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Confusion between dimethyl selenenyl sulfide and dimethyl selenone released by bacteria

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Dimethyl selenone [$(CH_3)_2SeO_2$] has been reported in the literature as a metabolite released by bacteria in contact with selenium metal or selenium salts. In this study, mass spectral, chromatographic, and boiling-point data are presented that show that dimethyl selenone has been confused with dimethyl selenenyl sulfide (CH_3SeSCH_3). In addition, the headspaces above monocultures of selenium-resistant bacteria were examined using gas chromatography followed by fluorine-induced chemiluminescence detection. A number of alkyl sulfur and selenium species were detected, along with dimethyl selenenyl sulfide. A pathway from oxidized selenium salts to reduced methylated selenides and dimethyl selenenyl sulfide is also presented.

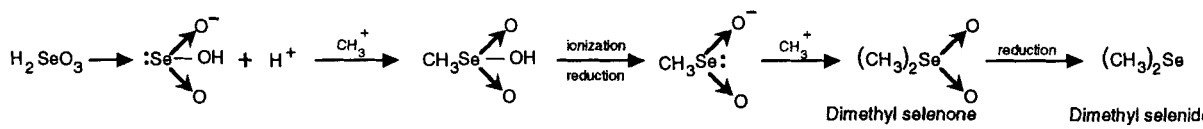
Keywords: Bioremediation, biodegradation, dimethyl selenenyl sulfide, biomethylation, pathway, chemiluminescence, micro-organism

INTRODUCTION

The interaction of selenium-resistant micro-organisms with selenium has been reported to yield dimethyl selenone or methyl methylselenite, $(CH_3)_2SeO_2$.[1–3] However, it appears that the compound that has been detected may actually be dimethyl selenenyl sulfide, CH_3SeSCH_3. Data are presented here that support the conclusion that $(CH_3)_2SeO_2$ and CH_3SeSCH_3 have been previously confused in reports published in the literature. This paper also reports the simultaneous detection of dimethyl selenenyl sulfide and a number of sulfur and selenium species in the microbial headspace above different bacterial monocultures.

The detection of the volatile products from the interaction of micro-organisms with toxic metalloids such as selenium and tellurium was reported in the first half of the 20th century;[4,5] however, the mechanism by which micro-organisms biomethylate these toxic materials is still not well understood. Challenger put forth a mechanism over 40 years ago that can be described as a series of ionization, methylation and reduction steps leading from an oxidized polyatomic anion of selenium to dimethyl selenide (DMSe)[6,7] (Scheme 1). It is important to note that $(CH_3)_2SeO_2$ is included as the last and possibly only volatile intermediate before fully reduced dimethyl selenide, the other components being ionic species in solution.

More recently, there has been speculation on an augmentation of this mechanism to include a possible pathway to dimethyl diselenide (DMDSe) that relies on a reversible oxidation/reduction step,[1,8] (Scheme 2). Again, these authors invoked $(CH_3)_2SeO_2$ as a possible intermediate and, indeed, they reported the detection of $(CH_3)_2SeO_2$ (as either dimethyl selenone or methyl methylselenite) as well as DMSe and DMDSe in gases released from sewage samples that were purged with high-purity nitrogen or air. Their analytical system consisted of packed-column gas chromatography (15% Carbowax 20M on 60/80 mesh Chromosorb-W, 1.7 m long by 21 mm i.d.) interfaced with a microwave discharge detector. The peak that they assigned to dimethyl selenone eluted between DMSe and DMDSe in their chromatograms. Their chromatographic conditions yielded peak elution orders that follow increasing boiling point for compounds of similar structure.[8] The confirmation of



