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PAPER

The mechanism of radical-trapping antioxidant activity of plant-derived thiosulfinates†‡

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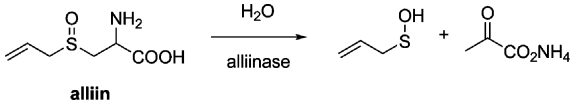
It has long been recognized that garlic and petiveria, two plants of the *Allium* genus—which also includes onions, leeks and shallots—possess great medicinal value. In recent times, the biological activities of extracts of these plants have been ascribed to the antioxidant properties of the thiosulfinate secondary metabolites allicin and *S*-benzyl phenylmethanethiosulfinate (BPT), respectively. Herein we describe our efforts to probe the mechanism of the radical-trapping antioxidant activity of these compounds, as well as *S*-propyl propanethiosulfinate (PPT), a saturated analog representative of the thiosulfinates that predominate in non-medicinal alliums. Our experimental results, which include thiosulfinate-inhibited autoxidations of the polyunsaturated fatty acid (ester) methyl linoleate, investigations of their decomposition kinetics, and radical clock experiments aimed at obtaining some quantitative insights into their reactions with peroxyl radicals, indicate that the radical-trapping activity of thiosulfinates is paralleled by their propensity to undergo Cope elimination to yield a sulfenic acid. Since sulfenic acids are transient species, we complement our experimental studies with the results of theoretical calculations aimed at understanding the radical-trapping behaviour of the sulfenic acids derived from allicin, BPT and PPT, and contrasting the predicted thermodynamics and kinetics of their reactions with those of the parent thiosulfinates. The calculations reveal that sulfenic acids have among the weakest O–H bonds known (*ca.* 70 kcal mol^{-1}), and that their reactions with peroxyl radicals take place by a near diffusion-controlled proton-coupled electron transfer mechanism. As such, it is proposed that the abundance of a thiosulfinate in a given plant species, and the ease with which it undergoes Cope elimination to form a sulfenic acid, accounts for the differences in antioxidant activity, and perhaps medicinal value, of extracts of these plants. Interestingly, while the Cope elimination of 2-propenesulfenic acid from allicin is essentially irreversible, the analogous reaction of BPT is readily reversible. Thus, in the absence of chain-carrying peroxyl radicals (or other appropriately reactive trapping agent), BPT is reformed.

Introduction

The genus *Allium* is among the most numerous of the plant kingdom. Garlic, onions, leeks and shallots are the most recognizable species of *Allium*, which share fascinating organosulfur chemistry that is used largely for their defense.[1] At the top of the metabolic cascade initiated upon invasion or tissue injury to these plants are non-proteinogenic amino acids, which can occur at up to 5% of the dried weight of the species. In garlic, the predominant such amino acid derivative is the *S*-allyl cysteine sulfoxide alliin, which is converted to the thiosulfinate allicin (**1**) upon disruption of vacuoles that contain alliinase, the enzyme responsible for catalyzing the reaction.[2,3] Alliinase is a pyridoxal-dependent enzyme that catalyzes the cleavage of one of the C–S bonds in alliin to yield ammonium pyruvate and 2-propenesulfenic acid (eqn (1)); the latter of which condenses with another molecule of 2-propenesulfenic acid to afford allicin (eqn (2)).[2] Allicin gives garlic its characteristic odour and flavour, and is believed to be the compound primarily responsible for garlic's medicinal properties.[2,4,5] The purported health benefits of garlic (some of which are more anecdotal than demonstrated on a sound scientific basis) include antibacterial, immunostimulant, anti-inflammatory, anti-atherogenic and anti-tumorigenic.[2,5]



alliin (1)

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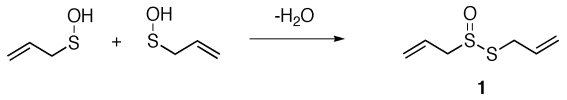
† This contribution is dedicated to the memory of the late Professor Athel Beckwith, a personal and professional inspiration to so many.

‡ Electronic supplementary information (ESI) available: Decomposition of allicin and PPT in the presence of ethyl propiolate, and complete details of all theoretical calculations (*i.e.* Cartesian coordinates of all relevant structures, energies and thermochemical corrections). See DOI: 10.1039/c1ob05192j



(2)

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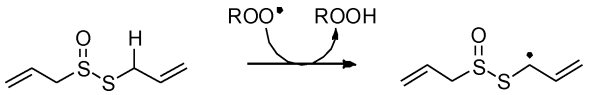
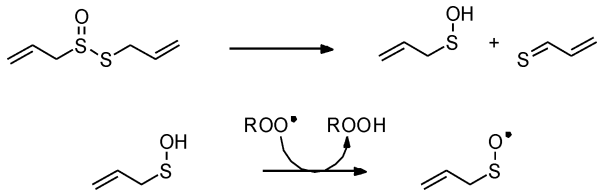
Garlic has long been known to possess potent antioxidant properties, and many investigators have ascribed garlic's biological activity to this reactivity.[6–16] Recently, Okada *et al.* have worked to understand the structure–activity relationships in garlic-derived organosulfur compounds in this context.[7,17] From antioxidant assays of several different disulfides and thiosulfinates, they determined that the thiosulfinate moiety combined with an allylic substituent on the divalent sulfur was essential.[17] With this information in hand, they proposed a mechanism for the radical-trapping activity which involved H-atom transfer to an autoxidation chain-carrying peroxyl radical from the methylene of the allyl group on the divalent sulfur (eqn (3)).[7]

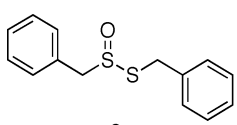


(3)

In a preliminary communication, we suggested an alternative mechanism; one wherein the radical-trapping antioxidant activity of allicin could be accounted for by the intervention of 2-propenesulfenic acid, which arises by Cope elimination from allicin, along with thioacrolein (eqn (4, 5)).[18]



Herein we elaborate on these initial studies, which we have subsequently expanded to include two other thiosulfinates: *S*-benzyl phenylmethanethiosulfinate (BPT, **2**) and *S*-propyl propanethiosulfinate (PPT, **3**). BPT is the primary thiosulfinate of *Petiveria alliacae*, a plant indigenous to the Amazon rainforest and tropical areas of Central and South America, the Caribbean and Africa. *Petiveria*—also widely called anamu or apacin among other names—has been used as an herbal medicine for centuries where it grows.[19–21] PPT is of interest since propyl thiosulfinates are abundant in chives, scallions, shallots and leeks, and it provides a saturated comparison to the essentially isostructural allyl substituent in allicin. Alongside the experimental results with **1**, **2** and **3**, which comprise inhibited autoxidations of the polyunsaturated fatty acid (ester) methyl linoleate, investigations of their decomposition kinetics, and radical clock experiments aimed at obtaining some quantitative insights into their reactions with peroxyl radicals, we present the results of theoretical calculations aimed at understanding the radical-trapping behaviour of the transient sulfenic acids derived from allicin, BPT and PPT, and contrasting the predicted thermodynamics and kinetics of these reactions with those of the parent thiosulfinates.



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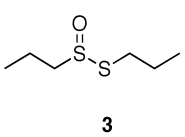

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Results

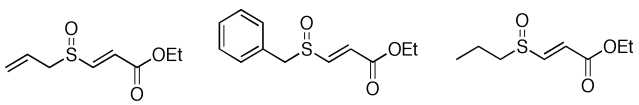
I. Thiosulfinate-inhibited autoxidations of methyl linoleate

Following the work of Okada *et al.*,[7,22] the thiosulfinates **1** and **2** were first investigated for their ability to inhibit the AIBN-initiated autoxidation of methyl linoleate—a model lipid—at 37 °C.[23] In addition to carrying out these experiments in chlorobenzene as Okada *et al.* did, we also modulated the H-bond donating and H-bond accepting properties of the medium through the addition of hexafluoroisopropanol (HFIP; 0.15 M ~ 1.6% by volume) and acetonitrile (1 M ~ 5% by volume), respectively, as co-solvents. The data, plotted as the concentration of methyl linoleate hydroperoxides determined by normal phase HPLC as a function of time, are shown in Fig. 1A and 1B, and the corresponding data for the saturated thiosulfinate **3** is shown in Fig. 1C. The data for **1** and **2** are very similar, with both compounds showing a clear inhibited period. Upon addition of acetonitrile, the inhibited period of ca. 40 minutes is no longer evident and a retarded autoxidation is observed. When HFIP is added, essentially no inhibition is observed. The data for **3** is quite different—no inhibition is observed, even in the absence of the co-solvents.

