MAGNETIC RESONANCE IN CHEMISTRY, VOL. 27, 669–675 (1989)

Two-Dimensional NMR Studies of Arsenical–Sulfhydryl Adducts

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British anti-lewisite (2,3-dimercaptopropanol) (BAL) has long been used as an arsenic antidote, but its therapeutic efficacy is limited by its inherent toxicity. Two less toxic potential replacements for BAL are dimercaptosuccinic acid and dimercaptopropanesulfonic acid. These two disulfhydryl compounds were compared with BAL by studying the structures of their adducts with two organic arsenicals, phenyldichloroarsine and *trans*-2-chlorovinylarsine oxide. The 1:1 adducts were synthesized and characterized by one- and two-dimensional NMR spectroscopy. ^{1}H and ^{13}C spectral data and resonance assignments, verified by spin simulation, are presented for the adducts. All were five-membered heteroatomic ring systems, with different solubility properties. When stereoisomers were detectable, the ratio of *anti* to *syn* isomers varied, indicating that the functional group of the antidote influenced stereochemical aspects of adduct formation.

KEY WORDS 2D NMR Phenyldichloroarsine Lewisite British anti-lewisite Dimercaptosuccinic acid 2,3-Dimercaptopropanesulfonic acid Adducts

INTRODUCTION

Arsenical toxicity is attributed to reactions with critical cellular sulfhydryls.[1] For 40 years (in the USA), the antidote has been 2,3-dimercapto-1-propanol (British anti-lewisite; BAL). BAL is able to extract tissue-bound arsenic and facilitate urinary excretion because the BAL–arsenic adduct is favored over cellular sulfur–arsenic adducts. It was developed originally as a topical antidote to counteract the blistering action of lewisite.[2] BAL is an excellent topical antidote, but is less than ideal for systemic arsenic poisoning. It is inherently toxic, which unfortunately limits its therapeutic usefulness. Recently, attention has been given to obtaining a replacement for BAL for systemic use.[3] The molecular design of effective, less toxic antidotes to organic arsenic requires a thorough understanding of the interaction of arsenic with disulfhydryl compounds.

We have previously studied the adduct of BAL and phenyldichloroarsine (PDA) by nuclear magnetic resonance.[4] In the study described here we compared the adducts of *trans*-2-chlorovinylarsine oxide (lewisite oxide; LO) and PDA with BAL and two candidate replacements, *meso*-2,3-dimercaptosuccinic acid (DMSA) and 2,3-dimercaptopropanesulfonic acid (DMPS).

EXPERIMENTAL

PDA was purchased from Research Organic/Inorganic Chemical (Sun Valley, CA, USA). It was purified by repeated distillation at 128 °C under 15 mmHg of nitrogen. Phenylarsine oxide (PAO), purchased from ICN Pharmaceuticals, Life Sciences Group (Plainview, NY, USA), was used interchangeably for PDA (with identical results). LO was synthesized by slowly hydrolyzing *trans*-2-chlorovinyldichloroarsine with aqueous sodium hydrogencarbonate in carbon tetrachloride. The product was removed from the organic phase, dehydrated in a vacuum desiccator and subsequently recrystallized from benzene to yield a polymer (m.p. 143 °C).[5] The purity of the above arsenicals was confirmed by IR and NMR spectroscopy. BAL, DMSA and DMPS were purchased from Sigma Chemical (St. Louis, MO, USA). Deuteriated solvents were purchased from Aldrich Chemical (Milwaukee, WI, USA) or Merck Sharpe and Dohme (West Point, PA, USA).