Scheme 1

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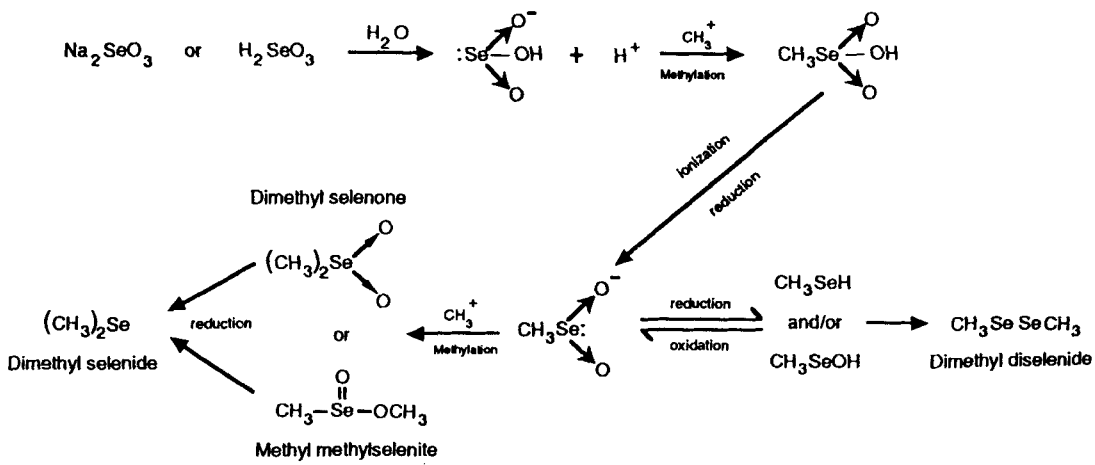


Scheme 2

$(CH_3)_2SeO_2$ by Reamer and Zoller was based upon comparison of the GC MS spectrum with the mass spectrum of dimethyl selenone in the literature.[9] Reamer and Zoller's is almost certainly the first report of dimethyl selenone release from a biomethylating system; however, Reamer and Zoller also noted the detection of a compound at the same retention time in previous work using similar chromatographic conditions[10] and speculated that this compound was also $(CH_3)_2SeO_2$.[1]

Since this initial report, dimethyl selenone has been reported by other workers: Jiang and coworkers reported the detection of dimethyl selenone downwind from a sewage plant, a smelter, and a lake in Belgium;[2,3] however, the identification of this compound was not based upon a reagent standard but instead was made by boiling-point considerations.[11,12] These workers used a gas-chromatographic system (2 m, 2 mm i.d., glass column, packed with 10% OV-101) coupled to an atomic absorption spectrometer. Similarly to the initial report in the literature, dimethyl selenone was reported to elute between DMSe and DMDSe under the chromatographic conditions used;[12] again, this chromatography yielded an elution order that reflected boiling-point relationships.

This work with selenium-resistant bacterial cultures dosed with selenate and selenite salts has also yielded detection of DMSe and DMDSe in the headspace above these organisms. In addition, a compound has been detected that elutes between DMSe and DMDSe in the chromatographic conditions used. However, the data suggest that this compound is not dimethyl selenone, but rather dimethyl selenenyl sulfide, CH_3SeSCH_3.

EXPERIMENTAL

Gas chromatography/mass spectrometry

The GC MS system used in this work consisted of a Hewlett–Packard 5890 gas chromatograph directly interfaced to a Hewlett–Packard 5988 A mass spectrometer. The 70-eV electron impact ionization spectra were collected by a Hewlett–Packard Chemstation Data System. The column used in all of the GC MS experiments was a Hewlett–Packard Ultra 1 capillary column coated with cross-linked 5% phenylmethylsilicone; the 25 m capillary had a 0.31 mm i.d. and a 0.52 μm film. Except for a thinner film, this column is the Hewlett–Packard analog of the J&W Scientific DB-5 column used in the experimentation with the chemiluminescence detector.