II. Decomposition of thiosulfinates

Thiosulfinates are well known to undergo Cope elimination to yield a thiocarbonyl and sulfenic acid (*i.e.* eqn (4)).[3,24] To provide some insight as to whether this reaction may play a role in the observed antioxidant activities of **1**, **2** and **3**, we looked at the rate of decomposition of the thiosulfinates under the same conditions as the autoxidations described above by reverse phase HPLC. The results are presented in Fig. 2, and reveal that allicin is by far the most labile thiosulfinate (A), followed by BPT (B) and PPT (C), which display similar profiles. The addition of HFIP slows the rate of decomposition, consistent with previous observations that H-bond donating solvents slow the Cope elimination through interaction with the sulfoxide moiety,[25] while the addition of acetonitrile has little effect.

The experiments were also carried out in the presence of ethyl propiolate (0.1 M), which yields the corresponding alkenoate sulfoxides **4**, **5** and **6** upon reaction of the sulfenic acids derived from **1**, **2** and **3**, respectively.[26] The sulfoxides were prepared independently and characterized as described in the Experimental Section.

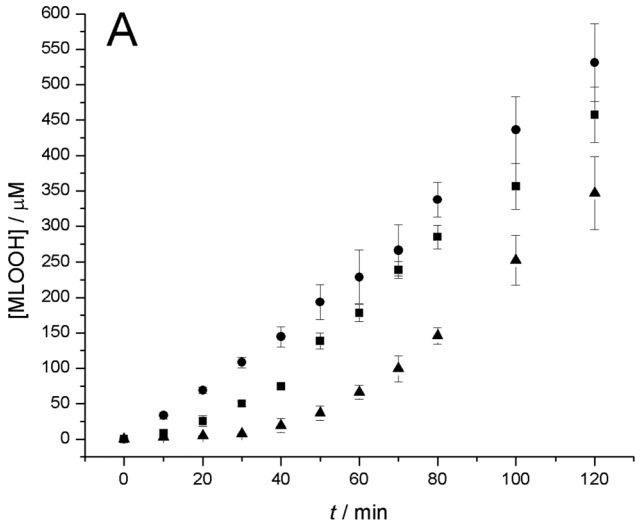


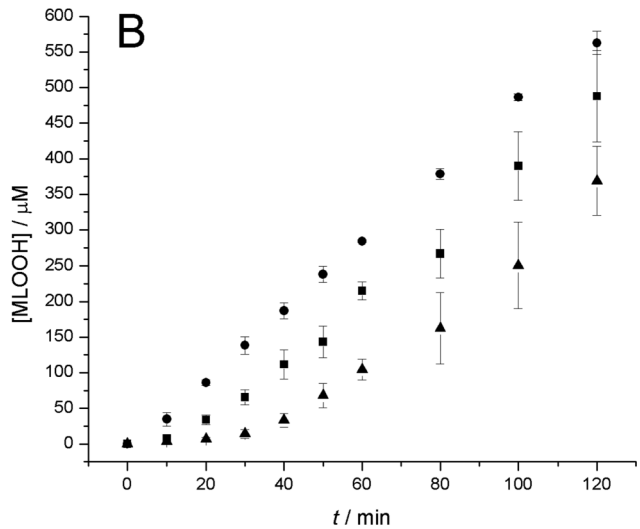
4 5 6

The addition of ethyl propiolate did not change the decomposition profiles of **1** or **3** (see the ESI‡ for the data), but had a marked effect on the rate of decomposition of **2**, wherein now the rate of decomposition of BPT is essentially indistinguishable from that of allicin, and coincides directly with the rate of formation of the (*E*)-ethyl 3-(benzylsulfinyl)acrylate **5** (Fig. 3).

III. Peroxyl radical clock experiments: effect of thiosulfinates on the *Z*,*E*/*E*,*E* ratios of methyl linoleate hydroperoxide products

The autoxidation of methyl linoleate gives rise to a regio- and stereoisomeric mixture of diene hydroperoxide products





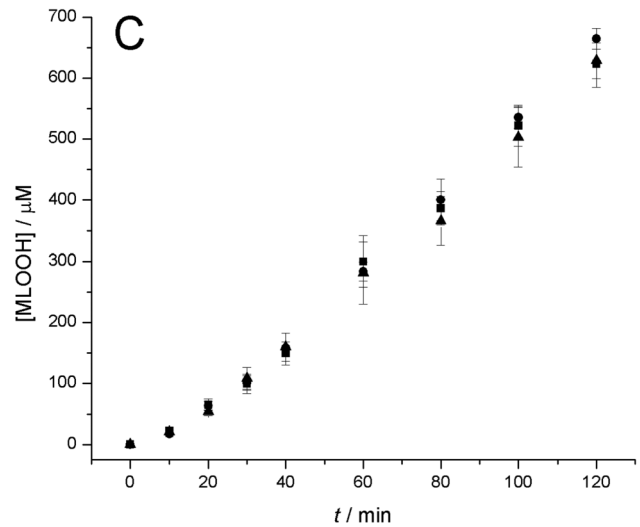
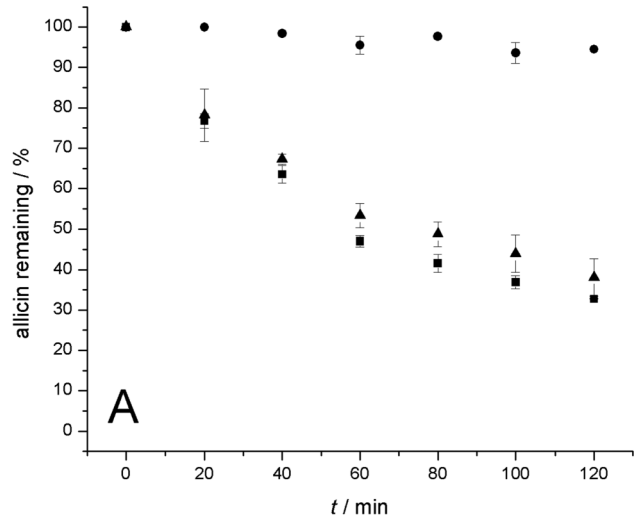
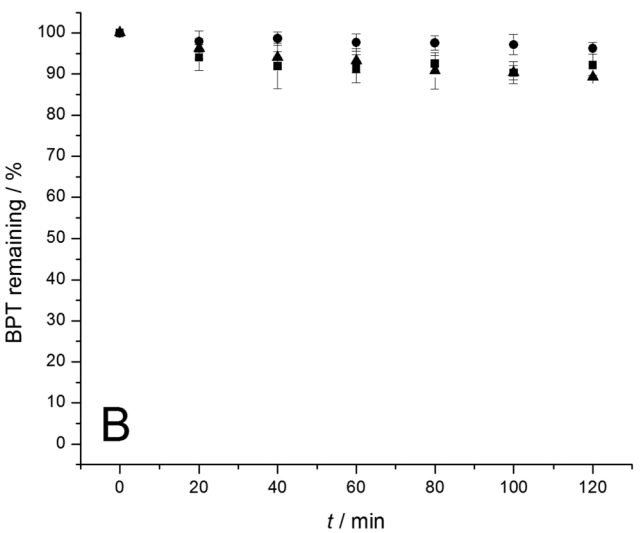


Fig. 1 Thermally initiated (2,2′-azobis(isobutyronitrile) (AIBN), 40 mM) autoxidation of methyl linoleate (91 mM) at 37 °C in chlorobenzene containing (A) 50 μM allicin **1**, (B) 50 μM BPT **2** and (C) 50 μM PPT **3** with HFIP (0.15 M, ●), CH_3CN (1 M, ■), or no additive (▲).





