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In Vitro Stability and Chemical Reactivity of Thiosulfinates

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A model reaction system was used to generate pure thiosulfinates (**3**) from *S*-alk(en)yl-L-cysteine sulfoxides (**1**) to facilitate studies on the intrinsic pH and thermal sensitivities of individual thiosulfinate species. Thiosulfinate decay could be characterized as first-order processes over the pH range of 1.2−9.0 and at 20−80 °C. The stability of thiosulfinates was greatest at pH 4.5−5.5, followed by pH 1.2, pH 6.5−7.5, and pH 8.0−9.0. Thiosulfinates with longer and saturated alk(en)yl groups were generally more stable than those with shorter and unsaturated alk(en)yl groups. Thiosulfinates underwent thioalkyl-exchange reactions at pH 8−9 without loss of total thiosulfinate levels within 60−90 min at 20 °C.

KEYWORDS: Thiosulfinates; Allium; organosulfur; stability; reactivity; thioalk(en)yl-exchange

INTRODUCTION

Epidemiological, laboratory and clinical investigations of the biological activity of tissue preparations of *Allium* species (e.g., onion, garlic) have sustained a widely held belief that endogenous organosulfur components exhibit pharmacological effects in humans (*1*−*3*). The precursors of these organosulfur compounds in intact *Allium* tissues are *S*-alk(en)yl-L-cysteine sulfoxides (**1**), which upon tissue disruption are transformed by an endogenous alliinase (E. C. 4.4.1.4) to yield the alkanesulfenic acid (**2**) scission products (**Figure 1**) (*4*−*6*). Compound **2** rapidly undergoes condensation reactions to form homologous (R = R′) or heterologous (R ≠ R′) thiosulfinates (**3**; RS(O)SR′; where R/R′ = methyl, propyl, 1-propenyl, or 2-propenyl residues (*6*), with trace levels of ethyl residues (*7*)). A competing pathway for the fate of **2** (R = 1-propenyl) is the rearrangement to form propanethial *S*-oxide (**4**). Both **2** and **4** are intermediates in the formation of virtually all other organosulfur compounds that arise in fresh and processed *Allium* tissues, and the centrality of these chemical species to organosulfur transformation has been established (*6*).

There is ample evidence that many organosulfur compounds found in *Allium* tissue preparations may possess various biological activities (*1*−*3, 6, 8*−*11*). Pending the unequivocal assignment of specific pharmacological effects to specific organosulfur compounds, one can anticipate the advantage of being able to direct organosulfur transformation along one of multiple and competing pathways, to attain an enrichment of a specific organosulfur component. Such a capability would facilitate the use of *Allium* tissue preparations as vehicles for delivering specific health-promoting benefits, and generally support technology development for "functional foods". To achieve this capability, it is of paramount importance to understand the chemical properties and reactivity of alkanesulfenic acid (**2**) and thiosulfinate (**3**), which are intermediates in the formation of other organosulfur compounds. Although there have been several reports on various chemical and biological properties of thiosulfinates in the past decade (*6, 9*−*11*), much remains to be learned of the structure−function relationships of **2** and **3**.

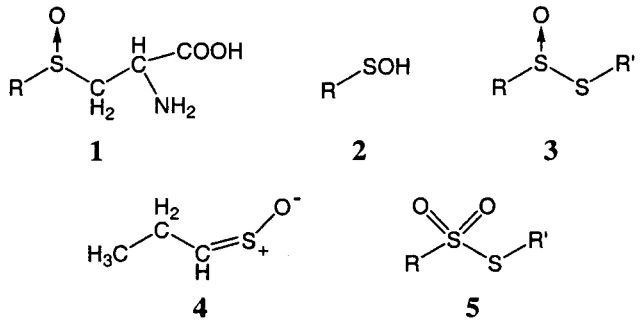

Figure 1. Chemical structures of selected organosulfur compounds found in *Allium* tissue preparations.

To address this need, we evaluated the intrinsic pH and temperature stabilities and selected reactivities of a series of homologous and selected heterologous species of **3**, containing R groups relevant to *Allium* species.

MATERIALS AND METHODS

Materials. 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).

Preparation of Immobilized Crude Onion Alliinase. A crude alliinase preparation was prepared using the steps of homogenization, 65% saturated ammonium sulfate fractionation and dialysis at 0−4 °C,

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and immobilization in alginate gels as described earlier (*16*). The resulting enzyme-loaded gel was sliced into 5−10-mm-thick disks and stored at 4 °C until used, as previously described (*17*).

Preparation of *S*-Alk(en)yl-L-cysteine Sulfoxide (1) Substrates. All methods for preparing diastereomeric (±) **1**, as well as product yields and ^{1}H NMR confirmation of structure, are described in detail in Shen and Parkin (*17*). Briefly, **1** (R = Me) was prepared by a modified method of Synge and Wood (*18*). Synthesis of **1** (R = Et) was essentially as described for **1** (R = Me) except that *S*-ethyl-L-cysteine was used as the starting material. Synthesis of **1** (R = Pr) was similar to the method described by Lancaster and Kelly (*19*). Synthesis of **1** (R = All) was similar to that described for **1** (R = Pr) except that 2-propenyl bromide was substituted for propyl bromide.

