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.

(4) T. J. Bardos, *Biochem. Pharmacol.*, **11**, 256 (1962).

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

(6) R. M. Johnstone in "Metabolic Inhibitors," Vol. II, R. M. Hochster and J. H. Quastel, Ed., Academic Press Inc., New York, N. Y., 1963, p 99.

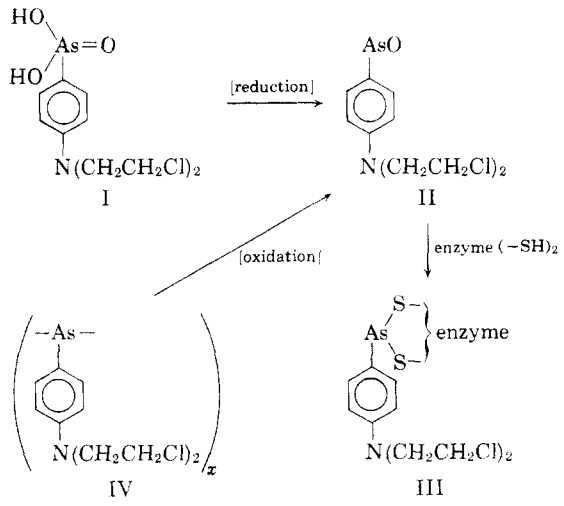
(7) Several clinical investigators reported on the use and apparent potentiating effect of certain arsenicals in combination with irradiation [F. E. Knock, *Arch. Surg.*, **86**, 489 (1963)] or alkylating agents [R. N. Ibbotson and C. W. Kingston, *Med. J. Australia*, **2**, 135 (1960)] in the chemotherapy of human cancer.

(8) T. J. Bardos, N. Datta-Gupta, P. Hebborn, and D. J. Triggle, *J. Med. Chem.*, **8**, 167 (1965).

(9) G. O. Doak and L. D. Freedman in "Medicinal Chemistry," A. Burger, Ed., Interscience Publishers, Inc., New York, N. Y., 1960, p 1027; H. Eagle and G. O. Doak, *Pharmacol. Rev.*, **3**, 107 (1951).

(10) The structure of the arseno compound IV is represented, according to present views, as a chain polymer: M. Y. Kraft, G. M. Borodina, I. N. Streltsova, and I. T. Struckkov, *Dokl. Akad. Nauk SSSR*, **131**, 1074 (1960).

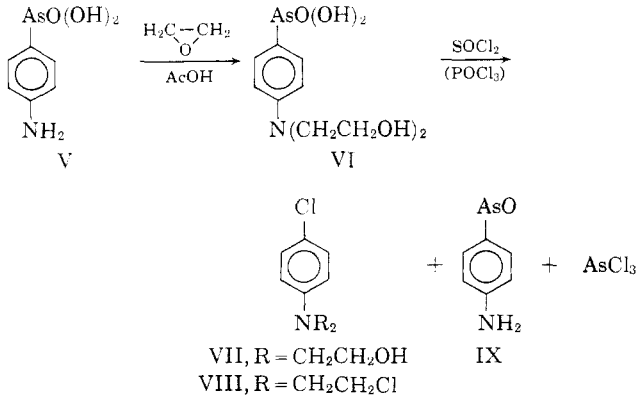
CHART I



usefulness in chemotherapy, it appeared of interest to make available such compounds in all three oxidation states, *i.e.*, arsonic acid (I), arsenoso (II), and arseno (IV), as well as in a "thiolated" form (XXIII) which could serve as a model compound for the arsenical–enzyme complex (III). In addition, some new, monoalkylating (XVII) and nonalkylating arsenicals having analogous structures were synthesized for comparative studies.

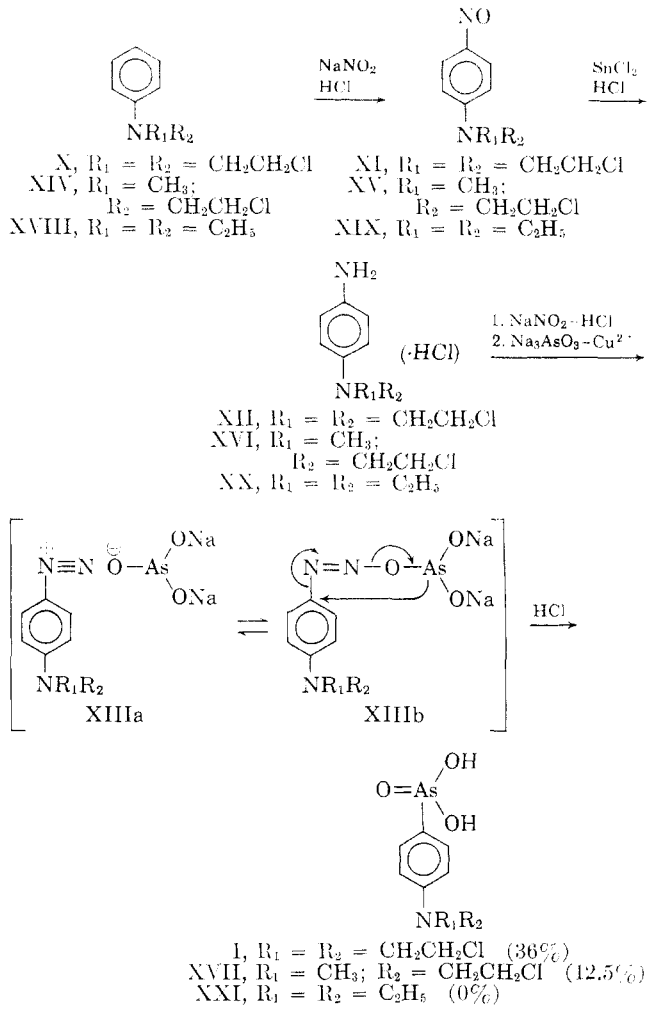
Synthesis of the arsonic acid mustard (I) was attempted first by conventional methods, using arsanilic acid (V) as the starting material. Reaction of V with ethylene oxide in aqueous acetic acid gave the bis-(β-hydroxyethyl) derivative (VI) in 88% yield. However, even mild treatment of VI with phosphorus oxychloride or thionyl chloride (at 0–25° in chloroform) apparently resulted in nucleophilic displacement of the arsenic group and/or elimination of the hydroxyethyl side chains.[11] *p*-Chloro-*N*,*N*-bis(2-hydroxyethyl)aniline (VII), *p*-chloro-*N*,*N*-bis(2-chloroethyl)aniline (VIII), *p*-arsenosoaniline (IX), and inorganic As(III) were identified as final products (Chart II).