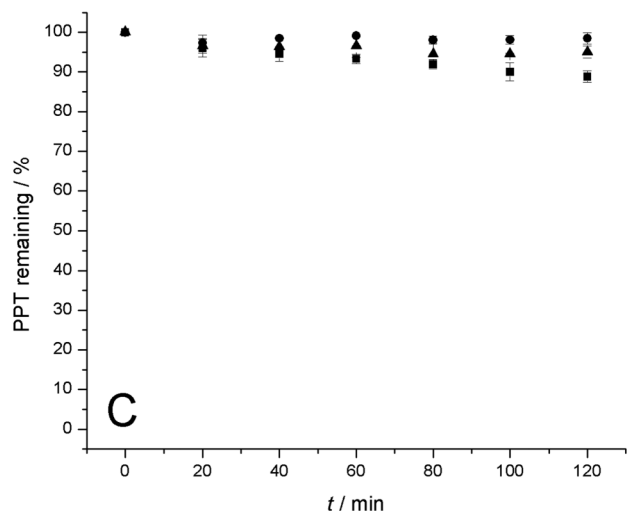


Fig. 2 Decomposition of (A) 50 μM allicin **1**, (B) 50 μM BPT **2**, and (C) 50 μM PPT **3** in chlorobenzene with HFIP (0.15 M, ●), CH_3CN (1 M, ▲), or no additive (■).

(see Scheme 1).[27] The 9- and 13-*Z*,*E*-isomers are kinetic products, whose corresponding peroxyls can undergo a conformational change followed by β-fragmentation and re-addition of O_2 to yield the 9- and 13-*E*,*E* isomers, the thermodynamic products. When

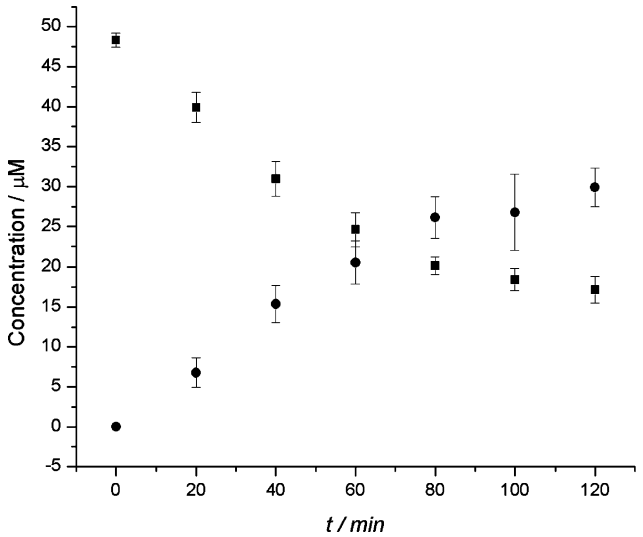
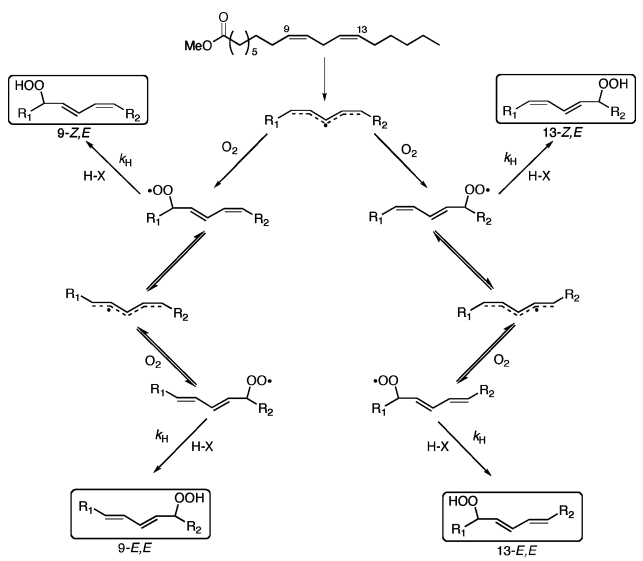


Fig. 3 Decomposition of 50 μM BPT **2** (■) in the presence of 0.1 M ethyl propiolate in chlorobenzene and formation of the corresponding (*E*)-ethyl 3-(benzylsulfinyl)acrylate **5** (●).

methyl linoleate autoxidations are carried out in the presence of a moderately reactive H-atom donor (X–H in Scheme 1) that can trap the intermediate peroxyl radicals in competition with their β-fragmentation, the ratio of *Z*,*E* to *E*,*E* products obtained as a function of the concentration of the H-atom donor can be used to obtain the rate constant for the reaction of the intermediate peroxyl radicals with X–H (k_H)[28] We used this kinetic competition to obtain effective inhibition rate constants for **2** and **3** in chlorobenzene, to which we added either acetonitrile or HFIP as above. The data are given in Table 1.



Scheme 1 General mechanism for the autoxidation of methyl linoleate.

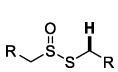
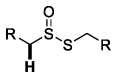
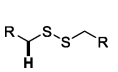
The results are consistent with the autoxidation data, and show that BPT is a good antioxidant, with an effective inhibition rate constant of 2.0×10^5 M^{-1} s^{-1}, while PPT is not, as its kinetics are *ca.* 1000-fold slower. While significant solvent effects are observed on the reactivity of BPT, with the inhibition rate constant dropping

Table 1 Inhibition rate constants determined for BPT **2** and PPT **3** in MeOAMVN(2,2′azobis(4-methoxy-2,4-dimethylvaleronitrile))-initiated kinetically-controlled autoxidations of methyl linoleate in the given solvent system at 37 °C

Thiosulfinate	PhCl	PhCl + CH_3CN[a]	PhCl + HFIP[b]
BPT	2.0×10^5	2.3×10^4	1.7×10^4
PPT	350	340	330

[a] 1 M (~ 5% by volume) acetonitrile in chlorobenzene. [b] 0.15 M (~ 1.6% by volume) hexafluoroisopropanol in chlorobenzene.

Table 2 CBS-QB3-Calculated C–H BDEs in allicin and PPT and O–H BDEs in the sulfenic acids derived therefrom, as well as from BPT

R				
CH═CH_2	81.8	87.2	82.4	70.3
Ph	*nd*[a]	*nd*[a]	*nd*[a]	69.8
Et	n/a[b]	96.3	95.0	68.7

[a] *nd* = not determined; [b] n/a = not available; the radical derived therefrom is unbound.

ca. 10-fold upon incorporation of either acetonitrile or HFIP, no solvent effect is observed on the reactivity of PPT.

IV. Thermochemistry of radical-trapping activities

The most labile H-atom in allicin has been reported to be that adjacent to the divalent sulfur atom. We have used the complete basis set approach of Petersson and co-workers at the CBS-QB3 level[29] to determine the C-H bond dissociation enthalpies (BDEs) at both methylene positions in allicin and PPT. The CBS-QB3 method has been demonstrated to predict C–H and O–H BDEs in excellent agreement with experiment, where comparison with high quality data has been possible.[30] The results are given in Table 2. Alongside are the O–H BDEs in the sulfenic acids that arise upon Cope elimination from the corresponding thiosulfinate, as well as the phenylmethanesulfenic acid that arises from BPT (**2**).