All NMR spectra were recorded on a Varian XL-300 FT NMR spectrometer using the 5 mm broad-band probe. Chemical shifts (^{13}C and ^{1}H) are referenced downfield relative to external $(Me)_4Si$. The 1:1 stoichiometry was verified by adding excess arsenical or sulfhydryl to the adduct in solution and observing both

0749–1581/89/070669–07 $05.00

Received 6 December 1988
Accepted (revised) 6 February 1989

^{13}C and ^{1}H NMR spectra. Phase-sensitive double quantum COSY (DQCOSY) and heteronuclear ^{1}H–^{13}C shift correlated (HETCOR) experiments were performed using the Varian programs. Windows of 16 500 and 4000 Hz were used for ^{13}C and ^{1}H spectra, respectively. The DQCOSY experiments were optimized with f_1 and f_2 domains containing a minimum of 1024 data points each. The HETCOR experiments were typically optimized for $^{1}J(CH) = 150$ Hz, while the $f_1(^{13}C)$ and $f_2(^{1}H)$ domains contained a minimum of 1024 and 256 data points, respectively.

PDA–BAL and PDA–DMSA adducts were prepared as previously reported.[6] LO–BAL was prepared by combining stoichiometric amounts of LO, HCl and BAL in methanol, followed by removal of all volatiles. LO–DMSA was prepared by combining stoichiometric amounts of LO, HCl and DMSA in acetone. The gradual addition of water precipitated the adduct, which was subsequently removed and dried. Both PDA–DMPS and LO–DMPS were prepared by adding stoichiometric amounts of DMPS to an aqueous solution of the arsenical, followed by aqueous recrystallization of the product. All NMR experiments were performed in methanol-d_4 for the BAL adducts, acetone-d_6 for the DMSA adducts and D_2O for the DMPS adducts. These solvents were chosen to minimize spectral interference, limited aqueous solubility constraints or side-reactions with the solvent (e.g. esterification of DMSA adducts with alcohols). The sample concentrations varied from a maximum of 0.100 M at 20 °C for the BAL adducts to 0.020 M at 60 °C for the DMPS adducts.

Exact values for coupling constants and chemical shifts in the ^{1}H spectra for the LO–BAL, PDA–DMPS and LO–DMPS adducts were determined by using the Varian spin simulation computer program, which is based on the FORTRAN program LAME (with magnetic equivalence added). Iteration was considered converged when the simulated spectrum matched the actual spectrum and the r.m.s. error was equal to or less than the resolution of the real spectrum.

RESULTS

Confirmation of 1 : 1 adducts between the arsenicals and the disulfhydryls was accomplished by sets of both proton and carbon NMR experiments. This can best be seen in Fig. 1, in which the arsenical was titrated with a disulfhydryl compound. The lower spectrum (1 : 0) is the ^{13}C spectrum of soluble lewisite oxide (the large peak at 49.00 ppm is due to the solvent). Addition of 0.5 molar equivalent of BAL resulted in the next spectrum up (1 : 0.5). The third spectrum (1 : 1) is that of approx-

