Garlic: Source of the Ultimate Antioxidants—Sulfenic Acids**

Vipraja Vaidya, Keith U. Ingold, and Derek A. Pratt*

Garlic, onion, and other members of the Allium spp., long believed to be of great medicinal benefit, contain up to about 5% dry weight of nonprotein sulfur amino acid secondary metabolites, such as (−)-S-allyl-L-cysteine S-oxide (alliin, 1). Alliin, which is found predominantly in garlic, is cleaved by allinase upon the homogenization of garlic to form ammonium pyruvate and 2-propenesulfenic acid [2, Eq. (1)]. The latter compound undergoes self-condensation to yield the diallyl thiosulfinate alliin [3, Eq. (2)]. Allicin provides garlic with its odor and flavor, and is believed responsible for its health benefits,[3] often ascribed to antioxidant activity.[4]

\[
\begin{align*}
1 & \text{alliin} \quad R = \text{vinyl} \\
2 & \text{alliinase} \quad R = \text{phenyl} \\
3 & \text{alliin} + \text{H}_2\text{O} \quad R = \text{vinyl} \\
4 & \text{alliin} + \text{HOS} \quad R = \text{phenyl}
\end{align*}
\]

In a recent series of papers,[5] Okada et al. presented results of kinetic studies aimed at elucidating the mechanism of allicin’s antioxidant activity. Therein, inhibited autoxidation of methyl linolate (ML) and cumene yielded inhibition of allicin/C29’s antioxidant activity. Therein, inhibited autoxidation results of kinetic studies aimed at elucidating the mechanism that requires the S(O)SCH2CH3 moiety was essential for the antioxidant activity of allicin.[5a] Okada et al. suggested a mechanism involving abstraction of the allylic H atom adjacent to the divalent sulfur atom [Eq. (3)]. Subsequent studies with the dibenzyl thiosulfinate 4 [Eq. (3)] from Petiveria alliacae L. afforded similar results, which prompted the suggestion that the mechanism for its reaction with peroxyl radicals was abstraction of the analogous benzyllic H atom.

The suggested mechanism for the scavenging of peroxyl radicals by thiosulfinates is unlikely for two reasons. First, rate constants for H-atom transfer from hydrocarbons to peroxyl radicals are much lower than those reported for allicin-inhibited autoxidation reactions, even when highly stabilized carbon-centered radicals are formed. For example, the rate constant for the reaction of peroxyl radicals with methyl linolate to give a highly delocalized pentadienyl radical is only 60 m−1 s−1.[7] Second, carbon-centered radicals generally undergo diffusion-controlled reactions with O2 to yield peroxyl radicals, which continue to propagate the autoxidation chain reaction.[8]

Amorati and Pedulli were similarly mystified by the suggested mechanism,[9] which prompted them to investigate the ability of diallyl disulfide (and allyl methyl disulfide) to inhibit the controlled autoxidation of cumene and styrene. As expected, they found these compounds to be ineffective inhibitors, with second-order rate constants for the reaction with peroxyl radicals of approximately 1 m−1 s−1. These results reinforced our view that a mechanism other than the H-atom transfer shown in Equation (3) must be responsible for the radical-trapping activities of 3 and 4, but nevertheless a mechanism that requires the S(O)SCH2CH=CH2 and S(O)SC(=CH2)Ph moieties, respectively.

In 1972, Koelewijn and Berger[10] demonstrated that thermally unstable dialkyl sulfoxides were effective inhibitors of hydrocarbon autoxidation at 60°C, because they decomposed by a Cope elimination to yield a sulfenic acid [and an olefin, Eq. (4)]. Indeed, from careful inhibited autoxidation reactions it was estimated that 2-methyl-2-propanesulfenic acid reacts with peroxyl radicals with a rate constant greater than 107 m−1 s−1, which suggests that sulfenic acids are among the most potent classes of peroxyl-radical-trapping agents.[11] Unfortunately, few studies on sulfenic acids as antioxidants have been reported in the intervening years.

Thiosulfinates also undergo Cope elimination to form sulfenic acids, along with thioaldehydes or thioketones.[12] This process does not require elevated temperatures, because the S–S bond in a thiosulfinate is much weaker than the S–C bond in a sulfoxide. Cope elimination is even more facile for allyl (and benzyl) thiosulfinates, such as allicin (and 4), because of the weak β C–H bond of the allyl (benzyl) moiety. Indeed, allicin is known to undergo Cope elimination readily.