The calculated BDE for the C–H bond adjacent to the divalent sulfur in allicin is 81.8 kcal mol^{-1}, whereas that for the C–H bond adjacent to the thionyl moiety is 87.2 kcal mol^{-1}. By comparison, the C–H BDE in diallyldisulfide is 82.4 kcal mol^{-1}. The BDEs calculated in the saturated derivative, PPT, are much higher, as expected, since they cannot benefit from the allylic stabilization of the incipient carbon-centered radical. We were unable to determine a BDE for the position adjacent to the divalent sulfur in PPT since the corresponding radical was not bound, *i.e.* no minimum energy structure could be determined, and instead the structure dissociated to the *n*-propyl sulfinyl radical and thiopropanal. While the corresponding radical derived from allicin was indeed a bound structure, we found that it underwent β-fragmentation with a very low activation energy (E_a = 3.3 kcal mol^{-1}) to yield the 2-propenesulfinyl radical and thioacrolein. The number of atoms in BPT and dibenzyl disulfide made calculations of their C–H BDEs at this level of theory prohibitively lengthy.

The calculated O–H BDEs in the sulfenic acids are much lower at *ca.* 69–70 kcal mol^{-1}, and essentially independent of the nature of the alkyl group on the intervening methylene unit. In fact,

sulfenic acids would appear to have some of the lowest O–H BDEs known, comparable to those in hindered hydroxylamines, such as *N*-hydroxy-2,2,6,6-tetramethylpiperidine (TEMPO-H), which has an O–H BDE of 69.6 kcal mol^{-1}.[31,32]

V. Kinetics of radical-trapping activities

Given the weak calculated C–H bond in allicin and O–H bond in the 2-propenesulfenic acid derived therefrom, we also calculated the transition state (TS) structures for the formal H-atom transfer (HAT) from allicin and 2-propenesulfenic acid to methylperoxyl, a model peroxidation chain-carrying peroxyl radical. We have done the same for the saturated analogs, PPT and *n*-propanesulfenic acid, to compare with the foregoing experimental data. The lowest energy TS structures for the formal HAT from allicin and PPT are shown in Fig. 4, while those for 2-propenesulfenic acid and *n*-propanesulfenic acids are shown in Fig. 5. The calculations were again carried out using the CBS-QB3 approach, and as such, all of the structures shown were optimized at the B3LYP/CBSB7 level of theory.

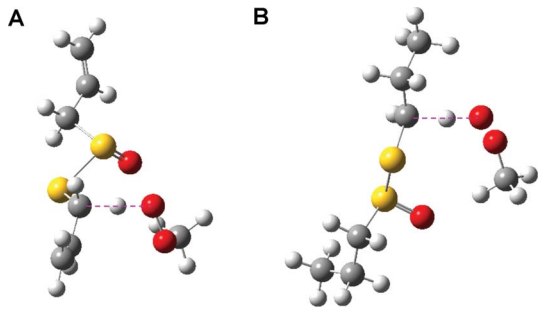


Fig. 4 Transition state (TS) structures for the formal H-atom transfer reactions between allicin (A) and PPT (B) and (methyl) peroxyl radicals.

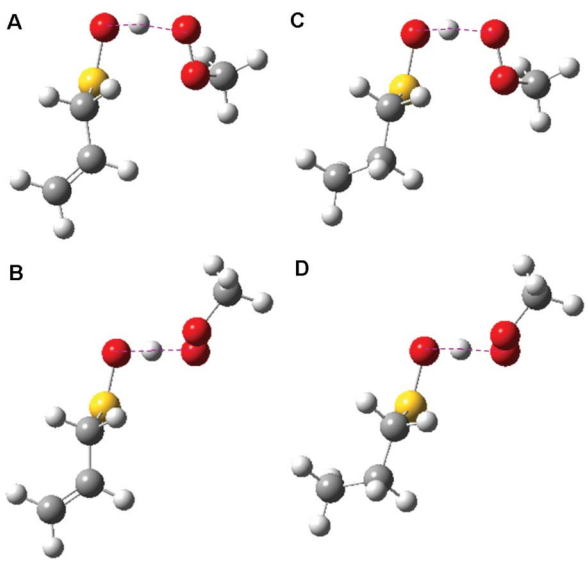


Fig. 5 Transition state (TS) structures for the formal H-atom transfer reactions between 2-propenesulfenic acid (A, B) and *n*-propanesulfenic acid (C, D) and (methyl) peroxyl radicals.

The TS structure for HAT from allicin to methylperoxyl sees the internal oxygen atom of the peroxyl radical lying over the top of the proximal side of the double bond of the allyl unit. This is similar to the TS calculated for HAT from unsaturated lipids to peroxyl radicals, and arises due to the secondary orbital interaction between the π* SOMO of the peroxyl radical and the π HOMO of the allyl unit.[33] In contrast, for the saturated analog (PPT), the TS structure for HAT is slightly different since the π*/π interaction is no longer available. Instead, the peroxyl moiety has rotated around such that the internal oxygen atom of the peroxyl radical is above the divalent sulfur atom, permitting a π*/n interaction. Given the foregoing C–H BDE calculations, it is perhaps not surprising that the calculated E_a for HAT from allicin (10.3 kcal mol^{-1}) is lower than that for PPT (11.1 kcal mol^{-1}). The same trend is evident upon comparison of the calculated E_as for HAT from diallyldisulfide and dipropyldisulfide (10.9 and 11.6 kcal mol^{-1}, respectively)—see the ESI‡ for these structures.

Two TS structures for the formal H-atom transfer from both 2-propenesulfenic acid and *n*-propanesulfenic acid were readily identified—one in which the substituents on the oxygen atoms between which the H-atom is being transferred have a *syn* orientation (Fig. 5A, C), and one in which they have an *anti* orientation (Fig. 5B, D). The *syn* structures are lower in energy by 6.7 kcal mol^{-1} for both 2-propenesulfenic acid and *n*-propanesulfenic acid, respectively. This can be attributed to the interaction of the sulfur lone pair with the peroxyl radical π* SOMO that is possible in the *syn* structure (Fig. 6), but not in the *anti* structure. These types of orbital interactions have been described to be typical of proton-coupled electron transfer reactions.[34,35] The corresponding calculated activation energies for O–H abstraction from 2-propenesulfenic acid and *n*-propylsulfenic acid are 1.8 and 1.0 kcal mol^{-1}, respectively.

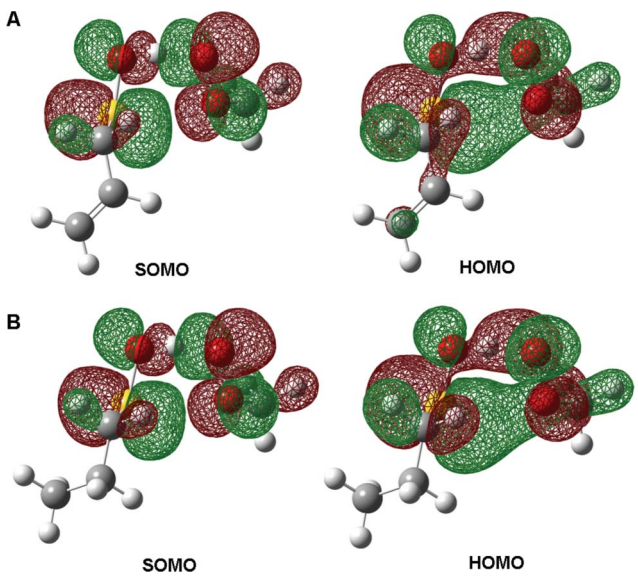


Fig. 6 Relevant molecular orbitals for the low energy TS structures for the reaction of 2-propenesulfenic acid (**A**) and *n*-propylsulfenic acid (**B**) with (methyl) peroxyl radicals.