Chemiluminescence detection

The chromatographic column used in this work was a DB-5, 30-m capillary with a 0.25-mm i.d. and 1-μm film (J&W Scientific, Folsom, CA, USA). The chromatograph was a Hewlett–Packard model 5890 with a Sievers Research Model 300 Sulfur Chemiluminescence Detector. In this instrument, molecular fluorine is produced in-line by a high-frequency electrical

discharge of a stream of SF_6. The dissociation of SF_6 and subsequent formation of molecular fluorine in this system is aproximately 10%.[13] Reaction of fluorine with an analyte in the gas phase results in chemiluminescence, which is detected by a red-sensitive photomultiplier tube (PMT, Hamamatsu model no. 42228, peak sensitivity at 600 nm) using photon-counting electronics. No filter between the PMT and reaction cell was used in this work. The pressure in the reaction cell was <1 torr (as measured by a calibrated capacitance manometer) and the residence time for gases in the cell was calculated to be approximately 100 ms. Excess fluorine and hydrogen fluoride produced in the reaction chamber are removed by a trap containing charcoal and Ascarite. The interface between the gas chromatograph and the detector was a heated nickel transfer line maintained at 150 °C. The capillary column was inserted through this transfer line directly into the reaction chamber of the detector. This detector is selective in that it does not respond to normal alkanes or atmospheric permanent gases, and only very poorly to alkenes; however, it responds very sensitively to alkyl sulfur and alkyl selenium compounds with routine detection limits in the lower-picogram range.

Microbial incubations

Bacterial cultures used in this work were collected at Kesterson and Volta Reservoirs.[14] Cells were grown overnight under aerobic conditions at 28–30 °C in R2A medium.[15] The following morning these cells were diluted with R2A in tubes with Teflon-lined septa, 1 ml of cells into a final volume of 2.5 ml, some with the addition of 1 mM selenium(VI) as the potassium or sodium selenate salt. The tubes were then incubated at 28–30 °C until they were analyzed (16–24 h). Details of the bacteria are given later in this paper.

Culture sampling procedure

The cultures grown for this study were usually 2.5-ml samples in 4-ml vials. For GC analysis, 1 ml of gas was removed from the vials by gas syringe. Since the gas chromatography involved a capillary column and low-boiling-point compounds, cryogenic trapping was used to freeze the samples at the head of the column upon injection: headspace gas samples from biological cultures were injected through a hot injector (263 °C) and cryogenically trapped on the capillary column maintained at −20 °C for 1 min prior to a fast temperature ramp of 20 °C min^{-1}.

Sample vials inoculated with a particular bacterial strain and medium, with no selenate salts added, were run with each group of samples as controls. This established which gases detected in the headspace were the result of the addition of metalloid salts and which were merely methylation products from medium components. Samples of headspace were removed by syringe after the desired phase of growth was obtained, and they were immediately analyzed. Sealed samples whose septa were pierced once by sampling were generally not sampled again in order to avoid analyzing air-contaminated vials. Syringe blanks were frequently run to ensure that there was no carry-over between sample injections.

In vitro synthesis of dimethyl selenenyl sulfide, methanethiol and methaneselenol

Dimethyl selenenyl sulfide was prepared by reaction of dimethyl disulfide and dimethyl diselenide with acidified, powdered zinc (Baker Analyzed Grade, J. T. Baker, Phillipsburg, NJ, USA); 25 μg of both DMDS and DMDSe (Strem Chemicals, Newburyport, MA, USA) were pipetted together into a glass vial. After addition of 500 mg of zinc powder, this mixture was acidified to pH 2 with aqueous 4 M HCl, sealed with a Teflon® lined septum and allowed to react for 10 min at room temperature. Then 1 ml of gas from this headspace was removed from the vial by a gas syringe and analyzed by GC MS. Methanethiol and methaneselenol were also produced in this reaction and their mass spectra were recorded in the same GC MS runs. The retention times of the species generated in this reaction and recorded in the total ion chromatogram of the GC MS were used for the identification of all the compounds unavailable from other sources. These included methanethiol, methaneselenol, and dimethyl selenenyl sulfide.

