Radical Model for Arsenic Toxicity: Experimental EPR Spin Trapping and Theoretical Studies

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Abstract

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Arsenic is one of the most environmentally significant toxins and a great global health concern. Long known for its acute toxicity, arsenic has also been discovered to be a potent carcinogen. Evidence links arsenic toxicity and carcinogenic actions to reactive oxygen species (ROS). In most animal including humans, arsenic undergoes a biomethylation to yield organic arsenic species, long assumed to be a process of detoxification. However, a growing body of evidence suggests that these organic arsenic species, particularly the reduced trivalent intermediates, may be just as if not more toxic than the inorganic analogs. Furthermore, current food safety regulations often monitor only inorganic arsenic levels. There is, therefore, a clear need for more literature and empirical data concerning the toxic mechanisms of arsenic.

In this study, we examined organic and inorganic arsenites capacity to generate ROS when exposed to common oxidizing agents via EPR spectroscopy and spin-trapping with the cyclic nitrone 5,5-dimethyl-1-pyrroline-N-oxide (DMPO). Experimental observations were theoretically rationalized using density functional theory approach.
Introduction

As a naturally occurring element abundant in the earth’s crust, arsenic and its compounds are environmentally pervasive health hazards. Long known for its acute toxicity, arsenic is also understood to act as a potent carcinogen, even at sublevel concentrations. Studies of long-term exposure in humans associate arsenic with skin, bladder, lung, liver, colon, prostate, and kidney cancers.\textsuperscript{1-5} Besides a possible role in causing cancer, arsenic exposure has also been linked to hypertension, cardiovascular diseases, diabetes, and the unique vaso-occlusive Blackfoot disease\textsuperscript{6,7} where undernourished populations are believed to be at a higher risk for developing these conditions.\textsuperscript{8} Hence, the monitoring of levels of arsenic in food, water, and soil is of tremendous importance, particularly in developing countries. The urgency of this global health initiative has renewed interest in arsenic chemistry, particularly for the purposes of elucidating the mechanism of its carcinogenic properties.

Inorganic arsenic in the environment typically exists as the pentavalent $\text{As}^V$ (arsenic acid, $\text{H}_3\text{AsO}_4$) or the trivalent $\text{As}^{\text{III}}$ (arsenous acid, $\text{H}_3\text{AsO}_3$).\textsuperscript{9} Of the two forms, $\text{As}^{\text{III}}$ (or as it is more commonly named by its conjugate base arsenite) is more toxic and mobile and remains in tissues longer.\textsuperscript{10} Once inhaled or ingested, inorganic arsenic is transformed to organic arsenic via a series of enzyme-catalyzed successive reduction and methylation steps (Scheme 1).\textsuperscript{11,12} Initially believed to be a detoxification mechanism, it has been realized that the organic trivalent arsenic species monomethylarsonous acid (MMA$^{\text{III}}$) and dimethylarsinous acid (DMA$^{\text{III}}$) are the most toxic of all arsenic metabolites detected in human and animal urine.\textsuperscript{13-17} Variations at certain points in the human arsenic methyltransferase ($\text{AS3MT}$) gene may cause differences in methylating ability between individuals,\textsuperscript{18} but data suggests a standard profile of urinary arsenic humans as 10-30% inorganic, 10-20% MMA$^{(\text{III}+\text{V})}$, and 60-80% DMA$^{(\text{III}+\text{V})}$.\textsuperscript{19} The acute toxicity
of inorganic As\textsuperscript{V} has been understood in its capacity to replace phosphate in biomolecules and to uncouple ATP \textit{via} so-called arsenolysis. Compounding the lack of information is the apparent paradoxical dual-action of arsenic as both carcinogen and chemotherapeutic agent. Despite the plethora of evidence linking arsenic exposure to the development of various cancers, inorganic arsenite remains one of the most effective treatments for certain cancers, including acute promyelocytic leukemia (APL).\textsuperscript{20-24}