We also considered the possibility that peroxyl radicals could add to the thiosulfinates. Indeed, we were able to find TS structures for the addition of methylperoxyl to both allicin and PPT, and in both cases, addition was concerted with the scission of the S–S bond. These structures are shown in Fig. 7. The calculated E_as

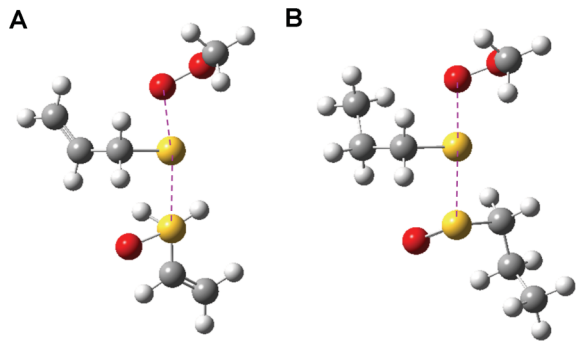
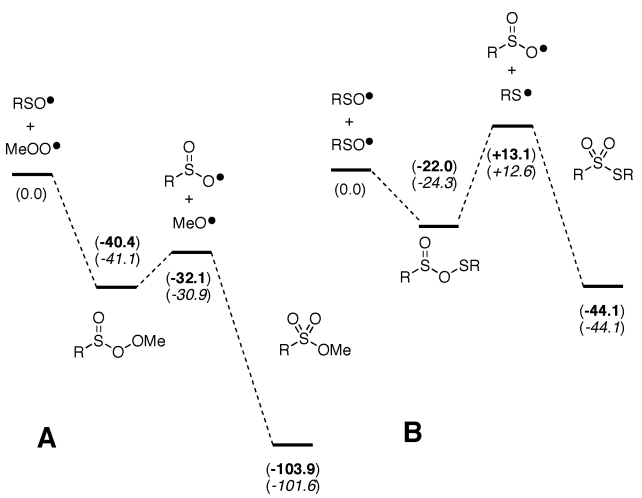


Fig. 7 Transition state (TS) structures for the homolytic substitution of (methyl) peroxyl radicals for sulfinyl radicals on allicin (A) and PPT (B).

for these reactions are 8.8 and 7.4 kcal mol^{-1}, respectively. The products of these reactions are the corresponding sulfinyl radicals and an RSOOMe species, the latter of which has an exceptionally weak O–O bond (calculated to be 6.1 and 5.6 kcal mol^{-1} for R = allyl and propyl, respectively). The resultant radical pairs can then recombine to yield sulfinate esters, which lie 54.1 and 53.6 kcal mol^{-1} lower in enthalpy, respectively.

VI. Fate of the sulfinyl radicals

We extended our CBS-QB3 calculations to include the possible fates of the sulfinyl radicals formed from the formal H-atom transfer from sulfenic acids to peroxyl radicals. Two possibilities are clearly evident, and are considered here: the trapping of a second peroxyl radical by a sulfinyl radical (Scheme 2A), and the dimerization of two sulfinyl radicals (Scheme 2B). Calculations were carried out for R = Me and R = ^{t}Bu simply to be able to assess any steric contributions to the reaction energetics.



Scheme 2 Two possible reaction paths of model sulfinyl radicals (R = Me, *t*-Bu) under autoxidizing conditions: (A) reaction with a (model) methylperoxyl radical to yield a sulfonate ester, and (B) dimerization to form a thiosulfonate ester. Relative enthalpies, calculated using CBS-QB3, are given in kcal mol^{-1} for R = Me (bold) and R = *t*-Bu (italics).

Two modes of combination of sulfinyl and peroxyl radicals can be envisioned: coupling between the two terminal oxygen atoms to yield a structure wherein all heteroatoms are connected linearly (*i.e.* RSOOOR), or coupling between the terminal oxygen atom of the peroxyl radical and the sulfur atom of the sulfinyl—which bears significant (*i.e.* 50%) unpaired electron spin density—to yield a peroxysulfinate ester, RS(O)OOR. While the formation of the former is endothermic, formation of the peroxysulfinate ester is *ca.* 40 kcal mol^{-1} exothermic. The peroxysulfinate ester is expected to have an exceptionally weak O–O bond, which was computed to be 8–10 kcal mol^{-1}. The sulfonyl and alkoxyl radical pair that results can then recombine to form the more stable sulfonate ester, which is predicted to lie ~60 kcal mol^{-1} lower in enthalpy than peroxysulfinate ester and ~100 kcal mol^{-1} lower than the starting sulfinyl and peroxyl radicals. Alternatively, one might expect a concerted rearrangement of the peroxysulfinate ester to a sulfonate ester. Despite several attempts, we failed to locate a transition state structure for this path.

The dimerization of two sulfinyl radicals can be envisioned to occur in three modes: *via* coupling between the two oxygen atoms to yield the linear adduct, RSOOSR; coupling between the two sulfur atoms to yield the vicinal disulfoxide, RS(O)S(O)R; or, coupling between an oxygen atom and a sulfur atom to yield RS(O)OSR. While the formation of RSOOSR was found to be endothermic with both R = Me and *t*-Bu, the formation of the vicinal disulfoxide and the RS(O)OSR species were exothermic by 18–21 and 22–24 kcal mol^{-1}, respectively. The latter can undergo a rearrangement to a thiosulfinate, similarly to the peroxysulfinate → sulfonate above, but now a much stronger O–S bond must be cleaved to give the radical pair (35–37 kcal mol^{-1}), compared to the O–O bond in the peroxysulfinate ester (8–10 kcal mol^{-1}). In this case, a transition state for the concerted rearrangement to the thiosulfonate ester could be located, which was characterized by a slightly larger barrier than the fragmentation/recombination pathway (see the ESI‡ for further details).

Discussion

The most common naturally-occurring radical-trapping antioxidants are phenols.[36] Phenols are particularly effective as antioxidants due to their relatively weak O–H bonds[37] and their ability to react by a proton-coupled electron transfer mechanism.[38] This provides good thermodynamics and kinetics for their reaction with autoxidation chain-carrying peroxyl radicals. For example, α-tocopherol, the most potent form of Vitamin E, has an O–H BDE of 78 kcal mol^{-1} (10 kcal mol^{-1} lower than the O–H BDE of an alkylhydroperoxide) and reacts with peroxyl radicals with a second order rate constant of 3×10^6 M^{-1} s^{-1} at 37 °C in benzene.[36]

Garlic, which has long been described to have potent radical-trapping antioxidant activity, does not have a high concentration of α-tocopherol, or other free lipid-soluble phenolic antioxidants,[39] leading investigators to suppose that the activity is due to indirect mechanisms, such as *via* the induction of the expression of antioxidant enzymes.[1] Garlic and other alliums contain high concentrations of non-proteinogenic amino acids derived from cysteine, which undergo further metabolic transformation to lead to thiosulfinates and other organosulfur compounds believed to impart medicinal activity to some of these plants.[3] The most well studied compound is allicin, which affords garlic its flavour and odour.

Recent work by Okada and co-workers indicated that allicin and the related thiosulfinate BPT from *Petiveria alliacae* are effective antioxidants.[7,17,22] Their results were particularly interesting since

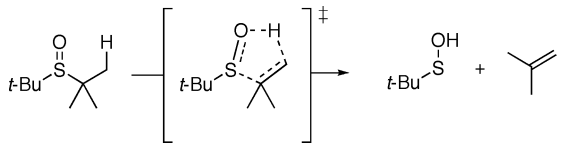
thiosulfinates do not have any labile O–H bonds, as do phenols. This prompted the investigators to suggest a mechanism involving the donation of the allylic H-atom adjacent to the divalent sulfur in allicin (or similarly activated benzylic H-atom in BPT) to the peroxyl radicals. In subsequent work, Amorati and Pedulli demonstrated that diallyldisulfide, which was expected to have a similar C–H bond strength to the allylic C–H bond in allicin and, therefore, H-atom donating ability, was not a good antioxidant.[40] This suggested that Okada *et al.*'s mechanism could not be correct, but an alternative explanation was not obvious—prompting our work in this area.