The disproportionation of a mixture of DMDSe and DMDS to produce dimethyl selenenyl sulfide was studied by mixing headspace gases from above solutions of these two reagents diluted with acetonitrile (Spectro Grade, Burdick & Jackson, Muskegon, MI, USA). Portions (25 μl) of each chalcogen reagent were separately pipetted directly into glass vials sealed with Teflon-lined septa, each containing 1 ml of acetonitrile. The concentrations of these diluted solutions were

Table 1 Peak-by-peak comparison of the mass spectra of alkyl selenide compounds

This work		Zoller (1978)		Rebane[9] [a]	
Peak *m/z*	Intensity (%)	Peak *m/z*	Intensity (%)	Peak *m/z*	Intensity (%)
142	100	142	100	127	100
127	60	127	60	142	87
140	59	140	48	15†	79
93	41	93*	39	97	78
125	37	125	28	95	77
144	25	94*	26	93	75
94	24	144	22	111	73
95	23	95*	20	94	63
138	20	138	19	125	49
139	19	91*	18	109	48

[a] i.e. $(CH_3)_2SeO_2$.
† *m/z* 15 mass peaks not reported in the other two spectra. * Denotes peaks corrected.

approximately 0.02 g ml^{-1}. After complete mixing, 1 ml of the headspace gas above each solution was removed by gas syringe and injected into the same 16 mm × 120 mm glass test-tube with a resealable Teflon-lined septum. After 15 min, 1 ml of this gas-phase reaction mixture was removed with a clean gas syringe and analyzed by GC with chemiluminescence detection. This experiment was performed with the reaction test-tube pre-purged either with nitrogen or oxygen before the introduction of the gas-phase reagents.

RESULTS AND DISCUSSION

Table 1 lists the 10 most prominent *m/z* peaks in order of relative abundance in the GC MS spectrum of the chromatographic peak that elutes betwen DMDS and DMDSe in the capillary chromatography reported here, Reamer and Zoller's GC MS spectrum of the compound identified as dimethyl selenone,[8] and Rebane's reference spectrum of dimethyl selenone.[9] Errors were found in the peak assignments for *m/z* peaks 93, 94, 95 and 91 in Reamer's spectrum; these have been correctly reported in Table 1 and are indicated with asterisks.

An important aspect of this comparison of the mass spectra, and where it is believed that the confusion starts, is the fact that the nominal molecular mass of both dimethyl selenone and dimethyl selenenyl sulfide is 142 (based on the most abundant ^{80}Se isotope). The most significant difference between these spectra is the base peak comparison: in the literature spectrum of dimethyl selenone the base peak and the parent peak (molecular ion) are not at the same mass-to-charge ratio. In Rebane's spectrum, the base peak is *m/z* 127 and the parent peak, *m/z* 142, is 87% of this intensity (relative abundance). (Although listed, the *m/z* 15 peak is of no relevance in this comparison because in neither of the other spectra are *m/z* values so small reported. This peak is therefore marked with a dagger (†) to denote this in Table 1.) In our spectrum and in Reamer and Zoller's spectrum the base peak and the parent peak are at the same *m/z*, and in both of these the base/parent is followed by the *m/z* 127 peak at 60% of the parent peak intensity. The next three most abundant peaks are in the same order (though different intensities) in both spectra. Small differences are apparent for the last five peaks in the table but the differences in intensities for the same *m/z* value are never more than 3% of the base peak. These last *m/z* peaks and intensities also strongly contrast with the peaks of the authentic dimethyl selenone spectrum. The Reamer and Zoller mass spectrum and the mass spectrum determined for this work are quite similar, yet they are quite different from the published literature spectrum of dimethyl selenone and methyl methylselenite. Furthermore, the mass spectrum of dimethyl selenenyl sulfide recorded here is comparable with the mass spectrum of structurally similar dialkyl selenides and sulfides that have been reported in the literature.[16–18]

Rebane synthesized dimethyl selenone for his mass-spectral work.[9] His reported melting point for $(CH_3)_2SeO_2$ is 152–153.5 °C. He did not report a boiling point. He has also recorded the