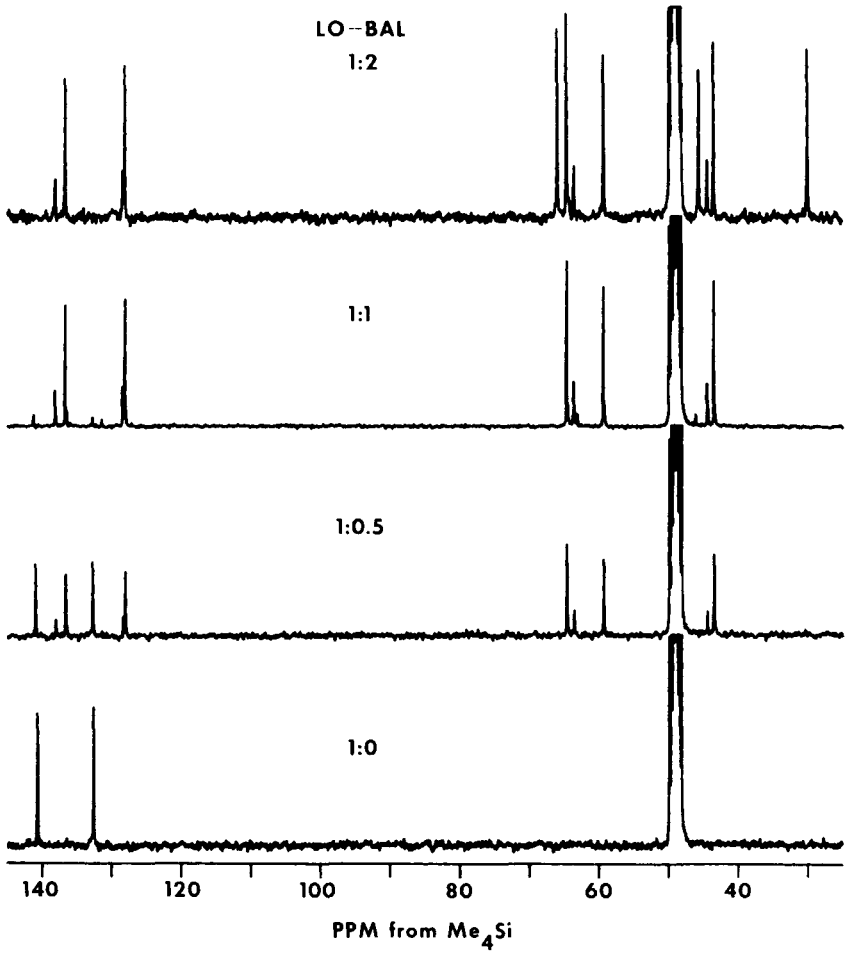


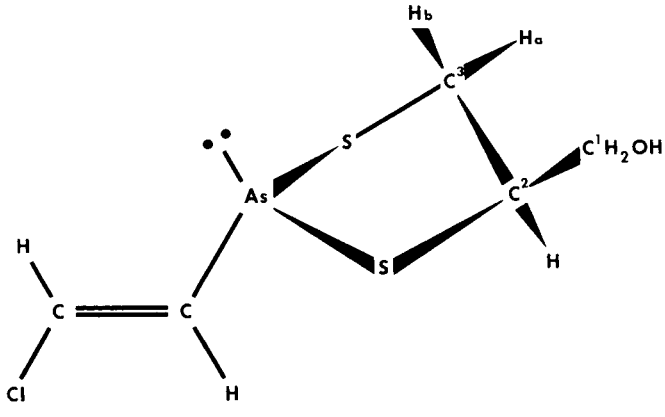
Figure 1. ^{13}C NMR spectra of Lewisite plus BAL showing the formation of a 1 : 1 adduct.

Table 1. ^1H and ^{13}C spectral data for the major LO–BAL adduct (methanol-d_4)

Proton or carbon	Chemical shift (ppm)	^2J(HH) (Hz)	^3J(HH) (Hz)
H-1	2.98	−14.35	8.97
H-1′	2.93	−14.35	4.32
H-2	3.66	—	8.97, 4.32, 3.10, 4.20
H-3a	3.57	−12.80	3.10
H-3b	2.68	−12.80	4.20
Hv-1	6.18	—	
Hv-2	6.18	—	
C-1	64.52		
C-2	59.23		
C-3	43.51		
Cv-1	136.52		
Cv-2	128.01		