A growing body of evidence suggests that both the apoptosis-inducing antitumor effects and the genotoxic effects of arsenite and other arsenic metabolites are mediated \textit{via} ROS and oxidative stress pathways.\textsuperscript{5, 25-35} Recent evidence also suggests that biomethylation of arsenic may be responsible for the observed carcinogenicity.\textsuperscript{36, 37} However, it is currently not known which specific form (or forms) of arsenite is responsible for the observed antitumor and genotoxic effects due to the various transformations arsenic undergoes \textit{in vivo}. Furthermore, there is a current lack of literature concerning the structural differences between the organic and inorganic arsenites and the effects of structure on capacity to generate ROS. Gaining greater understanding of the electronic properties, reactivities, and differences therein of the inorganic and organic arsenic radical species may help to elucidate arsenic’s toxic mechanisms. We used electron paramagnetic resonance (EPR) spectroscopy and the spin trap 5,5-dimethyl-1-pyrroline-N-oxide (DMPO) to study the free radical activity of As\textsuperscript{III} and DMA\textsuperscript{III} in aqueous solution. We also performed theoretical calculations on the structures and thermodynamics of the arsenic species involved in several proposed mechanisms of oxidation and radical generation as well as theoretical analysis of the spin adducts experimentally generated. We have identified trends in ROS activity that closely correlate to trends in the experimental data as a function of degree of methylation.
Experimental Procedures

**General Computational Methods.** All calculations were performed at the Ohio Supercomputer Center using Gaussian 09. Density functional theory was applied in this study to determine the optimized geometry, vibrational frequencies, and single-point energy of all stationary points. Single-point energies were obtained at the B3LYP/6-31+G** level based on the optimized B3LYP/6-31G* geometries, and the B3LYP/6-31+G**//B3LYP/6-31G* wave functions were used for Natural Population Analyses (NPA). The effect of solvation on the gaseous phase calculations was also investigated using the polarizable continuum model (PCM). The B3LYP level was employed based on previous studies demonstrating the appropriateness of this level for the study of H2AsO radical compared to other more powerful levels of theory. The 6-31G* basis set was chosen for its computational speed and its good agreement with experimentally determined bond lengths and angles for the third row atoms. These basis set calculations used the standard six Cartesian d functions. Vibrational frequency analyses (B3LYP/6-31G*) for each of the stationary points for all arsenic species and for DMPO and its spin adducts yielded no imaginary vibrational frequencies. A scaling factor of 0.9806 was used for the zero-point vibrational energy (ZPE) corrections with the B3LYP/6-31G*.

**Calculation of Isotropic Hyperfine Coupling Constants (hfcc).** The prediction of hfcc of the nitrogen atom in simple nitroxides was demonstrated in several benchmark works. Based on these previous studies, we employed similar models in the prediction of hfcc’s of the nitrogen, β-hydrogen and γ-hydrogens of possible DMPO adducts of radical species produced by the oxidation of AsIII and DMAIII in water. A discussion of the comparative study of the calculated hfcc’s for DMPO-O2H optimized at the B3LYP density functional and basis sets, 6-31+G**, 6-31G*, EPR-II and EPR-III, and the core-valence correlation-consistent cc-pCVDZ.
in the gas and aqueous phases can be found in pages S13-S18 of the Supplementary Material Section of our previous paper. In the same article, the hybrid PBE0 functional and EPR-II basis set was found to yield accurate $a_N$ values in simple nitroxides and was also employed in the calculation of hfcc’s for DMPO-O$_2$H. Although the levels of theory mentioned above gave accurate $a_N$ for 2,2,5,5-substituted pyrrolidine nitroxides, the calculated hfcc’s for DMPO-O$_2$H using the same levels of theory gave hfcc’s comparable to that predicted at B3LYP/6-31+G**//B3LYP/6-31G*. Hence, in this study, the $a_N$, $a_{\beta-H}$ and $a_{\gamma-H}$ for all of the spin adducts were only calculated at the B3LYP/6-31+G**//B3LYP/6-31G* level to show qualitative trends in the hfcc compared to the experimental.

**EPR Measurements.** EPR measurements were carried out on an X-band spectrometer with HS resonator at room temperature. General instrument settings are as follows unless otherwise noted: microwave power, 10 mW; modulation amplitude, 0.5 G; receiver gain 3.17-3.56 x $10^5$, time constant, 82 ms, time sweep 42 s. The hyperfine splittings (hfcc) of the spin adducts were determined by simulating the spectra using an automatic fitting program.

