; H, 9.69; N, 10.35; S, 23.73. Found: C, 45.79; H, 9.69; N, 9.38, 9.50; S, 23.01.

A mixture of 1.5 g of 3-aminopropyl-2-hydroxyethyl sulfide, 2.0 g of 4,7-dichloroquinoline, and 2.0 g of diethanolamine was heated and stirred at an internal temperature of 120–125° for 3 hr, cooled, taken up in dilute acetic acid, and filtered. The product was precipitated with alkali, filtered, and reprecipitated once more from dilute acetic acid to give 2.6 g, mp 142–146°. Crystallization from ethanol gave 2.18 g (73%) of D-2 (Table II).

2-Hydroxy-2′-(7-benz[*c*]acridinylamino)diethyl Sulfide Hydrochloride (B-2, Table II).—To a stirred solution of 1.55 g of mercaptoethanol in 20 ml of 1 *N* methanolic NaOH was added 1.6 g (4.8 mmoles) of 7-(2-chloroethylamino)benz[*c*]acridine hydrochloride. The mixture was heated for 2 hr on the steam cone, filtered, diluted, and cooled. Since the product resisted crystallization, the aqueous layer was decanted and the product was taken up in ether. The solution was extracted with 4.5 ml of 1 *N* HCl and the extract was concentrated *in vacuo* and taken up in a little ethanol, when the salt crystallized and was filtered; it weighed 1.7 g, mp 200–204°. Recrystallization gave the analytical sample in Table II.

N^1-(7-Chloro-4-quinolyl)-N^2-[S-(2-hydroxyethyl)thioglycolyl]-ethylenediamine (D-4, Table II).—To 100 ml of 1 *N* methanolic NaOH was added 7.8 g (0.10 mole) of mercaptoethanol. The solution was cooled and 12.2 g (0.10 mole) of ethyl chloroacetate was added with swirling. The solution was filtered, warmed for 2 hr on the steam cone, and refiltered. One-half of the resultant solution was added to 11 g of N-(7-chloro-4-quinolyl)ethylenediamine and the solution was warmed on the steam cone for 2 hr and cooled. The product was removed by filtration; it weighed 9.1 g, mp 151–160°. Crystallization from 4:1 ethanol–water gave a first crop of 4.5 g, mp 164–166.5°, and a second crop of 4.5 g, mp 165.5–168°.

Biological Results

Ascites tumors in the mouse are uniquely suitable for quantitative determinations of the effectiveness of analogs of the nitrogen and sulfur mustards. The administration of a variety of chemotherapeutic agents will substantially reduce the ascites cell count in the mouse, but a pronounced capacity to prolong the survival time of mice inoculated with ascites tumors is restricted to certain mustards.[5,9]

The procedures used to determine and interpret the results of our antitumor studies have been described in detail in earlier publications.[3,5,9] In brief, the compounds in saline solution or suspension were injected intraperitoneally into albino (ICR Swiss) mice which had been inoculated the previous day with 7×10^6 cells of the Ehrlich ascites tumor EF. Survival data were recorded daily for the experimental and control mice and the antitumor activity of the compound was calculated from these graphs. The tests on each compound utilized between 100 and 200 mice.

Under our experimental conditions, the control mice displayed a mean survival time of 16 ± 1 days. To maintain space in our animal quarters, all experiments were terminated at the end of the period that was three times the mean survival time of the control mice in each series of tests. A value of 3.0 for the degree of antitumor activity was assigned when every mouse in the experimental group of 16 given a certain dosage of compound lived to the time of sacrifice at about 48 days. In our experience with nitrogen mustards, nearly all of the mice surviving 7 weeks could be expected to live more than 15 weeks without signs of ascites tumor development. A degree of activity of 1.0 indicates that a compound had no effect on the survival time of the tumor-bearing mice. A significant increase in survival time is considered to have occurred when the degree of activity reached 1.8, an 80% increase over the controls.

