Sulfoxidation of the Soil Fumigants Metam, Methyl Isothiocyanate, and Dazomet†

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Oxidation of metam [$CH_3NHC(S)SNa$] with H_2O_2 produces the sulfenate [$CH_3NHC(S)SONa$] and sulfinate [$CH_3NHC(S)SO_2Na$], depending on the amount of oxidant, but the sulfonate [$CH_3NHC(S)SO_3Na$] and corresponding thiocarbamates [$CH_3NHC(O)SO_nNa$, $n = 0$, 1, 2, or 3] are not observed on the basis of comparisons with standards. Metam in aqueous solution is converted by mouse microsomes with NADPH to the sulfenate and methyl isothiocyanate (MITC), by the arachidonate/lipoxygenase and glucose oxidase systems to the sulfenate, by magnesium monoperoxyphthalate to MITC, methyl isocyanate (MIC), and methylamine, and by sunlight to MITC. Metam sulfenate decomposes readily to metam and the disulfide [$CH_3NHC(S)S]_2$, while both metam sulfenate and metam sulfinate react with glutathione to form the $CH_3NHC(S)SG$ adduct. *m*-Chloroperoxybenzoic acid in organic solvent converts MITC to MIC and dazomet to metam, MITC, and formaldehyde.

Keywords: *Dazomet; fumigants; metam; methyl isothiocyanate*

INTRODUCTION

Metam (**1**) (metham, metam sodium) (Figure 1) is a general biocide used in large amounts (6 million pounds for agriculture in California in 1990) and at high rates (62–310 lb of active ingredient per acre) primarily as a preplant treatment of soil to kill seeds, weeds, nematodes, fungi, and insects (California Environmental Protection Agency, 1992). It is a very important wood preservative (Miller and Morrell, 1990). Metam was largely unknown to the general public until July 1991 when an accidental spill of ~60 000 lb of active ingredient into the Sacramento River resulted in a massive kill of aquatic organisms and great concern for potential health effects from human exposure (California Environmental Protection Agency, 1992). Methyl isothiocyanate (MITC) (**2**) and dazomet (**3**) (Figure 1) are related soil fumigants with uses similar to those of **1**. MITC is of particular interest since it is a primary environmental degradation product and probably the ultimate toxicant of **1** and **3** in soil (Worthing and Hance, 1991; Ware, 1993).

The metabolic pathways and environmental fate currently proposed for **1**–**3** involve primarily hydrolysis, conjugation, and dissociation reactions (Alvarez and Moore, 1994; Draper and Wakeham, 1993; Lam *et al.*, 1993, and references cited therein). MITC (**2**) is detoxified in rats by conjugation with glutathione (GSH), leading to excretion of the mercapturate (Lam *et al.*, 1993; Mennicke *et al.*, 1983, 1987), which is also a major metabolite of **1** and **3** in rats but not in mice (Lam *et al.*, 1993). In addition, microsomal FAD-containing monooxygenases slowly oxidize **1** and **3**, but the products have not been identified previously (Smyser *et al.*, 1985).

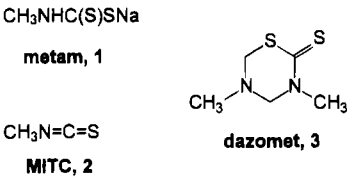

Figure 1. Structures of soil fumigants examined.

This investigation focuses on the possible role of oxidative activation of **1**–**3** to reactive species which may contribute to the overall toxicological profile of these pesticides. Oxidants including H_2O_2 and organic peroxides are used to produce intermediates which are evaluated for reactivity with biological nucleophiles such as GSH. This is the first report of the sulfenate and sulfinate derivatives of **1** and its thiolate analog metamoxon (**4**) (Figure 2) and of the characterization of an enzymatic oxidation product of **1** formed in the microsome–NADPH system.

MATERIALS AND METHODS

Spectroscopy. A Bruker AM-300 NMR spectrometer was used to acquire the ^1H and ^{13}C NMR spectra, the latter with nuclear Overhauser enhancement, at 300 and 75 MHz, respectively. Chemical shift values are relative to tetramethylsilane (0.00 ppm) in $CDCl_3$ or to HOD (4.80 ppm) in D_2O. $^{13}CH_3$-enriched reactants were used in a portion of the experiments.

Mass spectra of volatile compounds were acquired by GC/MS in the electron impact or chemical ionization (CH_4 as the reagent gas) modes with the Hewlett-Packard 5985B instrument (DB-5 column) for oxidation products and their derivatives or with the Hewlett-Packard 5971A instrument (MS-1 column) for metam impurities. Polar products were analyzed by LC/MS with positive or negative electrospray ionization (ESI) using a VG Bio-Q instrument with an Aquapore 300 column eluting with 0.1% trifluoroacetic acid (TFA) in water for compounds **5** and **6** or a VG Platform instrument with an ABI C_{18} Aquapore column eluting with a 1:1 mixture of 0.1% formic acid in water and 0.05% formic acid in ethanol/propanol

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(5:2) for compounds **1** and **8**–**10**. Fast atom bombardment (FAB) mass spectra were acquired on a Kratos MS-50 spectrometer.

