Chemical Research in Toxicology

SEPTEMBER 1995
VOLUME 8, NUMBER 6

Communications

Thiol Oxidation by 1,2,3-Oxadiazolinium Ions, Presumed Carcinogens

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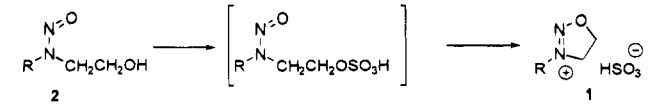
Received April 26, 1995[⊗]

3-Alkyl-1,2,3-oxadiazolinium ions **1** have been proposed as reactive intermediates in the activation of (2-hydroxyethyl)nitrosamines. The reaction of 3-methyl-1,2,3-oxadiazolinium tosylate (**1a**), 2-ethyl-1-methoxy-2-phenyldiazenium tetrafluoroborate (**3**), and 3-phenyl-1,2,3-oxadiazolinium triflate (**1b**) with thiols was investigated to determine the behavior of these compounds toward typical "cellular nucleophiles". Each of these substances oxidizes benzenethiol to diphenyl disulfide. The reaction in aqueous buffer at pH 7.4 is rapid. Reaction of **1b** with benzenethiol gives, in addition to the disulfide, benzene, biphenyl, azobenzene, diphenyl sulfide, aniline, and glycolaldehyde. Similar products are obtained from **3**. Phenyldiazene is postulated as an intermediate in this process, and its generation from phenyldiazoformate in the presence of benzenethiol gives similar products. Diazenes are presumed to arise by proton abstraction from the CH adjacent to N. The kinetics of reaction of **1a** with *N*-acetylcysteine to give the corresponding disulfide show first order dependence on each reactant and base catalysis. The data from these model chemical experiments suggest that 1,2,3-oxadiazolinium ions could react with abundant thiols in cells to lead to either their detoxification or radical processes emanating from diazenes. The occurrence of thiol–oxadiazolinium ion redox transformations could modulate the alkylation chemistry of these substances as well.

Introduction

3-Alkyl-1,2,3-oxadiazolinium ions **1** have been proposed as reactive intermediates in the activation of 2-(hydroxyethyl)nitrosamines **2** by a route which involves sulfate conjugation (*1–6*) as shown in Scheme 1. Initially, this hypothesis was advanced on the basis of purely chemical observations (*7, 8*), but has since been the subject of various biological experimental tests (*1–5*). 3-Alkyl-1,2,3-oxadiazolinium ion intermediates are postulated to alkylate DNA through nucleophilic attack at the carbons

Scheme 1



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bonded to N (*1–4*). In the case of (2-hydroxyethyl)-methylnitrosamine, DNA methylation and hydroxyethylation would result. Both of these adducts, along with unidentified ones, are produced in the DNA of animals treated with this carcinogen (*4, 5*). Alternatively, we have postulated that ethanol–nitrosamines are activated through their biooxidation to reactive α-nitrosamino

[⊗] Abstract published in *Advance ACS Abstracts*, July 15, 1995.

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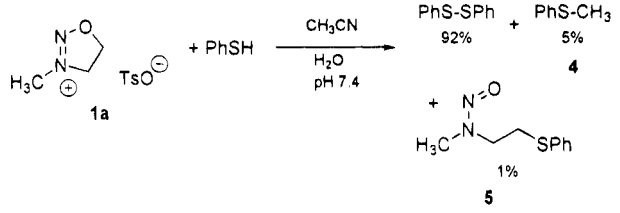
Scheme 2



aldehydes (*8, 10*). Some of the chemistry of these reactive aldehydes may also involve oxadiazolinium ions since we have shown that the α-nitrosamino aldehyde derived from (2-hydroxyethyl)methylnitrosamine cyclizes to 5-hydroxy-3-methyl-1,2,3-oxadiazolinium ion on treatment with acid (*11*). *N*-Acyl-1,2,3-oxadiazolinium ions have also been postulated as intermediates in the activation of chemotherapeutically employed β-chloro-*N*-nitrosoureas (*12*). Moreover, the chemical properties of the unstable oxadiazoline produced from the *N*-dealkylation or *N*-deacylation of 3-substituted 1,2,3-oxadiazolinium ions have remained a subject of speculation (*12, 13*).

In order to provide insight into the chemistry of 1,2,3-oxadiazolinium ions and test several hypotheses regarding their probable biologically relevant chemistry, we have probed their reactions toward "cellular" nucleophiles. The results of a computational chemical study of nucleophile-induced 1,2,3-oxadiazolinium ion transformations appeared during the course of this research (*13*). We report here, however, that 3-substituted-1,2,3-oxadiazolinium ions rapidly oxidize thiols to disulfides through a novel chemistry involving, at least in part, the participation of diazene intermediates derived from the 1,2,3-oxadiazolinium ions.