mass spectrum of methyl methylselenite, $CH_3Se(O)OCH_3$.[19] The spectrum of the methyl ester is almost identical to his published dimethyl selenone spectrum. No melting or boiling point for this compound was reported in his work; however, other researchers have synthesized and reported the boiling point of the sulfur analogs of these two selenium-containing compounds. Dimethyl sulfone (b.p. 238 °C) is available commercially and the boiling point of methyl methanesulfinate (b.p. 45.5 °C at 18 mm pressure, about 147 °C at 1 atm) has been reported in the literature.[20] Although it is probable that the analogous selenium compounds have this same order of boiling points, the ester with the lower boiling point of the pair, by analogy it is improbable that methyl methylselenite boils below 225 °C. These melting and boiling point data are obviously applicable to the chromatographic elution order of these compounds. The chromatographic conditions reported by Reamer and Zoller[8] and Jiang *et al.*[21] yield peak elution order roughly based on boiling point for compounds of similar structure. These workers report the elution of what they have identified as dimethyl selenone between dimethyl selenide and dimethyl diselenide.[8,12] A dimethyl selenone *melting point* of 153 °C, as reported by Rebane, renders unlikely an elution between two compounds of the boiling points of DMSe (b.p. 58 °C) and DMDSe (b.p. 154 °C) under these chromatographic conditions. Likewise, the estimated boiling point of methyl methylselenite derived above rules it out as a candidate for chromatographic elution between dimethyl selenide and dimethyl diselenide.

The chemiluminescence detection system that was used for this work detects reduced sulfur and reduced selenium compounds simultaneously in the same chromatogram.[22] Given the nonpolar chromatographic phases used, the peak order for this family of alkyl sulfur and selenium compounds follows boiling-point trends. In order from earliest to latest elution, the elution order of these compounds was routinely found to be CH_3SH, CH_3SeH, CH_3SCH_3, CH_3SeCH_3, CH_3SSCH_3, CH_3SeSCH_3, $CH_3SeSeCH_3$, and CH_3SSSCH_3. The elution order for these compounds on this kind of liquid chromatographic phase (nonpolar) has also been reported by other workers,[23] and duplicated this order. The elution of dimethyl selenenyl sulfide in both chromatographic systems reported in this work was intermediate between dimethyl disulfide (b.p. 109 °C) and dimethyl diselenide (b.p. ~154 °C). The boiling point of dimethyl selenenyl sulfide has not been reported in the literature; however, based on the chromatographic data, it is estimated that the boiling point of dimethyl selenenyl sulfide at 1 atm is approximately 132 °C. This is the first boiling-point estimation for dimethyl selenenyl sulfide that has been reported.

The microwave discharge detector used in Reamer and Zoller's work has a monochromator tuned to the 196 nm emission line of selenium to monitor this element in the plasma,[8] and specifically detects selenium-*containing* compounds. The detection system used in the work by Jiang *et al.* was a graphite furnace atomic absorption spectrometer (AA), also tuned to a spectral line for selenium, 196 nm.[21] Both of these detection systems will respond to a molecule like dimethyl selenenyl sulfide that contains both a selenium and a sulfur moiety, as well as to one containing selenium and oxygen moieties. It is also important that the reported chemical sources of these emissions were sulfur-rich environments such as smelters, sewage sludge, sewage plants, etc. These systems would also be strong sources of methylated sulfur species and provide the conditions for the production of dimethyl selenenyl sulfide.

The biological systems examined in Reamer and Zoller's work were sewage and soil samples containing polycultures. All of the work reported here is with cultures containing one organism at a time. A chromatogram of the headspace from an organism that produces dimethyl selenenyl sulfide has been published elsewhere.[22] Table 2 summarizes the experimental screening of some selenium-resistant bacteria isolated from Kesterson and Volta Reservoirs, California, USA[14] grown in the presence of 1 mM sodium selenate for 24 h. Kesterson Reservoir is a selenium pollution site; Volta Reservoir is a low-selenium site near Kesterson Reservoir that has a low but significant level of selenium-resistant bacteria. Along with the genus and species designation (when available) is a relative scaling of the concentrations of selenium compounds in the headspace above these organisms (see Table 2 footnote for experimental details). Also included is a listing of the major sulfur gases detected simultaneously in the same samples. In every sample reported here except one, CH_3SeSCH_3 was detected in the presence of methanethiol and often other sulfur compounds found in the headspace. It is probable that this determination of dimethyl selenenyl sulfide would be interpreted by other selenium-specific

Table 2. Selenium and sulfur gases detected in headspaces of bacterial cultures[a]