CHART II



The synthesis of I was finally accomplished by a different route starting from phenyl nitrogen mustard[8] (X) and using the Bart reaction in the last step (Chart III). The "aniline mustard" XII was prepared essentially by the method of Everett and Ross.[12] Various modifications of the Bart reaction[13] were tried for the conversion of XII to I, but the only successful procedure was one in which nearly neutral (pH <8) conditions were used in the coupling step.

CHART III



The *monofunctional nitrogen mustard arsonic acid* XVII was prepared by an analogous route (Chart III), starting from *N*-methyl-*N*-(β-chloroethyl)aniline[14] (XIV); in this case, the one-arm mustard XVI was subjected to the Bart reaction to give only a 12% yield of XVII. Several attempts to prepare the nonalkylating analog XXI, by application of the Bart reaction to *p*-aminodiethylaniline (XX) under similar conditions, were unsuccessful.

The Bart reaction,[15] under weakly basic conditions, probably proceeds through the formation of an intermediate XIIIb which then decomposes *via* internal nucleophilic displacement of the diazo group by the unshared electron pair of As and simultaneous cleavage of the *N*–O bond with transfer of its electron pair to

(11) One previous example could be found in the literature for similar displacement of an arsenic group with thionyl chloride [W. Steinkopf and S. Schmidt, *Ber.*, **61B**, 675 (1928)]; on the other hand, the facile elimination of the hydroxyethyl side chains (presumably by "reverse *N*-alkylation" of an intermediate dichloroarsine derivative) under these mild experimental conditions appears surprising and may warrant further study.

(12) J. L. Everett and W. C. J. Ross, *J. Chem. Soc.*, **121**, 2764 (1949).

(13) C. F. Hamilton and J. F. Morgan, *Org. Reactions*, **2**, 415 (1944).

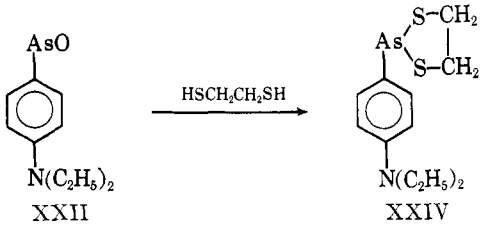
(14) K. H. Saunders, *J. Chem. Soc.*, **121**, 2674 (1922).

(15) Studies concerning the mechanism of the Bart reaction were not found in the modern organic chemical literature; the mechanism proposed here is analogous to the rearrangements of trialkylphosphites to dialkylalkylphosphonates and to other reactions involving valence-shell expansion of trivalent phosphorus.

the O–As bond; this results in expansion of the valence shell of As (As(III) → As(V)). Increasing basicity of the p-NR_1R_2 group would be expected to increase resonance stabilization of the diazonium ion (XIIIa) and thus favor dissociation of XIIIb rather than its conversion to the desired product. Moreover, it would decrease the electrophilicity of the phenyl ring, thus making it less susceptible to nucleophilic attack by the As.

Compound XXI was eventually synthesized by oxidation of the known arsenoso compound XXII, which was obtained by a modification of the literature[16] method, involving the reaction of diethylaniline with arsenic trichloride.

Synthesis of the arsenoso mustard II was accomplished by reduction of I in alcoholic solution with sulfur dioxide and 50% aqueous hydriodic acid, followed by precipitation with cold ammonium hydroxide. However, due to the alkylating side chain, the preparation of thiolated derivatives of II was particularly difficult. A cyclic disulfide derivative (XXIII) was obtained in impure form (as an oil) by the reaction of II with 1,2-ethanedithiol, while the same reaction of the nonalkylating analog XXII yielded the pure crystalline dithiarsenolane derivative XXIV.