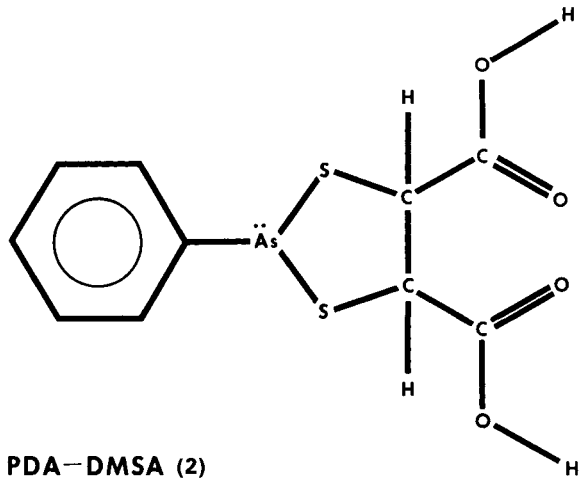
imately equal molar equivalents of lewisite and BAL. The top spectrum (1:2) represents 2 molar equivalents of BAL added to lewisite. This last spectrum is essentially identical with the previous spectrum, except for three new peaks at 65.77, 45.54 and 30.00 ppm, which correspond to unreacted BAL.

Spectral parameters for the LO–BAL adduct are given in Table 1. Assignments of the resonances were based on the respective chemical shifts, coupling constants, HETCOR and DQCOSY spectra and spin simulation results. The LO–BAL adduct (**1**) consisted of two structural isomers, with the hydroxymethyl group *syn* or *anti* to the *trans*-2-chlorovinyl group. The *anti* isomer was the more dominant, and integration of the proton resonances indicated the *anti/syn* ratio to be $\geq 4:1$.

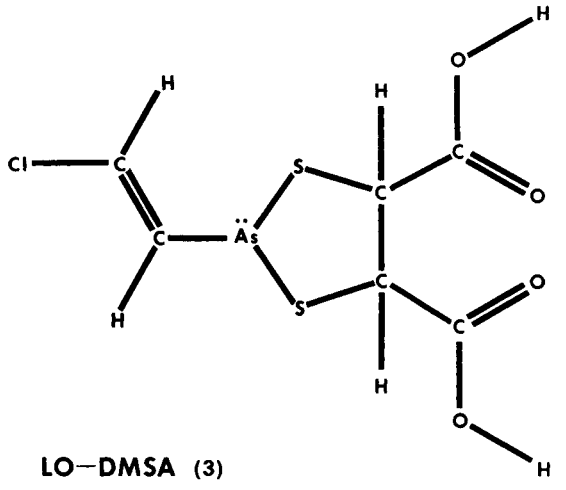


LO–BAL (1)

Because *meso*-DMSA was used, the PDA–DMSA (**2**) and LO–DMSA (**3**) adducts were spectroscopically very simple. In the aliphatic region of the proton spectra, H-2 and H-3 were magnetically equivalent and resonated at 4.56 ppm for PDA–DMSA and 4.86 ppm for LO–DMSA. The carbon spectra indicated that the aliphatic C-2 and C-3 were magnetically equivalent and resonated at 59.48 ppm for PDA–DMSA and 59.64 ppm for LO–DMSA. Similarly, the C-1 and C-4 carbonyl carbons were also magnetically equivalent and resonated at 170.16 and 170.08 ppm for the respective adducts.



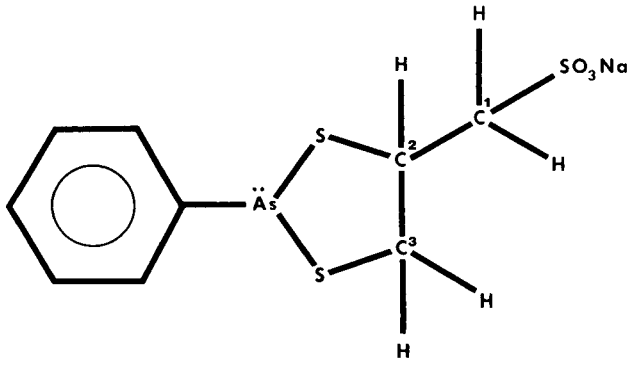
PDA–DMSA (2)