Bacterial strain	CH_3SeH	CH_3SeCH_3	$CH_3SeSeCH_3$	CH_3SeSCH_3	Major sulfur gases detected
Aeromonas sp. VS6	0	+	+	+	
Citrobacter freundii KS8	+	++	+++	+	CH_3SH CH_3SSSCH_3
Pseudomonas aeruginosa VS7	+	++	+	+	CH_3SH CH_3SCH_3
Pseudomonas cepacia KS5	+	+++	+++	0	CH_3SH
Pseudomonas fluorescens K27	0	+++	+++	+	CH_3SH CH_3SSCH_3
Pseudomonas sp. VW1	+	+++	+++	+	CH_3SH

[a] Each culture was incubated 24 h in R2A medium supplemented with 1 mM sodium selenate, and then headspace gases were analyzed. Volatile selenium gases were scored: 0, below detection limit (<7 ppbv); +, 7–50 ppbv; ++, 51–100 ppbv; +++, >100 ppbv (ppbv = parts per 10^9, by volume).

methods as $(CH_3)_2SeO_2$. It is very probable that the compound detected by Reamer and Zoller[1] in their work with soil and sewage samples, by Jiang *et al.* in their sampling of emissions from sewage and smelter plants,[2,3] and in this work with monocultures of selenium-resistant bacteria is dimethyl selenenyl sulfide.

The *in vitro* production of dimethyl selenenyl sulfide by the reaction of dimethyl disulfide and dimethyl diselenide in a reducing environment can be represented by Scheme 3.

$$CH_3SSCH_3 + CH_3SeSeCH_3 \xrightarrow[HCl]{Zn} CH_3SeSCH_3$$

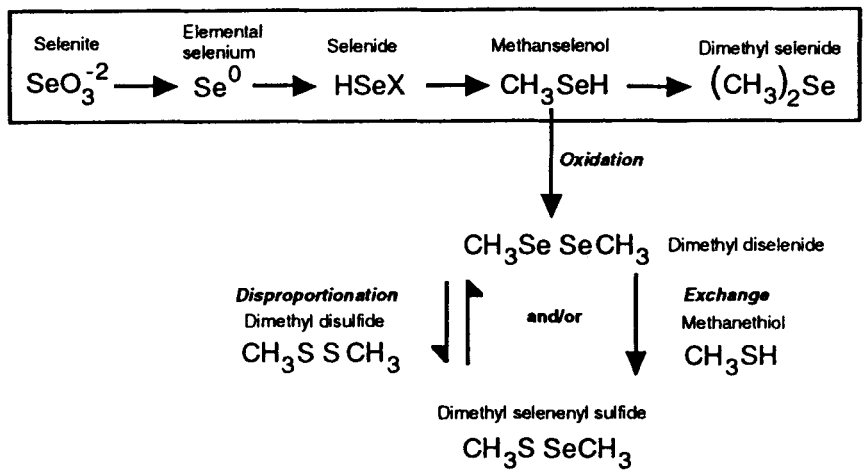
Scheme 3

More completely this process probably entails the reaction of methaneselenol, created by the reduction of the diselenide, with dimethyl disulfide and analogously, the reaction of methanethiol, created by disulfide reduction, with the diselenide.[24–27] Methaneselenol and methanethiol were detected in the headspace of this reaction mixture, demonstrating that these reductions had indeed occurred.

Another possible route to dimethyl selenenyl sulfide simply involves the disproportionation of the diselenide and disulfide to the selenenyl sulfide as reported by Bergson and Nordström;[28] however, these workers reported their reaction under alkaline conditions and in solution. Similarly, a mixture of diselenides and disulfides in solution reacted to form the selenenyl sulfide when heated above 80 °.[29] The nonreducing gas-phase disproportionation reaction carried out in this work between DMDSe and DMDS produced CH_3SeSCH_3 under both nitrogen-purged and oxygen-purged conditions. Since this gas-phase reaction was carried out in the absence of either selenol or thiol (and neither was detected in the reaction mixture), the exchange reactions must be excluded as the source of selenenyl sulfide in that experiment; however, this result does not preclude the possibility of disproportionation as the major route of selenenyl sulfide production in the acidified/reducing reaction described above or in bacterial cultures where thiol or selenol are present.