Chromatography. Reversed-phase HPLC with UV detection was performed as follows: Hewlett-Packard 1050 solvent delivery system and 1040M Series II photodiode array detector at 220 and 260 nm; Merck 100RP-18 column (5 μm, 0.4 × 12.5 cm); 0.1% TFA for 5 min then linear gradient of 0–50% methanol in water with constant 0.1% TFA over 20 min and finally gradient to 100% methanol over an additional 10 min, each at 1.5 mL/min. TFA is required because unprotonated **1** and its oxidation products elute with water at the solvent front, whereas acidification with 0.1% TFA results in sufficient retention to separate **1** from **5** and from the highly polar **6** and **8**–**10** (which are barely retained on the column). The diode array detector provided essential information on the UV chromophores.

Chemicals. Sources for the chemicals were as follows: GSH from Calbiochem (San Diego, CA); oxidized GSH (GSSG), arachidonic acid, GSH *S*-transferase (GST) (rat liver), lipoxygenase (soybean), and glucose oxidase (Type X-S from *Aspergillus niger*) from Sigma (St. Louis, MO); H_2O_2 (30% solution), *m*-chloroperoxybenzoic acid (MCPBA) (50–60%), and magnesium monoperoxyphthalate (MMPP) (80%) from Aldrich Chemical Co. (Milwaukee, WI). MCPBA was purified (99%) by washing a dichloromethane solution with pH 9 buffer.

Metam (**1**) as the dihydrate (Chem Service, Westchester, PA) was washed with dichloromethane to obtain a sample of >98% purity based on ^{1}H NMR. The organosoluble impurities, characterized by GC/MS (t_R, *m/z*), were as follows. Three major compounds were identified by comparison with standards: $CH_3NHC(S)NHCH_3$ (standard from Aldrich), 11.4 min, [MH^+ = 105]; $CH_3\overline{NC(S)SSC}{=}NCH_3$ and $CH_3\overline{NC(S)N(CH_3)C(S)}S$ (Miller and Morrell, 1990) (standards generously supplied by Drs. D. B. Miller and J. J. Morrell, Oregon State University, Corvallis, OR), 13.1 and 14.1 min, respectively (some isomerization occurs during GC), [MH^+ = 179]. Four minor compounds were tentatively identified by their molecular ions: $CH_3NHC(O)NHCH_3$, 6.5 min, [MH^+ = 89]; $CH_3NHC(S)OCH_3$, 5.2 min, [MH^+ = 106]; **2**, 8.5 min, [MH^+ = 74]; sulfur (S_8), [M^+ = 256]. Compounds **2** and **3** were from Aldrich and Chem Service, respectively. [$^{13}CH_3$]-**1** and [$^{13}CH_3$]-**3** were available from previous studies (Lam *et al.*, 1993). Metam-oxon (**4**) was prepared by bubbling COS for 1 h into a solution of CH_3NH_2 and NaOH (each 40% w/v) in H_2O according to the general procedure of Johanssen *et al.* (1989). To prepare metam disulfide (**11**), a solution of **1** (162 mg) in H_2O (1.5 mL) at 5 °C was treated with $(NH_4)_2S_2O_8$ (900 μL, 30% solution) followed by stirring for 20 min and recovery of the product by filtration, washing (water), and drying (44 mg, 41%) [M^+ = 212] [method based on that of Klöpping and van der Kerk (1951)].

Oxidation of Metam and Metam-Oxon with H_2O_2. Metam sulfenate (**5**) was generated by reacting [$^{12}CH_3$ or $^{13}CH_3$]-**1** (11 and 0.5 mg for direct NMR and LC/MS/ESI analyses, respectively) with equimolar H_2O_2 in D_2O (^{1}H and ^{13}C NMR) or H_2O (MS) (500 μL) for 10–20 min at 23 °C. Metam sulfinate (**6**), prepared from **1** as above but with 2.1 equiv of H_2O_2, was isolated by lyophilization prior to ^{1}H and ^{13}C NMR and LC/MS/ESI analyses. Metam-oxon sulfenate (**7**), generated from **4** (10 mg) in D_2O (500 μL) by addition of equimolar H_2O_2, was examined by ^{1}H NMR. Metam-oxon sulfinate (**8**), from reaction of **4** with 2 equiv of H_2O_2, was analyzed by ^{1}H and ^{13}C NMR and LC/MS/negative ESI. Metam sulfonate (**9**) and metam-oxon sulfonate (**10**) were prepared by reaction of **2** and methyl isocyanate (MIC), respectively, with $Na_2S_2O_5$ (Backer *et al.*, 1935), and the structures were confirmed by ^{1}H and ^{13}C NMR and LC/MS/negative ESI.