Preparation of Thiosulfinates (3). Typically, a single species of **1** (100−200 mg) was combined with 2.5 g of immobilized alliinase (∼1,500 Units g^{-1}) in 40 mL of 0.1 M Tris (pH 7.5) at 20−22 °C (*17*). After incubation for 2−4 h, thiosulfinate (**3**, RS(O)SR′) products were obtained by extraction into 20 mL of $CHCl_3$. The $CHCl_3$ extract was immediately dried over anhydrous sodium sulfate and then evaporated either at ∼80 mmHg or with a stream of $N_{2(g)}$ without temperature control (<22 °C because of evaporative cooling). The residue was promptly dissolved in water adjusted to pH 4−5, and quantified and verified for structure by ^{1}H NMR (model AM-300 NMR spectrometer, Bruker Instruments, Inc., Billerica, MA) operated at 300 MHz, using $CDCl_3$ (*17*).

Quantification of thiosulfinate profiles was based on the peak area (detection by $Abs_{254\ nm}$) and corresponding response factors using normal phase HPLC chromatography on a 250 mm × 4.6 mm, Microsorb 5-μm silica column (Rainin Instrument Co Inc., Woburn, MA) using gradient elution with 2-propanol/hexane (*17*).

Thiosulfinate Stability Studies. Pure thiosulfinates (at final concentrations of 0.37−5.0 mM) were dissolved in aqueous 0.1 M Tris at 20 °C, and four sub-samples were prepared at different pH values of 1.2, 5.5, 7.0, and 9.0 by addition of dilute NaOH or HCl. (The broad range of initial thiosulfinate concentrations used was caused by variable losses of thiosulfinates during the solvent ($CHCl_3$) evaporation step during their preparation. The perceived need to initiate stability studies before HPLC analyses could be completed, out of concern that thiosulfinate decay may proceed rapidly, prevented the opportunity to adjust thiosulfinates to similar starting levels for these studies). This approach was used for separate stability studies at each of the temperatures of 4, 20, 40, 60, and 80 °C. Immediately after pH adjustment, each sample was analyzed for thiosulfinate level at "zero-time" by HPLC, and analyses were repeated at predetermined intervals during subsequent incubation in closed vials (5−10 mL). At each interval, a 0.2-mL sub-sample was extracted with an equal volume of $CHCl_3$, and the $CHCl_3$ extract was subjected to HPLC analysis. First-order rate constants for decay were calculated as the slopes of Ln[thiosulfinate] versus time plots, and these constants were transformed into half-lives. Regression analysis for these semilog plots to determine decay rate constants afforded highly linear fits ($r^2 \geq 0.96$) with few exceptions (**Figure 2**).

It is acknowledged that Tris is an effective buffer only in the pH range of about 7.3−9.1 at 20 °C. The primary reason it was included was that it is a common buffer used for studies on onion alliinase, as well as in our model reaction systems (*17, 20*), because of the slightly alkaline pH optimum for this enzyme (*16, 21*−*23*). Also, it was not pragmatic to attempt to account for the ΔK_a of −0.031 °C^{1-} for Tris buffer when thiosulfinate solutions were incubated at different temperatures. For example, a thiosulfinate-containing Tris-buffered solution to be examined at 80 °C at pH 9.0 would have to be initially prepared at 20 °C at pH ∼10.8. This is outside of the pH buffering range of Tris, making appropriate pH adjustment uncertain. Furthermore, a pH of near 11 is also a condition where thiosulfinate decay could be initiated during the time taken to simply prepare the thiosulfinate samples for incubation under the specific conditions to be evaluated (*6, 20, 24*). As an alternative, samples were prepared at 20 °C at pH 1.2, 5.5, 7.5, or 9.0 in 0.1 M Tris, and then incubated at various temperatures. Actual pH values were measured and recorded at each temperature. As a result, different but similarly spaced intervals of pH values were used to examine thiosulfinate decay at different temperatures.

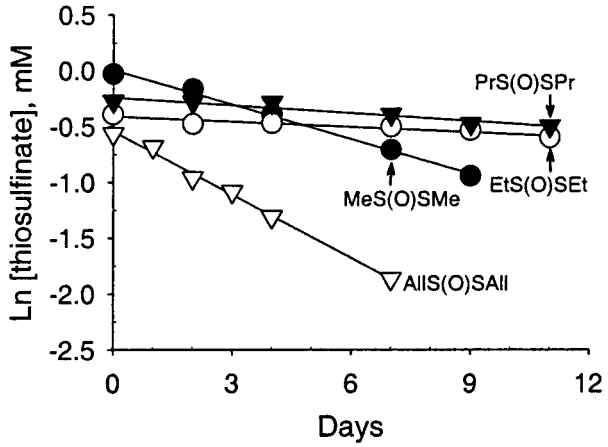


Figure 2. Homologous thiosulfinate decay at pH 9.5 in 0.1 M Tris at 4 °C. The results are reported as the mean values of two separate experiments where initial thiosulfinate levels ranged 0.70–3.2 mM, and % error in half-life estimations was ≤ 16%.

The temperature dependence of decay for each thiosulfinate was evaluated by presenting the estimated first-order rate constants on Arrhenius plots, and estimating an E_a value from the slope of these plots. Because pH values varied up to 1.0 unit for each interval and were not constant over the range of temperatures used for each of these plots, the E_a values obtained are referred to as "pseudo-E_a" values for the balance of this manuscript, and these values may differ from actual E_a values.

Thiosulfinate Thioalk(en)yl-Exchange Reactivity Studies. Two species of homologous thiosulfinates were combined at 20−22 °C in either 50 mM Tris or water, and pH was adjusted within the range of 7.5−11.0 by the addition of dilute NaOH or HCl. At "zero-time" and at predetermined intervals, a 0.3-mL sub-sample was extracted with an equal volume of $CHCl_3$, and the $CHCl_3$ extract was then subjected to HPLC analysis for thiosulfinates.

