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Fate and Kinetic Modeling of Reactivity of Alkanesulfenic Acids and Thiosulfinates in Model Systems and Onion Homogenates

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The dynamic changes in thiosulfinate profiles were studied in reaction systems containing a crude onion alliinase, *S*-alk(en)yl-L-cysteine sulfoxide substrates (**1**) and preformed thiosulfinates (**4**). Regioisomeric excesses of one of two possible heterologous **4** species (RS(O)SR′, where R ≠ R′) could be manipulated under conditions where alliinase, **1**, and **4** levels were varied. Regioisomeric excesses could be explained by a thiosulfinate (**4**)/alkanesulfenic acid (**2**) trapping mechanism, with the greatest control over product profile governed by the rate of **2** generation in the system. The series of reactions existing in this dynamic reaction system was kinetically modeled with reasonable fits to the experimental data. The application of the **4**/**2** trapping strategy to manipulate thiosulfinate and related organosulfur product profiles in diluted onion homogenates was demonstrated using exogenous MeS(O)SMe (**4a**), PrS(O)SPr (**4c**), and AllS(O)SAll (**4d**) as the preformed thiosulfinate.

KEYWORDS: Thiosulfinate; alkanesulfenic acid; alliinase; kinetics; trapping; Allium

INTRODUCTION

The immediate isolable reaction products of alliinase (EC 4.4.1.4) action on *S*-alk(en)yl-L-cysteine sulfoxides (**1**) are thiosulfinates (**4**) and propanethial-*S*-oxide (**3**), the compounds (**Figure 1**) responsible for the flavor of fresh-cut Alliums (*1, 2*). These organosulfur compounds have also been reported to be responsible for many other biological activities (*3*−*11*). It is reasonable to expect that a relationship between structural features of **4** and various biological activities may exist. If so, then the ability to manipulate the specific profiles of **4** in tissue derivatives of *Allium* spp. may be useful in differentiating and controlling any biological activities that these organosulfur compounds may possess.

A source of various species of pure **4** of defined structure would facilitate studies on structure−function relationships. As an alternative to traditional chemical synthesis (*12, 13*), we have developed the means to prepare pure homologous **4** as well as quaternary mixtures of homologous and heterologous **4** species by employing a biogenerating system founded on alliinase action on appropriate substrates **(1)** (*14*). The molar ratios of the four **4** species evolving in binary **1** reaction systems vary with the specific combination of **1** employed. Because there would appear to be limited opportunities to manipulate or differentially control the levels of specific **4** regioisomers or species in tissue matrixes, the in vitro biogenerating system may provide the means to prepare pure or enriched preparations of specific heterologous **4** species. This development may further enable the ability to

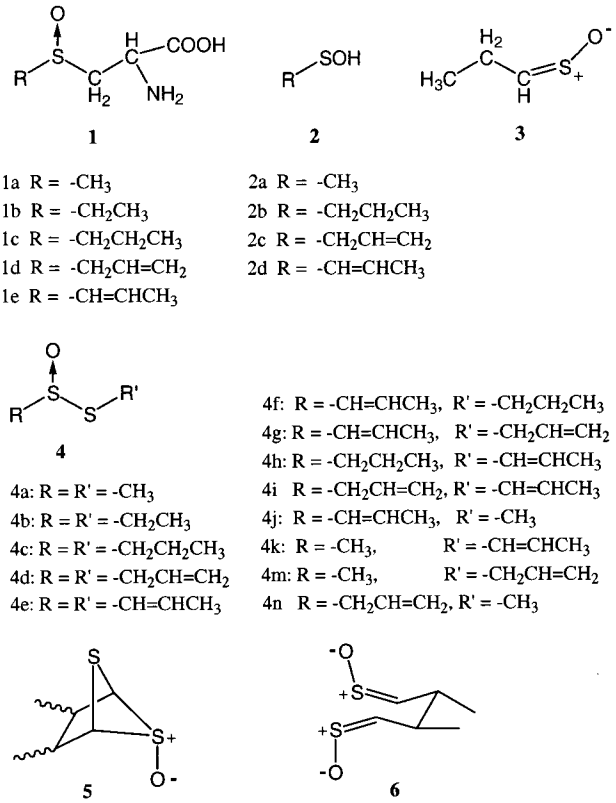

Figure 1. Structures of organosulfur compounds.

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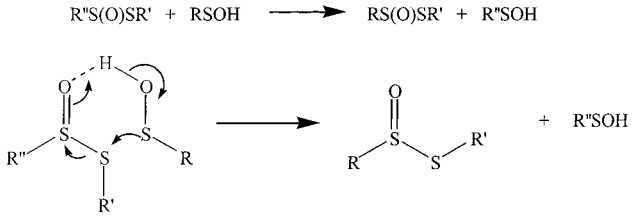
Scheme 1. Exchange Reaction of Sulfenic Acid and Thiosulfinate (proposed in ref. *20*)



evaluate structure−function relationships of these compounds. A similar biogenerating system was employed to yield a variety of sulfinyl disulfides (cepaenes) for the evaluation of structural features related to antithrombotic properties (*8*).

Two recognized alliinase isoforms (*15, 16*) as well as a crude alliinase preparation (*14*) from onion bulb show similar patterns of substrate selectivity in descending order of preference: **1e** > **1d** > **1c** > **1b** > **1a**. One consequence of this relationship is that in model reaction systems with binary mixtures of **1**, an uneven distribution of regioisomeric heterologous **4** species is observed (*14*) as it is in tissue preparations of garlic and other *Allium* species (*17, 18*). Another consequence of these kinetic relationships is that in onion much of the volatile **3** formed from the fast reacting **1e** is lost from the tissue matrix (*19*). Furthermore, bimolecular condensation of **2d** and/or **3** leads to zweibelanes (**5**), sulfinyl disulfides (cepaenes), and bis-sulfine (2,3-dimethylbutanedithial *S*,*S*′-dioxide; **6**) (*13, 20*). Thus, the recovery of 1-propenyl (Pren) residues in thiosulfinates of minced onion is less than predicted based on the original **1** profile in the tissue (*18, 21, 22*). If **3** is a reservoir or source of beneficial biological properties in *Allium* spp. tissue derivatives, then much of the potential benefit of **3** may be lost through the "unfavorable kinetic consequences" arising from the simple step of disrupting onion tissue.