Oxidation of Metam by Enzymes. Mouse microsomes (2.2 mg of protein; Cole *et al.*, 1991) were mixed with [$^{13}CH_3$]-**1** (2.9 mg) and NADPH (0 or 13 mg) in phosphate buffer (200 mM, pH 7.4, 450 μL) plus D_2O (200 μL). The vial was sealed with a septum and the air replaced with oxygen by three cycles of vacuum purging and gas replacement. ^{13}C NMR spectra were taken after the incubation mixture was shaken rapidly for 2 h at 37 °C and again after an additional 2 h of holding in the NMR tube for unstable products to decompose.

To examine possible oxidation by the arachidonate/lipoxygenase system, **1** (300 μg) was added to a medium containing arachidonic acid (181 μg) and soybean lipoxygenase type 1 (0 or 200 μg) in 50 mM sodium borate buffer (1 mL, pH 9.0) [reaction mixture based on that of Cashman *et al.* (1989)] with product analysis by HPLC following incubation for up to 200 min at 37 °C. Alternatively, glucose oxidase (0 or 100 μg) was added to **1** (100 μg) and glucose (3 mg) in 50 mM phosphate buffer (300 μL, pH 7.4) with product analysis by HPLC after incubation for 1 or 5 min at 37 °C.

Oxidation of Metam with Other Oxidants. [$^{12}CH_3$ or $^{13}CH_3$]-**1** (11 mg) was reacted with MMPP (1–3 equiv) in D_2O (or H_2O) (500 μL), and the reaction was monitored by ^{1}H and ^{13}C NMR. After 2 h, *n*-butylamine (15 mg) was added with stirring for 1 h and the products were extracted into dichloromethane for GC/MS analysis. To examine possible photooxidation, a solution of **1** (10 mg) and Rose Bengal (0 or 3 mg) in D_2O (500 μL) was exposed to midday sunlight (September) for 3 h and analyzed by ^{1}H NMR. Comparisons were made with the same reaction in D_2O/acetone (1:1) alone or with acetophenone (7 μL).

Decomposition of Metam Sulfenate, Metam-Oxon Sulfenate, and Metam Sulfinate. Sulfenates **5** and **7** and sulfinate **6**, formed in H_2O with 1 and 2 equiv of H_2O_2, respectively, were examined for recovery of starting material on lyophilization (HPLC analysis), decomposition products within 2 h at 23 °C (^{1}H NMR analysis), or reactions with **1** or 1 M HCl (^{1}H NMR analysis). Precipitated material following treatment with 1 M HCl was recovered by extraction into chloroform and subjected to electron impact/MS analysis.

Reactions of Metam Sulfenate and Metam Sulfinate with GSH. Sulfenate **5** with a minor amount of sulfinate **6** (from the reaction of 600 μg of **1** and equimolar H_2O_2 in 500 μL of 50 mM, pH 7.4, phosphate buffer) was treated *in situ* (no solvent removal) with GSH (2 or 4 equiv) for 5 min before HPLC analysis. Sulfinate **6** (500 μg) was reacted with 2 equiv of GSH in 50 mM, pH 7.4, phosphate buffer (500 μL) alone (control) or with rat liver GST (0.3 mg) at 37 °C with removal of aliquots at 5 min and 1, 2, and 3 h for analysis of $CH_3NHC(S)SG$ formation by HPLC.

Oxidation of MITC and Dazomet with H_2O_2 and MCPBA. MITC (6 mg) or [$^{12}CH_3$ or $^{13}CH_3$]-**3** (11 mg) was reacted with 1–3 equiv of H_2O_2 in CD_3CN (or CD_3OD) (500 μL) with monitoring by ^{1}H NMR. Alternatively, **2** (5 mg) or **3** (6 mg) was reacted with MCPBA (1–3 equiv) in $CDCl_3$ (500 μL), and the reaction was monitored by ^{1}H and ^{13}C NMR. Products from **2** and **3** were also analyzed after 10 min by GC/MS following derivatization with *n*-butylamine (15 mg) and diazomethane. To analyze for bound or free formaldehyde from oxidation, a solution of **3** (12 mg) in dichloromethane (500 μL) was treated with 3 equiv of MCPBA or *m*-chlorobenzoic acid (MCBA) (as a control). After 10 min, the solvent was evaporated and the residue or nonvolatile fraction was treated with 2,4-dinitrophenylhydrazine to form the hydrazone (2,4-DNP) (7.3 mg) (Shriner *et al.*, 1980) with verification by TLC comparison with the standard compound (silica gel; ethyl acetate/hexane 1:2; R_f = 0.58). Alternatively, free formaldehyde was determined by bubbling nitrogen through the same type of reaction mixture into the Hantzsch reagent (Nash, 1953) with adduct identification by UV comparison with a standard (λ_{max} = 414 nm).