**Spin Trapping Studies.** DMPO, As$_2$O$_3$, cacodylic acid ((CH$_3$)$_2$AsOOH), and 80% H$_2$O$_2$ were all commercially obtained. Dimethylarsinous acid was prepared by allowing dimethyliodoarsine, (CH$_3$)$_2$AsI, to hydrolyze in solution. Dimethyliodoarsine was prepared by reduction of cacodylic acid following a previously established procedure and purity of the product was confirmed by GC/MS. All solutions for spin trapping were prepared using Dulbecco’s phosphate buffered saline solution at pH 7.4. The total volume of each solution used for the EPR measurement was 50 $\mu$L, and was loaded into a 50 $\mu$L quartz micropipette. All samples contain 100 mM arsenite species concentration and 100 mM DMPO in aerobic PBS. Experimental conditions were as follows: DMPO and As$_2$O$_3$; DMPO, As$_2$O$_3$ and 0.2% H$_2$O$_2$;
DMPO, As$_2$O$_3$, 0.2% H$_2$O$_2$ and 3.5 mM Fe$^{II}$; DMPO, As$_2$O$_3$, and 3.5 mM Fe$^{II}$. Experiments were repeated with (CH$_3$)$_2$AsOH in place of arsenite. Negative controls used DMPO in pure PBS. Positive control used DMPO in the presence of H$_2$O$_2$ and irradiation with 254 nm light.

**Results and Discussion**

**Spin trapping.** Spin trapping with DMPO in aqueous solutions of trivalent arsenic yielded various spin adducts, however, no arsenic-centered radicals were detected in this way due to the absence of splitting pattern from $^{75}$As with $I_{As} = 3/2$. Trivalent arsenic is a demonstrably powerful reducing agent and single electron transfers to ROS such as HO’ have been demonstrated to be highly kinetically favorable.$^{62}$

Spin trapping of a solution containing DMA$^{III}$, H$_2$O$_2$, and in the presence or absence of Fe$^{II}$ yielded an acylnitroxide adduct (Figure 1)$^{63}$ that is likely formed from the hydrolysis of superoxide (O$_2^-$) or peroxyl (HO$_2^-$) radical adducts. Spin trapping of a solution containing DMA$^{III}$ and Fe$^{II}$ yielded predominantly HO$^{•}$ and C-centered adduct (Figure 2).$^{65}$ Spin trapping of a solution containing DMPO and DMA$^{III}$ yielded HO’ as the major species with minor species of alkyl radical adduct (Figure 3). Spin trapping of a solution containing As$^{III}$, H$_2$O$_2$, and Fe$^{II}$ yielded predominantly HO’ adduct and C-centered adduct, with open chain nitrone$^{63,66}$ and HO$_2^{•}$ adduct as minor species (Figure 4). The presence of the open chain nitrone is suggested as the source of the carbon-centered adduct.$^{67}$ Spin trapping of a solution containing As$^{III}$ and H$_2$O$_2$ yielded mostly HO’ adduct, and also open chain nitrone and C-centered adduct (Figure 5). Spin trapping of a solution containing As$^{III}$ and Fe$^{II}$ yielded mostly HO’ radical adduct and C-centered adduct, with minor products being open chain nitrone and HO$_2^{•}$ adduct (Figure 6). Spin trapping of a solution containing DMPO and As$^{III}$ gave mostly HO’, with a minor species of C-centered
adduct and possible open chain nitrone (Figure 7). We found no evidence of adducts formed between the nitrone and any As-containing radical compounds. We propose that the trapped ROS’s were generated as byproducts of a rapid oxidation of trivalent to pentavalent arsenic.

The appearance of open-chain nitrones and C-centered adducts is proposed to be the result of reductive DMPO•-OOH decomposition. This decomposition has been shown to occur via a ring-opening mechanism, leading to carbon-centered radicals. Once formed, such radicals would be rapidly trapped. Decomposition analysis of adducts formed in a solution containing As^{III}, H_{2}O_{2}, and Fe^{II} supports this hypothesis as O_{2}− adduct decomposes to form the open chain nitrone (Figure 8). However, one would expect a proportionate formation of C-centered radical adduct with ring-opening, but an inverse relationship exists there too, with C-centered adduct being present in detectable quantity only for the first 200s of the experiment.

