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Facile dimethylarsenic exchange and pyramidal inversion in its cysteine and glutathione adducts†

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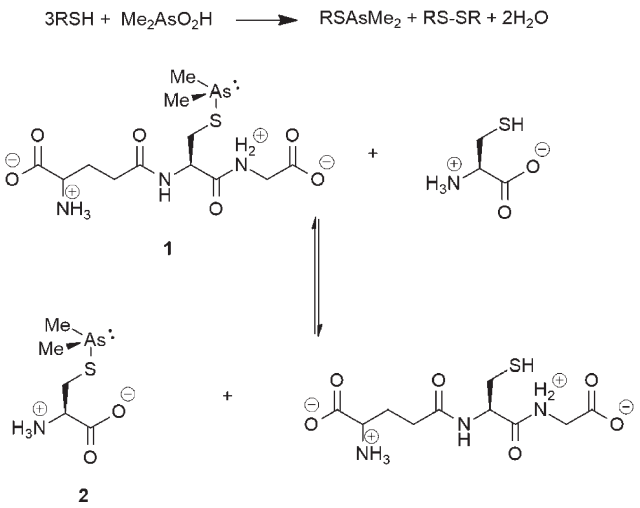
Rapid thiolate exchange of dimethylarsonium, Me_2As^+, is observed between two different thiolate species in solution. NMR is used to characterize the equilibrium constants for interthiol transfer as well the rapid intra molecular conformational dynamics which leads to the coalescence of diastereotopic methyl resonances. These rapid exchange kinetics have important consequences of arsenic's toxicity and pharmacology.

Regardless of its oxidation state or its substitution arsenic and its compounds are to varying degrees universally toxic.[1] Surprisingly though As_4O_6, arsenous oxide, is the FDA approved therapy for acute promyelocytic leukemia (APL).[2] As with many metals and metalloids our current understanding of arsenic detoxification centers on its methyl derivatives, their transport, and localization.[3] In addition to its methylation arsenic binding and transport frequently involves thiols and thiol containing proteins, which reflects arsenic's strong thiophilicity.[4] For example, arsenic(III) compounds form very strong bonds to glutathione, GSH, Scheme 1, and the tris glutathione adduct, $As(SG)_3$, has a high formation constant of log K_f = 32.0.[5] Nevertheless, thiolate exchange even from this tris-thiol adduct by either *meso*-2,3-dimercaptosuccinic acid or British Anti-Lewisite is well established.[6] The mechanisms for these facile exchange reactions have not been examined in any detail, but recent studies for As(III) species suggested that their Lewis basicity is a factor in their lability.[7] However the kinetics of arsenic-thiol exchange remains poorly characterised. In the course of characterising darinaparsin, dimethyl-arsino-glutathione, **1**, and dimethyl-arsino-cysteine, **2**,[8] as part of a program to understand the formers anticancer role in APL,[9] we have discovered examples of rapid thiolate exchange. Herein we report: (1) Equilibrium constants for the rapid exchange of dimethylarsenium groups between cysteine and glutathione; (2) Dynamic NMR studies for the self-exchange of the methyl sites in these dimethylarsenic thiolate adducts; and (3) The dependence of these self-exchange reactions on pH, concentration, and thiol. Taken together these studies lay a foundation for understanding biochemical arsenic lability and transport.



Scheme 1 Synthesis and equilibrium of **1** and **2**. In H_2O at 25 °C.

In aqueous solution GSH rapidly exchanges Me_2As^+ with **2** to give $GSAsMe_2$, **1**. ESI mass spectroscopy reveals the presence of the two dimethylarsenio derivatives in solution, with the peaks at 225.889 and 411.991 *m*/*z* corresponding to **1** and **2**. When handled under nitrogen atmosphere both **1** and **2** are stable for several days at room temperature and during the time course of the NMR experiments described here. In the presence of oxygen there is the slow evolution of cacodylic acid and the corresponding disulfide. In the ^1H NMR spectra of these solutions at room temperature and high field, 500 MHz, Fig. 1, there are total of 4 peaks between 1–2 ppm which correspond to the diastereotopic, non-equivalent methyl resonances of compounds **1** and **2**. Warming this mixture leads to reversible coalescence of first the methyls resonances of the cysteine

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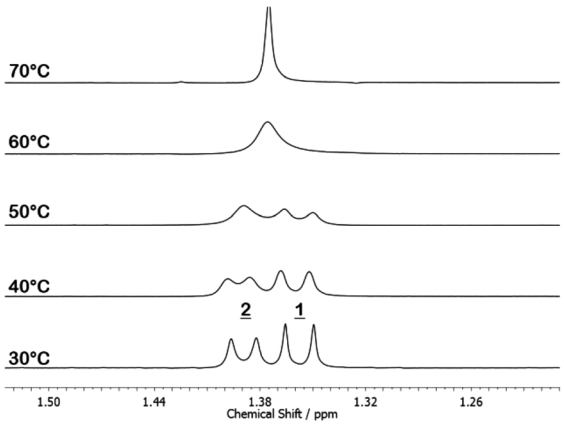


