Radical Scavengers

DOI: 10.1002/anie.200804560

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

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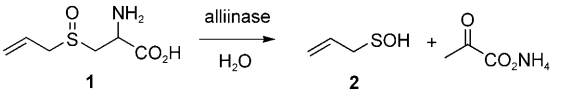
Garlic, onion, and other members of the *Allium* spp., long believed to be of great medicinal benefit, contain up to about 5% dry weight of nonprotein sulfur amino acid secondary metabolites, such as (+)-*S*-allyl-L-cysteine *S*-oxide (alliin, **1**).[1] Alliin, which is found predominantly in garlic, is cleaved by alliinase 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 allicin [**3**, Eq. (2)].[2] Allicin provides garlic with its odor and flavor, and is believed responsible for its health benefits,[3] often ascribed to antioxidant activity.[4]



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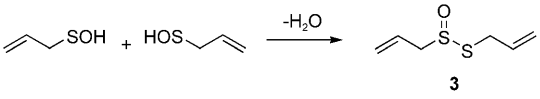

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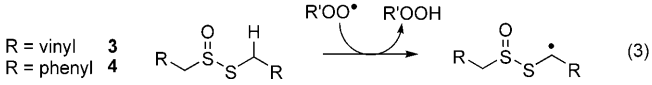
In a recent series of papers,[5] Okada et al. presented results of kinetic studies aimed at elucidating the mechanism of allicin's antioxidant activity. Therein, inhibited autoxidation of methyl linoleate (ML) and cumene yielded inhibition rate constants (k_{inh}) for the reaction of allicin with methyl linoleate- and cumene-derived peroxyl radicals of 1.6×10^5 and $2.6\times10^3\,M^{-1}s^{-1}$, respectively.[5b,6] Since previous structure–activity studies had indicated that the $S(O)SCH_2CH{=}CH_2$ 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 benzylic H atom.

The suggested mechanism for the scavenging of peroxyl radicals by thiosulfinates is unlikely for two reasons. First, rate

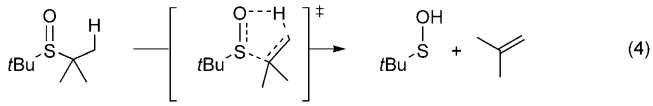


(3)

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 linoleate 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 O_2 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 $S(O)SCH_2CH{=}CH_2$ and $S(O)SCH_2Ph$ 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 $10^7\,M^{-1}s^{-1}$, 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.



(4)

Thiosulfinates also undergo Cope elimination to form sulfenic acids, along with thioaldehydes or thioketones.[1] 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

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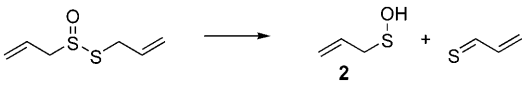
[**] We are grateful for the support of the Natural Sciences and Engineering Research Council of Canada and the Ontario Ministry of Innovation. D.A.P. acknowledges the support of the Canada Research Chairs program.

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

at room temperature to give 2-propenesulfenic acid (**2**) and thioacrolein [Eq. (5)].[1]



(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 thiosulfinate **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 1 b). Most importantly, when HFPA was added to the autoxidation reaction mixture, the induction period (previously ascribed to allicin itself)[5] was eliminated (see Figure 1 a). 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 CH_3CN (1 M) to a solution of allicin in chlorobenzene had little effect on the rate of decomposition of allicin (Figure 1 b), it markedly reduced the ability of allicin to inhibit the autoxidation of ML (Figure 1 a). Thus, the use of freshly purified (HPLC) allicin in chlorobenzene in the presence of CH_3CN (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 CH_3CN (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