For some of these studies, changes in thiosulfinate profiles and levels were calculated as a intermolecular exchange factor (K_{xch}), where

$$K_{xch} = \frac{[R_1S(O)SR_2] \times [R_2S(O)SR_1]}{[R_1S(O)SR_1] \times [R_2S(O)SR_2]} \quad (1)$$

This factor represents the thiosulfinate distribution as a ratio of the molar products of the heterologous/homologous species, in a manner analogous to an equilibrium constant.

The significance of differences among the mean K_{xch} values for different thiosulfinate pairs in exchange reactions at various pHs in media with and without Tris was determined at the $p \leq 0.05$ level, using one-way ANOVA followed by Bonferroni tests (GraphPad InStat Version 1989).

An effect of the aqueous medium was evaluated for the exchange reaction between 1 mM each AllS(O)SAll and MeS(O)SMe in 50 mM of each (separately) of the following adjuvants: Tris, dimethylamine, triethylamine, trihydroxymethylamine, 2-hydroxymethyl 1,3-propanediol, sodium phosphate, or water alone (control), adjusted to pH 8.0 by the addition of dilute HCl or NaOH. After 90 min incubation, the reaction mixture was extracted with an equal volume of $CHCl_3$ and analyzed for thiosulfinates by HPLC as already described.

Thiosulfinate Alkaline Decay Studies. MeS(O)SMe (∼10 mM) was dissolved in D_2O, and the pH was adjusted to 7.5 to ∼11 with 0.2 N NaOD (prepared from Na and D_2O). Samples were periodically removed for ^{1}H NMR analysis. ^{1}H NMR signals of standard compounds, representing anticipated intermediates and products of alkaline decay, were also obtained.

Calculation of Sulfenyl α−C−H Bond Energy of Thiosulfinates. The sulfenyl α−C−H bond energies of AllS(O)SAll, PrS(O)SPr, EtS(O)SEt, and MeS(O)SMe were calculated using the Gaussian 98, Revision A.5, program (Gaussian, Inc., Carnegie, PA). The basis set used for the elements (e.g., C, H, O, and S) was the Gaussian basis set

Table 1. Half-Lives of Thiosulfinates in 0.1 M Tris Buffer[a]

pH	1.2	5.5	7.5	9.0
temperature 20 °C				
AllS(O)SAll	20.7 d	52.3 d	3.2 d	5.8 h
PrS(O)SPr	180 d	stable	25.4 d	69.5 h
EtS(O)SEt	282 d	stable	19.0 d	22.3 h
MeS(O)SMe	69.6 d	179 d	4.9 d	13.0 h
pH	1.2	5.1	7.1	8.8
temperature 40 °C				
AllS(O)SAll	1.7 d	2.7 d	0.57 d	1.0 h
PrS(O)SPr	11.0 d	97.2 d	6.2 d	9.9 h
EtS(O)SEt	14.5 d	78.8 d	4.6 d	5.6 h
MeS(O)SMe	9.2 d	32.9 d	1.3 d	1.8 h
pH	1.2	4.8	6.8	8.4
temperature 60 °C				
AllS(O)SAll	5.0 h	6.9 h	2.5 h	0.45 h
PrS(O)SPr	45.2 h	355 h	19.9 h	3.8 h
EtS(O)SEt	66.0 h	254 h	15.2 h	2.6 h
MeS(O)SMe	30.2 h	170 h	5.4 h	0.70 h
pH	1.2	4.5	6.4	8.0
temperature 80 °C				
AllS(O)SAll	0.34 h	0.82 h	0.31 h	0.06 h
PrS(O)SPr	1.4 h	2.2 h	1.7 h	0.37 h
EtS(O)SEt	1.7 h	4.0 h	3.2 h	0.30 h
MeS(O)SMe	2.1 h	10.0 h	1.0 h	0.12 h

[a] Half-lives were calculated from k^{-1} values obtained from first-order plots of thiosulfinate decay. Stable signifies that no measurable loss (± 3%) was observed in 4 months. The results are from two experiments where initial thiosulfinate levels ranged 0.37–5.0 mM and % error of the mean for half-life estimations was ≤ 20%.

6-31+G*. The calculation method used was B3LYP (specifically, $^1/_3$ of the Becke exchange functional and $^2/_3$ of the Hartree−Fock exchange and Lee, Yang and Parr electron correlation functional; sometimes abbreviated ACM for adiabatic correction method). This analysis was done and contributed by Peter Woloham (Department of Chemistry, University of Missouri-St. Louis).

RESULTS AND DISCUSSION

Thiosulfinate Stability as a Function of pH. The decay of thiosulfinates in aqueous media could be fitted to first-order processes, as typified by the loss of thiosulfinate species in 0.1 M Tris (pH 9.5) at 4 °C (**Figure 2**). From estimates of first-order rate constants (k^{-1}), half-lives were calculated as 3.7 days for AllS(O)SAll, 6.7 days for MeS(O)SMe, 30 days for PrS(O)SPr, and 44 days for EtS(O)SEt under these conditions. (The apparent difference in stability between EtS(O)SEt and PrS(O)SPr may be an anomaly created by shallow slopes and relatively short time frames selected for the decay plots, situations that were avoided in more complete and subsequent studies conducted at 20 °C to 80 °C). At pH 7.9 and 4 °C, observed half-lives for these same thiosulfinate species were 18, 36, 672, and 703 days, respectively. At pH values of 5.9 and 1.5 at 4 °C, these homologous thiosulfinate species were stable within the limits of variability (± 3%) for 4 months, and half-lives could not be estimated.