Because of the differential reactivity of **1** species, some species of alkanesulfenic acids (RSOH; **2)** will be generated (from slower reacting **1** species) in the presence of homologous thiosulfinates (**4**) already derived from faster reacting **1** species. This situation is simplified in **Scheme 1** where the reaction of **2** directly with a preformed **4** (R″S(O)SR′) may be used to specifically produce one of the two possible regioisomers (RS(O)SR′) of newly generated **4**. This pathway of thiosulfinate genesis has not been studied as much as the simple process of bimolecular condensation of two **2** (like or unlike species) generated directly from the enzymic phase of the reaction (*1, 2, 20, 23*).

In this paper, the dynamics of reactivity of in vitro model systems containing a preformed thiosulfinate and alliinase-generated **2** or **3** from **1** were described using a kinetic modeling approach. Within the scope of this approach, attempts were also made to (1) demonstrate how the thiosulfinate profile (especially heterologous species) could be manipulated; (2) trap **2d** derived from the action of alliinase upon **1e** as components of various **4** species at the expense of forming **3**; and (3) demonstrate proof-of-concept of trapping **3** in situ.

MATERIALS AND METHODS

Materials. All chemicals were obtained from Sigma (St. Louis, MO) or Aldrich (Milwaukee, WI) Chemical Companies unless otherwise noted. All solvents used were chromatography grade. White onion bulbs were purchased from a local retail market (variety and source unknown). The preparation of alliinase and substrates **1a**−**e** and their general use in model reaction mixtures (all incubated at 20−22 °C) to yield **4a**−**d** was described earlier (*14*). To simplify the kinetic modeling, the crude alliinase was incorporated into the model reaction mixtures as a potassium phosphate-buffered (pH 7.5) solution instead of an alginate-immobilized form. The activity of this preparation was about 0.35 unit (U) mL^{-1} (where one U is defined as that capable of transforming 1 μmol *S*-propyl-L-cysteine sulfoxide min^{-1} at 20−22 °C).

Alkanesulfenic Acid (2)-Trapping Reaction in Model Reaction Systems. Different levels of a single homologous thiosulfinate (**4a** or **4d**), alliinase substrate (**1a** or **1d**), and alliinase were included in a total reaction volume of 3.5 mL of 100 mM Tris buffer (pH 7.5). The progress of changes in thiosulfinate profile was determined by extracting a 0.2-mL sub-sample of reaction mixture into an equal volume of $CHCl_3$ and subjecting the latter to HPLC analysis (*14*).

Quantitation of all organosulfur compounds in this study was done using internal and external standards and published response factors (*14, 18*). In instances for which the response factor was not known, estimates were made on the basis of similarity of structure (for example, the response factor for EtS(O)SPren was considered identical to that of other RS(O)SPren species, all of which have $\epsilon_{M,\ 254\ nm}$ of 21; (*18*). In cases where multiple components coeluted, a mean response factor was used to estimate composite analyte levels.

Medium Effect on Trapping Efficiency of 1-Propenesulfenic acid (2d) by Thiosulfinates (4) in Model Reaction Systems. Reaction mixtures were made to contain 1.5 mM thiosulfinate (**4a** or **4d)**, 2.2 mM *S*-1-propenyl-L-cysteine sulfoxide (**1e)**, and alliinase (0.09 U mL^{-1}) in 100 mM Tris buffer (pH 7.5) or 100 mM potassium phosphate buffer (pH 5.0). The total reaction mixture was extracted with 1 mL of $CHCl_3$ at the desired time point and subjected to HPLC analysis.

Trapping of 1-Propenesulfenic Acid (2d) from *S*-1-Propenyl-L-cysteine Sulfoxide (1e) in Model Reactions. Thiosulfinates **4a**, **4b**, **4c**, or **4d** (at 1.3−1.5 mM) were included individually with 3 mM **1e** and alliinase (0.15 U mL^{-1}) in 100 mM Tris buffer (pH 7.5). At predetermined intervals, 0.2 mL of reaction mixture was extracted with an equal volume of $CHCl_3$ and subjected to HPLC analysis.

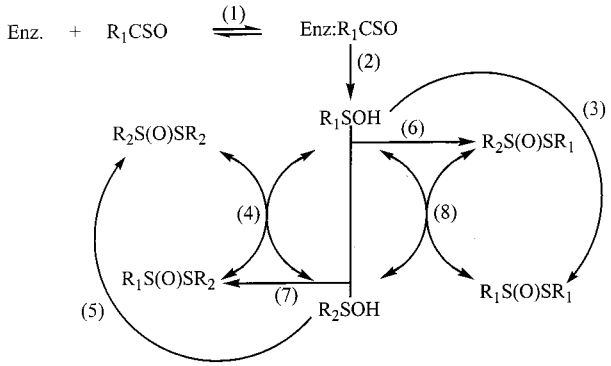
All of the aforementioned model systems "trapping" experiments were conducted as duplicate trials. Standard statistical procedures were not used to analyze the primary data because of the complication that on occasion the presence of a reaction product at a particular time point was observed for only one, but not both experimental trials. This problem was particularly evident for analytes present at levels near the limit of detection. As an alternative, the range in % variation of mean values for each experiment is noted in the figure legends.

Trapping of 1-Propenesulfenic Acid (2d) in situ with Freshly Minced, Diluted Onion Tissue. A mixture of 50 g of white onion bulb tissue, 1.05 U of alliinase, 1 mL of thiosulfinate stock solution (**4a**, **4c**, or **4d** individually at 12−22 mM), and 80 mL of distilled water was homogenized for 1 min (a sample without added thiosulfinate served as a control and the homogenate pH ranged from 5.2 to 5.5). Although the onion bulb tissue was not analyzed, alliinase activity is expected to be in the range of 15−70 U g^{-1} (fresh weight) depending on the specific substrate (**1)** (*14, 24, 25*), the substrate profile may range between 2 and 11 μmol g^{-1} **1a**, 1−2 μmol g^{-1} **1c**, and 2−33 μmol g^{-1} **1e**, depending on cultivar and soil sulfur fertility (*21, 22, 26*), and the total levels of thiosulfinates (**4**) after maceration may range between 0.14 and 0.35 μmol g^{-1} (*18*). Thus, although the added alliinase is negligible, the **4a**, **4c**, or **4d** is added at a level similar to that expected in disrupted onion bulb tissue.