RESULTS

Oxidation of Metam and Metam-Oxon with H_2O_2 and Assignment of the Sulfenates and Sulfinates Formed (Figure 2). Dithiocarbamate **1** is oxidized with H_2O_2 in water to yield two primary products evident by ^{1}H NMR and HPLC in a ratio dependent on the amount of oxidant (Figure 3). The principal product with 1 equiv of H_2O_2 is assigned as sulfenate **5** and that with 2 equiv as sulfinate **6**. As expected on this basis, **5** reacts with equimolar H_2O_2 to form **6**, which undergoes no further reaction with excess H_2O_2. These

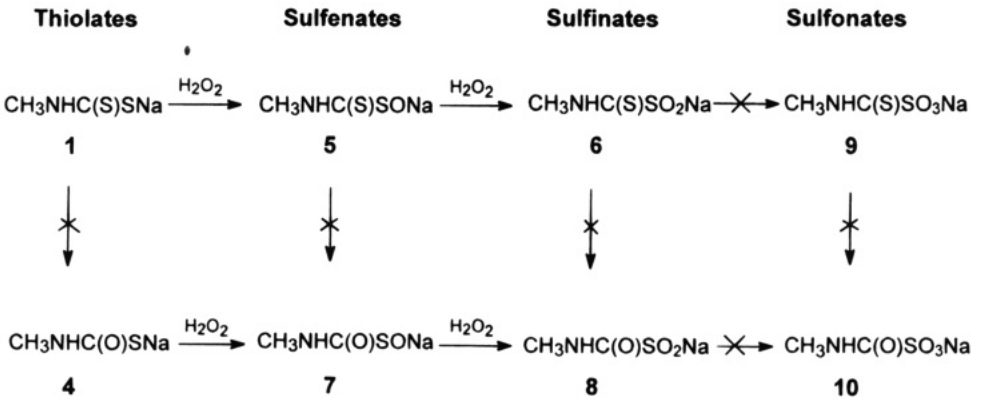

Figure 2. Oxidation of metam (**1**) and metam-oxon (**4**) with H_2O_2 to the corresponding sulfenates and sulfinates but not to the sulfonates or desulfuration products.

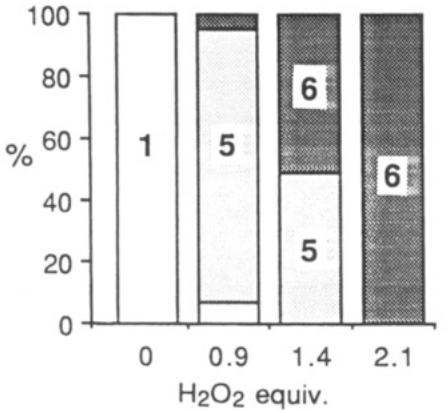

Figure 3. Product distribution on oxidation of metam (**1**) to its sulfenate (**5**) and sulfinate (**6**) with up to 2.1 equiv of H_2O_2 as analyzed by ^1H NMR.

assignments are based on appropriate HPLC, ^1H and ^{13}C NMR, LC/MS, and UV data (Table 1). Increasing polarity on HPLC is observed through the oxidation sequence **1** to **5**, **6**, and **9**. The ^1H and $^{13}CH_3$ NMR chemical shifts of **6** are between those of **5** and **9**, as expected for the successive oxidation states. Diagnostic MS ions observed are as follows: MH^+ for **1**, **5**, and **6**; $[M - H]^-$ for **9**; $[CH_3NH{=}C{=}S]^+ = 74$ as the base ion from **1** and **5**; $[M + Na]^+$ for **6**. The UV spectra are also distinctive for **1**, **5**, and **6**, and the latter is similar to **9**.

Thiocarbamate **4**, as with dithiocarbamate **1**, is oxidized with H_2O_2 to two principal products assigned as sulfenate **7** (^1H NMR) and sulfinate **8** (^1H and ^{13}C NMR; $[M - H]^-$ and fragment ion $[HSO_2]^-$ as the base peak on LC/MS). The NMR (^1H and ^{13}C) and MS data ($[M - H]^-$ and $[HSO_3]^-$) for sulfonate **10** also support the assignments for **7** and **8**. No **10** is formed in the H_2O_2 oxidation of **1** or **4** on the basis of comparison with the standard.

Oxidation of Metam by Enzymes. $[^{13}CH_3]$-**1** is metabolized by the microsome/NADPH system on incubation in an oxygen atmosphere for 2 h to sulfenate **5** and isothiocyanate **2** with ^{13}C NMR chemical shifts the same as those of standards in D_2O (Figure 4). Continuing the incubation for 2 h in the NMR tube results in loss of **5** with an increased amount of **2** and methylamine (^{13}C NMR δ 27.6). The identity of **5** formed by the microsome/NADPH system was confirmed by coincidence of the signal with authentic standard generated from residual **1** by addition of H_2O_2 following the second period of incubation. No metabolism took place in the absence of NADPH or when the microsomal oxidases were inactivated by preheating at 60 °C before the incubation. A signal for the microsomes appeared at δ 35.7 in every case.

The arachidonate/lipoxygenase system oxidizes **1** to **5**, requiring both arachidonate and the enzyme, with no evidence of other oxidation products (Figure 5). Glucose oxidase converts **1** to **5** in about 75% yield within 1 min, and at 5 min no **1** is left and a small amount of **5** is oxidized to sulfinate **6**.