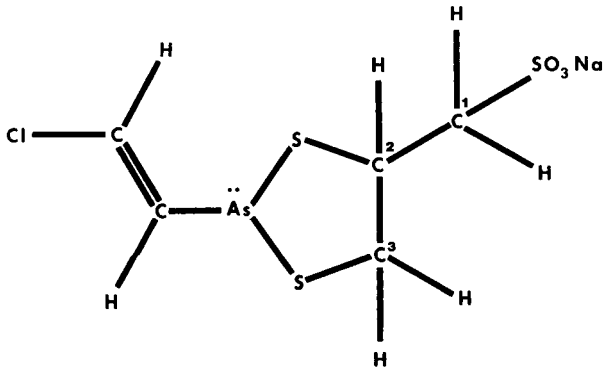
LO–DMSA (3)

The DQCOSY spectra for the PDA–DMSA and LO–DMSA adducts were straightforward, as were their HETCOR spectra. The only assignments corroborated by spin simulation were the two protons of the *trans*-2-chlorovinyl group of the LO–DMSA adduct. The chemical shifts of 6.80 and 6.89 ppm and the coupling constant of 14.27 Hz revealed that these two protons were *trans*.

The final two adducts investigated were PDA–DMPS (**4**) and LO–DMPS (**5**), which were similar to the adducts of BAL except for replacement of the hydroxyl with a sulfonic acid group. Each adduct contained two



PDA–DMPS (4)



LO–DMPS (5)

structural isomers in which the sulfonic acid group was *syn* or *anti* to the phenyl or *trans*-2-chlorovinyl group attached to arsenic. The ratio of the *anti* to *syn* isomers was 2.0:1.0 for PDA–DMPS and, initially, 2.0:1.0 for LO–DMPS. After repeated recrystallization in water, the ratio of the *anti* to *syn* isomers for LO–DMPS increased to 3.6:1.0, indicating that the *syn* isomer was more soluble.

Spectral assignments for the PDA–DMPS and LO–DMPS adducts are given in Tables 2 and 3, respectively. The challenge of unraveling the complex spectra is apparent in the DQCOSY and HETCOR spectra of the LO–DMPS adduct (Figs 2 and 3). The overlap of the H-1 (3.19 ppm) and H-3b (3.22 ppm) splitting pattern of the *anti* isomers was further compounded by the H-1 (3.14 ppm), H-3b (3.15 ppm) and H-3a (3.37 ppm) splitting pattern of the *syn* isomers. The proton assignments were based not only on the DQCOSY and HETCOR spectra, but also on exhaustive spectral spin simulations. The spectra for both structural isomers (*anti* and *syn*) were simulated (Fig. 4(A) and (B)) and then added together (Fig. 4(C)) in order to compare them with the original spectrum (Fig. 4(D)). The final iteration for the *anti* isomer actually allowed five chemical shifts and six coupling constants to be variables, and resulted in an r.m.s. error (in relation to line assignment) of less than the real resolution of the actual spectrum.

DISCUSSION

Aksnes and co-workers[7,8] have shown that arsenicals will react with vicinal sulfhydryls to form five-membered heteroatomic ring system. The organic arsenicals and disulfhydryl compounds in this study all formed the expected 1:1 cyclic adducts. No 1:2 adduct was detectable, indicating that the arsenicals clearly prefer to react with both sulfhydryls on a single molecule, rather than with sulfhydryls from two separate molecules. The proportion of geometric isomers (*anti*:*syn*) varied from 2:1 to greater than 4:1, depending on the functional groups of the antidotes. The adducts formed with BAL have an *anti*:*syn* ratio of at least 4:1. This would seem to indicate that the hydroxyl group prefers a stereochemical arrangement such that it is as distant as possible from the phenyl or *trans*-2-chlorovinyl attached to the central arsenic atom. However, when a sulfonic group is substituted for the

Table 2. ^{1}H and ^{13}C spectral data for the PDA–DMPS adducts (D_2O)