A more comprehensive analysis of the pH-dependence of thiosulfinate decay as a function of temperature, using the approach shown in **Figure 2**, was obtained at 20−80 °C (**Table 1**). Stability of the homologous thiosulfinates tested was dependent on pH, with a descending order of stability at pH ranges: 4.5−5.5 > 1.2 > 6.4−7.5 > 8.0−9.0 in the presence of 0.1 M Tris. Thus, acidic media were more conducive to stabilizing thiosulfinates than were the neutral or alkaline pH media, consistent with earlier reports (*6*, *25*, *26*). The present results are also consistent with the reported 7-fold greater stability of MeS(O)SMe over AllS(O)SAll in an aqueous medium at 23 °C (*24*). What was most surprising was just how stable some thiosulfinates were, in view of the reported in situ instability of various thiosulfinates at temperatures of ∼20 °C (*10*, *25*, *27*). For example, a complete retention (± 3%) of PrS(O)SPr and EtS(O)SEt levels was observed over 4 months at 20 °C and pH 5.5. Thus, the lack of persistence of thiosulfinates in comminuted *Allium* tissue preparations in likely mediated through reactions with other endogenous components rather than by an intrinsic instability or intramolecular decay of thiosulfinates. This contrast between intrinsic stability and reactivity is most exemplified by MeS(O)SMe, which had a 179-day half-life at pH 5.5 in 0.1 M Tris at 20 °C (**Table 1**), compared to a half-life on the order of hours in a fresh garlic homogenate (*10*).

Table 2. Half-Lives of Thiosulfinates as Quaternary Mixtures in 0.1 M Tris Buffer[a]

thiosulfinate (quaternary mixture components)	temperature and pH 7.5 20 °C	6.8 60 °C	6.8 60 °C
MeS(O)SMe	3.2 d		4.5 h
MeS(O)SAll	1.8 d		2.5 h
AllS(O)SMe	4.3 d		3.3 h
AllS(O)SAll	1.8 d		2.4 h
AllS(O)SAll		3.0 h	
AllS(O)SEt		5.9 h	
EtS(O)SAll		5.7 h	
EtS(O)SEt		19.8 h	

[a] Half-lives were calculated from k^{-1} values obtained from first-order plots of thiosulfinate decay; the quaternary mixture components are separated by group. The results are the mean values from two experiments where initial thiosulfinate levels ranged 0.70–3.2 mM, and % error in half-life estimations was ≤ 20%.

The present results also indicated a correlation of stability with longer and saturated substituent alk(en)yl substituent groups within the series tested, and the relative stabilities generally were as follows: PrS(O)SPr ∼ EtS(O)SEt > MeS(O)SMe > AllS(O)SAll. However, there were some exceptions to this pattern of stability. For example, PrS(O)SPr was more stable than EtS(O)SEt at pH 7.5−9.0, but the reverse was true at pH 1.2, and this relationship held for the full range of temperatures examined. In addition, as temperature was increased from 60 °C to 80 °C, MeS(O)SMe became the most stable thiosulfinate under acidic conditions (pH 1.2−4.5).

A limited study of stability was conducted with selected quaternary mixtures of homologous and heterologous thiosulfinates (prepared through a thioalk(en)yl-exchange reaction between two homologous thiosulfinates; see later section on "Thiosulfinate thioalk(en)yl-exchange reactions"). Half-life calculations from first-order decay estimates for each species in the mixture are shown in **Table 2**. For each of the three situations evaluated, thiosulfinates with the thioallyl group (−S−CH_2−CH=CH_2) functional unit were the most unstable members of each group of thiosulfinates evaluated. This may be attributed to the ability of this functional unit to undergo an elimination reaction to yield thioacrolein (CH_2=CH−CH=S) (*6*). However, the stability of the RS(O)SAll species was impacted by the R group, as the R = Et derivative was more

Table 3. Pseudo-Activation Energies (E_a) for Thiosulfinate Decay in 0.1 M Tris Buffer at 20–80 °C[a]

	pH			
thiosulfinate	1.2	4.5–5.5	6.4–7.5	8.0–9.0
AllS(O)SAll	103	104	75.4	72.9
PrS(O)SPr	111	158	91.5	76.2
EtS(O)SEt	113	140	87.0	78.1
MeS(O)SMe	93.8	83.9	72.1	73.1

[a] Pseudo-E_a values were determined from Arrhenius plots from the data in **Table 1**. Values are expressed in kJ mol^{-1}.

than twice as stable as the methyl derivative. The stability of the MeS(O)SMe species was reduced in these mixtures (in the presence of AllS(O)SAll) relative to its stability when evaluated alone under the same conditions, although this pattern of stability was not observed for the EtS(O)SEt species (**Table 1**). The destabilizing influence of one thiosulfinate species on another may be related to the ability of thiosulfinates to undergo thioalk(en)yl-exchange reactions, an issue addressed later in this report.

The temperature-dependent shift in order of stability of homologous thiosulfinates prompted an estimation of "pseudo-E_a" values, despite the limitations of lack of precise pH control over the full range of temperatures (see Methods and Materials for explanation). Considering that pH ranged as much as 1.0 unit for each interval tested, linear regression fits for Arrhenius-type plots were reasonable, at $r^2 \geq 0.97$ for AllS(O)SAll, $r^2 \geq 0.92$ for PrS(O)SPr, $r^2 \geq 0.93$ for EtS(O)SEt, and $r^2 \geq 0.96$ for MeS(O)SMe for each of the four pH ranges evaluated for each thiosulfinate. Generally, "pseudo-E_a" values were greater in the acidic pH range compared to those in the alkaline pH range for all thiosulfinates (**Table 3**). Different E_a values are consistent with different dominant mechanisms (multiple mechanisms may occur at a given pH range) of thiosulfinate decay at different pH intervals. Possible mechanisms for each pH range are addressed in the next section.