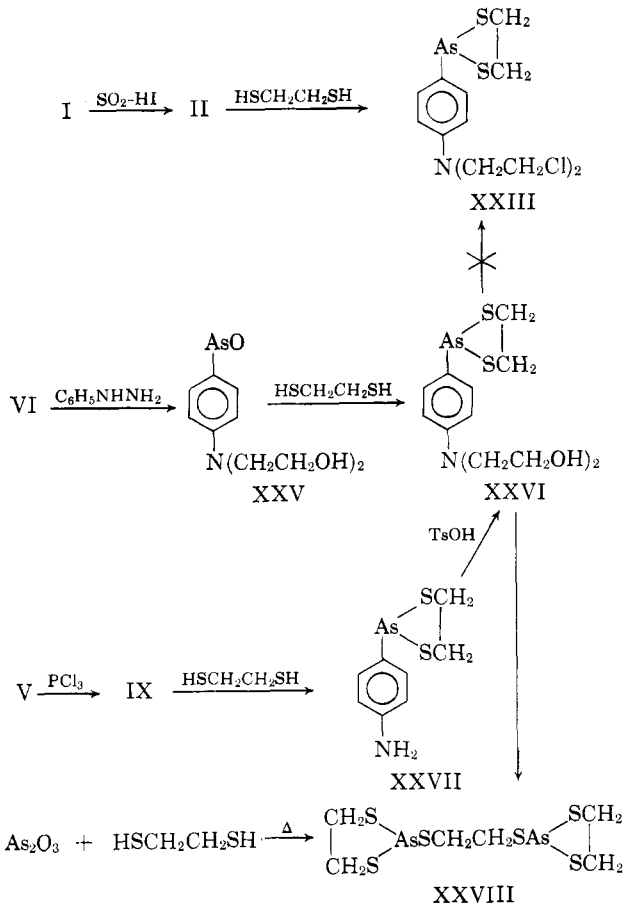
XXII XXIV

In exploring alternative routes to XXIII, the synthesis of the corresponding cyclic disulfide (XXVI) of 4-arsenosobis(β-hydroxyethyl)aniline (XXV) was attempted (by two different routes, Chart IV) in the hope that the dithiolate group might protect the arsenic during subsequent chlorination of the β-hydroxyls. Compound XXVI, however, could not be isolated in pure form. Prolonged crystallization in the presence of some toluenesulfonic acid led to the formation of a crystalline product which gave an elemental analysis and infrared spectrum consistent with the novel structure XXVIII; the alternative possibility of an entirely symmetrical bicyclo[4.4.4] structure, with the As atoms as bridgeheads, was excluded by the pmr spectrum which showed two sharp peaks at τ 6.88 (exocyclic protons) and 6.50 (ring protons) in the integrated ratio of 1:2. The same compound was subsequently obtained from the reaction of arsenic trichloride with 1,2-ethanedithiol (Chart IV). The arseno mustard IV was prepared by reduction of I with stannous chloride and hydrochloric acid.

Infrared Spectra.—A review of the literature of organic arsenicals revealed almost complete lack of spectral data; therefore, in addition to the new compounds reported in this paper, samples of other, known arsenicals were prepared or otherwise acquired for determination of their infrared spectra. The band assignments given below are based on comparison of a substantial number of aromatic arsenicals with related structures.

(16) A. Michaelis and J. Robinerson, *Ann.*, **270**, 139 (1892).

CHART IV



The following characteristic absorption peaks were observed in the spectra of all arsonic acid derivatives: strong broad bands at 2800 and 2350 cm^{-1} attributed to the $As(OH)\overset{-}{O}\overset{+}{N}\leqslant$ (bonded ion pair of an inner salt), a very strong sharp peak at 1090–1102 cm^{-1} which we assigned to the C_6H_5–As bond, a strong-to-medium intensity peak at 880–910 cm^{-1} assigned to As=O, and a medium-to-strong peak at 760–780 cm^{-1} (As–O). The remaining spectral bands are those expected for the p-disubstituted phenyl, primary or tertiary amine, COH, or CCl groups of the various compounds studied.

The arsenoso compounds have a sharp, medium intensity peak at 3000 cm^{-1} (with absence of the broad 2800–2350-cm^{-1} bands); they show a slight shift in the position of the C_6H_5–As band to 1080–1090 cm^{-1}. Furthermore, they have a characteristic, very broad and intense absorption throughout the 780–680-cm^{-1} region which may be attributed to AsOAs linkages of the dimeric, trimeric, or tetrameric structures, but the 880–910-cm^{-1} absorption peak attributed to As=O is absent, thus supporting the current view[17] concerning the structure of arsenoso compounds.

The spectra of the dithiarsenolane derivatives are similar to those of the corresponding arsenoso compounds, but their C_6H_5–As absorption peak is slightly further shifted to 1065–1082 cm^{-1}, the characteristic broad absorption of the arsenoso compounds at 680–780 cm^{-1} is absent, and two new weak-to-medium intensity peaks appear at 930 and 820–835 cm^{-1}.

(17) C. K. Banks, J. Controulis, D. F. Walker, and J. A. Sultzaberger, *J. Am. Chem. Soc.*, **69**, 5 (1947).

The only significant difference between the spectra of the arsenobenzene derivatives and those of the corresponding arsenoso compounds is the absence of the broad 780–680-cm^{-1} absorption (AsOAs) in the former. This difference is, however, sufficiently striking to permit clear distinction between the two series.

Alkylating Activities.—The comparative chemical alkylating activities (SN2 reactivities) of the arsenical nitrogen mustards were determined by a previously published method,[8] and the results are given in Table I. Since the arseno mustard (IV) and the "thiolated" arsenical mustard (XXIII) were insoluble in alcohol, dioxane was used for these compounds instead of alcohol, and the k_{80}' values were calculated with reference to the k_{80}' value of the unsubstituted phenyl nitrogen mustard which was used as a reference standard for the alkylating-activity determinations in both solvents.

TABLE I

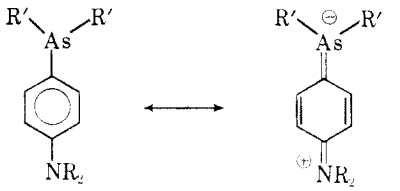
COMPARATIVE CHEMICAL ALKYLATING ACTIVITIES OF ARSENICAL NITROGEN MUSTARDS

Compd	k_{80}''[a]	k_{80}'[b]	Relative alkylating activities, % of std[c]
I	1.2		9.2
XVII	1.0		7.7
II	1.2		9.2
IV	(3.4)[d]	1.0	26.6
XXIII	(2.7)[d]	0.8	21.6
Standard[c]	13.0	3.8	100

[a] "Comparative alkylating activity" at 80°, determined according to the previously described method.[8] [b] Comparative alkylating activity at 80°, using dioxane as the solvent (see text). [c] Unsubstituted phenyl nitrogen mustard (X) was used as the reference standard, and the "relative alkylating activities" are expressed as percentages of k_{80}' or k_{80}'' values. [d] Calculated from the per cent values (last column), based on the k_{80}' value of the standard.