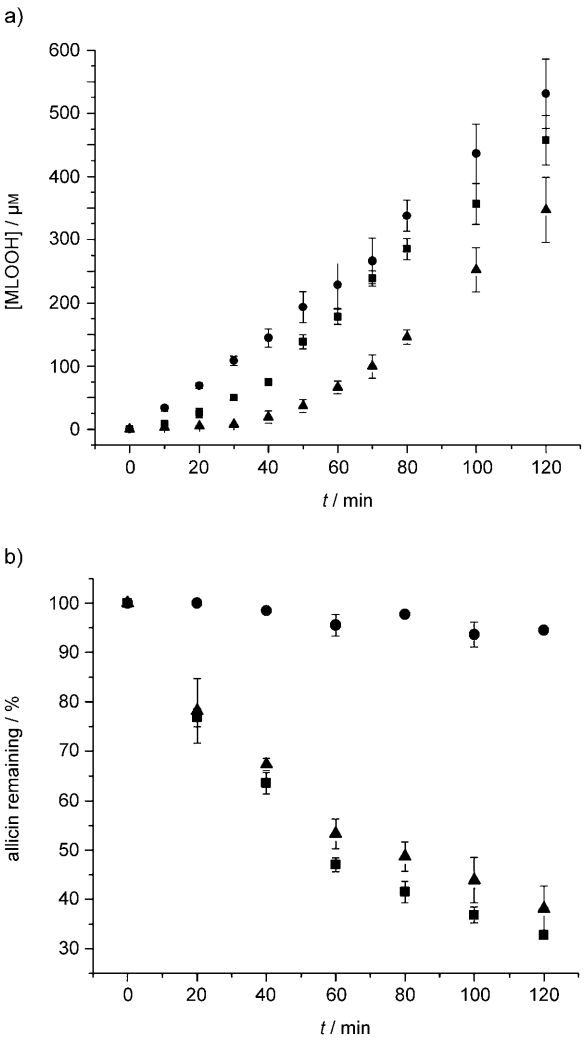
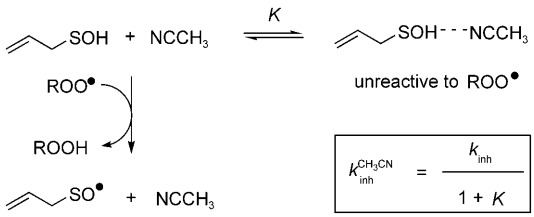


Figure 1. a) Thermally initiated (azobisisobutyronitrile (AIBN), 40 mM) autoxidation of methyl linoleate (91 mM) at 37 °C in chlorobenzene containing allicin (50 μM) and HFPA (0.15 M, ●), CH_3CN (1 M, ■), or no additive (▲). b) Decomposition of allicin (50 μM) at 37 °C in chlorobenzene containing HFPA (0.15 M, ●), CH_3CN (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–$10^3\,M^{-1}s^{-1}$). 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,[16] the complete-basis-set approach of Petersson and co-workers (Table 1).[17]

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

	O–H BDE[a]		O–H BDE
HSO-H	73.1	HOO-H	87.3
MeSO-H	68.4	MeOO-H	86.2
$C_2H_3CH_2SO$-H	68.6	$C_2H_3CH_2OO$-H	86.2
*t*BuSO-H	68.6	*t*BuOO-H	84.8
$C_6H_5CH_2SO$-H	68.6	$C_6H_5CH_2OO$-H	86.1

[a] G3 calculations[18] yielded 72.8 and 68.2 kcal mol^{-1} for HSO-H and MeSO-H, respectively.