Oxidation of Metam with Other Oxidants. Reaction of $[^{13}CH_3]$-**1** with 1−3 equiv of MMPP gives 13 ^{13}C NMR peaks within the first 10 min. The most prominent peak is from **2** (δ 32.4), and methylamine (δ 27.2) is one of the minor products; these structural assignments are verified by spiking with authentic compounds. Isothiocyanate **2** and a minor product MIC formed on oxidation were confirmed also by derivatization with *n*-butylamine to the corresponding thiourea [$MH^+ = 147$] and urea [$MH^+ = 131$], respectively (Figure 6).

Photooxidation of **1** by sunlight yields **2** with enhanced conversion on addition of acetone or particularly Rose Bengal (a singlet oxygen sensistizer) (Table 2). A major unknown is formed when acetone is present.

Decomposition of Metam Sulfenate, Metam-Oxon Sulfenate, and Metam Sulfinate. Sulfenate **5** completely decomposes on attempted isolation from water by lyophilization, whereas sulfinate **6** can be obtained in this manner with only a little decomposition. Sulfenates **5** and **7** decompose within 2 h at 23 °C, partially for **5** and completely for **7**, each reverting in part to the parent **1** or **4**, respectively, with additional products identified by ^1H NMR as **2** and methylamine from **5** and as dimethylurea and methylamine from **7** (Figure 7). Disulfide **11** [$M^+ = 212$] forms quickly as a precipitate and is the only major product on reaction of **5** with **1** and on acidification of **5** with 1 M HCl.

Reactions of Metam Sulfenate and Metam Sulfinate with GSH. Sulfenate **5** reacts with GSH to give primarily **1** and GSSG (Figure 8A). With 2 equiv of GSH some **5** remains but all GSH is consumed, whereas with 4 equiv of GSH all **5** is eventually consumed with regeneration of much more **1** than with 2 equiv of GSH. $CH_3NHC(S)SG$ is a minor product from reaction of **5** with 2−4 equiv of GSH. Sulfinate **6** also reacts with GSH to form $CH_3NHC(S)SG$ (Figure 8B) with the rate enhanced by GST (Figure 9).

Oxidation of MITC and Dazomet with H_2O_2 and MCPBA. Neither **2** nor **3** reacts with H_2O_2 in CD_3CN or CD_3OD. Treatment of **2** with 1−3 equiv of MCPBA in $CDCl_3$ gives two characterized products: MIC identified by ^1H NMR (δ 3.02), spiking with authentic compound, and derivatization with *n*-butylamine to the corresponding urea [$MH^+ = 131$]; the mixed anhydride of MCBA and methylcarbamic acid (^1H NMR δ 2.98; doublet, $J = 4.1$ Hz, which collapses to a singlet by D_2O

Table 1. Characterization of the Sulfenate, Sulfinate, and Sulfonate Derivatives of Metam and Metam-Oxon

	$CH_3NHC(S)SO_nH$ (or Na salt)				$CH_3NHC(O)SO_nH$ (or Na salt)			
parameter	**1** ($n = 0$)	**5** ($n = 1$)	**6** ($n = 2$)	**9** ($n = 3$)	**4** ($n = 0$)	**7** ($n = 1$)	**8** ($n = 2$)	**10** ($n = 3$)
HPLC t_R, min	7.5	6.1	1.2	1.1		[a]	0.9	0.8
NMR, δ[b]								
^1H, CH_3	2.99	3.23	3.18	3.15	2.62	2.90	2.83	2.81
^{13}C								
CH_3	37.3	33.5	34.8	35.8	30.7	[c]	29.2	29.4
C(S) or C(O)	213.6	208.2	212.7	197.6	186.8		180.7	169.0
LC/MS, m/z (rel int)[d]								
$[MH]^+$	108 (60)	124[e] (68)	140 (100)					
$[M - H]^-$				154 (48)			122 (40)	138 (30)
UV								
λ_{max}, nm	210				[a]	[a]	[a]	~200
	235	230						
	265	280	270	271				

[a] Not detected due to low absorbance at 220 and 260 nm. [b] Sodium salts in D_2O relative to HOD at δ 4.80. [c] Data not available since unstable and no $^{13}CH_3$ precursor. [d] Diagnostic ions (relative intensity) (M = free acid): **1** and **5**, $[CH_3NH{=}C{=}S]^+ = 74$ (100); **5**, $[MH - H_2O]^+ = 106$ (17); **6**, $[M + Na]^+ = 162$ (44); **8**, $[HSO_2]^- = 65$ (100); **9** and **10** $[HSO_3]^- = 81$ (100). [e] The diagnostic ion $[MH^+ = 124]$ was of low intensity by FAB-MS relative to other unassigned ions.