A closer look at **Table 3** indicates that the energetics of decay were similar for the thiosulfinate pair of MeS(O)SMe and AllS(O)SAll, and as well for the pair of PrS(O)SPr and EtS(O)SEt. The MeS(O)SMe and AllS(O)SAll species also exhibited lesser "pseudo-E_a" values than did the PrS(O)SPr and EtS(O)SEt species, although similar values were observed among all thiosulfinate species at pH 8.0−9.0. Decay kinetics of the MeS(O)SMe species were generally the least temperature-dependent of the thiosulfinates tested.

Mechanisms of Decay as a Function of pH. A common mechanism of thiosulfinate decay is disproportionation to yield disulfide (RSSR′) and thiosulfonate (**5**, RS(O$_2$) SR′), and this can take place under acidic, alkaline, and neutral conditions (*6, 28*−*31*). Under acidic conditions, thiosulfinate decomposition may also take place by an H$^+$-catalyzed cycloelimination (fragmentation) or condensation reactions, or direct hydrolysis to yield various products, such as disulfides and trisulfides. An H$^+$-catalyzed cycloelimination (Schemes 10 and 31 in ref. *6*) reaction may help explain the unexpected order of stability of MeS(O)SMe > EtS(O)SEt > PrS(O)SPr at 80 °C at pH 1.2−4.5, because the bond dissociation energies for the sulfenyl α−C−H bond of thiosulfinates calculated were to be 364.08, 362.86, and 361.80 kJ/mol, respectively (see Methods and Materials section). A sulfenyl α−C−H bond energy of 350.17 kJ/mol was calculated for AllS(O)SAll, consistent with the observation that AllS(O)SAll was the least stable thiosulfinate tested. A cycloelimination reaction appears to be a favored pathway of decay over disproportionation specifically for AllS(O)SAll (*6*).

Under near-neutral pH conditions, the principal mode of thiosulfinate decay is likely disproportionation (when R = saturated alkyl groups) to form disulfides and **5** (*6*). For the specific AllS(O)SAll species, elimination reactions may prevail at near-neutral pH, and may account for the accumulation of allyl disulfide, allyl trisulfide, vinyl dithiins, and 2-propen-1-ol in heated garlic juice (*6, 32*).

Base-mediated (OH$^-$) decay of thiosulfinates at alkaline pH is caused by nucleophilic attack on either sulfinyl (RS(O)−) or sulfenyl (RS−) sulfur atoms of the thiosulfinate leading to disulfides (RSSR) and alk(en)ylsulfinate ions (RSO$_2^-$) as principal products (*30*), although SO$_2$ has also been reported (*26, 33*). To study the mechanism of alkaline decay, MeS(O)SMe (as a model thiosulfinate) was incubated at pH 8.5 and periodically analyzed by ^{1}H NMR (**Figure 3**). Proton signals corresponding to those of the standard compounds, methyl sulfinate ion (MeSO$_2^-$) and dimethyl disulfide (MeSSMe) progressively increased throughout the time frame studied. Proton signals corresponding to the methyl sulfide ion (MeS$^-$) and an unknown peak at 3.312 ppm (this signal was also present during the incubation of thiosulfonate **5** (R = R′ = Me) under the same conditions and was not extractable by chloroform) reached a steady-state or maximum level within 20 min, whereas the MeS(O)SMe continually decayed throughout the entire time period. These results are consistent with the mechanism shown in **Scheme 1**, where the progressive accumulation of MeSSMe and MeSO$_2^-$ would be predicted during the initial stages of decay. At pH ∼10.5, proton signals were only observed for these latter two compounds after 15 min incubation, whereas no proton signals for MeS$^-$ and the unknown peak at 3.312 ppm were evident (data not shown).

Thiosulfinate Thioalk(en)yl-Exchange Reactions. During preliminary studies on stability of binary homologous thiosulfinate systems under alkaline conditions, the evolution of heterologous thiosulfinate species was observed in aqueous 0.1 M Tris-buffered systems (*20*). A similar phenomenon of thioalk(en)yl-exchange was observed earlier for neat preparations and benzene solutions of thiosulfinates (*34*). These observations prompted an evaluation of the pH dependence of thioalk(en)yl-exchange between pairs of homologous thiosulfinates. The kinetics of alkyl-exchange reactions were pH-dependent, as illustrated for the binary system containing PrS(O)SPr and EtS(O)SEt (**Figure 4**). At pH 9.0, a nearly equimolar ratio of heterologous/homologous thiosulfinate species ($K_{xch} \sim 1.0$) was reached within about 30 min, whereas the same binary pair incubated at pH 8.5 had not approached $K_{xch} \sim 1.0$ even after 240 min incubation. These trends indicated that this thioalk(en)yl-exchange or "scrambling" process is random, and a true equilibrium may be reached when the molar distribution of heterologous/homologous species reaches 1:1 ($K_{xch} \sim 1.0$). An important caveat is that maximum or "equilibrium" K_{xch} values of >1.0 or <1.0 could be observed if any of the homologous or heterologous thiosulfinate species (respectively), was preferentially subject to decay during the incubation period. Although a net 20% thiosulfinate decay took place at pH 9.0 in the test system (**Figure 4A**), no one thiosulfinate species appeared to be more sensitive to decay than another, and K_{xch} remained ∼1.0. In contrast, <3% decay of total thiosulfinate levels was observed for the same thiosulfinate pair after 240 min at pH 8.5 in Tris buffer (**Figure 4B**). These results indicate that there was a delicate balance between the influence of pH