Dosages are expressed as the number of micromoles of compound injected per kilogram of body weight of mouse. The effective range of a compound covers the lowest to the highest dosages that caused at least an 80% increase in survival time over that of the controls. At one or more intermediate levels, the maximum degree of antitumor activity (3.0) was often observed with the highly effective analogs of nitrogen and sulfur mustard. The degree of activity given in Table I, however, is the average of all the values obtained at the low, high, and 3–6 intermediate levels of effective dosage and consequently, by definition, cannot reach 3.0. Average values of 2.2–2.5 in Table I for the degree of antitumor activity indicate a highly effective compound; values of 1.8–2.1 indicate that the compound was moderately active since the average survival time of the treated mice was at least twice that of the controls.

The ratio of the highest to the lowest dosages in the active range is an expression of therapeutic index since it relates the dosage at which toxicity is beginning to appear to the lowest dosage that causes significant prolongation of survival time. The reference compound for the nitrogen half-mustards is N-(2-chloroethyl)-N-ethyl-1,3-propanediamine dihydrochloride; it displayed a degree of antitumor activity of 2.3 at 25–60 μmoles/kg. The reference compound for the sulfur mustard series, 3-(2-chloroethyl)mercaptopropylamine hydrochloride, had a degree of antitumor activity of 2.0 at 250–450 μmoles/kg.

Lines A-1 and A-2 of Table I compare the nitrogen half-mustard and sulfur mustard of 2-methoxy-6-chloroacridine, which is substituted at the 9-position with an aminoethylene linkage, to the mustard moiety. Both compounds are highly effective against the ascites tumor with the nitrogen half-mustard being more active on a molar basis. The presence of an aminopropylene linkage instead of an aminoethylene linkage (compound A-3 and A-4) increases the degree of activation of both mustard moieties caused by the quinacrine nucleus. The sulfur mustard exhibited a wide range between the low and high effective dosage levels. This same relationship was found for the compounds with the amide linkage in the side chain (A-5 and A-6). The next series of compounds contained the benz[*c*]acridine nucleus (B-1 to B-4) which also was found to be a strong activator of the mustard moiety. An almost identical influence was exhibited by the aminoethylene and aminopropylene side chains of both heterocyclic structures, and the sulfur mustards again had wider dosage ranges than the nitrogen half-mustards. The phenanthridine nucleus was also a satisfactory heterocyclic structure in combination with the aminopropylene sulfur mustard and nitrogen half-mustard (C-2 and C-1). Although the nitrogen half-mustard of 7-chloroquinoline has been found to be ineffective against ascites tumors, but highly toxic (D-1), the corresponding sulfur mustard displayed relatively high activity within a narrow range of dosages (D-2). Satisfactory antitumor activity was shown by the 7-chloroquinoline derivatives containing the amide

(9) H. J. Creech, T. S. Hauschka, R. F. Hankwitz, Jr., B. J. Littleton, and J. Andre, *Cancer Res. Suppl.*, **3**, 47 (1955).

side chains although the required dosages were unusually high with both types of mustard (D-3 and D-4). Even higher dosages were needed to obtain a display of activity with the mustards combined with the 3,7-dichloroquinoline nucleus (E-1 and E-2). Since the nitrogen half-mustard reference compound had an activity degree of 2.3 at 25–60 μmoles/kg, it is obvious that 7-chloroquinoline and 3,7-dichloroquinoline do not cause any activation of the nitrogen half-mustard moiety, but, in fact, depress its antitumor effectiveness. In the case of the sulfur mustards, however, compounds containing these quinoline nuclei display a high order of antitumor activity, and although the molar dosage is high, that of the sulfur mustard reference compound, 3-(2-chloroethyl)mercaptopropylamine hydrochloride, whose average activity degree is 2.0 over a range of 250–450 μmoles/kg, is even higher. Therefore potentiation by these less active carriers has been detected by use of the 2-chloroethyl sulfide moiety.