**Theoretical Studies.** Theoretical analysis of the electronic structures of various proposed As^{IV} radical intermediates was carried out at the PCM/B3LYP/6-31G**//B3LYP/6-31G* level of theory (Figure 7). For a more systematic model of the effect of methylation on arsenic toxicity, monomethyl arsenic species were included in the calculations. Trends in structure and reactivity were readily observed. The neutral tetravalent species (formed by one electron oxidation of the corresponding trivalent species via radical addition of hydroxyl radical to the As atom) exhibited a see-saw molecular geometry with methyl groups always located equatorially. An analysis of electron spin-density revealed the axial oxygen atoms to have greater spin density compared to the nonexistent spin on the equatorial oxygens (as in As^{III} and MMA^{III}). Additionally, increasing methylation character was proportional to an increase in spin density on the As atom. This may be explained by the increased As-OH axial bond lengths with methylation character caused by the bulky equatorial methyl groups and by the electron donating effect of the methyl groups. The
cationic tetravalent intermediates exhibited the same trend in increasing As spin density with increasing methyl substitution, although spin density is evenly distributed across the oxygen atoms arranged in a trigonal pyramidal molecular geometry.

We examined (at the same level of theory) the mechanisms of generation of the tetravalent arsenic radical intermediates and subsequent oxidation to the pentavalent arsenics with radical products including superoxide and hydroperoxyl radical. The mechanisms examined used common oxidants including O₂, O₂⁻, and H₂O₂/HO⁺ which have been previously established as causal agents in the oxidation of As^{III} to As^{V}, with As^{IV} the proposed intermediate. The mechanisms and results, shown in Tables 1-3, reveal an important trend: increasing methyl character of the starting trivalent arsenic species is proportional to increasing exoergicity of reaction. Thus, oxidation from trivalent to pentavalent arsenic and subsequent generation of reactive oxygen species byproducts is more energetically favorable with increasing methyl substitution.

Increasing evidence suggests that the organic trivalent arsenics are the most toxic of all common arsenic species. It has been proposed that this increased toxicity is due to the high membrane permeability of organic trivalent arsenics, with DMA^{III} being more permeable than MMA^{III} or As^{III}. However recent studies present evidence that non-methylating urothelial cells are less susceptible to As^{III} exposure than methylating heptocytes. This is interesting because it means that the methylation occurs only after the inorganic arsenite has already permeated the membrane and is inside of the cell, suggesting that the increased toxicity of the methylated arsenites is due to its intrinsic toxic character rather than greater membrane permeability. Recent experiments have also shown that hepatocytes of catalase-knockout mice exposed to H₂O₂ or MMA^{III} exhibit significantly higher occurrences of DNA strand breaks than
in wild type mice with the intact gene for catalase (catalase catalyses the conversion of \( \text{H}_2\text{O}_2 \) to oxygen and water, thus limiting the production of damaging hydroxyl radical).\(^{75}\) In the same study, EPR spin trapping with DMPO of the cell contents showed the presence of •OH in hepatocytes of mice exposed to \( \text{H}_2\text{O}_2 \) or MMA\(^{\text{III}}\), the signal for which was greatly reduced in wild type mice versus catalase-knockout mice. This suggests that the genotoxic effects of organic arsenite exposure are mediated by ROS, in particular •OH. In consideration of recent studies that link arsenic exposure to oxidative stress and ROS generation, we hold our study to be a timely and relevant contribution. Our findings show that arsenites generate various ROS when exposed to common oxidants in solution. Both \( \text{H}_2\text{O}_2 \) and \( \text{O}_2 \) have previously been demonstrated to oxidize As\(^{\text{III}}\) to As\(^{\text{V}}\) and DMA\(^{\text{III}}\) to DMA\(^{\text{V}}\)\(^{68, 76}\) and our theoretical calculations suggesting a basis for the increased redox activity of methylated arsenites can be applied to the observed trends in arsenic methylation and toxicity. That methylation appears to localize electron spin density on arsenic centered radicals may explain the increasing exoergicity of radical oxidation proportional to increasing methyl substitution of arsenite. In turn the increasing exoergicity of methylated arsenites oxidation in water and thus increasing ROS production may help to explain the trends in arsenic toxicity. Of additional interest is the application of inorganic arsenite as an effective chemotherapeutic agent in the treatment of acute promyelocytic leukemia,\(^{20, 22}\) which may seem counterintuitive as arsenite is known to be a potent human carcinogen. However, evidence suggests that the dose-response relationship induced by arsenic exposure is not linear, rather it is biphasic. Thus at low concentrations arsenite acts as a carcinogen, causing tumorigenesis by stimulating transcription factors that express genes promoting cell proliferation and malignant transformation and causing apoptosis at higher concentrations.\(^{77, 78}\) However, at higher concentrations arsenic acts to promote apoptosis by causing genomic instability.\(^{79, 80}\) More recent
evidence pinpoints at least one biphasic mechanism by which arsenic acts to promote and suppress proliferation with increasing concentrations: in human keratinocytes exposed to low levels or arsenite, there is inactivation of the tumor suppressor protein p53 (an inhibitor of survivin) and correspondingly heightened levels of survivin (mediator of apoptosis resistance, highly expressed in cancer cells) while higher concentrations sees the opposite effect: high activation of p53 and negative regulation of survivin. However, it must be noted that these studies cannot pinpoint the exact species (or multiples species) of arsenic (be it inorganic or biomethylated, tri- or penta-valent) responsible for these effects. For example, the urine of acute promyelocytic leukemia patients treated with inorganic arsenite has been found to contain considerable levels of methylated arsenic.