After the samples incubated for 20 min at 20−22 °C, they were filtered through cheesecloth to remove coarse tissue particles. Each sample was then extracted with 50 mL of $CHCl_3$. The $CHCl_3$ extracts were subjected to HPLC analysis after being concentrated to ∼1 mL by evaporation at ambient conditions (<22 °C) under a stream of $N_{2(g)}$. 1-Propenyl (Pren)-containing thiosulfinates were identified on the basis of the elution patterns observed for the reaction mixtures in the preceding sections as well as from previous studies (*14, 18, 27*). Because considerable variance in specific analyte levels was evident in these studies (likely because of the extensive sample workup and solvent evaporation required), representative observations (and not averaged values) are reported for each in situ system studied.

Kinetic Modeling. The overall series of reactions that was considered to take place between preformed **4** and alliinase-generated **2** is provided in **Scheme 2**. The reaction of **1** with alliinase was treated as a

Scheme 2. Reaction Network for the Mixture of Homologous Thiosulfinate ($R_2S(O)SR_2$), Alliinase Substrate (R_1CSO), and Onion Alliinase (Enz)



Scheme 3. Component Reactions for the Mixture of MeS(O)SMe (**4a**), **1d**, and Onion Alliinase

(1) 2-PeCSO + E $\underset{k_{-1}=20}{\overset{k_1=3.5}{\rightleftharpoons}}$ 2-PeCSO:E

(2) 2-PeCSO:E $\xrightarrow{k_2=0.96}$ AllSOH:E + others

(3) AllSOH:E + AllSOH:E $\xrightarrow{k_3=0.018}$ AllS(O)SAll + H_2O

(4) AllSOH + MeS(O)SMe $\underset{k_{-4}=0.035}{\overset{k_4=0.035}{\rightleftharpoons}}$ AllS(O)SMe + MeSOH

(5) MeSOH + MeSOH $\xrightarrow{k_5=0.14}$ MeS(O)SMe + H_2O

(6) All-SOH + MeSOH $\xrightarrow{k_6=0.10}$ AllS(O)SMe + H_2O

(7) All-SOH + MeSOH $\xrightarrow{k_7=0.16}$ MeS(O)SAll + H_2O

(8) MeSOH + AllS(O)SAll $\underset{k_{-8}=0.035}{\overset{k_8=0.035}{\rightleftharpoons}}$ MeS(O)SAll + AllSOH

conventional enzymic reaction mechanism, in which the substrate first forms a reversible complex with the enzyme (step 1), and this complex undergoes dissociation to release the product species (step 2) or substrate (step 1). For the enzymic reaction, the Michaelis−Menten rate expression was used to model the kinetic relationships among reaction components. The RSOH (**2**) evolved from the enzymic reaction can subsequently react in bimolecular condensation reactions (step 3, which exclusively represents the pool of **2** species derived from the enzyme reaction) or with a thiosulfinate ($R_2S(O)SR_1$) species (step 4), the latter being a reversible reaction. The subsequent chemical reactions (steps 5−8) were also assumed to conform to conventional bimolecular elementary reaction rate laws (viz., the reaction order of each species is identical to the stoichiometric coefficient of that species for the reaction as written). Reactions 5−7 are bimolecular condensation reactions of species of **2**, and reaction 8 is another reversible "exchange" reaction between species of **4** and **2**.

Specifically, for the model that included **1d** (2-PeCSO), **4a** (MeS(O)SMe), and alliinase, the network of possible reactions (rate constants and species) is depicted in **Scheme 3**. Referring back to **Scheme 2**, the 2-propenyl group is assigned as R_1 and the methyl group is assigned as R_2 for this model reaction. This network gives rise to the following rate expressions for component reactions in a manner that accounts for the fate of each chemical species incorporated into the model (the "*y*" term was incorporated to account for any fractional degree of enzyme decay, possibly by reaction of enzyme sulfhydryl groups with **4** (*11*) or inhibition (*26*, *28*) during the course of reaction). The % enzyme decay in the model was in the range 33−36% in **Figure 2** (**Scheme 3**), and 38−39% in **Figure 3** (**Scheme 4**), and this decay parameter is likely to be affected by enzyme purity. The reaction network for **Scheme 3** is

$$d[2-\text{PeCSO}]/dt = (-k_1[2-\text{PeCSO}][\text{E}]) + (k_{-1}[2-\text{PeCSO:E}])$$

$$d[\text{E}]/dt = (-k_1[2-\text{PeCSO}][\text{E}]) + (k_{-1}[2-\text{PeCSO:E}]) + (yk_2[2-\text{PeCSO:E}])$$

$$d[2-\text{PeCSO:E}]/dt = (k_1[2-\text{PeCSO}][\text{E}]) - (k_{-1}[2-\text{PeCSO:E}]) - (k_2[2-\text{PeCSO:E}])$$

$$d[\text{AllSOH}]/dt = (k_2[2-\text{PeCSO:E}]) - (2k_3[\text{AllSOH:E}][\text{AllSOH:E}]) - (k_4[\text{MeS(O)SMe}][\text{AllSOH}]) + (k_{-4}[\text{AllS(O)SMe}][\text{MeSOH}]) - (k_6[\text{MeSOH}][\text{AllSOH}]) - (k_7[\text{MeSOH}][\text{AllSOH}]) + (k_8[\text{MeSOH}][\text{AllS(O)SAll}]) - (k_{-8}[\text{MeS(O)SAll}][\text{AllSOH}])$$

$$d[\text{AllS(O)SAll}]/dt = (k_3[\text{AllSOH}][\text{AllSOH}]) - (k_8[\text{MeSOH}][\text{AllS(O)SAll}]) + (k_{-8}[\text{MeS(O)SAll}][\text{AllSOH}])$$

$$d[\text{MeS(O)SMe}]/dt = -(k_4[\text{MeS(O)SMe}][\text{AllSOH}]) + (k_{-4}[\text{AllS(O)SMe}][\text{MeSOH}]) + (k_5[\text{MeSOH}][\text{MeSOH}])$$

$$d[\text{AllS(O)SMe}]/dt = (k_4[\text{MeS(O)SMe}][\text{AllSOH}]) - (k_{-4}[\text{AllS(O)SMe}][\text{MeSOH}]) + (k_6[\text{MeSOH}][\text{AllSOH}])$$

$$d[\text{MeS(O)SAll}]/dt = (k_7[\text{MeSOH}][\text{AllSOH}]) + (k_8[\text{MeSOH}][\text{AllS(O)SAll}]) - (k_{-8}[\text{MeS(O)SAll}][\text{AllSOH}])$$

$$d[\text{MeSOH}]/dt = (k_4[\text{MeS(O)SMe}][\text{AllSOH}]) - (k_{-4}[\text{AllS(O)SMe}][\text{MeSOH}]) - (2k_5[\text{MeSOH}][\text{MeSOH}]) - (k_6[\text{MeSOH}][\text{AllSOH}]) - (k_7[\text{MeSOH}][\text{AllSOH}]) - (k_8[\text{MeSOH}][\text{AllS(O)SAll}]) + (k_{-8}[\text{MeS(O)SAll}][\text{AllSOH}])$$