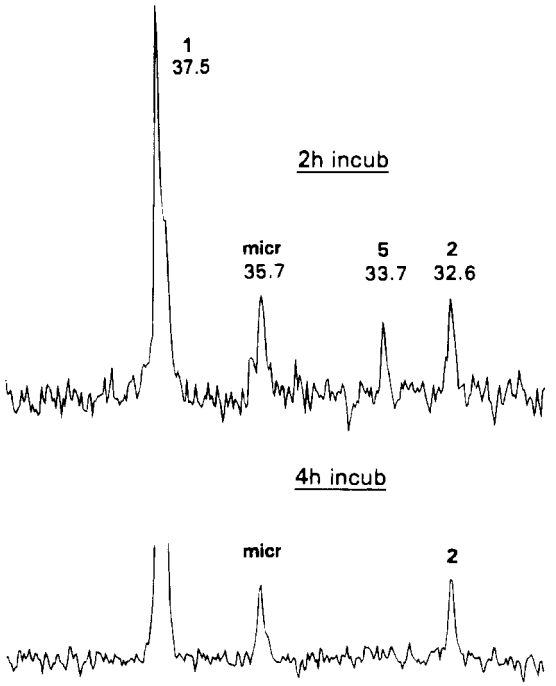

Figure 4. Oxidation of metam (**1**) to its sulfenate (**5**) and MITC (**2**) on incubation for 2 h with mouse microsomes (micr) and NADPH and subsequent loss of **5** with continued incubation until 4 h.

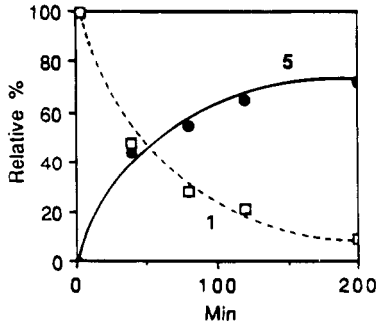

Figure 5. Oxidation of metam (**1**) to its sulfenate (**5**) with the arachidonate/lipoxygenase system.

exchange) (Figure 10). In addition, there are 14 other ^{13}C signals for CH_3 groups of unidentified products from the reaction of **2** and MCPBA.

Reaction of $[^{13}CH_3]$-**3** (^{13}C NMR δ 40.5 and 39.2) with equimolar MCPBA in $CDCl_3$ gives an intermediate (^{13}C NMR δ 48.4 and 38.9) that reverts to **3** over a period of several hours and can be re-formed by addition of more

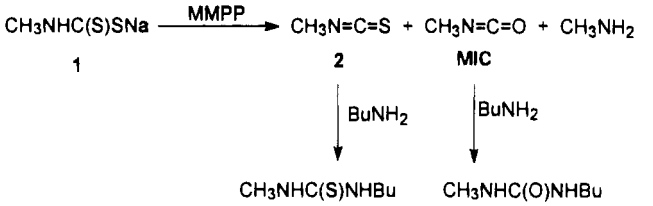

Figure 6. Reaction of metam (**1**) with magnesium monoperoxyphthalate and derivatization of two products with *n*-butylamine.

Table 2. Product Distribution from Photooxidation of Metam by Sunlight (3 h)

	% yield (or remaining)[a]		
solvent	**1**	**2**	unknowns
D_2O	92	1	7
D_2O, acetone[b]	34	5	61
D_2O, Rose Bengal	58	37	5

[a] Average of duplicate determinations. [b] The same products and ratios are observed on addition of acetophenone. ^1H NMR CH_3 signals for the unknowns appear at 3.24, 3.11 (major), and 2.68 ppm.

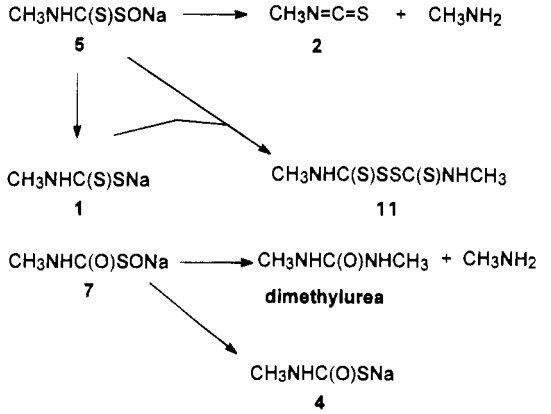

Figure 7. Decomposition of metam sulfenate (**5**) and metam-oxon sulfenate (**7**) in water.