Another study compared gene expression and cell viability between iAs\textsuperscript{III}, MMA\textsuperscript{III}, and DMA\textsuperscript{III} in human keratinocytes.\textsuperscript{83} After analyzing trends in cell proliferation, apoptosis, and the expression of genes promoting cellular oxidative defense, apoptosis factors and growth factors, the study’s authors concluded that MMA\textsuperscript{III} was the most cytotoxic species (LC\textsubscript{50} = 2.8\(\mu\)M), followed by DMA\textsuperscript{III} (LC\textsubscript{50} = 5.6\(\mu\)M), and then iAs\textsuperscript{III} (LC\textsubscript{50} = 17\(\mu\)M). In human bladder cancer cells, treatment with DMA\textsuperscript{III} or DMMTA\textsuperscript{V} (dimethylmonothioarsinic acid) drastically reduced GSH and p53 levels compared to control cells, while iAs also decreased p53 it was not as drastic as DMA\textsuperscript{III} and treatment actually increased GSH levels compared to control.\textsuperscript{15} One should not compare these results with the previously described study in human keratinocytes as the cancer cells have already demonstrated a compromised oncosuppressive system so further toxic insult is not likely to produce the response seen in normal cells exposed to high levels of arsenic. The Authors of this study suggest that the increased toxicity of the organic arsenicals was occurring via a cycle of GSH depletion from catalyzed reduction of DMA\textsuperscript{V} to DMA\textsuperscript{III} and subsequent
regeneration of the trivalent form. Our theoretical results corroborate this finding, we found the oxidation of DMA$_{\text{III}}$ to DMA$_{\text{V}}$ to be highly exoergic in multiple redox pathways. Our results also suggest a similar ROS speciation between iAs$_{\text{III}}$ and DMA$_{\text{III}}$, with no difference in signal intensity (as would be expected from our calculations, the oxidation from tri to pentavalent arsenical is highly exoergic for both species).
Scheme 1. Biomethylation of arsenic in humans. The reduction steps are catalyzed by $\text{As}^\text{V}$ reductase, the oxidative methylation steps are catalyzed by $\text{As}^\text{III}$ methyltransferase, with $S$-adenosyl methionine (SAM) as the methyl donor demethylated to $S$-adenosyl-$L$-homocysteine (SAH). Reduced glutathione (GSH) serves as the electron donor.\textsuperscript{17}
Figure 1. Simulated (top) and experimental (bottom) EPR spectrum of a solution containing DMPO, DMA$^{\text{III}}$, and H$_2$O$_2$ showing a spectral profile and splitting parameters characteristic of the oxidized DMPO radical adduct DMPOX: $a_N = 9.1$ G, $a_{\beta-H} = 5.1$ G, $a_{\gamma-H} = 5.0$ G.
Figure 2. (a) Experimental and (b) simulated EPR spectrum of a solution containing DMPO, DMA$^{\text{III}}$, and Fe$^{\text{II}}$. Simulation of the spectrum components (c) hydroxyl radical adduct (76.4%): $a_N = 15.1$ G, $a_{\beta-\text{H}} = 14.5$ G; (d) alkyl radical adduct (23.6%): $a_N = 15.5$ G, $a_{\beta-\text{H}} = 22.5$ G.
Figure 3. (a) Experimental and (b) simulated EPR spectrum of a solution containing DMPO and DMA\textsuperscript{III}. Simulation of the spectrum components (c) hydroxyl radical adduct (81.6\%): $a_N = 15.0$ G, $a_{\beta,H} = 14.5$ G; (d) alkyl radical adduct (18.4\%): $a_N = 14.5$ G, $a_{\beta,H} = 22.8$ G.