The nitrogen- or oxygen-rich conditions used in the disproportionation reaction basically reproduce the experimental conditions used by Reamer and Zoller in their work with sewage and soil samples that were swept by either nitrogen or air purge gases, conditions that yielded the compound the authors determined was dimethyl selenone.[1] Since it is probable that this was actually dimethyl selenenyl sulfide, together these results suggest that the production of dimethyl selenenyl sulfide does not depend on reducing conditions *per se*.

The confusion of dimethyl selenone for dimethyl selenenyl sulfide does not preclude the existence of dimethyl selenone as an intermediate in biomethylating systems. Also, the detection of dimethyl selenenyl sulfide in microbial headspace does not assure that this compound is actually a metabolite released by bioremediation. It is still possible that dimethyl selenenyl sulfide could simply be a product of disproportionation between dimethyl disulfide and dimethyl diselenide or exchange between diselenide and methanethiol and/or disulfide and methaneselenol. As



Scheme 4

Table 2 shows, methanethiol and methaneselenol were detected in some of our culture samples, although not in our gas-phase disproportionation experiments.

Doran has proposed a pathway for the reduction and methylation of an inorganic selenium salt to dimethyl selenide that proceeds through elemental selenium.[30] Many of the cultures of selenium-resistant bacteria that have been examined in this report, and in work by others,[14,31] produced pink or reddish cultures after being dosed with selenium salts. This indicates the reductive production of metallic selenium and lends support to the Doran pathway (shown in Scheme 4 in the box). Elemental selenium was not included in either the Challenger mechanism or the Reamer and Zoller modification, though these last workers did dose some of their samples with elemental selenium. The results of the experiments reported here lead us to the next step in determining the products of the reduction and methylation of selenium by micro-organisms; therefore a further augmentation of the Doran mechanism is proposed (Scheme 4) which includes the additional products that have been detected in bacterial cultures dosed with selenium salts. The proposed modification is seen below the boxed Doran pathway and is initiated by the oxidation of methaneselenol to dimethyl diselenide.

The two possible paths to the mixed sulfur/selenium product are expressed as exchange and disproportionation reactions. Since there is no strong evidence of which reversible fork is actually occurring, both are included. However, the experimental gas-phase production of dimethyl selenenyl sulfide in the disproportionation reaction with no selenol or thiol present suggests that disproportionation is quite probable. The analogous production of dimethyl selenenyl sulfide via the exchange between methaneselenol and dimethyl disulfide is not shown but is implied by this mechanism, and since methaneselenol and dimethyl disulfide have been detected in some of the bacterial cultures examined here, this exchange reaction should likewise remain under consideration.

The proposed mechanism as shown in Scheme 4 is initiated by the introduction of selenium(IV) as selenite; however, experiments have been performed with both selenite and selenate [Se(VI)]. Except for the toxicity differences of these two chemical species that affect bacterial output of the reduced gases that have been detected, there is no reason to expect that this mechanism is not applicable to both selenate and selenite reduction and methylation.

CONCLUSIONS

Selenium-resistant bacteria were examined to determine the products released into the headspace above cultures dosed with selenium salts. These bacteria emitted sulfur gases such as CH_3SH, CH_3SCH_3, CH_3SSCH_3 and CH_3SSSCH_3. The composition of methylated selenium gases detected in these samples was also complex, including mixtures of monomethylated (CH_3SeH) and dimethylated (CH_3SeCH_3, $CH_3SeSeCH_3$) molecules and a mixed selenium/sulfur compound dimethyl selenenyl sulfide, CH_3SeSCH_3. This last compound was identified by synthesizing

it *in vitro* using a mixture of dimethyl diselenide and dimethyl disulfide in a reducing environment, sampling the headspace above this mixture, and determining the mass spectrum of the compound by GC MS.

It is probable that a compound found in bacterial headspace and previously reported in the literature[1–3,10] has been erroneously identified as dimethyl selenone (or methyl methylselenite) and is in fact dimethyl selenenyl sulfide. This conclusion is based upon mass-spectral data and chromatography and boiling-point considerations.