Similarly, rate expressions for the network of reactions that include **1a** (MCSO), **4d** (AllS(O)SAll), and alliinase (**Scheme 4**), where referring back to **Scheme 2**, the methyl group is assigned as R_1 and the 2-propenyl group is assigned as R_2 for this model reaction, are as follows:

$$d[\text{MCSO}]/dt = (-k_1[\text{MCSO}][\text{E}]) + (k_{-1}[\text{MCSO:E}])$$

$$d[\text{E}]/dt = (-k_1[\text{MCSO}][\text{E}]) + (k_{-1}[\text{MCSO:E}]) + (yk_2[\text{MCSO:E}])$$

$$d[\text{MCSO:E}]/dt = (k_1[\text{MCSO}][\text{E}]) - (k_{-1}[\text{MCSO:E}]) - (k_2[\text{MCSO:E}])$$

$$d[\text{MeSOH}]/dt = (k_2[\text{MCSO:E}]) - (2k_3[\text{MeSOH:E}][\text{MeSOH:E}]) - (k_4[\text{AllS(O)SAll}][\text{MeSOH}]) + (k_{-4}[\text{MeS(O)SAll}][\text{AllSOH}]) - (k_6[\text{AllSOH}][\text{MeSOH}]) - (k_7[\text{AllSOH}][\text{MeSOH}]) + (k_8[\text{AllSOH}][\text{MeS(O)SMe}]) - (k_{-8}[\text{AllS(O)SMe}][\text{MeSOH}])$$

$$d[\text{MeS(O)SMe}]/dt = (k_3[\text{MeSOH}][\text{MeSOH}]) - (k_8[\text{AllSOH}][\text{MeS(O)SMe}]) + (k_{-8}[\text{AllS(O)SMe}][\text{MeSOH}])$$

$$d[\text{AllS(O)SAll}]/dt = -(k_4[\text{AllS(O)SAll}][\text{MeSOH}]) + (k_{-4}[\text{MeS(O)SAll}][\text{AllSOH}]) + (k_5[\text{AllSOH}][\text{AllSOH}])$$

$$d[\text{MeS(O)SAll}]/dt = (k_4[\text{AllS(O)SAll}][\text{MeSOH}]) - (k_{-4}[\text{MeS(O)SAll}][\text{AllSOH}]) + (k_6[\text{AllSOH}][\text{MeSOH}])$$

$$d[\text{AllS(O)SMe}]/dt = (k_7[\text{AllSOH}][\text{MeSOH}]) + (k_8[\text{AllSOH}][\text{MeS(O)SMe}]) - (k_{-8}[\text{AllS(O)SMe}][\text{MeSOH}])$$

$$d[\text{AllSOH}]/dt = (k_4[\text{AllS(O)SAll}][\text{MeSOH}]) - (k_{-4}[\text{MeS(O)SAll}][\text{AllSOH}]) - (2k_5[\text{AllSOH}][\text{AllSOH}]) - (k_6[\text{AllSOH}][\text{MeSOH}]) - (k_7[\text{AllSOH}][\text{MeSOH}]) - (k_8[\text{AllSOH}][\text{MeS(O)SMe}]) + (k_{-8}[\text{AllS(O)SMe}][\text{MeSOH}])$$

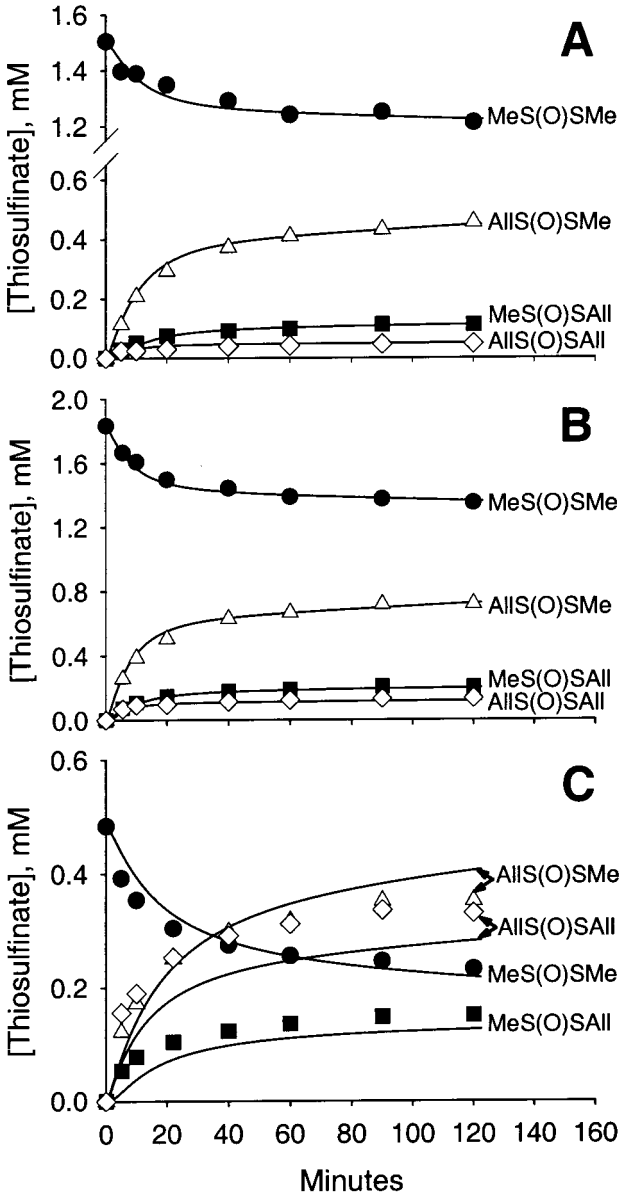


Figure 2. Trapping of 2-propenesulfenic acid (**2c**) with MeS(O)SMe (**4a**). Symbols represent experimental data and lines represent kinetic model for reactions between **4a**, **1d**, and alliinase at respective levels of (**A**) 1.4 mM, 18.5 mM, 0.05 U mL^{-1}; (**B**) 1.8 mM, 31.3 mM, 0.10 U mL^{-1}; (**C**) 0.5 mM, 31.3 mM, 0.10 U mL^{-1}. Respective error of the mean was 13.4, 9.6, and 7.6% for duplicate trials, with r^2 values for fit of modeled to experimental data = 0.92.