Fig. 1 Variable temperature ^{1}H NMR spectra for the diastereotopic methyl resonances on **1** and **2**. The observed coalescence of the inequivalent methyls is caused by dynamic inter and intramolecular exchange.

derivative, and then at higher temperature the glutathione, and finally all four methyls. Up until 50 °C the ratio of **1** : **2** remains constant. Subsequent titration and integration of related samples gives the equilibrium constant (K_{eq}) for Scheme 1 to be 0.6 and favoring the glutathione derivative. This corresponds to a relatively small free energy difference (ΔG) of 1.4 kJ mol^{-1} between the two species and suggests similar arsenic–sulfur bond energies. Raising the pH to between 5.5 and 7.0 also results in similar spectroscopic changes as shown in Fig. 1. More basic conditions promote methyl site exchange. Attempts to also measure the kinetics of these reactions have been hampered by the lack of useful UV-vis chromophores, in **1** and **2**, and that the reaction occurs in the mixing time (ESI†), of a typical NMR experiment. We conclude that this coalescence is due facile methyl exchange, and to test this facile exchange we opted to perform the study of **2** in isolation.

Individually the ^{1}H and ^{13}C NMR spectra of **1** and **2** are markedly temperature, pH, and concentration sensitive. For example in Fig. 2 the solution spectra for a 5 mM solution of **2** in 0.1 M phosphate buffer at pD = 4.5 exhibited a reversible

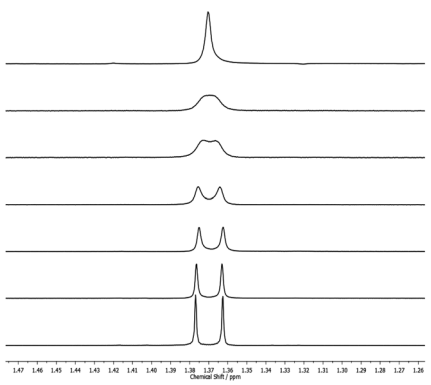
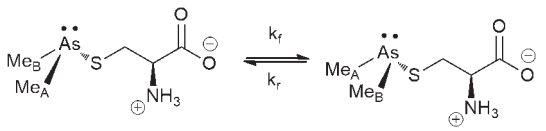


Fig. 2 Variable temperature ^{1}H NMR for the diastereotopic methyl signals in **2** in 0.1 M phosphate buffer at pD = 4.5. Bottom trace is at 10 °C, followed by 20, 30, 40, 45, 50, 55, and 70 °C, at top.



Scheme 2 Methyl site exchange in **2**.

coalescence of the two methyl resonances. Formally this corresponds to the two site exchange shown in Scheme 2, which corresponds to an effective inversion of the arsenic stereochemistry. Although this mechanism may not be the same as interthiol exchange of Me_2As^+, both reactions suggest a markedly unexpected lability for the otherwise thermodynamically stable Me_2As–S bond.

The ^{1}H NMR spectra measurements of **2** were performed on a 5 mM phosphate buffered solution at pH = 4.6 between the temperatures of 270 K to 335 K. The rate constant $(d[\mathbf{2}])/dt = -k_f[\mathbf{2}]$ was calculated using the chemical shift difference between the methyl peaks using the Sandstrom's equation[10] $k = \{\pi/\sqrt{2}\}[\sqrt{(\delta\nu^2 - \delta\nu_\varepsilon{}^2)}]$. Peak width was also used to independently calculate the rate; both methods yielded the same activation parameters.

The data give good linear Arrhenius fits for all data above 10 °C with only slight deviations being found for the temperatures just above the freezing point of water. The activation energy (E_a) is 14 kJ mol^{-1}, indicating that very little energy is required to cause the coalescence. In terms of the activation parameters from the Eyring equation, $\Delta G^\ddagger$ = 73 kJ mol^{-1}, $\Delta H^\ddagger$ = 11 kJ mol^{-1} and $\Delta S^\ddagger$ = −190 J mol^{-1} K^{-1}. The relatively small $\Delta G^\ddagger$ suggests that As–S bond dissociation is an unlikely mechanism as the bond enthalpy of the As–S bond is around 380 kJ mol^{-1}.[11] In addition, the $\Delta S^\ddagger$ is negative indicating a markedly more ordered transition state, this suggests that there might be an associative mechanism for this exchange.