Isomer	Proton or carbon	Chemical shift (ppm)	^{2}J(HH) (Hz)	^{3}J(HH) (Hz)
Major isomer (*anti*)	H-1	2.63	−14.20	8.35
	H-1′	2.49	−14.20	5.20
	H-2	3.69	—	8.35, 5.20, 4.23, 4.69
	H-3a	3.21	−12.92	4.23
	H-3b	2.46	−12.92	4.69
	C-1	56.35		
	C-2	52.81		
	C-3	45.49		
	C_ϕ-1	143.51		
	C_ϕ-2,6	130.97		
	C_ϕ-4	129.92		
	C_ϕ-3,5	129.18		
Minor isomer (*syn*)	H-1	2.61	−11.96	6.97
	H-1′	2.60	−11.96	7.01
	H-2	2.46	—	6.97, 7.01, 4.40, 8.51
	H-3a	3.21	−12.94	4.40
	H-3b	2.32	−12.94	8.51
	C-1	55.44		
	C-2	54.85		
	C-3	45.78		
	C_ϕ-1	143.99		
	C_ϕ-2,6	130.97		
	C_ϕ-4	129.92		
	C_ϕ-3,5	129.18		

Table 3. ^{1}H and ^{13}C spectral data for the LO–DMPS adduct (D_2O)

Isomer	Proton or carbon	Chemical shift (ppm)	^{2}J(HH) (Hz)	^{3}J(HH) (Hz)
Major isomer (*anti*)	H-1	3.19	−14.46	8.30
	H-1′	3.02	−14.46	5.24
	H-2	4.34	—	8.30, 5.24, 3.94, 4.27
	H-3a	3.88	−13.04	3.94
	H-3b	3.22	−13.04	4.27
	Hv-1	6.58	—	14.22
	Hv-2	6.62	—	14.22
	C-1	64.52		
	C-2	59.23		
	C-3	43.51		
	Cv-1	136.52		
	Cv-2	128.01		
Minor isomer (*syn*)	H-1	3.14	−13.25	9.28
	H-1′	3.84	−13.25	3.85
	H-2	3.94	—	9.28, 3.85, 6.92, 6.34
	H-3a	3.37	−14.36	6.92
	H-3b	3.15	−14.36	6.34
	Hv-1	6.61	—	14.22
	Hv-2	6.72	—	14.22
	C-1	55.59		
	C-2	54.89		
	C-3	46.11		
	Cv-1	136.58		
	Cv-2	128.50		