Our own efforts began with the reproduction of the inhibited periods observed by Okada and co-workers in the AIBN-initiated autoxidation of solutions of methyl linoleate containing either allicin[7] or BPT.[17] Inhibited autoxidations are a convenient means for determining both the kinetics (k_{inh}) and stoichiometry (n) of the radical-trapping chain-breaking activities of antioxidants, provided that a clear inhibition period is observed. As can be seen in Fig. 1, a clear inhibition period is indeed observed under the experimental conditions used by Okada *et al.*, confirming that allicin and BPT are effective antioxidants. However, it should be pointed out that inhibition rate constants (k_{inh}) cannot be derived from the rate of the inhibited part of the autoxidation (R_{inh}) using eqn (6)—as was done by Okada and co-workers—because the kinetic chain length ($\nu = R_{inh}/R_i$) is less than the 5–10 required for the autoxidation to be a true radical chain mechanism.[23] Eqn (6) is therefore not valid under these conditions, and the information that can be gleaned from these experiments is purely qualitative.

$$R_{inh} = \frac{\mathrm{d[ROOH]}}{\mathrm{d}t} = \frac{k_p[\mathrm{RH}]R_i}{nk_{inh}[\mathrm{IH}]} \quad (6)$$

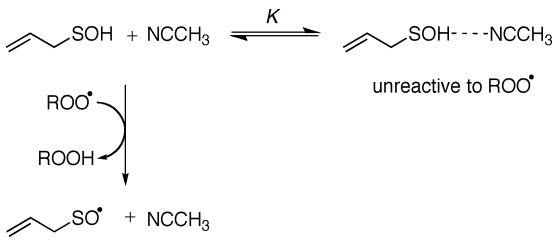
Nevertheless, the experiments are useful as they have allowed us to qualitatively probe the kinetic solvent effects on the radical-trapping activities of these compounds in order to provide some mechanistic insight. The addition of hexafluoroisopropanol (HFIP) as a co-solvent (0.15 M ~ 1.6% by volume) to the autoxidations resulted in the complete abrogation of the inhibition period ascribed to the action of allicin or BPT, with only a slight deviation from a thoroughly uninhibited autoxidation observed near the beginning of the autoxidation. Likewise, the addition of acetonitrile as a co-solvent (1 M ~ 5% by volume) to the autoxidations slowed the inhibitory action of both allicin and BPT as evidenced by the greater rate of inhibited autoxidation (R_{inh}) in the presence of acetonitrile as compared to its absence. It is difficult to rationalize these results on the basis of a mechanism involving H-atom abstraction from the methylene group adjacent to the divalent sulfur as Okada *et al.* have suggested. A mechanism that is consistent with these solvent effects is necessary; *i.e.* one which accounts for the observation that both HBA and HBD solvents render allicin and BPT less effective as an antioxidant.

In 1972, Koelewijn and Berger demonstrated that di-*tert*-butyl sulfoxide is capable of inhibiting hydrocarbon (tetralin) autoxidation at 60 °C. This activity was ascribed to the trapping of chain-carrying peroxyl radicals by 2-methylpropanesulfenic acid produced by the thermal decomposition of the sulfoxide *via* a Cope elimination (eqn (7)).[41] From their inhibited autoxidation data, Koelewijn and Berger estimated that 2-methylpropane sulfenic acid reacted with peroxyl radicals with a rate constant in excess of 10^7 M^{-1} s^{-1}, making sulfenic acids perhaps the most effective class of peroxyl radical-trapping agents known.



(7)

Thiosulfinates, like sterically-crowded sulfoxides, are known to undergo decomposition *via* Cope elimination to give rise to a sulfenic acid and a thiocarbonyl (eqn (4)). Therefore, it seemed reasonable for us to suggest that the mechanism of antioxidant activity of allicin[18] and BPT is the result of peroxyl radical-trapping, not by the thiosulfinates themselves, but by the 2-propenesulfenic acid and phenylmethanesulfenic acid that arise due to Cope elimination from them. It is known that H-bond donating solvents slow the rate of elimination of sulfenic acids from thiosulfinates[25]—and this would account for the lesser antioxidant activity of allicin and BPT when HFIP is added to the autoxidation. The effect of HBA solvents on the antioxidant activity of allicin and BPT is also readily explained by a mechanism involving radical-trapping by sulfenic acids. It is now well established that the radical-trapping activities of phenols are diminished in HBA solvents due to H-bond formation between the phenol and solvent, which precludes transfer of the phenolic H-atom to the radical.[42] For example, the rate constant for the reaction of α-tocopherol with peroxyl radicals decreases from 2.7×10^6 to 3.9×10^5 M^{-1} s^{-1} on changing the medium from chlorobenzene to acetonitrile.[43] Since the sulfenic acid will undoubtedly engage in H-bonding interactions with H-bond accepting solvents (*e.g.* acetonitrile), the same type of kinetic solvent effect can be expected on its radical-trapping activity (Scheme 3).

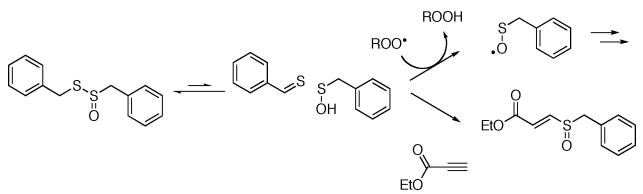


Scheme 3 The expected effect of a H-bond accepting co-solvent on the H-atom donating ability of 2-propenesulfenic acid.

The role of sulfenic acids in the radical-trapping activity of thiosulfinates is also supported by the results obtained with PPT, the third thiosulfinate we examined. Since PPT does not have an activated C–H bond adjacent to the divalent sulfur atom, as does allicin and BPT, which have allylic and benzylic C–Hs, respectively, it undergoes Cope elimination to yield the corresponding propanesulfenic acid much more slowly (*cf.* Fig. 2). Hence, there is simply less sulfenic acid available to inhibit the autoxidation.

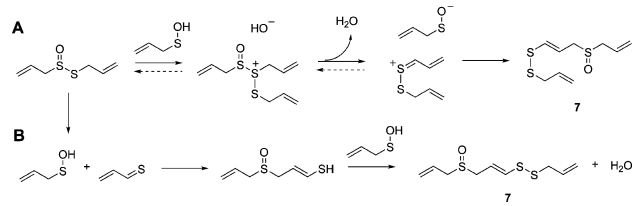
To provide a direct link to our autoxidation experiments, we studied the effects of added acetonitrile and HFIP on the rate of decomposition of allicin and BPT in chlorobenzene. The results with allicin followed our expectations nicely; allicin decomposes steadily in chlorobenzene, and the addition of HFIP slows this process whereas the addition of acetonitrile does little. On the other hand, we found that BPT was stable to decomposition

under all the experimental conditions—initially suggesting that our mechanistic proposal must be incorrect. However, when we incorporated ethyl propiolate, a good electrophilic trap for the nucleophilic phenylmethanesulfenic acid that is formed from BPT in the reaction mixture, BPT decomposed at a rate scarcely different from allicin. Moreover, the addition of HFIP and acetonitrile now produced the same effects on the decomposition of BPT as we observed for allicin. These data indicate that the Cope elimination of phenylmethanesulfenic acid from BPT is reversible, and that the equilibrium lies toward the thiosulfinate. When large quantities of a good electrophile (*i.e.* ethyl propiolate) or potent oxidant (a chain-carrying alkylperoxyl) are present, they can outcompete the rate of the reverse reaction with thiobenzaldehyde that reforms BPT (Scheme 4).



Scheme 4 The reversible Cope elimination of phenylmethanesulfenic acid from BPT.