---

[5] Dr. V. Vaidya, Prof. D. A. Pratt
Department of Chemistry, Queen’s University
90 Bader Lane, Kingston, ON, K7L 3N6 (Canada)
Fax: (+1) 613-533-6669
E-mail: pratt@chem.queensu.ca
Dr. K. U. Ingold
National Research Council of Canada, Ottawa (Canada)

[6] We are grateful for the support of the Natural Sciences and Engineering Research Council of Canada and the Ontario Ministry of Innovation. D.A.P. acknowledges the support of the Canada Research Chairs program.

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/anie.200804560.

DOI: 10.1002/anie.200804560


157
at room temperature to give 2-propenesulfenic acid (2) and thioacrolein [Eq. (5)].

Hence, we considered it probable that the antioxidant activity ascribed to allicin (and garlic, in general) is actually due to the trapping of peroxyl radicals by 2-propenesulfenic acid—both its decomposition product [Eq. (5)] and its precursor [Eq. (2)]! Likewise, α-toluenesulfenic acid is probably the inhibitor in autoxidation reactions involving the analogous dibenzyl thiiosulfinate 4.

If 2-propenesulfenic acid is responsible for the trapping of peroxyl radicals in allicin-inhibited autoxidation reactions, then the inhibition should be less effective under reaction conditions which retard the decomposition of allicin. It is well documented that hydrogen-bond-donor (HBD) solvents retard allicin decomposition.[1,12] Accordingly, the half-life of allicin (50 μm) in chlorobenzene was found to be approximately 1 h, but very little of the allicin (a few percent) decomposed in 1 h when hexafluoroisopropanol (HFPA, 0.15 m) was added to the solution in chlorobenzene (Figure 1b). Most importantly, when HFPA was added to the autoxidation reaction mixture, the induction period (previously ascribed to allicin itself)[5] was eliminated (see Figure 1a). No such solvent effect would be observed if the trapping of peroxyl radicals involved the (suggested)[5] abstraction of an allylic H atom. Clearly, allicin is not directly responsible for the inhibition of ML autoxidation; instead, an allicin decomposition product must be responsible.[13]

We also explored the effects of added hydrogen-bond-acceptor (HBA) solvents on allicin-inhibited autoxidation reactions. Although the addition of CH₃CN (1 m) to a solution of allicin in chlorobenzene had little effect on the rate of decomposition of allicin (Figure 1b), it markedly reduced the ability of allicin to inhibit the autoxidation of ML (Figure 1a). Thus, the use of freshly purified (HPLC) allicin in chlorobenzene in the presence of CH₃CN (1 m) led to an almost eightfold increase in the rate of inhibited autoxidation, and the stoichiometric factor dropped from n = 0.95 to 0.69.[14] Since allicin itself is not the radical-trapping antioxidant in these autoxidation reactions, we cannot derive an inhibition rate constant (k inh) from the autoxidation data in the customary way,[9] however, the drop in antioxidant activity in HBA solvents is informative. This type of solvent effect is well documented for reactions of peroxyl radicals with H-atom donors that are HBDs, such as phenols.[15] The decomposition product of allicin, 2-propenesulfenic acid, is expected to be a strong HBD that will form strong hydrogen bonds with CH₃CN (Scheme 1) and thus lead to a decrease in the rate of the inhibited autoxidation, as observed.

Although these experiments demonstrate that 2-propenesulfenic acid is highly likely to be the peroxyl-radical scavenger in allicin-inhibited autoxidation reactions, the reason that sulfenic acids appear to be such effective antioxidants[10] is currently unclear. Since the O–H bond-dissociation enthalpy (BDE) of an H-atom donor, XOH (e.g., phenols, X = Ar), is the key thermodynamic parameter connected with the rate of reaction of XOH with peroxyl radicals, it would be desirable to compare the O–H BDE of 2-propenesulfenic acid and other sulfenic acids with the O–H BDEs of their isoelectronic cousins, the hydroperoxides (which react with peroxyl radicals much more slowly, with Figure 1. a) Thermally initiated (azobisisobutyronitrile (AIBN), 40 mM) autoxidation of methyl linoleate (91 mM) at 37 °C in chlorobenzene containing allicin (50 μm) and HFPA (0.15 m, •), CH₃CN (1 m, ●), or no additive (▲). b) Decomposition of allicin (50 μm) at 37 °C in chlorobenzene containing HFPA (0.15 m, •), CH₃CN (1 m, ●), or no additive (▲).