Results and Discussion

The reaction of 3-methyl-1,2,3-oxadiazolinium tosylate (**1a**) with benzenethiol in aqueous acetonitrile at pH 7.4 gives the products shown in Scheme 2. The time course of the transformation is shown in Figure 1. The high yield of diphenyl disulfide (the yields shown in Scheme 2 are based on thiophenol)[1] indicates that redox processes rather than nucleophilic transformations are primarily occurring. While the material balance for benzenethiol is excellent, the fate of the carbons in the 1,2,3-oxadiazolinium ion is not well accounted for in the products, possibly due to the formation of small water soluble or gaseous products. To circumvent these problems, using HPLC and GCMS with authentic standards, we investigated the reaction of two related compounds, 2-ethyl-1-methoxy-2-phenyldiazenium tetrafluoroborate (**3**) and 3-phenyl-1,2,3-oxadiazolinium triflate (**1b**),[2] with benzenethiol under similar conditions.[3] Both of these compounds readily oxidize benzenethiol to diphenyl disulfide, and each reaction produces significant yields of benzene along with minor amounts of azobenzene, biphenyl, and diphenyl sulfide, all of which give significant clues to the reaction course (Scheme 3).[4] Benzene, azobenzene, and biphenyl are all decomposition products of phenyldiazene (PhN=NH) (*14, 15*), and their presence in our reaction mixture strongly suggests a possible role for an intermediate diazene.

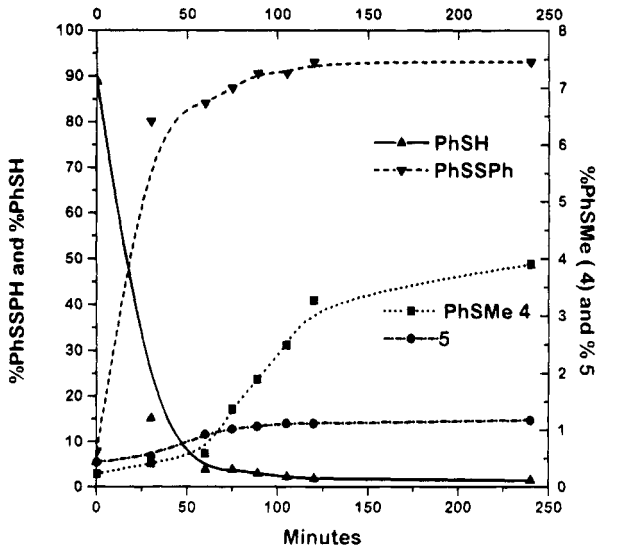
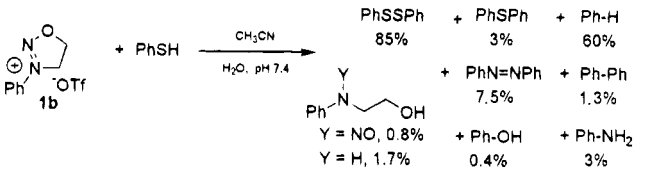


Figure 1. Percent yields of products/reactants for the reaction of 3-methyl-1,2,3-oxadiazolinium tosylate (**1a**) (0.097 M) with thiophenol (0.205 M) in 20% acetonitrile–pH 7.4 phosphate buffer solution (0.05M) at 25 °C. Each point represents a separate batch from which product determination was done by GC following extraction into CH_2Cl_2. The right-hand scale is expanded to show the minor products. Thioanisole formation appears to lag the redox process.

Scheme 3



Alkoxydiazenium ions, prepared from the *O*-alkylation of nitrosamines, are the acylic analogs of 1,2,3-oxadiazolinium ions and rearrange upon base treatment to 1-hydroxyalkyldiazenes through the likely intermediacy of an alkyldiazene (*16*). While a number of 1,2-disubstituted diazenes are well-known to oxidize thiols to disulfides (*17, 18*), we have been unable to find reports of the reactions of thiols with monoalkyl- or aryldiazenes. One report suggests that methyl phenylazoformate depletes thiols *in vivo* by their oxidation with phenyldiazene derived from that substrate (*19*).