The results show that not only the pentavalent arsonic acid mustards (I and XVII) have very low alkylating activities (as was expected) but also the three trivalent arsenical mustards have lower alkylating activities than the unsubstituted or even than the *p*-COOH-substituted phenyl nitrogen mustard.[5] Since the chemical alkylating activities (as well as the biological activities) of the aromatic nitrogen mustards were shown to be directly related to the basicity of the nitrogen which in this series depends solely on the electron-attracting or -releasing effect of the *para* substituent,[8] we must conclude that the trivalent arsenic has considerable *electron-attracting* character whether it is in the form of an arsenoso, arseno, or dithiarsenolane group.

Banks, *et al.*,[17] suggested that arsenoso compounds form "coordination complexes" with anions in which the arsenic has an "inert pair" of electrons in the 4s orbital and accepts the electron pair of the anion in its 5s orbital, thereby acquiring a negative charge. It is possible that the electron-attracting effect of trivalent arsenic is due to its tendency to participate in resonance structures with similar expansion of its electron shell to accept an additional pair of electrons and to acquire a negative charge, in contrast to the behavior of tertiary nitrogen which tends to release its unshared electron pair and participate in resonance



A B

structures in its positively charged form. The negative charge may be dispersed over the dithiarsenolane ring, the OAsO ring of the trimeric or tetrameric[17] arsenoso compound, and the polymer chain[10] of the arseno compound.

This electron-attracting character of the arsenic groups is also indicated by the pmr spectra of the corresponding diethylamino compounds (in deuterated pyridine). The doublets (at τ 2.10) corresponding to the benzene hydrogens *ortho* to the arsenoso group of XXII and to the arseno group of XXIX are only slightly upfield from the doublet of the hydrogens *ortho* to the arsonic acid group in XXI (τ 2.00); the doublets for the hydrogens *ortho* to the diethylamine groups are identical for all three compounds (τ 3.30). This is almost the same pattern as shown by *p*-diethylaminobenzaldehyde in the same solvent [τ 2.15 (*o*-CHO) and 3.32 (*o*-N(C_2H_5)$_2$)]. The corresponding dithiarsenolane derivative (XXIV) shows a somewhat higher field absorption for the hydrogens *ortho* to the arsenic (τ 2.40) but still considerably downfield from the chemical shifts of the *ortho* hydrogens of all electron-releasing and of many more weakly electron-attracting substituents such as Cl, CF_3, S→O, etc. in the same solvent.

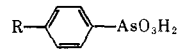
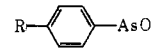
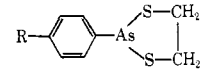
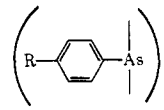
The results given in Table I indicate (1) that only a very moderate "activation" of the alkylating functions of compounds I and XVII can be expected to occur *in vivo* as a consequence of their reduction and combination with sulfhydryl groups, and (2) that the arseno mustard (IV) has only slightly higher chemical alkylating activity than the other arsenical nitrogen mustards.

Pharmacological Studies.—Toxicity and preliminary antitumor activity data are presented in Table II. It was important to determine whether the toxicity of the alkylating arsenicals was due entirely to the arsenic moiety or whether the alkylating function had a significant contributory effect. The LD_{50} values for the non-alkylating analogs XXI, XXII, and XXIV were equal to or even less than the values for the alkylating arsenicals, I, II, and XXIII. Moreover, the data in Table III indicate that BAL (2,3-dimercaptopropanol) was an effective protective agent against toxicity due to I raising the LD_{50} value by a factor of 6. Little or no protective action was observed against HN2. Sodium thiosulfate, a known protective agent against HN2 and some other (but not all) alkylating agents,[5] had no protective action against I. These data suggest that the toxicity of the alkylating arsenicals was, in the main, due to the arsenic moiety.

Additional evidence concerning the relative ineffectiveness of the alkylating moiety *per se* was obtained from studies of the leucotoxic properties of the alkylating arsenicals. Although alkylating agents are known to have a marked leucopenic effect, particularly at

TABLE II

TOXICITY AND ANTITUMOR ACTIVITIES

Compd	R	Acute toxicity, μmole/kg[a]	Antitumor activity: Ehrlich[b]	Antitumor activity: Walker 256[c]
				
I	$N(CH_2CH_2Cl)_2$	26	−	−
XVII	$N(CH_3)CH_2CH_2Cl$	7		
XXI	$N(C_2H_5)_2$	26	−	
XVII	$N(CH_2CH_2OH)_2$	3450	−	
V	NH_2	1340	−	
				
II	$N(CH_2CH_2Cl)_2$	18	+++	−
XXII	$N(C_2H_5)_2$	12	+	
XXV	$N(CH_2CH_2OH)_2$	28	−	−
IX	NH_2	22	−	
				
XXIII	$N(CH_2CH_2Cl)_2$	38	−	
XXIV	$N(C_2H_5)_2$	52	−	
				
IV	$N(CH_2CH_2Cl)_2$	1280	+++	−
XXIX	$N(C_2H_5)_2$	200–2000[d]		