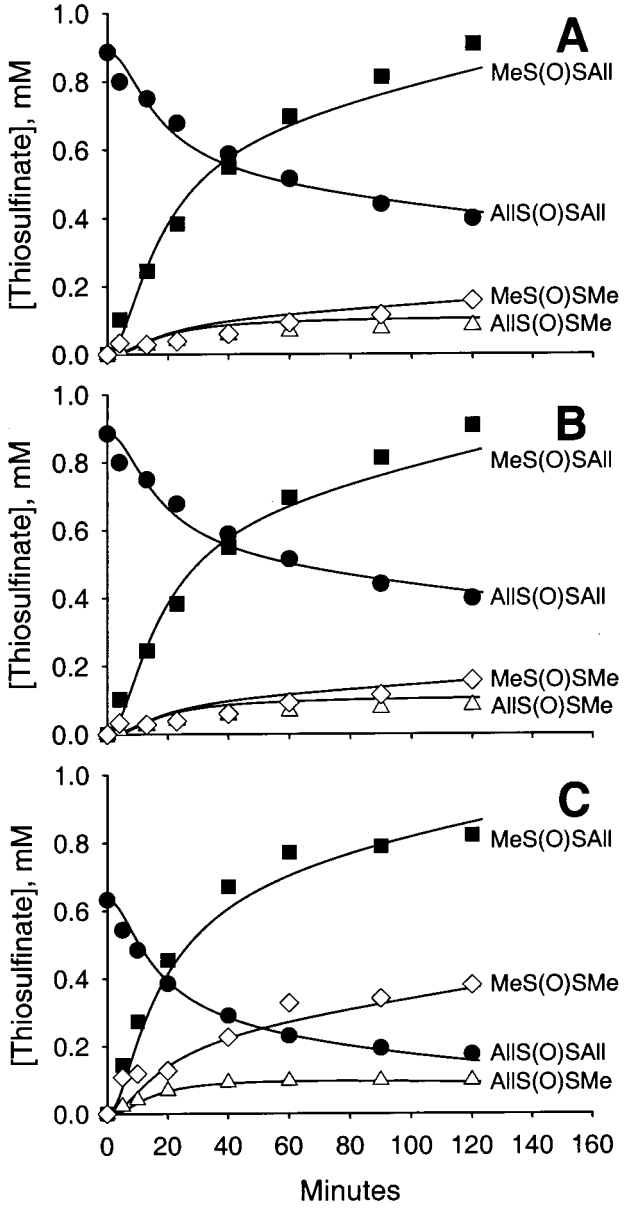


Figure 3. Trapping of methanesulfenic acid (**2a**) with AllS(O)SAll (**4d**). Symbols represent experimental data and lines represent kinetic model for reactions between **4d**, **1a**, and alliinase at respective levels of (**A**) 3.2 mM, 29.2 mM, 0.10 U mL^{-1}; (**B**) 0.9 mM, 29.2 mM, 0.10 U mL^{-1}; (**C**) 0.6 mM, 58.2 mM, 0.15 U mL^{-1}. Respective error of the mean was 24.5, 9.9, and 8.6% for duplicate trials, with r^2 values for fit of modeled to experimental data = 0.95.

All of the above differential equations were solved numerically using Mathematica Version 2.2 by Wolfram Research, Inc. (Champaign, IL; 1993). Wide ranges of values for rate constants were empirically tested. Rate constants represented by k_1 and k_{-1} for step 1 in **Scheme 2** and reaction 1 in **Schemes 3** and **4** were initially set to reflect affinity (dissociation) constants (K_s value or k_{-1}/k_1) of 6 mM and 15 mM, for **1d** and **1a**, respectively, in recognition of the K_m determinations made by previous studies under similar reaction conditions *(24, 28, 29)*. Values of rate constants obtained for the analysis of **Scheme 3** were retained for analysis of **Scheme 4** in the case of like reactions where rate constants were expected to be identical (**Schemes 2**−**4**: reactions/steps 4, 6, 7, and 8). The rate constants for each step were calculated to best fit the experimental results. Sensitivity of the modeled reactions was also tested by manually varying the rate constants and reevaluating fit of the model to the experimental results.

RESULTS AND DISCUSSION

Kinetic Modeling of Reactivity (Trapping) of Alkanesulfenic Acid (2) Generated by Alliinase in the Presence of Thiosulfinates (4). It was previously noted that the generation of **2** in the presence of a preformed homologous species of **4** could lead to the preferential accumulation of one of two regioisomers of heterologous species of **4** (*14*, *17*). Because of the wide range of reaction conditions that could be empirically explored to evaluate the chemical dynamics of the processes depicted in **Scheme 2**, it was judged to be more suitable to attempt a kinetic modeling of these reactions instead.

Two sets of trapping reactions were investigated, and within each set three conditions were evaluated where the levels of **4**,

Scheme 4. Component Reactions for the Mixture of AllS(O)SAll (**4d**), **1a**, and Onion Alliinase

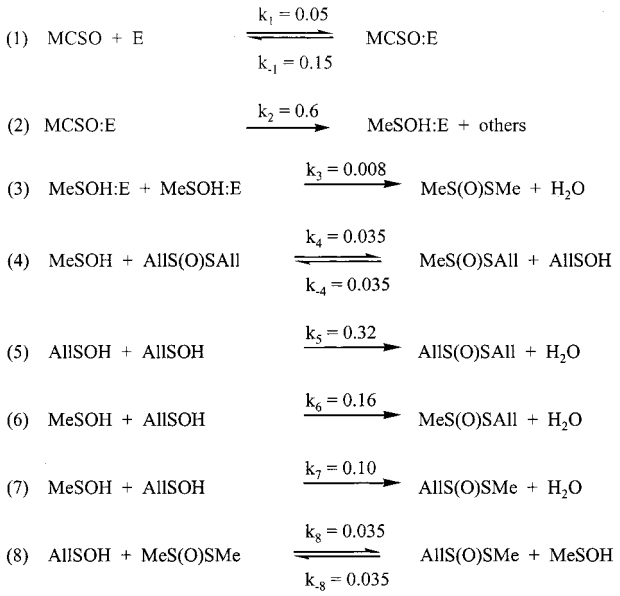


Table 1. Distribution of Alk(en)yl Groups Generated from (**1a**, **1c**) in Thiosulfinate-Containing Reactions[a]