In order to test the hypothesis that phenyldiazene is capable of rapid thiol oxidation, we generated it from the acidification of potassium phenylazoformate, PhN=N-$CO_2^-K^+$, (*20*) in the presence of benzenethiol. The reaction, which could not be performed exactly under the

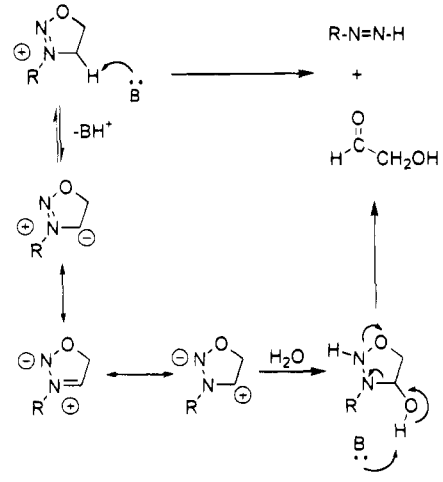
[1]Conditions: **1a,** 1 mmol, in 4 mL of CH_3CN added to PhSH, 1.4 mmol, in 7 mL of sodium phosphate buffer at pH 7.4 (0.05 M); 25 °C; 4 h; mixture extracted into CH_2Cl_2 which was washed with Na_2CO_3 solution prior to GCMS and HPLC analysis.

[2]Synthesis: **2-Ethyl-1-methoxy-2-phenyldiazenium tetrafluoroborate (3)** was prepared by the alkylation of ethylphenylnitrosamine (7 mmol) with 1 equiv of trimethyloxonium tetrafluoroborate in CH_2Cl_2. After trituration with ether and recrystalization, a white solid was obtained, mp 62–63 °C. ^{13}C NMR δ 12.1, 54.4, 72.1, 131.3, 134.9, 138.6. Anal. Calcd for $C_9H_{13}F_4N_2O_4$: C, 42.85; H, 5.16; N, 11.11. Found: C, 43.1; H, 5.16; N, 10.81. **3-Phenyl-1,2,3-oxadiazolinium trifluoromethanesulfonate (1b)** was prepared by treating 7.3 mmol of phenyl(hydroxyethyl)nitrosamine with 12 mmol of triflic anhydride in 10 mL of CH_2Cl_2 followed by heating. Trituration with ether gave a white solid, mp 110–111 °C. ^{13}C NMR δ 57.9, 79.1, 122.5, 131.4, 135.7. Anal. Calcd for $C_9H_9F_3N_2O_4S$: C, 36.24; H, 3.02; N, 9.39. Found: C, 36.26; H, 3.02; N, 9.21.

[3]Conditions: **1b** (as triflate), 3.3 mmol, in 10 mL of CH_3CN added to 40 mL of sodium phosphate buffer (pH 7.4, 0.05 M) containing 5 mmol of PhSH; 4 h, 25 °C.

[4]All yields except diphenyl disulfide, which is based on thiophenol, are based on **1b**.

Scheme 4



conditions in which **1b** or **3** were used,[5] nevertheless produced diphenyl disulfide 74% (yields calculated in a manner similar to footnote 4), diphenyl sulfide 9%, azobenzene 5%, biphenyl 3%, and benzene 43%, a product spectrum similar to what is observed for the reaction of **1b** with benzenethiol (see Scheme 3). If **1b** takes the decomposition pathway suggested by Huenig's observations *(16)*, then glycolaldehyde should be a product of this transformation. Trapping experiments utilizing phenylhydrazine show that 75% of **1b** results in the formation of glyoxal osazone, a known product of the reaction of phenylhydrazine with glycolaldehyde.

While the UV-absorbing characteristics of **1b**, thiophenol, and many of their products are very useful in HPLC-based analyses, thiophenol is much more acidic than typical cellular thiols related to cysteine. Consequently, we have used *N*-acetylcysteine in aqueous phosphate buffer (0.05 M) to probe the ability of **1a** to oxidize this thiol and, in turn, using kinetic methods, to gauge the lifetime of **1a** under conditions more likely to occur in biological media. The sulfur-containing product of this transformation is *N,N'*-diacetylcystine. Using pseudo-first order kinetic methods,[6] we established that the oxidation is first order in **1a** and first order in *N*-acetylcysteine. The observed rate constants (k_{obs} (M^{-1} m^{-1}, [pH]): 3.1 [6]; 7.4 [7.4]; 10.1 [8.5]) are a function of the base concentration at constant buffer concentration, but the specific form of this rate dependence awaits additional data. While **1a** exhibits a $t_{1/2}$ = 5 d in distilled water, it decomposes much more rapidly in buffer at pH 7.4. Similar observations have been made by Ohannesian and Keefer for related alkoxydiazenium ions *(21)*. The kinetic data and the similarity of the products generated from the reaction of benzenethiol with phenyldiazene and **1b** are consistent with a rapid, base (or buffer) assisted formation of a diazene intermediate from the 1,2,3-oxadiazolinium ion, which then reacts in a slow step with the thiol to give the disulfide. The conversion of **1a** to $CH_3N{=}NH$ and glycolaldehyde consumes a mole of base (see Scheme 4). 1-Substituted diazenes are highly air sensitive and decompose by both radical and non-radical pathways *(14, 15)*. The formation of diphenyl sulfide suggests the participation of radicals. Thiol oxidation by 1,2,3-oxadiazolinium ions could occur by thiol H-atom donation to radicals, a process which could explain the formation of benzene, which is also generated by phenyldiazene decomposition. The absence of phenylhydrazine in the reaction mixtures produced from the thiol reduction of **1b** suggests that this transformation is quite different from thiol oxidation by 1,2-disubstituted diazenes, which occurs by nucleophilic addition to the N=N and produce hydrazines or their derivatives.