[a] Approximate LD_{50} values (see Experimental Section). [b] −, T/C > 0.6; +, T/C = 0.2–0.6; ++, T/C = 0.01–0.2; +++, T/C < 0.01. See Experimental Section for definition of T/C. Antitumor activities are listed for the maximum tolerated dose of each compound. [c] T/C always exceeded 0.8. [d] The LD_{50} for this compound varied.[18]

TABLE III

EFFECT OF PRETREATMENT WITH BAL OR SODIUM THIOSULFATE ON TOXICITY OF ARSONIC ACID MUSTARD I

Pretreatment[a]	LD_{50}, mg/kg	Fiducial limits (95%)	DRF[b]
None	9.0	7.5–10.8	...
BAL, sc, 20 mg/kg at −1 hr and −0.25 hr	54.0	45–61	6.0
$Na_2S_2O_3$, sc, 2 g/kg at −0.5 hr and 1 g/kg at 0 hr	9.4	7.9–10.3	1.04

[a] Times of pretreatment are related to time of administration of I (at 0 hr). [b] DRF = dosage-reduction factor, the ratio of LD_{50} after pretreatment to LD_{50} without pretreatment.

doses approaching the lethal range, no such effect was observed with the alkylating arsenicals.

The antitumor activities of some of the compounds were determined against two alkylating agent sensitive tumors (Ehrlich ascites carcinoma and Walker carcinosarcoma 256). None of the compounds tested affected the growth of the Walker tumor. The Ehrlich carcinoma was not affected by the nonalkylating arsonic acid derivatives V and XXI or by the alkylating arsonic acid derivative I. The alkylating arsenoso compound II showed significant antitumor activity against the Ehrlich tumor. However, some activity was also apparent in the case of the nonalkylating analog XXII. The "thiolated" compounds XXIII and XXIV were inactive, but the alkylating arseno compound IV had significant antitumor action.[18] Thus, there is no consistent correlation between antitumor activity and alkylating potential. It is likely that the chemical reactivity of the alkylating moiety in these compounds is generally too low to permit a marked antitumor effect against the alkylating agent sensitive tumors at maximum tolerated doses. Data previously presented[8] indicate that, in the absence of a special mechanism of action, a dose in the range 500–1000 μmoles/kg would be necessary to exert a marked tumor-inhibitory effect (against Walker 256) in the case of alkylating agents with alkylating activities (k_{80}' values) in the range 1.0–3.4 (Table I). Except in the case of the arseno compound IV it is impossible to approach this dose range because of the toxic effects of the arsenic moiety.

The increased antitumor effectiveness of II against the Ehrlich ascites tumor compared with the nonalkylating analog XXII may indicate a possible potentiation of the antitumor effect of the arsenoso group by the alkylating group. Since the combination of trivalent arsenic with thiols is a reversible reaction[6] and, therefore, arsenicals may be considered as reversible enzyme inhibitors, it is possible that the reaction of the alkylating group with nearby nucleophilic centers of the enzyme may result in the formation of an irreversible enzyme–arsenical complex held together by additional covalent bonds in addition to the As–S linkages. However, if this were the case, one might expect an increase of the general toxicity of the arsenical. Since II is, in fact, somewhat *less* toxic than XXII, the increased selectivity of II against the tumor might be better explained on the basis of a synergism between the alkylating and sulfhydryl-inhibitory functions of this compound acting at two different sites of the same metabolic reaction sequence, *i.e.*, on the basis of its possible action as a dual antago-

(18) The nonalkylating arseno analog XXIX could not be tested because of its rapid oxidation in solution to the highly toxic arsenoso derivative.

nist. The significant antitumor activity of the arseno mustard IV, presumably after *in vivo* oxidation, may also be explained by either of the above two mechanisms. Clear distinction between these alternative mechanisms is not possible at the present time; however, a comparative study of the pharmacologic activities of II and IV with those of the *p*-arsenoso and *p*-arseno derivatives of iodoacetanilide (which are currently being prepared for testing) may shed some light on this problem. Because of the significant antitumor effect of the arsenoso and arseno mustards in the Ehrlich ascites test system, further studies are being conducted with these and other compounds described in this paper.

Experimental Section[19]

N,N-Bis(2-hydroxyethyl)-*p*-arsanilic Acid (VI).—Arsanilic acid (V, 3 g, 0.01 mole), was suspended in 60 ml of distilled water and 1 ml of glacial acetic acid, and the mixture was cooled in an ice–salt bath. Ethylene oxide gas was passed into the flask until all of the arsanilic acid dissolved. The solution was concentrated under reduced pressure to one-third volume, then acetone was added until the solution became turbid. A white crystalline solid (3.7 g, 88%) was isolated which, after two recrystallizations from water, melted at 163–164°. The infrared spectrum was in agreement with the structure.

Anal. Calcd for $C_{10}H_{16}AsNO_5$: C, 39.47; H, 4.93; N, 4.60. Found: C, 39.29; H, 5.03; N, 5.15.

N,N-Bis(2-chloroethyl)-*p*-arsanilic Acid (I).—N,N-Bis(2-chloroethyl)-*p*-phenylenediamine[12] (XII, 10 g, 0.04 mole) was suspended in 400 ml of water, and 8 ml of concentrated HCl was added. The mixture was heated for 3 min (stirring) to 60° and rapidly cooled; most of the solids were dissolved. The solution was further cooled in an ice–salt bath to −2°, and 2.75 g (0.04 mole) of $NaNO_2$ in 330 ml of water was added while maintaining the temperature below −1°.