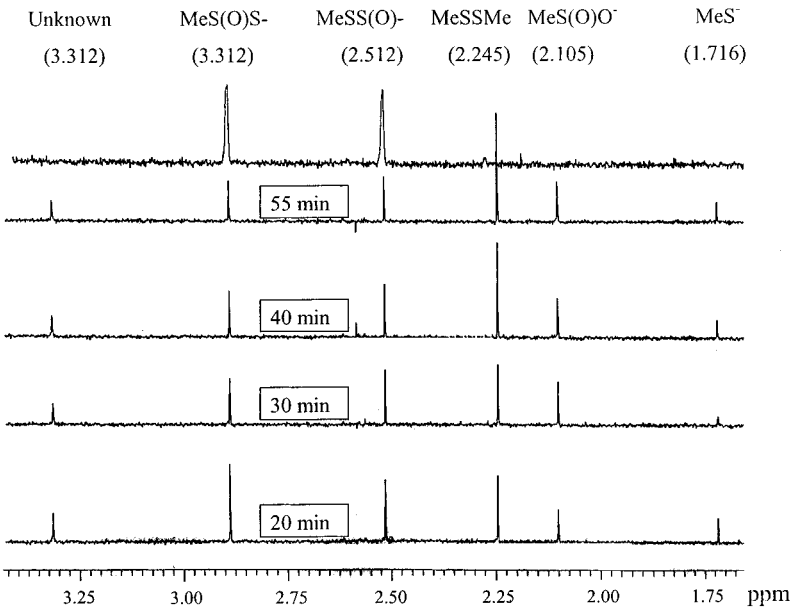
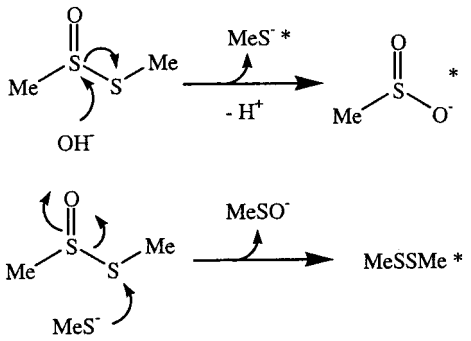


Figure 3. Time course of change in ^{1}H NMR spectrum during MeS(O)SMe decay in aqueous medium at pH 8.5. Top scan represents "zero-time".

Scheme 1. Proposed Mechanism for MeS(O)SMe Decay under Alkaline Conditions[a]



[a] Modified from ref *31*. *Proton signals of these species corresponded to those of the respective standard compounds.

on reactivity (thioalk(en)yl-exchange) and net decay of thiosulfinates, especially in the alkaline pH range.

The pH-dependence of thioalk(en)yl-exchange reactions was evaluated for several pairs of homologous thiosulfinates in the presence and absence of 50 mM Tris. The results obtained with the AllS(O)SAll/MeS(O)SMe binary mixture serves as a typical example of the pH dependence of thioalk(en)yl-exchange reactions (**Figure 5**). The kinetics thioalk(en)yl-exchange (rate of increase in K_{xch} value) changed abruptly within a narrow pH range (viz., ~1 pH unit), and the pH at which the change occurred shifted ~1.5 pH units in the presence of 50 mM Tris compared to that of the aqueous solution.

From the preceding example (**Figure 5**), it was judged to best capture differences in thioalk(en)yl-exchange reactivity among specific thiosulfinate pairs by determining K_{xch} values after a 90-min incubation period (**Figure 6**). Of the five thiosulfinate pairs tested, it was evident that reactivity of the AllS(O)SAll/MeS(O)SMe pair required the least alkaline pH conditions, whereas reactivity of the PrS(O)SPr/EtS(O)SEt pair required the most alkaline conditions, with other thiosulfinate pairs exhibiting an intermediate pH-dependence (**Figure 6**). At both pH 7.5 and 8.0 in Tris buffer (**Figure 6A**), the K_{xch} values for AllS(O)SAll/ MeS(O)SMe pair was significantly different from those for the other pairs ($P \leq 0.01$). At pH 8.5 in Tris buffer, the K_{xch} value for PrS(O)SPr/EtS(O)SEt pair was

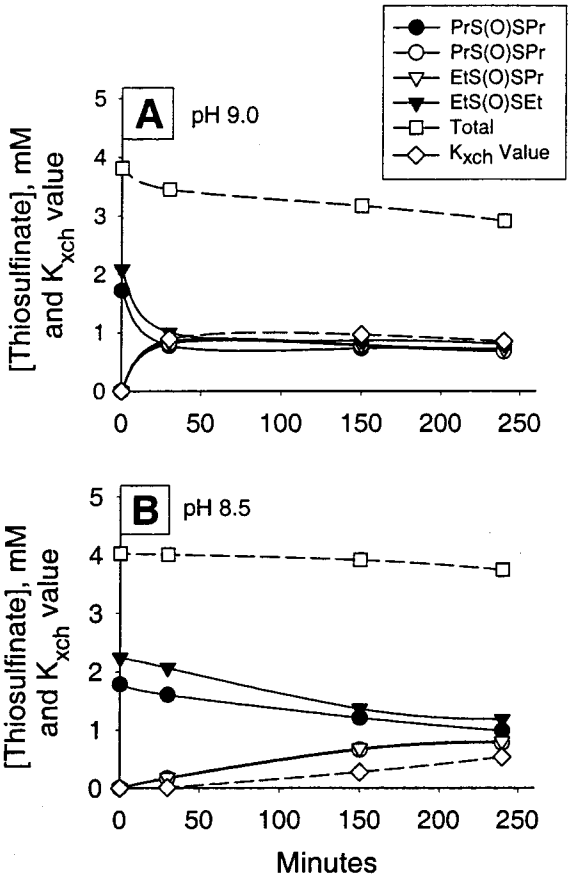


Figure 4. Progress of alkyl-exchange between homologous thiosulfinates at pH 9.0 (A) and pH 8.5 (B) in 50 mM Tris at 20–23 °C. Results are reported as the mean values of the two separate experiments where initial thiosulfinate levels ranged 0.70–3.2 mM, and % error in K_{xch} values was ≤ 13%.

significantly less than those for the other pairs ($P \leq 0.01$). There was no statistical difference between K_{xch} values in the pH range of 9−10.5 in Tris-buffered systems.