In contrast, the Cope elimination of 2-propenesulfenic acid from allicin is irreversible under the same reaction conditions. One reason could be that thioacrolein is a much more reactive byproduct. Block and co-workers have studied the decomposition of allicin in organic solvents and have found the dithins and (*E*)-ajoene (**7**) to be the major isolable products.[3] The dithins arise simply due to [4 + 2] cycloaddition of thioacrolein to itself. Ajoene is thought to arise from the initial *S*-thiolation of allicin with 2-propenesulfenic acid followed by either a concerted or step-wise (fragmentation/addition) 1,4-migration of the sulfenate moiety (Scheme 5A). Replacement of the allyl substituent of allicin with the benzyl substituent of BPT would be unlikely to affect either the *S*-thiolation step or the subsequent loss of sulfenic acid/sulfenate, suggesting that these steps must be reversible themselves. Another possible mechanism involves the 1,4-addition of 2-propenesulfenic acid to thioacrolein to form a sulfoxide, which could form (*E*)-ajoene through reaction with 2-propenesulfenic acid (Scheme 5B). Of course, the initial step in this sequence would be difficult upon replacing allyl with benzyl as it would require addition to the thiobenzaldehyde at the cost of aromaticity. We are currently exploring the mechanism of BPT decomposition further and will contrast it with allicin decomposition in a future report.



Scheme 5 Two possible mechanisms of (*E*)-ajoene formation from allicin.

Sulfenic acids are unstable, owing to their high reactivities as both nucleophiles and electrophiles. They readily self-condense to yield thiosulfinates, rapidly yield disulfides in the presence of thiols, and tend to be somewhat unstable in air. This instability, and the lack of any commercially-available sulfenic acids, precluded our ability to directly determine any kinetic or thermodynamic parameters for the reactions of sulfenic acids with peroxyl radicals. Therefore, we turned to theory.

We first carried out calculations to determine the O–H BDE in sulfenic acids to provide some insight into the thermodynamic basis for their apparently facile reactions with chain-carrying peroxyl radicals. Using the complete basis set approach at the CBS-QB3 level, we calculated O–H BDEs in 2-propenesulfenic acid and phenylmethanesulfenic acid of 70.3 and 69.8 kcal mol^{-1}, respectively. This is ~16 kcal mol^{-1} lower than the corresponding values calculated for alkyl hydroperoxides, of *ca.* 86 kcal mol^{-1}.[18] In comparison, the C–H bond that is broken in Okada *et al.*'s mechanism is predicted to have a BDE of ~82 kcal mol^{-1}, which would be far less exothermic. Of course, this tells us nothing of the kinetics of the reaction, so we moved on to consider the transition state structures and corresponding barriers for the reactions of peroxyl radicals with sulfenic acids *versus* the thiosulfinates themselves.

While transition states for the formal H-atom transfer from both allicin and PPT to methylperoxyl radicals were readily located, the calculated activation energies of 10.3 and 11.1 kcal mol^{-1}, respectively, were scarcely different from the predicted barriers for the diallyl and dipropyl sulfides of 10.9 and 11.6 kcal mol^{-1}, respectively. This suggests that the oxidation of a disulfide to a thiosulfinate does not impart any significant enhancement in its radical-trapping ability. In fact, combining the calculated activation energy for the reaction of diallyldisulfide with methylperoxyl with the typical A-factor for H-atom abstractions of 2×10^8 M^{-1} s^{-1},[44] yields a predicted rate constant of 2 M^{-1} s^{-1}, at 298 K, scarcely different from the experimental value of 1.6 ± 0.8 M^{-1} s^{-1}determined at 303 K by Amorati and Pedulli two years ago.[40] In contrast, the transition states for the formal H-atom transfer from both the 2-propenesulfenic acid and *n*-propanesulfenic acid to methylperoxyl radicals were characterized by very low barriers of 1.8 and 1.0 kcal mol^{-1}, respectively.[45] When taken with the same generic pre-exponential factor, these barriers yield predicted rate constants on the order of 10^7 M^{-1} s^{-1}, in very good agreement with Koelwijn and Berger's estimate for 2-methylpropanesulfenic acid derived from pyrolysis of di-*tert*-butyl sulfoxide,[41] and nicely explaining the observed inhibitory activities of allicin and BPT in the methyl linoleate autoxidations.

Our peroxyl radical clock experiments provide some quantitative information for the reactions of BPT and PPT with peroxyl radicals. Indeed, these are only effective inhibition rate constants which depend on a prior equilibrium to make sulfenic acid available to react, but if we assume that this equilibrium is fast with respect to the consumption of the sulfenic acid by reaction with peroxyls, the rate constant for the radical-trapping reaction can be derived from the effective inhibition rate constant if we know the equilibrium constant. We calculated the equilibrium constant from the ΔG obtained by the same CBS-QB3 calculations we have used throughout obtaining 1.5×10^{-3} and 6.4×10^{-7} for allicin (as a model for BPT) and PPT, respectively. When combined with the observed rate constants (in chlorobenzene), inhibition rate constants of 1.3×10^8 M^{-1} s^{-1} and 5.5×10^7 M^{-1} s^{-1} are obtained, consistent with the values above.

From the length of the inhibited period (τ) observed in the autoxidations in Fig. 1, the rate of initiation (R_i), and the concentration of the thiosulfinate, we can calculate the stoichiometry of the inhibition reaction,[46] *i.e.* the number of chain-carrying peroxyl radicals that are trapped per molecule of thiosulfinate, as in eqn (8), to be *ca.* 1 for both allicin and BPT.

$$n = \frac{R_i \times \tau}{[\text{thiosulfinate}]} \quad (8)$$

This differs from the stoichiometry for inhibition of autoxidation by phenols, wherein two peroxyls are trapped by a single molecule of phenol, first *via* formal H-atom transfer from the phenol to the peroxyl radical (eqn (9)), and second by the addition of a peroxyl to the phenoxyl radical to yield non-radical products (eqn (10)):

$$ArOH + ROO^{\bullet} \rightarrow ArO^{\bullet} + ROOH \quad (9)$$

$$ArO^{\bullet} + ROO^{\bullet} \rightarrow \text{non-radical products} \quad (10)$$

The result implies that the sulfinyl radical that results from the formal H-atom transfer from the sulfenic acid to the peroxyl radical does not break a second autoxidation chain. While our theoretical calculations indicate that the reaction of a sulfinyl radical and a peroxyl radical is highly exothermic to yield a sulfonate ester, this is predicted to occur by a step-wise rearrangement from the initial radical combination product peroxysulfinate, RS(O)OOR. Upon cleavage of the weak O–O bond in this species, the resultant radicals can recombine to yield a sulfonate ester, or the alkoxyl can escape the solvent cage to initiate another peroxidation chain reaction. The low stoichiometric factor suggests that cage escape predominates over recombination. The stoichiometric factor can also be reduced from the ideal value of 2 by any competitive reactions that would deplete the thiosulfinate or the sulfenic acid derived therefrom. This may include the slower direct reactions of the thiosulfinate with peroxyl radicals, which would not contribute to the inhibition period, the oxidation of the thiosulfinate by the product hydroperoxides to the thiosulfonate or oxidation of the sulfenic acid to its corresponding sulfinic acid. Indeed, we find that 2-propenesulfinic acid has an O–H BDE of 78.6 kcal mol^{-1} (8.3 kcal mol^{-1} higher than in 2-propenesulfenic acid), and has a calculated activation energy for reaction with (methyl) peroxyl of 9.9 kcal mol^{-1} (8.1 kcal mol^{-1} higher than for 2-propenesulfenic acid); see the ESI‡ for further details.