MCPBA. With a 3:1 molar ratio of MCPBA:**3**, another major product is evident (^{13}C NMR δ 48.0 and 40.3) which reverts to **3** over time but also decomposes to **1** (^{13}C NMR δ 37.6) and other peaks (Figure 11). Attempts to isolate these two products were not successful. A minor product at the 3:1 ratio of oxidant:**3** is **2** (^{13}C NMR δ 30.2). Product identities were verified by addition of *n*-butylamine and diazomethane and then GC/MS analysis of the resulting $CH_3NHC(S)SCH_3$ $[MH^+ = 122]$ from **1** and the $CH_3NHC(SCH_3){=}NBu$ $[MH^+ =$

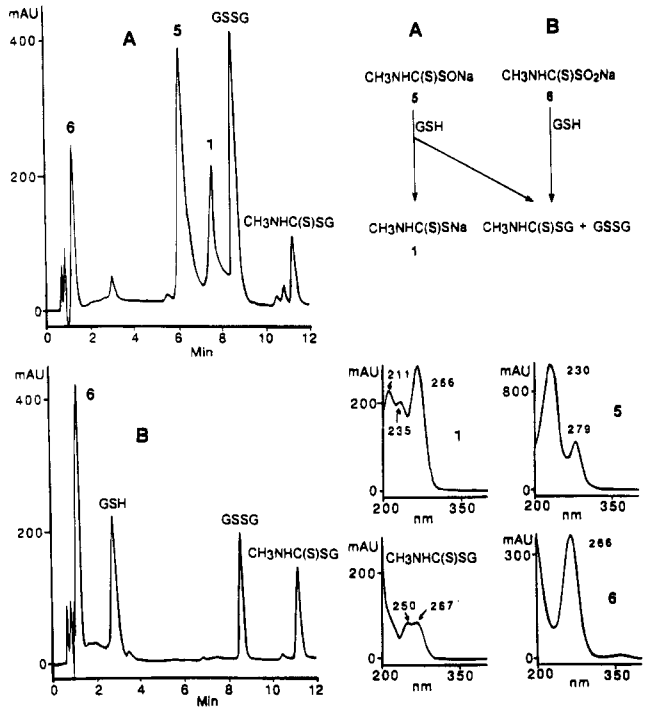

Figure 8. (A) Reactions of metam sulfenate (**5**) with GSH (**6** is present as an impurity, not a reaction product). (B) Reactions of metam sulfinate (**6**) with GSH. Also shown are HPLC chromatograms (A_{220}) and spectra of individual eluted products.

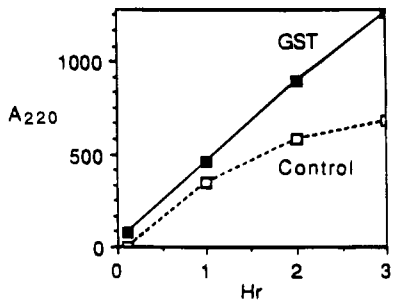

Figure 9. Effect of GSH *S*-transferase (GST) on $CH_3NHC(S)SG$ formation from reaction of metam sulfinate (**6**) with GSH as analyzed by HPLC.

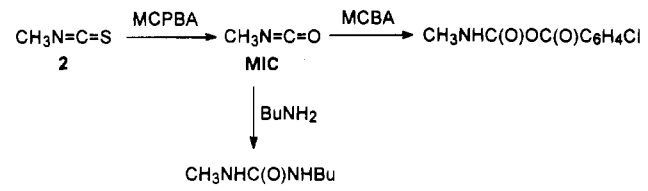

Figure 10. Reaction of MITC (**2**) with *m*-chloroperoxybenzoic acid and derivatization of one product with *n*-butylamine.

161] from **2**. Formaldehyde, probably both free and bound, is another identified product with 3 equiv of MCPBA as confirmed by formation of the Hantsch and 2,4-DNP adducts.

DISCUSSION

Metam reacts rapidly with aqueous H_2O_2 to form two principal oxidation products (Figure 2). The first is identified as sulfenate **5** on the basis of four observations: (1) LC/MS establishes the addition of one oxygen, which is probably on the thiolo sulfur rather than the thiono sulfur or nitrogen on the basis of the major fragment ion of $[CH_3NH{=}C{=}S]^+$. (2) It readily reverts to **1** and forms disulfide **11**. (3) A similar product is

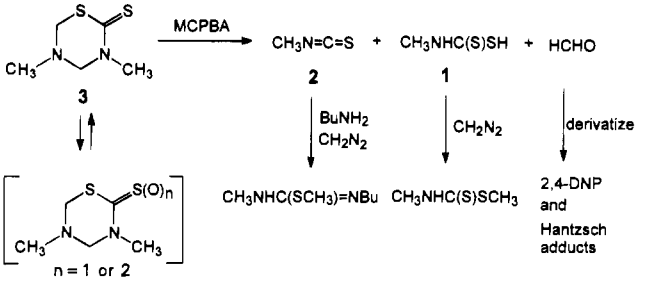

Figure 11. Oxidation of dazomet (**3**) with *m*-chloroperoxybenzoic acid and derivatization reactions for confirmation of product identifications.

formed from **4**, establishing that the oxidation occurs on the thiolo rather than the thiono sulfur. (4) Oxidation of *N,N*-diethyldithiocarbamate with H_2O_2 yields an analogous sulfenate (Watanabe and Ishimura, 1988). The present paper appears to be the first to report sulfenates of *N*-monoalkyldithiocarbamates and *N*-monoalkylthiocarbamates. Sulfinate **6** is identified as a dioxide since it is a further oxidation product of sulfenate **5** with one additional oxygen (LC/MS). The two oxygens are on the thiolate sulfur since it decomposes to MITC and oxon **4** yields an analogous dioxide product. The oxidation of **1** and **4** proceeds only to the sulfenates and sulfinates but not to the sulfonates with H_2O_2 on the basis of NMR comparison with authentic sulfonate standards. Failure to observe the oxidation of **1** by H_2O_2 in a previous study (Draper and Wakeham, 1993) is probably the result of using very dilute oxidant (10^{-4} M *vs* much higher levels in the present investigation) at less than 1 equiv with long reaction times.