Scheme 1. Mechanistic scheme to explain the observed increase in the rate of allicin-inhibited autoxidation in the presence of acetonitrile.
rate constants of approximately $10^2$–$10^3\text{M}^{-1}\text{s}^{-1})$. Unfortunately, no sulfenic acid O–H BDE has ever been reported. As the reliable determination of a sulfenic acid O–H BDE appeared to be experimentally impractical (owing to the transient nature of most known sulfenic acids), we calculated these BDEs by using a method known to accurately predict O–H BDEs\textsuperscript{[19,20]} the complete-basis-set approach of Petersson and co-workers (Table 1).\textsuperscript{[27]}

Table 1: O–H BDEs (in kcal mol\textsuperscript{-1}) calculated by the CBS-QB3 method for some sulfenic and hydroperoxides.

<table>
<thead>
<tr>
<th></th>
<th>O–H BDE\textsuperscript{[a]}</th>
<th>O–H BDE</th>
</tr>
</thead>
<tbody>
<tr>
<td>HSO-H</td>
<td>73.1</td>
<td>HOO-H</td>
</tr>
<tr>
<td>MeSO-H</td>
<td>68.4</td>
<td>MeOO-H</td>
</tr>
<tr>
<td>C\textsubscript{2}H\textsubscript{3}CH\textsubscript{2}SO-H</td>
<td>68.6</td>
<td>C\textsubscript{2}H\textsubscript{3}CH\textsubscript{2}OO-H</td>
</tr>
<tr>
<td>tBuSO-H</td>
<td>86.8</td>
<td>tBuOO-H</td>
</tr>
<tr>
<td>C\textsubscript{6}H\textsubscript{5}CH\textsubscript{2}SO-H</td>
<td>68.6</td>
<td>C\textsubscript{6}H\textsubscript{5}CH\textsubscript{2}OO-H</td>
</tr>
</tbody>
</table>

\textsuperscript{[a]} G3 calculations\textsuperscript{[27]} yielded 72.8 and 68.2 kcal mol\textsuperscript{-1} for HSO-H and MeSO-H, respectively.

Table 2: Activation energies (in kcal mol\textsuperscript{-1}) calculated by the CBS-QB3 method\textsuperscript{[a]} for H-atom transfer between sulfenic acids and peroxyl radicals.

<table>
<thead>
<tr>
<th></th>
<th>$E_1$ (cisoid TS)</th>
<th>$E_1$ (transoid TS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HSO-H/OOH</td>
<td>6.7</td>
<td>13.6</td>
</tr>
<tr>
<td>HSO-H/OMe</td>
<td>7.7</td>
<td>15.1</td>
</tr>
<tr>
<td>MeSO-H/OOMe</td>
<td>4.6</td>
<td>11.0</td>
</tr>
<tr>
<td>C\textsubscript{2}H\textsubscript{3}CH\textsubscript{2}SO-H/OOMe</td>
<td>4.3</td>
<td>10.9</td>
</tr>
<tr>
<td>tBuSO-H/OOMe</td>
<td>4.2</td>
<td>9.8</td>
</tr>
</tbody>
</table>

\textsuperscript{[a]} Determined from a common hydrogen-bonded prereaction complex.

To provide additional insight into the reactions of sulfenic acids with peroxyl radicals, we also calculated the transition-state (TS) structures (Figure 2) and associated activation energies of some representative reactions (Table 2). Two TS structures were identified: one with a cisoid geometry with respect to the oxygen atoms between which the H atom is being transferred (Figure 2a) and one with a transoid geometry (Figure 2b). The cisoid TSs were found to be lower in energy by approximately 6–7 kcal mol\textsuperscript{-1} than the transoid TSs (Table 2). The highest occupied molecular orbitals (HOMOs) of the cisoid and transoid TS structures (which comprise the four possible combinations of the two $\pi^*$ orbitals on the sulfanyl and peroxyl fragments between which the H atom is transferred) reveal why the cisoid geometry is favored. Whereas the singly occupied (SO) HOMO and HOMO-1 in the cisoid (Figure 2c) and transoid TS structures are very similar, HOMO-2 and HOMO-3 are quite different in the two TS geometries: There is significant bonding overlap between the sulfenic acid sulfur atom and the internal oxygen atom in the peroxyl radical in the cisoid structure, an interaction that is absent in the transoid structure. This bonding overlap suggests that the electron to be transferred from the sulfenic acid to the peroxyl radical is partly localized on the peroxyl radical in the cisoid TS and thus indicates a mechanism based on proton-coupled electron transfer (PCET).\textsuperscript{[28]}