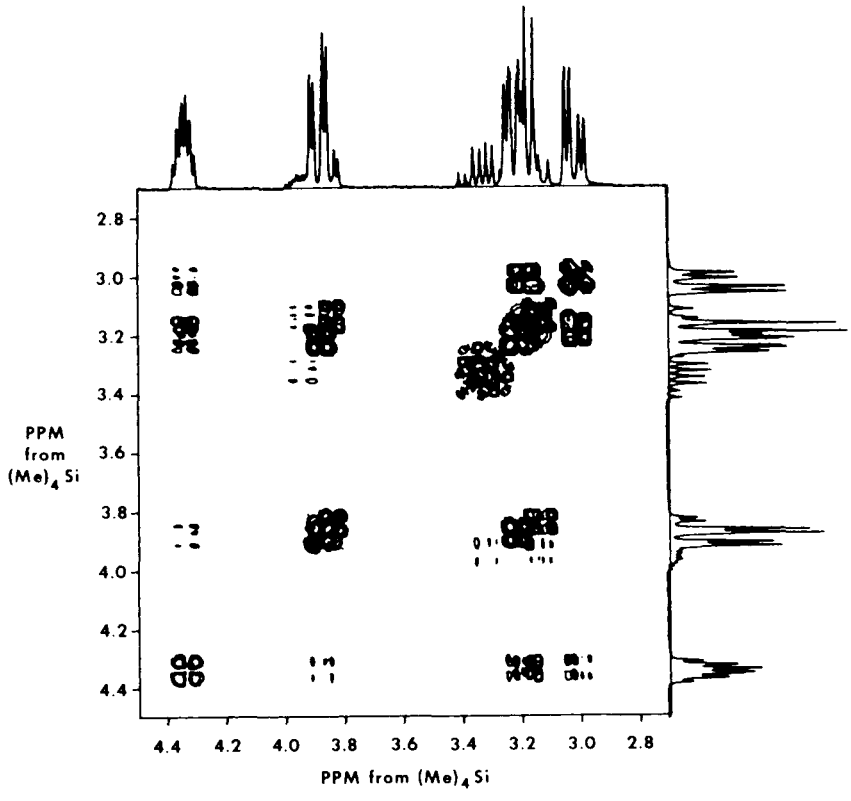


Figure 2. DQCOSY contour plot of LO–DMPS adduct (aliphatic region).

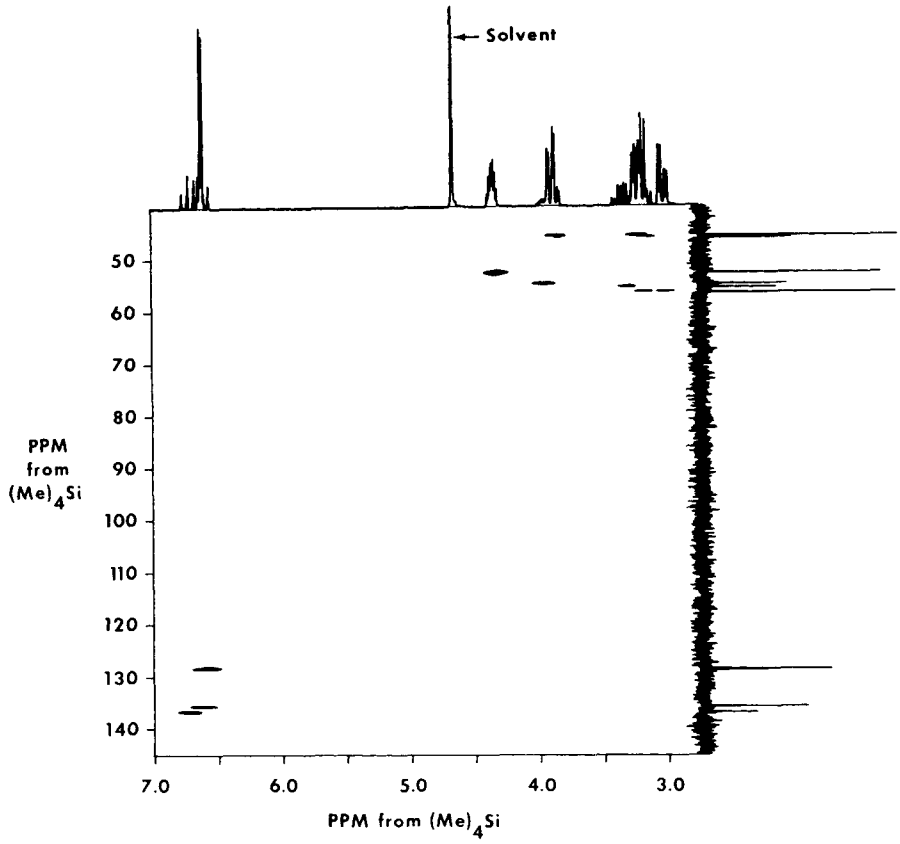
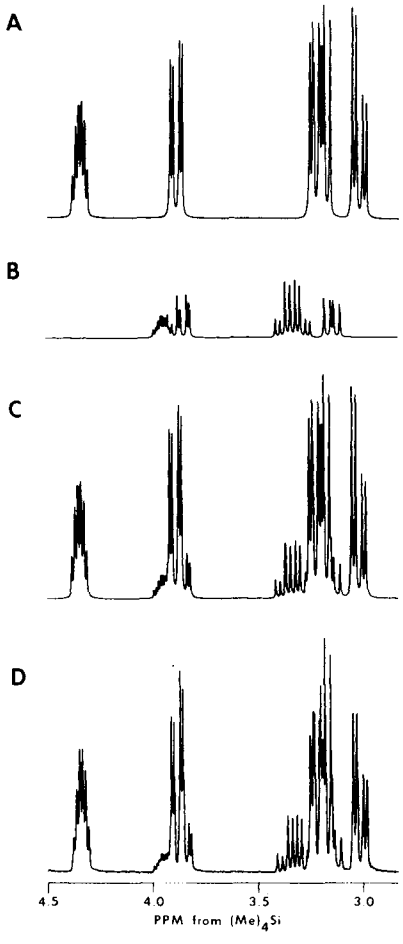