An arsenite solution was prepared by dissolving 10 g (0.09 mole) of anhydrous Na_2CO_3, 5 g (0.025 mole) of arsenous oxide, and 0.5 g (0.002 mole) of $CuSO_4 \cdot 5H_2O$ in 250 ml of water. To this was added slowly (stirring) the above diazonium salt solution and sufficient $NaHCO_3$ to bring the pH close to 8.0. Some gummy precipitate was formed; it was removed by filtration. The clear filtrate was slowly acidified by dropwise addition of concentrated HCl under vigorous stirring with a glass rod. A crystalline material separated; yield 4.65 g (36.7%). It was purified by dissolving in the theoretical amount of cold 0.1 *N* NaOH followed by gradual acidification of the solution. The gummy material appearing first was separated. The clear solution on further acidification gave yellow crystals. After recrystallization from dioxane, the pure compound melted at 158–161°; ν_{max}^{KBr} (cm^{-1}) 2800–2350 (vs, ion pair), 1600 (vs), 1550 (w, phenyl), 1510 (s, $C_6H_5R_2NH^+$), 1360 (s, $C_6H_5N<$), 1102 (vs, C_6H_5–As), 875 (s, As=O), 812 (vs, *p*-C_6H_5), 775 (vs, As–O), 735 (m, CCl).

Anal. Calcd for $C_{10}H_{14}AsCl_2NO_3$: C, 35.10; H, 4.09; Cl, 20.8; N, 4.09. Found: C, 35.15; 34.86; H, 4.16, 4.11; Cl, 20.9, 20.63; N, 4.10, 4.15.

N-Methyl-N-(2-chloroethyl)-*p*-arsanilic Acid (XVII).—N-Methyl-N-(2-hydroxyethyl)aniline[14] (22.0 g, 0.145 mole) was mixed with $POCl_3$ (14.5 ml, 0.16 mole) and stirred for 30 min. The brown, viscous liquid was poured into 500 ml of ice–water, and 10% NaOH solution was added to bring the pH to 6–7. The aqueous solution was extracted with benzene, and the benzene layer was washed with water and dried ($CaCl_2$). After evaporation of the solvent *in vacuo*, 20 g (81%) of a viscous product was obtained which gave an infrared spectrum compatible with the structure of **N-methyl-N-(2-chloroethyl)aniline (XIV)**, bp 132–134° (12–13 mm), lit.[20] 124° (10 mm).

Nitrosation[21] of XIV gave ***p*-nitroso-N-methyl-N-(2-chloroethyl)aniline (XV)** in 95% yield, mp 75–76°, lit.[21] 69°. This was reduced with $SnCl_2$ and HCl to give a 60% yield of oily **N-methyl-N-(2-chloroethyl)-*p*-phenylenediamine (XVI)**[21] which was then converted to XVII by the Bart reaction in the same manner as described for the synthesis of I; light brown crystals, mp 139–141° dec, yield 1 g (12.5%). Recrystallization from acetone (charcoal) gave pure XVII in large, needle-shaped crystals, mp 141–142°. Its infrared spectrum was similar to that of I.

Anal. Calcd for $C_9H_{13}AsClNO_3$: C, 36.80; H, 4.42; As, 25.5; Cl, 12.1; N, 4.79. Found: C, 37.00; H, 4.40; As, 25.5; Cl, 12.0; N, 4.82.

N,N-Diethyl-*p*-arsanilic Acid (XXI).—A mixture of diethylaniline, (18.6 g, 0.125 mole) and $AsCl_3$ (11.6 ml, 0.14 mole) was heated on a boiling-water bath with vigorous stirring for 30 min. The thick brown syrup was poured into 600 ml of ice-cold water and stirred until a pale yellow solution resulted. This solution was poured into a large excess of concentrated NaOH at 0° and extracted three times with ether. The aqueous solution was added dropwise to solid NH_4Cl and the precipitate obtained was washed with ice-cold water; yield 21 g (70%) of **4-arsenoso-N,N-diethylaniline (XXII)**,[22] mp 57–58° (lit.[16] 58°).

The above compound (XXII, 1.5 g, 0.06 mole) was dissolved in 20.4 ml of 5% aqueous NaOH and treated with 10 ml of 30% H_2O_2. When oxygen evolution had completely ceased, the solution was acidified with 50% aqueous acetic acid; yield of solid 1.1 g (64.5%). After crystallization from boiling water (charcoal), XXI was obtained in the form of needles, mp 167–169°. The infrared spectrum was similar to that of I and of XVII; nmr (in deuterated pyridine), benzene protons at τ 3.30 and 2.00 (doublets).

Anal. Calcd for $C_{10}H_{16}AsNO_3$: C, 43.9; H, 5.86; As, 27.4; N, 5.13. Found: C, 44.05; H, 5.80; As, 27.3; N, 5.23.

4-Arsenoso-N,N-bis(2-chloroethyl)aniline (II).—To a solution of 5 g (0.015 mole) of I in 50 ml of alcohol was added 20 ml of 50% aqueous HI, followed by SO_2 gas. When all of the iodine formed in the reaction was reconverted by the SO_2 to HI, an excess of concentrated NH_4OH was added slowly under cooling. The granular precipitate was washed with water; yield 4.5 g (100%), mp 30–50°. It showed the characteristic, infrared absorption bands of the arsenoso compounds (see discussion).

Anal. Calcd for $C_{10}H_{12}AsCl_2NO$: C, 38.89; H, 3.89; Cl, 23.0; N, 4.55. Found: C, 38.72; H, 4.04; Cl, 23.0; N, 4.60.

Conversion of II to I.—A small sample of the above compound was suspended in 1% NaOH and oxidized by treatment with a few drops of 30% H_2O_2 as described for the preparation of XXI. Upon acidification, some needle-shaped crystals formed; mp 153–155°. The infrared spectrum was identical with that of I prepared by the Bart reaction.