As in other PCET reactions,\textsuperscript{[21,22]} H-atom transfer between a sulfenic acid and a peroxyl radical is predicted to involve initial formation of a hydrogen-bonded complex, RSOH--‘OOR’. For the reactions we investigated, in which R and R’ were alkyl groups, these complexes lie some 4.5 to 5.0 kcal mol\textsuperscript{-1} lower in energy than the separated reactants. This result implies that the corresponding TSs are slightly lower in energy than the separated reactants. These reactions are, therefore, predicted to be diffusion-controlled, consistent with Koelewijn and Berger’s estimate\textsuperscript{[18]} that the rate constant for the reaction of 2-methyl-2-propanesulfenic acid with

![Figure 2](Image 333x569 to 515x782)

Figure 2. a) Cisoid and b) transoid transition-state structures for the HSOH/OOH reaction. c) The four highest occupied molecular orbitals of the structure in (a) showing the overlap between the sulfenic acid S atom and the internal peroxyl O atom.
Communications

tetrayl peroxy radicals was $>10^7 \text{m}^{-1}\text{s}^{-1}$. Thus, sulfenic acids are very probably the most potent of all peroxy-radical-trapping antioxidants.$^{[23,24]}

In conclusion, we suggest that the peroxy-radical-trapping activity of garlic is primarily due to 2-propenesulfenic acid formed by the decomposition of allicin. It is highly likely that the decomposition of thiosulfonates from other Allium spp. (e.g., onion) gives rise to sulfenic acids that are equally reactive towards peroxy radicals. Since the reactions of alkanesulfenic acids with peroxy radicals are predicted to be diffusion-controlled and to occur by a common proton-coupled-electron-transfer mechanism, both the abundance and stability of the thiosulfonate precursors may account for the different antioxidant activities of extracts of different species.

Received: September 16, 2008
Revised: October 10, 2008
Published online: November 28, 2008

Keywords: allicin · antioxidants · garlic · lipid peroxidation · peroxy radicals · proton-coupled electron transfer

[4] See, for example, references 11–20 in Ref. [5b].
[6] Such a large difference in the rate constants of reactions of radical-trapping antioxidants with secondary and tertiary peroxy radicals is unprecedented. Also, the inhibition rate constants were based on the assumption that one molecule of allicin reacts with one chain-carrying peroxy radical in the autoxidation of each substrate (stoichiometric factor: $n = 1$), an assumption that is invalid on the basis of the actual inhibition periods observed,$^{[69]}$ which correspond to stoichiometric factors of approximately 0.3.
[8] Appropriately substituted carbon-centered radicals, such as that derived from HP-136, are an exception; see: J. C. Scaino, A. Martin, G. P. A. Yap, K. U. Ingold, Org. Lett. 2000, 2, 899.
[13] Owing to its transient nature, 2-propenesulfenic acid is normally observed,$^{[5b]}$ which correspond to stoichiometric factors of 1. Thus, sulfenic acids derived from HP-136, are an exception; see: J. C. Scaiano, A. Martin, G. P. A. Yap, K. U. Ingold, Org. Lett. 2000, 2, 899.
[14] Values of $n < 1$ led to the suggestion$^{[69]}$ that sulfenic acids undergo other reactions, such as reaction with oxygen, during autoxidation processes.
[20] The measured value of 71.8 kcal mol$^{-1}$ must be decreased by 1.1 kcal mol$^{-1}$ because of a revised heat of formation of (E)-azobenzene.$^{[66]}$
[23] In contrast, the PCET pathway for hydroperoxide/peroxyl self-exchange reactions is characterized by much higher activation energies, for example, 9.0 kcal mol$^{-1}$ for the MeOOH/OOMe couple (or 6.0 kcal mol$^{-1}$ from the separated reactants, in very good agreement with experiment$^{[25]}$); therefore, the rate constants for these reactions are much smaller (generally on the order of $10^5$–$10^6$ m$^{-1}$ s$^{-1}$).
[24] Sulfonic and sulfonic acids, the oxidation products of sulfenic acids, would not be expected to be good peroxy-radical scavengers, since the lone pair of electrons on the sulfur atom would either be too low in energy (former) or unavailable (latter) for the five-center PCET reaction.