We have also investigated the reaction of benzenethiol with **1a** and 1-methoxy-2-ethyl-2-phenyldiazenium tetrafluoroborate (**3**) in CH_2Cl_2. In each case diphenyl disulfide is produced, but the yields, 13% and 51% (based on thiol), respectively, are much lower than is observed in protic solvent. In both cases, nucleophilic attack by PhSH at the *O*-bound substitutent is a major reaction path, giving methyl[2-(phenylthio)ethyl]nitrosamine (**5**) (33%) in the former and thioanisole (**4**) (24%) in the latter. Thioanisole (**4**) is also produced from the *N*-demethylation of **1a** (13%).[7] The production of azobenzene, biphenyl, and diphenyl disulfide from **3** demonstrates that diazene chemistry is also operative in CH_2Cl_2, but nucleophilic processes play a larger role than they do in buffer where the base-mediated abstraction of an α-hydrogen (Scheme 4) is the predominant reaction pathway.

While there has been no unequivocal demonstration of the role of 1,2,3-oxadiazolinium ions in the bioactivation of (2-hydroxyethyl)nitrosamines or in the biologically relevant chemistry of chemotherapeutic (β-chloroethyl)-nitrosoureas, the chemical rationale which suggests their formation is reasonable and has merited further exploration of their properties under conditions likely to exist *in vivo*. The data presented here clearly show that 1,2,3-oxadiazolinium ions are rapidly reduced by thiols at physiological pH and that diazenes are likely intermediates in these processes. Because the concentrations of thiols, such as reduced glutathione, in many cells is high, the model chemistry described here suggests that should β-hydroxynitrosamines produce 1,2,3-oxadiazolinium ions in an environment rich in thiols, purely chemical reactions will ensue. Diazenes produced in these processes could decompose by radical pathways, and the presence of oxygen could result in the formation of peroxy radicals and peroxides. Cellular damage could occur through these pathways as well as through the alkylation transformations previously proposed, although thiol interception of 1,2,3-oxadiazolinium ions could reduce the extent of the latter. Conversely, 1,2,3-oxadiazolinium ions could be detoxified through their thiol reactions. The data presented here also demonstrate that the alkylation chemistry of 1,2,3-oxadiazolinium ions is highly sensitive to solvent and only becomes more important in nonaqueous media. In no case were hydroxyethyl sulfides, products which would arise from nucleophilic attack at C-4 of the 1,2,3-oxadiazolinium ion, observed. Michejda and co-workers have also discounted hydroxyethylation as a reaction channel for **1a** *(5, 6, 13)*. The data presented here show that the chemistry of 1,2,3-oxadiazolinium ions is much more extensive than originally perceived and that reactions with thiols could play a role in modulating the reactivity of these substances, leading to additional considerations regarding them and their

[5]Conditions: $PhN{=}NCO_2^-K^+$, 5.6 mmol; PhSH, 4.8 mmol, in 50 mL of 20% CH_3CN/sodium phosphate buffer (pH 7.4, 0.05 M) followed by the addition of 2 mL of 2 M H_2SO_4.

[6]Typical conditions: The appearance of the disulfide of *N*-acetylcysteine was monitored spectrophotometrically using the Ellman reagent. [*N*-Acetylcysteine] = 8.4×10^{-4} M; [**1a**] = 8.44×10^{-3} M.

[7]It has been reported that **1a** reacts with 3,4-dichlorothiophenol in CH_2Cl_2 to give a 90% yield of 3,4-dichlorophenyl methyl sulfide, but no experimental documentation is provided *(2, 6)*.

role in the carcinogenic activation of (2-hydroxyethyl)-nitrosamines.

Acknowledgment. The support of this research by a grant from the National Institutes of Environmental Health Sciences, ES 03953, is gratefully acknowledged.

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