Figure 4. (a) Experimental and (b) simulated EPR spectrum of a solution containing DMPO, As$^{\text{III}}$, H$_2$O$_2$, and Fe$^{\text{II}}$. Simulation of the spectrum components (c) hydroxyl radical adduct (32.2%): $a_N = 15.9$ G, $a_{\beta-H} = 15.7$ G; (d) alkyl radical adduct (27.5%): $a_N = 16.2$ G, $a_{\beta-H} = 23.0$ G; (e) open chain-nitron (30.6%): $a_N = 15.8$ G, $a_{\beta-H} = 2.1$ G; (f) hydroperoxyl radical adduct (9.7%): $a_N = 14.6$ G, $a_{\beta-H} = 9.8$ G, $a_{\gamma-H} = 1.4$ G.
Figure 5. (a) Experimental and (b) simulated EPR spectrum of a solution containing DMPO, As$^{\text{III}}$, and H$_2$O$_2$. Simulation of the spectrum components (c) hydroxyl radical adduct (37.5%): $a_N = 15.9$ G, $a_{\beta-H} = 16.2$ G; (d) open chain-nitron (31.6%): $a_N = 15.5$ G, $a_{\beta-H} = 2.5$ G; (e) alkyl radical adduct (30.9%): $a_N = 16.1$ G, $a_{\beta-H} = 22.9$ G.
Figure 6. (a) Experimental and (b) simulated EPR spectrum of DMPO, As$^{III}$, and Fe$^{II}$ in aqueous solution. Simulation of the spectrum components (c) hydroxyl radical adduct (44.3%): $a_N = 15.9$ G, $a_{\beta-H} = 15.9$ G; (d) alkyl radical adduct (37.4%): $a_N = 15.8$ G, $a_{\beta-H} = 22.8$ G; (e) open chain-nitronate (13.3%): $a_N = 15.4$ G, $a_{\beta-H} = 3.4$ G; (f) hydroperoxyl radical adduct (5.0%): $a_N = 15.6$ G, $a_{\beta-H} = 10.3$ G, $a_{\gamma-H} = 2.2$ G.
Figure 7. (a) Experimental and (b) simulated EPR spectrum of DMPO and As$^{III}$ in aqueous solution. Simulation of the spectrum components (c) hydroxyl radical adduct (72.4%): $a_N = 16.0$ G, $a_{\beta-H} = 16.2$ G; (d) alkyl radical adduct (20.4%): $a_N = 15.4$ G, $a_{\beta-H} = 23.1$ G; (e) open chain-nitron (7.2%): $a_N = 15.3$ G, $a_{\beta-H} = 2.8$ G.
Figure 8. Decomposition of the various radical adducts in a solution containing DMPO, As$^{\text{III}}$, H$_2$O$_2$, and Fe$^{\text{II}}$. Notice the decomposition of DMPO'-OOH adduct in relationship to formation of the open-chain nitrone.
**Figure 7.** Aqueous phase bond distances, spin and charge (in parentheses) densities of various radicals at the PCM/B3LYP/6-31+G**//B3LYP/6-31G* level. Note the trend of increasing spin density on the As atom with increasing methyl substitution. Also note the absence of spin density on the equatorial oxygen atoms on the seesaw geometries of As$^{IV*}$ and MMA$^{IV*}$. 
Table 1. Free energies (in kcal/mol) of hydroxyl radical-initiated radical oxidation of arsenites at the PCM/B3LYP/6-31G**//B3LYP/6-31G* level of theory.

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\[ \text{As}^{\text{III}} \xrightarrow{\text{OH}} \text{MMA}^{\text{III}} \xrightarrow{\text{O}_2} \text{DMA}^{\text{III}} \]

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Table 3. Free energies (in kcal/mol) of oxygen-initiated radical oxidation of arsenites at the PCM/B3LYP/6-31G**/B3LYP/6-31G* level of theory.

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\begin{array}{c|c|c|c|c|c|c}
 & As^{III} & MMA^{III} & DMA^{III} & As^{V} & MMA^{V} & DMA^{V} \\
R₁, R₂ & & & & & & \\
\hline
OH,OH & \downarrow & \downarrow & \downarrow & \uparrow & \uparrow & \uparrow \\
\hline
\Delta G_{298K, I + II + III} & 8.6 & -2.6 & -7.9 \\
\end{array}
\]
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