In the headspace above selenium-resistant bacterial systems dosed with selenium salts that release a complex mixture of sulfur and selenium gases, dimethyl selenenyl sulfide is not uncommon. It has not been determined whether or not dimethyl selenenyl sulfide is indeed a microbial metabolite in the systems that have been studied. It may be a disproportionation or reaction product created in the headspace gases or in the aqueous media solution. A pathway for the reduction and methylation of salts of selenium has been proposed that augments that suggested by Doran[30] and incorporates steps of disproportionation and exchange based upon the detection of selenols, thiols and dimethyl selenenyl sulfide in the headspace above cultures of selenium-resistant bacteria.

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REFERENCES

1. Reamer, D and Zoller, W *Science*, 1980, 208: 500
2. Jiang, S, Robberecht, H and Adams, F *Atmos. Environ.*, 1983, 17: 111
3. Jiang, S, Robberecht, H and Adams, F *Appl. Organomet. Chem.*, 1989, 3: 99
4. Challenger, F and North, H *J. Chem. Soc.*, 1934, 68
5. Bird, M and Challenger, F *J. Chem. Soc.*, 1939, 163
6. Challenger, F *Chem. Rev.*, 1945, 36: 315
7. Challenger, F *Adv. Enzymol.*, 1951, 12: 429
8. Reamer, D Ph.D. Dissertation, 1978, Dept of Chemistry, University of Maryland, College Park, MD, USA
9. Rebane, E *Chem. Scr.*, 1974, 5: 65
10. Chau, Y, Wong, P, Silverberg, B, Luxon, P and Bengert, G *Science*, 1976, 192: 1130
11. Adams, F University of Antwerp, personal communication, 1990
12. Jiang, S Hebei University, personal communication, 1990
13. Chasteen, T G Ph.D. Dissertation, 1990, Dept of Chemistry and Biochemistry, University of Colorado, Boulder, CO, USA
14. Burton, G, Jr, Giddings, T, DeBrine, P and Fall, R *Appl. Envir. Microbiol.*, 1987, 53: 185
15. Maki, J S, La Croix, S J, Hopkins, B S and Staley, J T *Appl. Environ. Microbiol.*, 1986, 51: 1047
16. Budzikiewicz, H, Djerassi, C and Williams, D *Mass Spectrometry of Organic Compounds*, Holden-Day, San Francisco, CA, USA, 1967, pp 276–96
17. Agenäs, L Physicochemical investigation of selenium compounds. In: *Organic Selenium Compounds: Their Chemistry and Biology*, Klayman, D and Günther, W (eds), Wiley, New York, 1973, pp 961–88
18. Dill, J and McLafferty, F *J. Am. Chem. Soc.*, 1979, 101: 6526
19. Rebane, E *Chem. Scr.*, 1974, 5: 5
20. Douglass, I *J. Org. Chem.*, 1965, 30: 633
21. Jiang, S, De Jonghe, W and Adams, F *Anal. Chim. Acta*, 1982, 136: 83
22. Chasteen, T G, Silver, G, Birks, J and Fall, R *Chromatographia*, 1990, 30: 181
23. Hillen, L and Werner, R *J. Chromatogr.*, 1973, 79: 318
24. Eldjarn, L and Pihl, A *J. Biol. Chem.*, 1957, 225: 499
25. Günther, W *J. Org. Chem.*, 1967, 32: 3931
26. Ganther, H and Kraus, R *Anal. Biochem.*, 1984, 138: 396
27. Killa, H and Rabenstein, D *Anal. Chem.*, 1988, 60: 2283
28. Bergson, G and Nordström, G *Ark. Kemi*, 1961, 17: 569
29. Kostiner, E, Reddy, M, Urch, D and Massey, A *J. Organomet. Chem.*, 1968, 15: 383
30. Doran, J *Adv. Microb. Eco.*, 1982, 6: 1
31. Moore, M and Kaplan, S *J. Bacteriol.*, 1992, 174: 1505