MeS(O)SMe (**4a**) + **1d**

4a/**1d**/enzyme[b]	AllS(O)SMe (**4n**) (%)[c]	MeS(O)SAll (**4m**) (%)	AllS(O)SAll (**4d**) (%)	molar ratio **4n**:**4m**[d]
1.4 mM/18.5 mM/0.05 U mL^{-1}	68.8	16.7	14.5	4.1
1.8 mM/31.3 mM/0.10 U mL^{-1}	60.3	17.0	22.7	3.5
0.5 mM/31.3 mM/0.10 U mL^{-1}	30.2	13.0	56.8	2.3

AllS(O)SAll (**4d**) + **1a**

4d/**1a**/enzyme[a]	AllS(O)SMe (**4n**) (%)[b]	MeS(O)SAll (**4m**) (%)	MeS(O)SMe (**4a**) (%)	molar ratio **4m**:**4n**[c]
3.2 mM/29.2 mM/0.10 U mL^{-1}	5.0	90.1	4.9	18
0.9 mM/29.2 mM/0.10 U mL^{-1}	6.3	69.6	24.1	11
0.6 mM/58.2 mM/0.15 U mL^{-1}	5.8	48.9	45.3	8.4

[a] Results are representative of two trials. [b] Relative levels of **4**, **1**, alliinase. [c] Mol percent measured after 2 h reaction. [d] Molar ratio of $R_1S(O)SR_2$:$R_2S(O)SR_1$ (see also **Scheme 2**).

1, and alliinase were varied (**Table 1**). The distribution (mol %) of alk(en)yl groups arising from the **2** generated from the **1** species after 2 h in the model reactions was dependent on the relative levels of reactants employed, and both systems behaved similarly. In every case, the dominant regioisomer of the heterologous **4** species was the one depicted as $R_1S(O)SR_2$ where R_1 evolved from the **1** and R_2 evolved from the preformed **4** species employed in the reaction mixture (refer to **Scheme 2**).

Other general tendencies in the behaviors of both of these model reaction systems were evident. The molar ratio of $R_1S(O)SR_2$:$R_2S(O)SR_1$ increased as alliinase and/or **1** levels were reduced, or in other words, as the rate of **2** generation in the system was attenuated. Apparently this allowed for greater efficiency in "trapping" the R_1SOH (**2**) species as a heterologous thiosulfinate (and specifically as the $R_1S(O)SR_2$ species), as did increasing the level of preformed **4**. In contrast, as the reaction rate was accelerated, the mol ratio of $R_1S(O)SR_2$:$R_2S(O)SR_1$ decreased and there was a progressive increase in the mol % of the homologous **4** species ($R_1S(O)SR_1$), which was not originally present in the reaction system. It was evident that the rate of flux of R_1SOH (**2**) into the reaction mixture was the most important factor controlling the profile of various **4** species evolving in the reaction system (**Scheme 2**).

The kinetic modeling was intended to derive rate constants for each component reaction and fit calculated parameters to the experimental results (**Figures 2** and **3**). The close agreement of calculated parameters with experimental data indicated that the proposed component reactions of the reaction network (**Scheme 2**) were representative of the model reactions in a manner that could be used to predict the outcomes of these reaction systems (and perhaps for any other set of reactant concentrations). In general, the total **4** generated in the model reactions was determined by the extent of the enzymic reaction, whereas the distribution of **4** among various species was determined by the relative rate constants of competing reactions in the reaction networks. The actual rate constants obtained from modeling are shown with the equations in **Schemes 3** and **4**. These rate constants should be considered only as relative values, because the absolute amount of alliinase enzyme(s) (viz., concentration of active sites) was unknown, as was the level of impurities (potential inhibitors) in the substrate preparations.

Aside from the ability to predict reaction outcomes, the other focus of the kinetic modeling was to infer certain characteristics regarding various reactions that control the fate of organosulfur compounds in dynamic systems that simulate disrupted *Allium* tissues. On the basis of the results obtained from modeling, the rate constants of condensation reactions of **2** (steps 5−7) are about 3−4 times greater than for reactions between **2** and **4** (exchange reactions, steps 4 and 8).

An exception was that the rate constant of the condensation of the "primary" **2** species (derived directly from the enzymic reaction) to form a homologous **4** species (step 3 in **Schemes 2**−**4**) was unexpectedly slow given the fact that **2** are considered transient and nonisolable intermediates (*20*). An explanation for this behavior is that the "primary" **2** species may remain as an enzyme/product complex (RSOH:E) and delay **2** participation in homo-condensation reactions relative to the reactivity of the other **2** species derived from exchange reactions with **4** in solution phase. On balance there would be a great molar excess of preformed **4** available to react with the "primary" **2** to yield heterologous **4** species and "secondary" **2** species (reaction 4 in **Scheme 2**). These secondary **2** species can condense to form homologous and heterologous **4** (reactions 5−7 in **Scheme 2**). As further evidence of differences in reactivity of the "primary" and "secondary" **2** species, the kinetic model was very sensitive to changes in the calculated values of k_3 and k_5, inferring quantifiable differences between these rate constants.

The modeling also indicated that the rate constant for forming MeS(O)SAll (**4m**)was 1.6-fold greater than that of forming AllS(O)SMe (**4n**) in the condensation reaction between **2c** and **2a** (steps 6 and 7 in **Schemes 3** and **4**). This may be explained by the existence of a preferred orientation of these two **2** species upon condensation. Although this subtle difference would be difficult to verify experimentally given the transient nature of **2** species (*20*), it should be noted that the kinetic model was very sensitive to changes in the calculated values of k_6 and k_7, indicating quantifiable differences between these rate constants.