Enzymatic oxidation of **1** to sulfenate **5** is observed in the present investigation with microsomes and NADPH (probably attributable to FAD-containing monooxygenases; Smyser *et al.*, 1985). This oxidation also takes place with the arachidonate-derived hydroperoxides or reactive peroxy radicals formed by soybean lipoxygenase and with H_2O_2 generated in the glucose/oxidase system.

GSH probably plays an important role in the ultimate biological fate of the sulfenate since GSH and **5** readily react at equimolar concentrations to form GSSG and **1** as major products. With 4 equiv of GSH, **5** is completely decomposed and a small amount of $CH_3NHC(S)SG$ is formed. Sulfinate **6** also reacts with GSH, but more slowly and with catalysis from GST, which also facilitates the direct reaction of **1** with GSH (Lam *et al.*, 1993). The reactions of **1**, **5**, and **6** with GSH indicate the possibility of analogous derivatizations for thiol sites in enzyme targets.

The urine of a rat 0−18 h after ip treatment with $[^{13}CH_3]$-**1** contains the mercapturate derived from $^{13}CH_3NHC(S)SG$ and $^{13}CH_3NH_2$ evident by ^{13}C NMR on a sample that has been lyophilized and reconstituted in D_2O [procedure of Lam *et al.* (1993)]. Although not detailed here, the urine as collected contains two additional products, $[^{13}CH_3]$-**1** and $[^{13}CH_3]$-**2**, which are reduced in content or lost on lyophilization. The detection of **2** in the urine does not necessarily mean that it is an excretory product since it might form in the urine following excretion due to decomposition of **1** or the mercapturate. The oxidation products of $[^{13}CH_3]$-**1**, *i.e.*, **5**, **6**, and **9**, are not detected by ^{13}C NMR analysis of the urine. This suggests but does not in itself establish that **6** and **9** are not present *in vivo*, whereas **5**, if formed, would probably decompose before analysis. In general, sulfenates are highly reactive, and the first evidence for their formation in mammals was the

urinary excretion of 1,1,2,3,4-pentachloro-1,3-butadienylsulfenic acid in rats dosed with hexachlorobutadiene (Nash *et al.*, 1984; Tomisawa *et al.*, 1993).

Metam undergoes photodegradation at 310–410 nm to **2** and 1,3-dimethylthiourea (Draper and Wakeman, 1993). MITC is also a photoproduct of **1** in sunlight in the present study, with higher yields with the singlet oxygen sensitizer Rose Bengal. The presence of similar natural sensitizers may have environmental relevance to formation of **2** from **1**. Metam is also converted to **2**, which in turn forms MIC on treatment with MMPP in H_2O and with MCPBA in chloroform, respectively. The toxicology of MIC has been extensively studied following a catastrophic leak in Bhopal, India (Mehta *et al.*, 1990). It is not clear to what extent sulfenate **5** is an intermediate in the environmental degradation of **1** to **2** and other products. There is also an alternative pathway for environmental degradation of **2** in the vapor phase at solar wavelengths involving photodissociation to CH_3-NC and atomic sulfur (Alvarez and Moore, 1994).

Dazomet oxidation with MCPBA in chloroform yields many products including **1** and **2** plus two compounds that revert to **3** over time, suggesting that they may be the thiono mono- and di-*S*-oxides. The major pathway of **3** metabolism in rats involves **1** and/or MITC as intermediate(s) in forming $CH_3NHC(S)SG$ and the corresponding mercapturate (Lam *et al.*, 1993). Formaldehyde is also released in model oxidation systems and probably on metabolism as well.

The agricultural use of **1** and **3** as progenitors for **2** formed on hydrolysis provides many possibilities for oxidation reactions which undoubtedly play a role in the metabolism and environmental fate of these compounds. The toxicological relevance of these oxidation products remains unknown.

ABBREVIATIONS USED

$CH_3NHC(S)SG$, *S*-[(*N*-methylthio)carbamoyl]-GSH; ESI, electrospray ionization; FAB, fast atom bombardment; GSH, glutathione; GSSG, oxidized glutathione; GST, glutathione *S*-transferase; MCBA, *m*-chlorobenzoic acid; MCPBA, *m*-chloroperoxybenzoic acid; MIC, methyl isocyanate; MITC, methyl isothiocyanate; MMPP, magnesium monoperoxyphthalate; TFA, trifluoroacetic acid; t_R, retention time; 2,4-DNP, 2,4-dinitrophenylhydrazone.

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