Table 1 reveals two important features of the O–H BDEs of sulfenic acids. First, the values are much lower than those for the analogous hydroperoxides. For the simplest members of the series (hydrosulfenic acid and hydrogen peroxide), the difference is 14.2 kcal mol^{-1}. For the alkanesulfenic acids and alkyl hydroperoxides, the difference is even larger: almost 18 kcal mol^{-1} for methane-, 2-propene-, and α-toluenesulfenic acids and the corresponding hydroperoxides. Thus, the sulfur atom imposes two effects: It stabilizes the radical to a greater extent, largely by delocalizing the unpaired electron onto itself (the unpaired spin distribution is close to 50:50 between the oxygen and sulfur atoms in sulfinyl radicals and 70:30 between the terminal and nonterminal oxygen atoms in peroxyl radicals), and this results in greater interactions with substituents that are bonded to the sulfur atom. This effect leads to a greater difference between hydrosulfenic acid and the alkanesulfenic acids (ca. 4.5 kcal mol^{-1}) than between hydrogen peroxide and the alkyl hydroperoxides (ca. 1 kcal mol^{-1}). The O–H BDEs of sulfenic acids are among the weakest known and comparable to those of hydroxylamines, such as TEMPO-H (70.7 kcal mol^{-1}; TEMPO = 2,2,6,6-tetramethylpiperidin-1-oxyl).[19, 20]

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^{-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 π* orbitals on the sulfinyl and peroxyl fragments between which the H atom is transferred) reveal why the cisoid geometry is favored. Whereas the singly occupied (SO) HOMO and

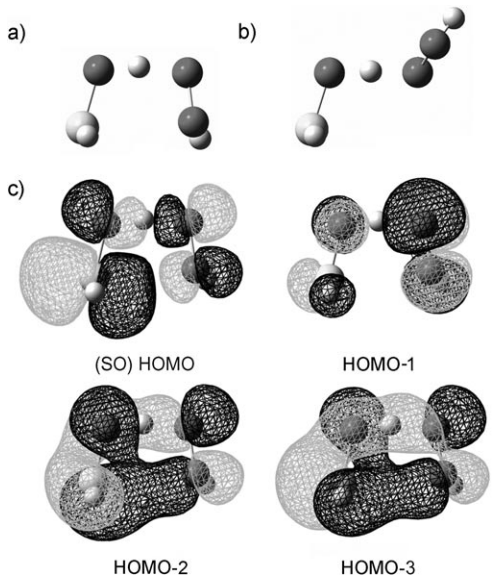


Figure 2. a) Cisoid and b) transoid transition-state structures for the HSOH/$^{\bullet}$OOH reaction. c) The four highest occupied molecular orbitals of the structure in (a) showing the overlap between the sulfenic acid S atom and the internal peroxyl O atom.

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

	E_a (cisoid TS)	E_a (transoid TS)
HSO-H/$^{\bullet}$OOH	6.7	13.6
HSO-H/$^{\bullet}$OOMe	7.7	15.1
MeSO-H/$^{\bullet}$OOMe	4.6	11.0
CH_2=$CHCH_2SO$-H/$^{\bullet}$OOMe	4.3	10.9
*t*BuSO-H/$^{\bullet}$OOMe	4.2	9.8

[a] Determined from a common hydrogen-bonded prereaction complex.

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 of 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).[21]

As in other PCET reactions,[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···$^{\bullet}$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^{-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[10] that the rate constant for the reaction of 2-methyl-2-propanesulfenic acid with

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

In conclusion, we suggest that the peroxyl-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 thiosulfinates from other *Allium* spp. (e.g., onion) gives rise to sulfenic acids that are equally reactive towards peroxyl radicals. Since the reactions of alkanesulfenic acids with peroxyl 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 thiosulfinate 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 · peroxyl radicals · proton-coupled electron transfer

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[13] Owing to its transient nature, 2-propenesulfenic acid is normally detected indirectly, for example, following its reaction with ethyl propiolate to yield ethyl (*Z*)-2-thioformyl-4-pentenoate *S*-oxide.[1] In our inhibited autoxidation reactions, the addition of ethyl propiolate to the reaction mixture did not prevent inhibition, presumably since the reaction of 2-propenesulfenic acid with peroxyl radicals is much faster than its addition to ethyl propiolate.
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[24] Sulfinic and sulfonic acids, the oxidation products of sulfenic acids, would not be expected to be good peroxyl-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.
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