The kinetic model also predicted that the enzymic step ($[k_2 + k_{-1}]/k_1$, equivalent to k_{cat}/K_m, the specificity constant) for the reactivity of **1d** was about 4-fold greater than that for **1a**. This is in close agreement with the experimental kinetic analysis of the enzymic reaction phase provided in a previous report (*14*). Considering the generation of "primary" **2** from **1d** was faster than that from **1a**, it stands to reason that there is less preference for one regioisomer of heterologous **4** over the

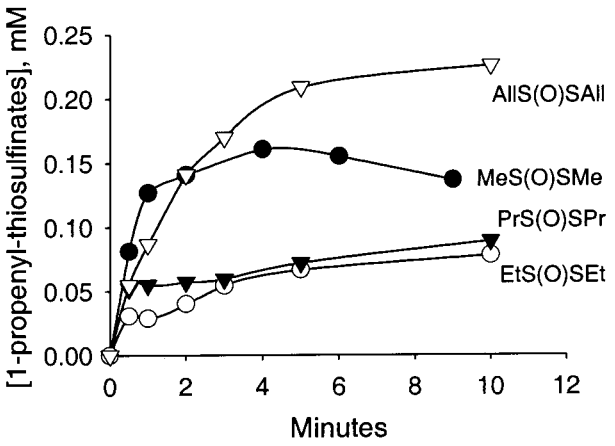


Figure 4. Trapping of 1-propenesulfenic acid (**2d**) with homologous thiosulfinates (**4**). Symbols and lines represent experimental data obtained for the evolution of 1-propenyl-containing thiosulfinates in separate reactions with individual homologous thiosulfinates (**4**, at 1.3–1.5 mM) with 3 mM **1e** and alliinase in 100 mM Tris (pH 7.5). For reactions with MeS(O)SMe (**4a**), EtS(O)SEt (**4b**), PrS(O)SPr (**4c**), and AllS(O)SAll (**4d**), respective errors of the mean were 4.4, 12.1, 8.9, and 9.7% for duplicate trials.

other in the former compared to the latter reaction system (**Table 1**). This can also be accounted for by the rate of flux of **2c** formation being sufficient to support reaction steps 6 and 7 in addition to being "trapped" in step 3 of **Scheme 3**, whereas the generated **2a** is more effectively "trapped" in step 3, leaving little to participate in steps 6 and 7 of **Scheme 4**.

Of all of the rate constant estimates made during the various iterations of kinetic modeling, steps 4 and 8 of **Schemes 3** and **4** (the reversible alkanesulfenic acid−thiosulfinate exchange reactions) were least sensitive in terms of fitting the experimental results.

Trapping of (2d) Generated in Model Reactions. Thiosulfinates **4d** and **4a** were more effective in trapping 1-propenyl (Pren) residues than were **4c** and **4b** (**Figure 4**). However, in all cases, the PrenS(O)SR species was formed at levels in excess of its regioisomer RS(O)SPren as one would expect from **Scheme 2**. Furthermore, the rapid rearrangement of any **4e** (to yield **5)** (*13, 20*) would also limit RS(O)SPren accumulation according to **Scheme 1**. One reviewer also suggested that the bimolecular condensation of **2d** with either **2a**, **2b**, or **2c** would favor the formation of PrenS(O)SR over RS(O)SPren because the greater acidity of **2d** would protonate **2a**, **2b**, or **2c** (to yield $RSOH_2^+$ and rendering this species subject to elimination of water) while maintaining the PrenS(O)− configuration. The regioisomeric (molar) excess of PrenS(O)SR/RS(O)SPren reached 5:1 (data not shown) for the systems employing **4d** and **4a** as the trapping agent. The same regioisomeric excess was noted but was less estimable in the case where **4c** and **4b** were employed, because of the apparent lower levels of heterologous **4** accumulated (in some cases, the lesser abundant species was fugitive and intermittently detected during reaction progress). Although the lesser reactivity or 1-propenyl-"trapping" ability of the **4c** and **4b** species is difficult to explain mechanistically, other evidence was obtained to suggest that this was an accurate conclusion. For reaction systems employing these latter two thiosulfinates, greater steady-state levels of both **3** and a product tentatively identified as bis-sulfine (**6**, a homocondensation and rearrangement product of **3**; ref. *20*) were observed by HPLC, indicating a relative lack of reaction of **3** with these thiosulfinates. The decline of the total Pren-thiosulfinate "trapping" products with the **4a**-based reaction system (**Figure 4**) may also

Table 2. Medium Effect on the Trapping 1-Propenyl Groups Generated from (**1e)** in the Presence of AllS(O)SAll (**4d**) or MeS(O)SMe (**4a**) in Model Reactions[a]

MeS(O)SMe (**4a**) + **1e**

	3	PrenS(O)SMe (**4j**)	MeS(O)SPren (**4k**)	molar ratio **4j:4k**
KH_2PO_4/K_2HPO_4 (40 min)	1.78	0.039	n.d.[b]	
Tris (40 min)	0.068	0.113	0.021	5.4
Tris (5 min)	0.669	0.248	0.038	6.5

AllS(O)SAll (**4d**) + **1e**

	3 + **4d**[c]	PrenS(O)SAll (**4g**)	AllS(O)SPren (**4i**)	molar ratio **4g:4i**
KH_2PO_4/K_2HPO_4 (40 min)	∼4.8	0.051	0.011	4.7
Tris (40 min)	∼1.2	0.233	0.053	4.4
Tris (5 min)	∼4.7	0.562	0.093	6.6

[a] Levels of organosulfur compounds are expressed as mM in reaction mixtures. Reaction conditions were 1.5 mM of **4d** or **4a**, 2.2 mM of **1e**, and 0.09 U mL^{-1} of alliinase in Tris buffer (100 mM, pH 7.5) or potassium phosphate buffer (100 mM, pH 5.0). [b] n.d. = Not detected. [c] Levels are considered estimates because an average response factor was used to quantify this mixture of components. Results are representative of two trials.

be attributable to further reactivity or instability of PrenS(O)-SMe (**4j**). This species is less stable in situ than PrenS(O)SAll (**4g**) (*18*), the latter which is the major product formed in Pren-trapping reactions employing **4d**.

The nature of the reaction medium also influenced the ability of **4a** or **4d** to trap 1-propenyl fragments originating from **1e** (and subsequently **2d** and **3**) (**Table 2**). The presence of potassium phosphate (at pH 5.0) facilitated the accumulation of **3** (as was noted in ref. *14*), although this may be primarily an effect of pH relative to the Tris-buffered (pH 7.5) system. In contrast, the Tris buffer at pH 7.5 was more conducive to accumulating Pren-thiosulfinate species, with the expected regioisomeric excess. Extended incubation of the Tris-buffered reaction systems resulted in a net loss of both **3** and Pren-thiosulfinates. This is likely caused by a combined volatility of **3,** lability of Pren-thiosulfinates species (*18*), and chemical reactivity of specific thiosulfinates near pH 7.5 (*27*).

Trapping of (2d) Generated from Freshly Minced and Diluted Onion Tissue. An attempt to employ the same strategy used in the model reaction systems to "trap" 1-propenyl fragments as **4** in freshly minced onion tissue yielded favorable results (**Table 3**). Peak identification was based on the results and product profiles obtained from the model reaction systems described earlier in this manuscript as well as published data (*14, 19, 27*).