2-*p*-[Bis(2-chloroethyl)amino]phenyl-1,3,2-dithiarsenolane (XXIII).—A mixture of 1 g (0.004 mole) of II, ethanedithiol (0.5 g, 0.005 mole), and 10 ml of dry ethanol was refluxed for 10 min. On cooling, a yellow oil separated. The alcohol was removed *in vacuo*, and the residue was quickly washed with three portions of ether. All attempts to crystallize this oil failed. Since the infrared spectrum indicated that the desired product was obtained (disappearance of the characteristic AsOAs absorption of II at 780–680 cm^{-1}, and appearance of two new medium-intensity peaks at 930 and 822 cm^{-1}, as in the spectra of XXIV and XXVII), a sample was dried for 8 hr over P_2O_5 and submitted for analysis.

Anal. Calcd for $C_{12}H_{16}AsCl_2NS_2$: C, 37.50; H, 4.17; Cl, 18.5; N, 3.65; S, 16.7. Found: C, 35.90; H, 4.21; Cl, 17.5; N, 4.31; S, 17.9.

All further attempts to purify this compound and to obtain more satisfactory analytical values failed.

2-(*p*-Diethylaminophenyl)-1,3,2-dithiarsenolane (XXIV).—A mixture of 1 g (0.004 mole) of XV, ethanedithiol (0.59 g, 0.006 mole), and 20 ml of ethanol was refluxed for 10 min. On cooling, shining white needles were obtained; yield 0.85 g (65%). A sample was recrystallized from ethanol; mp 74–75.5°.

(19) Melting points were taken on a Drechsel melting point apparatus and are uncorrected. The infrared spectra were recorded on Perkin-Elmer Model 137 (Infracord) and Model 237 spectrophotometers, using polyethylene reference standards. The nmr spectra were determined on a Varian Associates Model A-60 spectrometer, in $CDCl_3$ or deuterated pyridine (as indicated) with tetramethylsilane as the internal standard. Microanalyses are by Dr. S. Nagy, Massachusetts Institute of Technology, Cambridge, Mass., and Galbraith Laboratories, Knoxville, Tenn.

(20) G. R. Clemo and W. H. Perkin, Jr., *J. Chem. Soc.*, **121**, 648 (1922).

(21) J. V. Braun and G. Kirschbaum, *Ber.*, **52**, 1716 (1919).

(22) When the synthesis was carried out according to the literature procedure,[16] the yield varied from 0–36%.

Anal. Calcd for $C_{12}H_{18}AsNS_2$: C, 45.57; H, 5.72; As, 23.8; N, 4.45; S, 20.3. Found: C, 45.90; H, 5.95; As, 23.6; N, 4.32; S, 20.4.

Attempted Synthesis of 2-*p*-[Bis(2-hydroxyethyl)amino]-1,3,2-dithiarsenolane (XXVI). A.—A mixture of VI (30.5 g, 0.1 mole), phenylhydrazine (21.6 g, 0.2 mole), and 120 ml of methanol was refluxed for 1 hr. The methanol was distilled and the yellow viscous liquid was treated with 120 ml of 2 *N* NaOH and extracted with ether. The product was precipitated from the aqueous layer by the addition of solid NH_4Cl; yield 14.0 g (51.7%) of **4-arsenoso-N,N-bis(2-hydroxyethyl)aniline (XXV).** An analytical sample was prepared by dissolving the compound in 2 *N* NaOH and precipitation with solid NH_4Cl; mp 245–250° dec. The infrared spectrum was in agreement with the structure.

Anal. Calcd for $C_{10}H_{16}AsNO_3$: C, 44.30; H, 5.16; As, 27.7; N, 5.16. Found: C, 44.37; H, 4.84; As, 27.8; N, 5.39.

The above compound (XXV, 2 g, 0.007 mole) was mixed with ethanedithiol (0.76 g, 0.008 mole) and 50 ml of absolute ethanol; the mixture was refluxed for 2.5 hr. On cooling, an oil separated. The alcohol was distilled, and the residue was washed three times with ether and dried under vacuum. Comparison of the infrared spectrum with the spectra of XXIV and XXVII indicated that the desired compound (XXVI) was obtained, but this oily substance could not be sufficiently purified to give acceptable elemental analyses.

B.—4-Arsenosoaniline[23] (IX, 8 g, 0.044 mole), ethanedithiol (5 g, 0.053 mole), and 25 ml of absolute ethanol was heated under reflux for 10 min to give after chilling in Dry Ice–acetone 9.6 g (89%) of **2-*p*-aminophenyl-1,3,2-dithiarsenolane (XXVII).** A sample was crystallized from absolute ethanol; mp 69–70.5°.

Anal. Calcd for $C_8H_{10}AsNS_2$: C, 37.05; H, 3.86; As, 28.9; N, 5.46; S, 24.7. Found: C, 37.03; H, 3.70; As, 28.9; N, 5.29; S, 24.7.

A suspension of XXVII (15 g, 0.043 mole) in 150 ml of ethanol was chilled in Dry Ice–acetone. Ethylene oxide (10 ml, 0.2 mole) and 0.5 g of *p*-toluenesulfonic acid was added to the reaction mixture which was then stirred for 12 hr at room temperature. After concentration *in vacuo*, a thick syrup was obtained. This showed an infrared spectrum essentially identical with that of the crude XXVI prepared by procedure A. In an attempt to crystallize the syrup, it was dissolved in alcohol and left at room temperature for a month. Shining needles gradually separated. They were washed with alcohol; mp 79.5–80°; ν_{max}^{KBr} (cm^{-1}) 2950 (s), 1410 (s), 1285 (s), 1195 (s), 930 (m), 835 (vs), 722 (w), 670 (m); nmr (in $CDCl_3$), two single peaks at τ 6.88 and 6.50, in the integrated ratio of 1:2. The infrared and nmr spectra and the elemental analyses were compatible with the novel structure **XXVIII**, *i.e.*, **2,2′-(ethylenedithio)bis-1,3,2-dithiarsenolane.**

Anal. Calcd for $C_6H_{12}As_2S_6$: C, 16.80; H, 2.82; As, 35.2; S, 45.0. Found: C, 17.09; H, 3.07; As, 34.8; S, 44.8.