The zwitterionic ionization of the amine and carboxylic acid groups in **1** and **2** play an important role in the mechanism of the exchange. Exchange kinetics as a function of pH and substrate concentration are shown in Fig. 3. The slowest methyl site exchange kinetics correspond to a singly protonated species. This is in accord with a prior potentiometric titration result for **1** which was suggested to be particularly labile at pH ≥ 7.[5] While under basic conditions there may be significant formal dissociation of Me_2As^+ through an associative nucleophilic hydrolysis, for the pH used in these studies there is no substantial build-up of side products or other indications of competing side reactions.

The concentration dependence for the methyl site exchange, as reflected in its coalescence temperature, is shown in Fig. 3b. The marginal decrease in coalescence temperature with increased concentration suggest that in addition to a rapid intramolecular mechanism there is second intermolecular, bimolecular, pathway. This second pathway is consistent with the **1**/**2** exchange results shown in Fig. 1. However, the rate and thus contribution, of this second pathway makes to methyl site exchange is minor compared to basal unimolecular rate of site exchange. There are several mechanisms for methyl

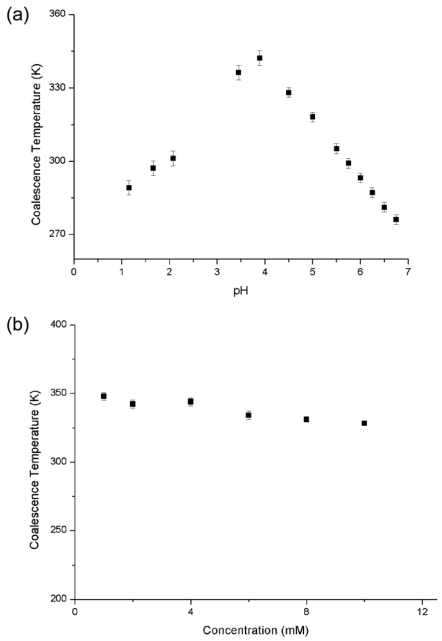
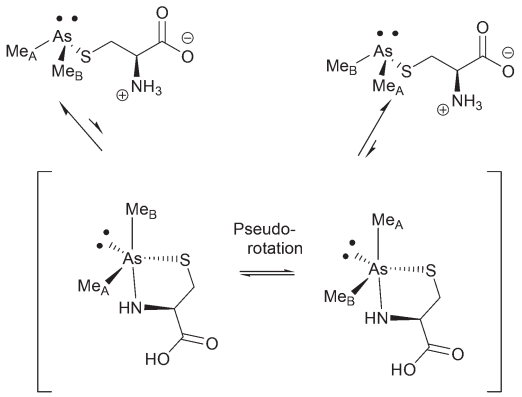


Fig. 3 Dependence of coalescence temperature upon (a) the pH of a 5 mM solution of **2** and (b) concentration of **2** in 5 mM phosphate buffer.

site exchange, with most obvious, a formal inversion of the arsenic geometry, being unlikely. Experimentally, arsenic(III) pyramidal inversion through a trigonal planar transition state has a high barrier, 176 kJ mol^{-1} for PhEtMeAs.[12] Theoretical calculations[13] also suggest these transition states should be in excess of 150 kJ mol^{-1}, which is much higher than our experimentally determined barrier of 80 kJ mol^{-1}. Surprisingly facile racemisation at arsenic of the diastereomeric methylphenylarsinic acid adduct with glutathione was observed by Edmonds *et al.*, and interpreted in terms of an unexpected and unaccountably low inversion barrier.[14]

To account for the rapid methyl site exchange in **1** and **2** we note that As(III) species are of course ambiphilic being potent nucleophiles and ligands as well being as metalloids with latent Lewis acidity. It is this latter character which would allow for an associative chelation of the amine to the arsenic to give a net five coordinate intermediate with four substituents and a stereochemically active lone pair, Scheme 3. For this geometry Berry pseudorotation barriers are expected to be low, and their action will lead to rapid methyl site exchange. This mechanism is in accord with the negative entropy of activation and the rate enhancement at higher pH. The increase in rate at lower pH may be due to a separate acid catalyzed exchange, but the generally low of solubility of these species limits a more extensive study under these conditions.

In conclusion, we have shown that the As–S bond is kinetically labile and can be interact with other thiols in aqueous solutions. In addition to being more stable to oxidation, **1** is 1.4 kJ mol^{-1} more stable than **2** in aqueous solutions. This type of facile thiol exchange has important implications for the activity of methylarsenic species in cells and proteins. Also given the enhanced electrophilicity of the AsO_2 moiety in



Scheme 3 Proposed fluxionality in **2**.

arsenous oxide suggests that its exchange rates may be considerably faster than those reported here. In what may be a helpful analogy, the facile dimethylarsenium transfer reactions discovered here have many parallels with the *trans*-nitrosylation chemistry of the nitrosylated thiolates, RSNO, which have been more extensively studied.[15]

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