A similar pattern of behavior held for binary thiosulfinate mixtures in the absence of 50 mM Tris (**Figure 6B**). At pH 10 in the aqueous system, the K_{xch} value of the PrS(O)SPr/

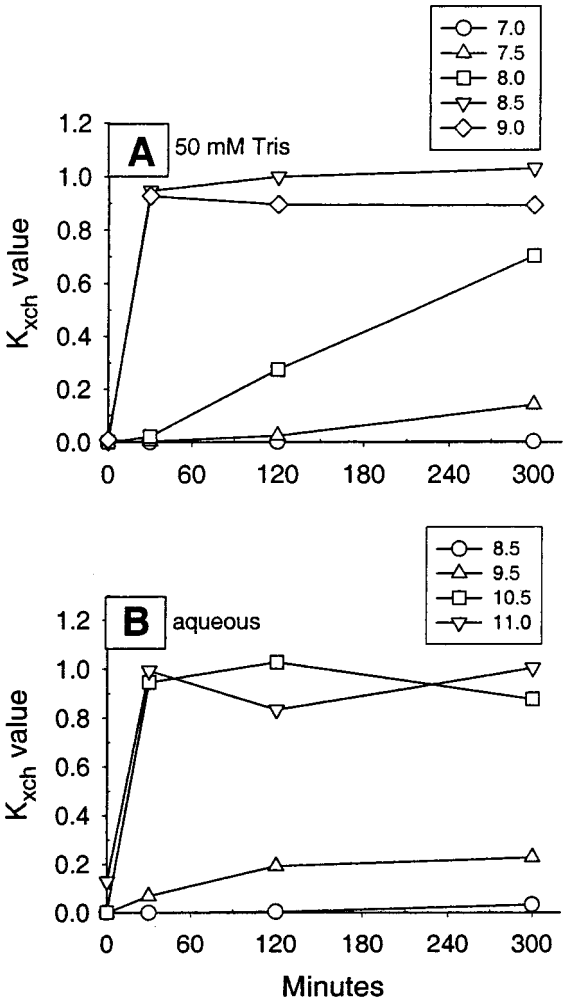


Figure 5. Progress of alk(en)yl-exchange reaction of AllS(O)SAll/MeS-(O)SMe as influenced by pH in the presence (A) and absence (B) of Tris. Initial thiosulfinate levels ranged 1.1–2.0 mM.

EtS(O)SEt pair was not significantly different from that of the AllS(O)SAll/ EtS(O)SEt pair. However, both of the K_{xch} values for these pairs were significantly less ($P \leq 0.01$) than the K_{xch} values observed for other thiosulfinate pairs. Generally, K_{xch} values reached levels of ~1.0 for all pairs, with the AllS(O)-SAll/MeS(O)SMe pair being the exception in that maximum K_{xch} values were ~0.7 in the absence of Tris buffer ($P \leq 0.01$ at pH 11). The inability to attain K_{xch} values ~1.0 for this pair may be caused by the preferential decay of a heterologous thiosulfinate species or a partially nonrandom nature of the exchange process. It was not possible to assess relative stabilities of specific thiosulfinate species during the dynamics of the exchange process. However, on the basis of the half-lives results obtained for the AllS(O)SAll/MeS(O)SMe mixture (**Table 2**), one would not expect that the relative sensitivities to decay of these thiosulfinates would yield K_{xch} values other than ~1.0. Overall, the relative sensitivity of the binary thiosulfinate systems to undergo exchange and decay correlated well with the relative sensitivities reported in **Table 1**, and it appeared that the relative ease or sensitivity to undergo exchange reactions was dictated by the presence of the least stable thiosulfinate species.

It is revealing to juxtapose the patterns in **Figure 4** with the half-lives of stability of PrS(O)SPr and EtS(O)SEt in 50 mM Tris (pH 9.0) at 20 °C (**Table 1**). Although one can consider these thiosulfinates to be fairly stable under these conditions,

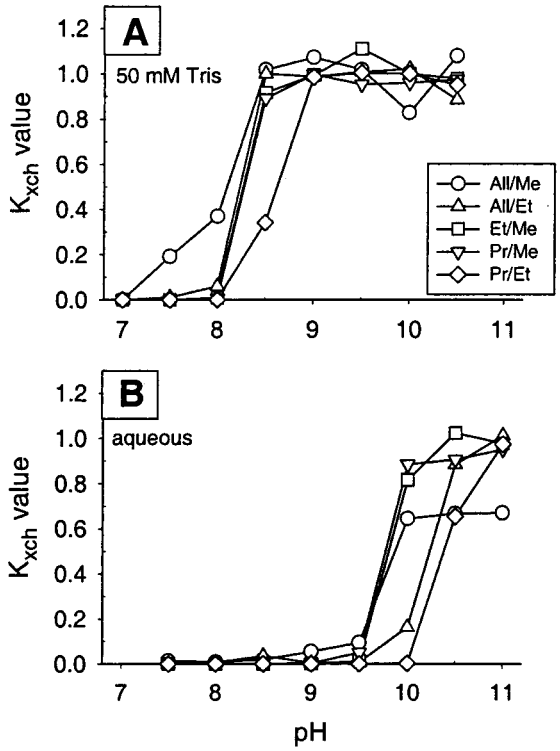


Figure 6. pH-Dependence of K_{xch}-values in the exchange reaction (90 min) of homologous thiosulfinates in the presence (A) and absence (B) of Tris. Results are reported as the mean values of the two separate experiments where initial thiosulfinate levels ranged 1.1–3.5 mM, and % error in K_{xch} values was ≤ 24%.