In the presence of preformed homologous **4**, compound **3** accumulation in freshly minced onion tissue was reduced with a reciprocal accumulation of Pren-containing thiosulfinates. This was not as obvious in the case of trapping with AllS(O)SAll (**4d**), which could not be chromatographically resolved from **3**. In terms of total levels of compounds that could represent "trapped" 1-propenyl groups (compounds **4f**, **4g**, **(*E,Z*)-4h**, **4j**, and **4k**), the systems containing the exogenous thiosulfinate were similarly effective and caused an elevation in the levels of 1-propenyl-containing thiosulfinates relative to that of the control.

Interestingly, the level of the bis-sulfine (**6**) appeared to increase in all cases where exogenous thiosulfinate was added compared to the control. Also, MeS(O)SPren (**4k**), a major thiosulfinate observed in freshly homogenized onion tissue (*18*), was not found in our control sample. However, compound **10**

Table 3. Trapping of 1-Propenyl Groups Generated from (**1e**) with Homologous Thiosulfinates in Freshly Homogenized Onion Bulb Tissue[a]

Control: 50 g white onion + 80 mL H_2O + 3 mL enzyme

compd[b]	**4f**	**4g**	Σ **4c**, (*Z*)-**4h**[c]	**3**	**6**
mM	0.294	0.025	0.847	31.7	0.6374

Sample 1: 50 g white onion + 1 mL AllS(O)SAll + 80 mL H_2O + 3 mL enzyme

compd	**4f**	**4g**	Σ **4c**, (*Z*)-**4h**[c]	Σ **4d**, **3**[e]	**4k**	**6**
mM	0.226	0.540	0.638	83.7	0.252	0.889

Sample 2: 50 g white onion + 1 mL PrS(O)SPr + 80 mL H_2O + 3 mL enzyme

compd	**4f**	**4g**	Σ **4c**, **4i**, (*Z*)-**4h**[d]	**3**	(*E*)-**4h**	**?**[f]	**4k**	**6**	**??**[f]
mM	0.240	0.040	5.42	1.09	0.368	0.208	0.257	1.12	0.161

Sample 3: 50 g white onion + 1 mL MeS(O)SMe + 80 mL H_2O + 3 mL enzyme

compd	**4f**	**4g**	Σ **4c**, (*Z*)-**4h**[c]	**3**	**4j**	**?**[f]	**4k**	**6**	**4a**	**??**[f]
mM	0.217	0.044	0.561	6.01	2.36	0.758	0.268	1.032	3.04	0.226

[a] Homogenates were extracted with $CHCl_3$ after 20 min, concentrated, and analyzed by HPLC, using response factors provided in (*14*). [b] Structures of compounds (compd) are listed in **Figure 1**. [c−f] Values are reported as mM in the homogenate. For compounds that could not be resolved, an average response factor ($\epsilon_{M,\ 254\ nm}$) was calculated as 16[c], 17.7[d], and 18[e], and for unknown compounds **?** and **??**[f] a response factor of 16 was used.

was generated to similar levels in the three samples with exogenous thiosulfinate added. Both of these results are somewhat surprising, because these compounds would not be expected to accumulate by virtue of a thiosulfinate−alkanesulfenic acid trapping reaction (depicted in **Scheme 2**). The enhanced levels of **4k** and **6** may be explained by the persistence of **2d** (or **3**) residues in homogenates supplemented with thiosulfinates through reversible steps 4 and 8 after the initial "trapping" in step 4 of **Scheme 2**.

Further evidence of trapping alkanesulfenic acids by preformed thiosulfinate was derived from the appearance of new compounds and the specific enrichment of some existing compounds relative to that of the control sample. The major new compounds formed by trapping with preformed AllS(O)SAll (**4d**) were PrenS(O)SAll (**4g**) and AllS(O)SPren (**4i**), both of which were not detected in the control sample. In the case of trapping with preformed PrS(O)SPr (**4c**), although the appearance of **(*Z*)-4h** needs to be further confirmed because of partial coelution with **4c**, the new peaks of **(*E*)-4h** and two unknown compounds (designated "**?**" and "**??**") on the HPLC chromatogram were clearly seen. Compound **(*E*)-4h** is not the regioisomer that would be expected to evolve from a thiosulfinate−alkanesulfenic acid trapping mechanism, but regioisomers **4f** and **(*E*)-4h** were found at similar levels.

In the onion juice with exogenous MeS(O)SMe (**4a**), the compound PrenS(O)SMe (**4j**), which was not found in the control, was formed as the third dominant organosulfur species after **3** and compound **6**. Only in the onion homogenate supplemented with **4a** (but not **4c** or **4d**), did the regioisomeric excess of PrenS(O)SR (**4j**) over PrenSS(O)R **(4k**) appear, at a ratio of about 5:1, similar to that of the model reaction systems (**Figure 4**).

The onion homogenates supplemented with MeS(O)SMe (**4a**) behaved more like the model systems than did homogenates supplemented with PrS(O)SPr (**4c**) or AllS(O)SAll (**4d**) (**Table 3**). Many reasons can account for differences in behavior between species of **4** used and the reaction system. Levels of preformed thiosulfinates in the homogenates were about 20% of those used in model systems. Compound **4a** may be at a greater solution level, and potential reactivity, because it partitions more into the water phase than do **4c** and **4d** (*14*). There may also be differences in reactivity of **4a**, **4c**, and **4d** with **3** generated in the onion homogenates (as indicated by **Figure 4**). The presence of γ-glutamyl-linked **1** species and transpeptidase action (*30, 31*) may provide an additional source of some organosulfur components and impact the dynamics of the production and trapping of **2** in tissue homogenates. Last, differences between the in vitro and in situ systems may be attributable to the reactivity of components endogenous to onion tissue as well as the presence of Tris in the model reactions systems, as Tris has also been shown to affect reactivity of **3** and **4** (*14, 27*; **Table 2**).

Supporting Information Available: Elution patterns of organosulfur compounds on HPLC chromatogram. This material is available free of charge via the Internet at http://pubs.acs.org.

ABBREVIATIONS USED

RS(O)SR′, thiosulfinate; Pren, 1-propenyl; RSOH, alkanesulfenic acid; 2-PeCSO, *S*-2-propenyl-L-cysteine sulfoxide; MCSO, *S*-methyl-L-cysteine sulfoxide

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