Synthesis, Chemistry, and Preliminary Pharmacology of Arsenical Nitrogen Mustards and Structurally Related Nonalkylating Arsenicals[1]

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The synthesis of phenyl nitrogen mustards substituted in the *para* position with arsonic acid, arsenoso, arseno, and dithiarsenolane groups and the synthesis of the corresponding arsenic derivatives of diethylaniline and bis(β-hydroxyethyl)aniline, representing nonalkylating structural analogs of the former, are described. In relation to this work, some novel aspects of the chemistry of organic arsenicals are discussed. All of the arsenical nitrogen mustards synthesized show very low chemical alkylating activities; this fact, in corroboration with the nmr spectra, indicates that not only the arsonic acid group but also the trivalent arsenic groups have strong electron-attracting character. All arsenical nitrogen mustards, except the arseno compound, are highly toxic in mice, but their toxicities are apparently due to their arsenic groups rather than their alkylating moieties. Preliminary screening results indicate that the arsenoso and arseno mustards have significant activities in the Ehrlich ascites test system, both showing complete inhibition at nontoxic dose levels.

Previous work directed toward the synthesis of compounds designed to incorporate the biologically essential structural features of two different but synergistic inhibitors into a single molecule resulted in several types of new antineoplastic agents,[2] some of which proved to be of clinical interest.[3] The rationale of this so-called "dual antagonist" approach to chemotherapy has been discussed.[4] Since cysteine, glutathione, and several other sulfhydryl compounds are known to antagonize the toxic effects of ionizing radiation as well as of some "radiomimetic" alkylating agents (*e.g.*, HN2 and aromatic nitrogen mustards[5]), it was thought that "sulfhydryl inhibitors" such as organic arsenicals[6] could, conversely, potentiate the effects of alkylating agents.[7] It appeared possible that new types of dual antagonists could be designed by combining the structural features of "sulfhydryl inhibitors" with those of alkylating agents. The synthesis and properties of such compounds, a series of "arsenical nitrogen mustards," are reported in this paper.

The effects of various substituents on the chemical and biological activities of phenyl nitrogen mustard (X) were discussed in a previous publication.[8] In designing the arsenical nitrogen mustards, it was anticipated that the bis(β-chloroethyl)amino group in compound I would have relatively low-alkylating activity, due to the electron-attracting *p*-arsonic acid group.[8] However, *in vivo* reduction[9] of the arsonic acid group to the arsenoso state (II) and its subsequent reaction with sulfhydryl groups[6,9] was expected to lead to a substantial increase of electron density on the nitrogen, which should result in an increase of alkylating activity.[8] Thus, (1) *in vivo* reduction of I to II was assumed to be necessary for its activation as a "sulfhydryl inhibitor" as well as an alkylating agent, and (2) the reaction of II with sulfhydryl groups (to give III) was necessary for the further activation of the alkylating function. In contrast, the arseno mustard (IV) was expected to be a more active alkylating agent than I, and only its action as a "sulfhydryl inhibitor" was expected to require *in vivo* oxidation to II (see Chart I).[10]

In order to explore the chemical and biological activities of arsenical nitrogen mustards and their potential

(1) (a) This investigation was supported by Public Health Service Research Grants No. CA-06695 and CA-06645 from the National Cancer Institute. (b) A preliminary report, covering a small portion of this work, was presented before the Division of Medicinal Chemistry, 147th National Meeting of the American Chemical Society, Philadelphia, Pa., April 1964; Abstracts, p 26M.

(2) T. J. Bardos, A. K. Barua, Z. F. Chmielewicz, G. E. Crevar, J. P. Dailey, S. Divald, and Z. B. Papanastassiou, *J. Pharm. Sci.*, **54**, 187 (1965), and previous articles of the series.

(3) D. V. Razis, J. L. Ambrus, C. A. Ross, L. Stutzman, J. E. Sokal, A. M. Rejali, and T. J. Bardos, *Cancer*, **14**, 853 (1961), and other publications.

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(5) T. A. Connors and L. A. Elson, *ibid.*, **11**, 1221 (1962).

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