Table 4. K_{xch} Values for Thiosulfinate Thioalk(en)yl-exchange as Influenced by Nucleophilic Adjuvants in Aqueous Systems[a]

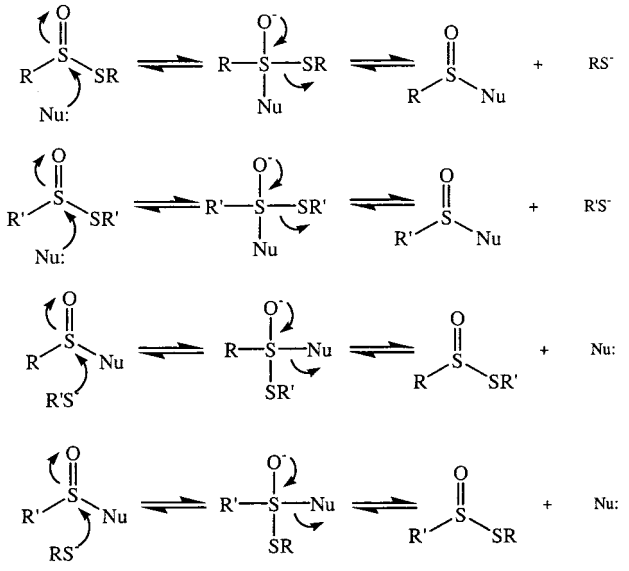
adjuvant	K_{xch} value
none (water control)	0.000
potassium phosphate	0.221
dimethylamine	0.046
triethylamine	0.007
tris(hydroxymethyl)aminomethane	1.086
trihydroxyethylamine	1.054
2-hydroxymethyl-1,3-propanediol	0.003

[a] K_{xch} values were determined after 90 min incubation in 50 mM of the adjuvant at pH 8.0 and 20–23 °C. Results are reported as the mean values of two separate experiments with the binary thiosulfinate system of AllS(O)SAll and MeS(O)SMe (initial thiosulfinate levels ranged 1.0–3.1 mM) and % error in half-life estimations was ≤ 6.3%.

they are not unreactive or chemically static. A dynamic intermolecular thioalkyl-exchange may take place under these conditions, although no net gain or loss of pure thiosulfinate in solution may be detected by traditional analytical procedures (the latter result is often interpreted as a sign of stability or "chemical inertness", which would be mistaken in the case of thiosulfinates under these conditions).

The prospect that the presence of Tris buffer may facilitate thioalk(en)yl-exchange (**Figure 6**) by serving as a nucleophilic catalyst prompted a limited survey of the capability of other potential nucleophiles to mediate this reaction (**Table 4**). It was apparent that solutes with amino groups catalyzed thioalk(en)yl-exchange reactions, and those solutes composed of both amino and hydroxyl functional units were most effective in this regard (**Table 4**). We suggest that Tris and trihydroxyethylamine function best in causing thioalk(en)yl-exchange by virtue of their ability to form hydrogen bonds (between hydroxyl and sufoxide

Scheme 2. Suggested Mechanism for the Nucleophilic-Assisted Exchange Reaction of Homologous Thiosulfinates under Alkaline Conditions[a]



[a] Net Equation: $RS(O)SR + R'S(O)SR' \overset{Nu:}{\rightleftharpoons} RS(O)SR' + R'S(O)SR$

O atom) with thiosulfinates, thereby increasing the local concentration of the nucleophilic N atom to mediate exchange reactions. Potassium phosphate may be effective in an analogous manner by virtue of H-bonding increasing the proximity of the nucleophilic P atom to the −S(O)S-functional group of thiosulfinates. **Scheme 2** represents a proposed mechanism to account for thioalk(en)yl-exchange, wherein the first two steps represent the nucleophile-mediated scission of the S(O)−S bond to yield the corresponding sulfinic acid derivatives and sulfide ions from two homologous thiosulfinates.

CONCLUSIONS

An aqueous alliinase-based system affords the preparation of pure thiosulfinates which are stabilized through H-bonding with water (*6*, *24*, *26*). Such preparations can be used to study the intrinsic chemical properties of thiosulfinates. Thiosulfinate decay was characterized by first-order processes, and the alk(en)yl substituent group(s) of the thiosulfinate was important in conferring the relative pH and thermal stability. Intrinsic stabilities of pure thiosulfinates in vitro were considerably greater than they appear to be in situ. Thus, while thiosulfinates are intrinsically stable, they remain reactive, and many endogenous components within *Allium* tissue preparations may mediate thiosulfinate transformation. Identifying these other chemical components will be important to developing strategies to control the fate of thiosulfinates in foods during or as a consequence of processing. Some effective processing strategies may prove to be rather simple, and it was demonstrated that brief pH adjustment could be used to modify the thiosulfinate profile through thioalk(en)yl-exchange reactions.

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