Direct Synthesis of XXVIII.—A mixture of As_2O_3 (3 g, 0.015 mole), 1,2-ethanedithiol (3 g, 0.032 mole), and 22 ml of absolute ethanol was heated under reflux for 20 min. A heavy oil separated. The alcohol layer was decanted, and the oil was washed with 20-ml portions of absolute ethanol. The oil crystallized slowly; yield 2.5 g (53%), mp 78–80°. A mixture melting point with the breakdown product obtained above showed no depression. The infrared spectra of the two samples were identical.

4-[N,N-Bis(2-chloroethyl)amino]arsenobenzene (IV).—I (0.5 g, 0.0015 mole) was dissolved in 5 ml of methanol and added to a solution of $SnCl_2$ (0.75 g, 0.0033 mole), concentrated HCl (3 ml), and a few crystals of KI in 3 ml of methanol. After stirring for 20 min, a yellow precipitate was obtained. This was separated by centrifugation and repeatedly washed with methanol; yield 0.25 g (59%). The crude product was readily soluble in tetrahydrofuran or dioxane but insoluble in alcohol or water; attempts to recrystallize it were unsuccessful. The compound was purified[24] by dissolving in tetrahydrofuran and precipitation with methanol; yellow powder, mp 120–121° dec. Its infrared spectrum was very similar to that of XXIX (below).

Anal.[24] Calcd for $(C_{10}H_{12}AsCl_2N)_x$: Cl, 24.3; N, 4.79. Found: Cl, 23.6; N, 4.33.

4-(N,N-Diethylamino)arsenobenzene (XXIX) was prepared according to the literature[16] procedure, for the purpose of spectral comparison with XXIV; yellow powder, mp 180°, lit.[16] 180°; ν_{max}^{KBr} (cm^{-1}) 3000 (m), 1585 (vs), 1495 (s), 1395 (m), 1370 (m), 1350 (s), 1282 (vs), 1195 (vs), 1075 (vs), 802 (vs); nmr (in deuterated pyridine), τ 8.92 triplet (CH_3), 6.80 multiplet (CH_2), 3.30 doublet, and 2.10 doublet (benzene protons).

Toxicity Determinations.—Male, Swiss mice, 22–26 g, were fed a pelleted diet (Purina Laboratory Chow) and tap water *ad libitum*. Animal quarters were maintained at a temperature of 23.3–24.4°. Compounds dissolved in 1% $NaHCO_3$, 0.1 *N* HCl, or dimethyl sulfoxide were administered intraperitoneally to groups of six mice/dose level. All deaths within a 21-day period were recorded and approximate LD_{50} values were estimated graphically from per cent mortality/log dose plots using the method of Litchfield and Wilcoxon.[25]

Tumor Inhibition Studies.—Ehrlich ascites carcinoma cells were harvested from male Swiss mice bearing a 10-day-old tumor. The cells were suspended in cold 0.85% NaCl at a concentration of 2×10^7 cells/ml, and 1×10^7 cells were injected intraperitoneally into recipient mice. Twenty-four hours later, the compound was administered intraperitoneally to groups of eight mice/dose level. Animals surviving on the tenth day were killed, and the ascites fluid was collected in graduated centrifuge tubes. After centrifugation, the packed-cell volume was determined and the degree of tumor inhibition was assessed by comparing the mean packed-cell volume of treated animals with that of control animals which received the solvent only (T/C).

Walker carcinosarcoma 256 was implanted subcutaneously in the flank region of male Holtzman rats using a trochar and cannula. Five days later when the tumor had grown to about 5 g, the compound was injected intraperitoneally to groups of 3–5 rats/dose level. Doses were repeated on the following 3–5 days, and the tumor volume was estimated on the tenth day from measurements taken with calipers. The ratio of the mean volume of treated tumors to the mean volume of control tumors (T/C) was determined.

Leucopenic Studies.—Blood was collected from a tail vein of normal male Swiss mice, 22–26 g, and the total leucocyte count was determined using a Neubauer counting chamber after dilution. On the same day, the compound was injected intraperitoneally to groups of eight mice/dose level. Four days later, the total leucocyte count was again taken and the mean change in the count was determined for each treated and control group. The results of this study are not tabulated because no leucopenic effect was observed in any of the drug-treated animals, even at doses approaching the LD_{50}.

(23) P. Ehrlich and A. Bertheim, *Ber.*, **43**, 917 (1910).

(24) Purification of the arseno compounds is very difficult, probably due to their polymeric structures (see ref 10). Most of the known arsenobenzenes are reported in the literature without complete or satisfactory elemental analysis results. For general references, see G. W. Regis and J. L. Gavron, "Organic Arsenical Compounds," American Chemical Society Monograph Series, The Chemical Catalog Co., Inc., New York, N. Y., 1923; M. Dub, "Organometallic Compounds," Vol. III, Springer-Verlag, Berlin, 1962.

(25) J. T. Litchfield and F. Wilcoxon, *J. Pharmacol. Exptl. Therap.*, **96**, 99 (1949).