Figure 3. HETCOR contour plot of LO–DMPS adduct.



hydroxyl, the *anti*:*syn* ratio drops to 2:1, indicating that the size of the group substituted is not the only factor. The *anti*:*syn* ratio of these five-membered heteroatomic ring systems does not appear to be appreciably affected by the organic group on the arsenical. Substitution of the phenyl ring for the *trans*-2-chlorovinyl group has little effect on the *anti*:*syn* ratio of these heteroatomic systems.

The *meso*-DMSA adducts presumably have the *anti* (both carboxyl groups relative to the phenyl or *trans*-2-chlorovinyl group) and *cis* (carboxyl groups to each other) configuration. The fact that only one aliphatic proton resonance is observed indicates that the two ring conformations of the five-membered ring system are interconverting rapidly on the NMR time scale.

Usually, in the case of the dithioarsolanes, interconversion of the two half-chair ring conformations is slow relative to the motions of the phenyl ring and the overall molecular reorientation of the molecule.[8] However, the two neighboring carboxyl groups could affect the stability and/or interconversion of the two ring conformations. Our unpublished results indicate that the adducts of *meso*-DMSA are indeed unstable over a period of several days.

In a related study of various potential antidotes, Aposhian *et al.*[9] observed that racemic DMSA was more effective in reactivating arsenite-inhibited enzymes. The

Figure 4. Spin simulated and actual proton spectra for the two LO–DMPS adducts (aliphatic region). (A) and (B), simulated spectra for the *anti* and *syn* isomers, respectively; (C), summation spectrum of (A) and (B); (D), authentic spectrum of the adduct.

adduct formed with racemic DMSA would be *trans* (with one carboxyl *anti* and one *syn*) and would have a conformation with a carboxyl close to the functional group of the arsenical. Again, the fact that we did not initially observe a DMSA adduct with a *syn* orientation could be interpreted as meaning that the size of the functional groups on the disulfhydryl is important, but far from being the only consideration.

Other factors to be considered in choosing an antidote include aqueous solubility and stability in physiological fluids. We found limited aqueous solubility for the adducts: approximately 0.5 mM for the BAL adducts and 10 mM for both the DMSA and DMPS adducts. We have also noted that new resonances appear in the spectra of the *meso* DMSA adducts with time, possibly because of partial dissociation of the adduct.[6] In these complexes the two carboxyl groups can be *syn* or *anti* to the organic moiety on the arsenical. It is possible that in fact we observed a shift in equilibrium from the kinetically favored product to the thermodynamically favored product. Thus, one geometric arrangement is favored over another, and the stereochemistry of the two charged carboxyl groups may play a role in this phenomenon.

Our work clearly indicates that there may be a variety of factors which need to be considered in the development of new antidotes for heavy metal poisoning. For a systemic antidote, these must include a low toxicity, a reasonable solubility in a physiological situation, and that the adduct formed be soluble enough to be removed from the system. The electrostatics and stereochemistry appear to have some consequence as to the efficacy of various antidotes. The relative binding constants of the various arsenical–antidote complexes and the factors that influence their magnitude will be treated in another paper.

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