Conclusions

The plant-derived thiosulfinates allicin, BPT and PPT exhibit peroxyl radical-trapping chain-breaking antioxidant activities that reflect their propensity to undergo Cope elimination to yield corresponding sulfenic acids. Sulfenic acids, which are predicted to have some of the weakest O–H bonds ever reported, are calculated to undergo a near diffusion-controlled reaction with peroxyl radicals. BPT would appear to be the most interesting of the thiosulfinates studied here, as we find that its decomposition to yield phenylmethanesulfenic acid and thiobenzaldehyde is reversible. Thus, in the absence of chain-carrying peroxyl radicals (or other appropriate electrophile) BPT is reformed. The stoichiometry of the radical-trapping reactivity of both allicin and BPT is *ca.* 1. This result may reflect that the fate of the sulfinyl radical following H-atom donation from the sulfenic acid is to first combine with another peroxyl radical, but that the resultant peroxysulfinate ester is unstable, and liberates a chain-initiating alkoxyl radical under the experimental conditions. Our results prompt further studies on the reactivities of these compounds under more physiologically-relevant conditions, and whether the structures can be modified for increased stability and/or activity.

Experimental section

General techniques

All chemicals were purchased from Aldrich except MeOAMVN, which was purchased from Wako. AIBN (recrystallized from methanol) and MeOAMVN were placed under high vacuum for two hours directly before use. Methyl linoleate (MeLin) was purified by preparative HPLC. Allicin, BPT and PPT were synthesized and purified as described below and used immediately. Chlorobenzene, acetonitrile and hexafluoroisopropanol were distilled before use.

Allicin (1). Diallyl disulfide was placed under vacuum at 0 °C to remove traces of allyl disulfide. To a solution of diallyl disulfide (1.46 g, 10.0 mmol) in chloroform (30 mL) was added *m*-chloroperbenzoic acid (77%, 2.35 g, 10.5 mmol) solution in chloroform (5 mL) dropwise at 0 °C. The reaction mixture was stirred at 0 °C for 1 h. Anhydrous sodium carbonate (8 g) was added in small portions with vigorous stirring. The reaction mixture was stirred for an additional 1 h at 0 °C and then filtered through a pad of celite and magnesium sulfate. The filtrate was concentrated under reduced pressure to give 1.41 g (87% yield) of crude allicin, which was purified by preparatory TLC (pentane–EtOAc, 80 : 20) immediately prior to use. The freshly prepared allicin was stored at −80 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.76–3.90 (m, 4H), 5.22–5.50 (m, 4H), 5.90–5.99 (m, 2H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ 35.03, 59.89, 119.06, 124.02, 125.84, 132.91 ppm. Spectral data are in accordance with those reported elsewhere.[47]

***S*-Benzyl phenylmethanethiosulfinate (2).** To a solution of dibenzyl disulfide (2.46 g, 10.0 mmol) in dichloromethane (30 mL) was added *m*-chloroperbenzoic acid (*m*-CPBA) (77%, 2.35 g, 10.5 mmol) in dichloromethane (5 mL) dropwise at 0 °C. The reaction mixture was stirred at 0 °C for one hour. Sodium carbonate (8 g) was added in small portions with vigorous stirring. The reaction mixture was stirred for an additional 1 h at 0 °C. The reaction mixture was then filtered through magnesium sulfate. The filtrate was concentrated under reduced pressure yielding crude BPT, which was recrystallized from hexanes–ethyl acetate (80 : 20) to yield pure BPT as a white solid (87%). Pure BPT was stored at −30 °C. ^{1}H NMR (400 MHz, $(CD_3)_2CO$): δ 4.31 (s, 2H), 4.36 (d, 1H, J = 12.9 Hz), 4.44 (d, 1H, J = 12.9 Hz), 7.28–7.40 (m, 10H) ppm; ^{13}C NMR (100 MHz, $(CD_3)_2CO$): δ 35.9, 62.8, 128.3, 129.2, 129.4, 129.5, 130.0, 131.4, 132.0, 138.5 ppm; HRMS (EI) calcd for $C_{14}H_{14}OS_2$ m/z 262.0486, found 262.0483. Spectral data are in accordance with those reported elsewhere.[48]

***S*-Propyl propanethiosulfinate (3).** A solution of *m*-CPBA (77%, 2.35 g, 10.5 mmol) in dichloromethane (5 mL) was added dropwise to a solution of dipropyl disulfide (1.50 g, 10.0 mmol) in dichloromethane (30 mL) at 0 °C. The reaction mixture was

stirred at 0 °C for one hour. Sodium carbonate (8 g) was added in small portions with vigorous stirring. The reaction mixture was stirred again at 0 °C for one hour. The reaction mixture was then filtered through magnesium sulfate. The filtrate was concentrated under reduced pressure to give crude PPT, which was then purified by column chromatography (pentane–ethyl acetate, 80 : 20). Purified PPT was stored at −30 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.03–1.13 (6H), 1.74–1.96 (4H), 3.04–3.27 (4H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ 13.1, 17.1, 24.2, 34.8, 57.9 ppm; HRMS (EI) calcd for $C_{14}H_{14}OS_2$ m/z 166.0489, found 166.0486. Spectral data are in accordance with those reported elsewhere.[49]

(*E*)-Ethyl 3-(allylsulfinyl)acrylate (4). Ethyl propiolate (0.66 mL, 3.3 mmol) was added dropwise to a solution allicin (0.25 g, 1.5 mmol) in 30 mL of benzene and the mixture was stirred at 37 °C under N_2 for 24 h. The solvent was removed under reduced pressure and the resulting oil was purified by flash chromatography (70 : 30 hexanes–ethyl acetate) to afford a colourless oil (74%). ^{1}H NMR (400 MHz, ($CDCl_3$) δ 1.35 (t, 3H), 3.16 (m, 2H), 4.26 (m, 2H), 5.20 (m, 2H), 5.78 (m, 1H), 6.72 (d, J = 15.0 Hz 1H), 7.80 (d, J = 15.0 Hz, 1H) ppm; ^{13}C NMR (100 MHz, ($CDCl_3$) δ 14.2, 61.4, 62.5, 119.3, 125.8, 132.3, 148.8, 165.1 ppm; HRMS (ESI) calcd for $C_8H_{12}O_3S$ m/z188.0507, found 188.0515.

(*E*)-Ethyl 3-(benzylsulfinyl)acrylate (5). Ethyl propiolate (0.22 mL, 1.1 mmol) was added dropwise to a solution of *S*-benzyl phenylmethanethiosulfinate (0.5 g, 1.91 mmol) in 30 mL of benzene and the mixture was stirred at 37 °C under N_2 for 24 h. Solvent was removed under reduced pressure and the resulting oil was purified by flash chromatography (65 : 35 hexanes–ethyl acetate) to afford a white solid (87%). ^{1}H NMR (400 MHz, $(CD_3)_2CO$) δ 1.28 (t, J = 7.1 Hz, 3H), 4.08 (d, J = 12.9 Hz, 1H), 4.21 (q, J = 7.1 Hz, 2H), 4.37 (d, J = 12.9 Hz, 1H), 6.29 (d, J = 15.0 Hz, 1H), 7.37 (m, 5H), 7.89 (d, J = 15.0 Hz, 1H) ppm; ^{13}C NMR (100 MHz, $(CD_3)_2CO$) δ 14.4, 59.3, 61.6, 126.4, 129.0, 129.3, 131.3, 131.4, 151.9, 164.1 ppm; HRMS (ESI) calcd for $C_{12}H_{15}O_3S$ m/z 239.0741, found 239.0747

(*E*)-Ethyl 3-(propylsulfinyl)acrylate (6). Ethyl propiolate (0.66 mL, 3.3 mmol) was added dropwise to a solution *S*-propyl propanethiosulfinate (0.25 g, 1.5 mmol) in 30 mL of benzene and the mixture was stirred at 37 °C under N_2 for 24 h. Solvent was removed under reduced pressure and the resulting oil was purified by flash chromatography (90 : 10 hexanes–ethyl acetate) to afford a yellow oil (16%). ^{1}H NMR (400 MHz, ($CDCl_3$) δ 1.07 (t, 3H), 1.29 (t, J = 7.1 Hz, 3H), 1.82 (m, 2H), 2.75 (m, 2H), 4.22 (q, J = 7.1 Hz, 2H), 6.62 (d, J = 15.0 Hz, 1H), 7.53 (d, J = 15.0 Hz, 1H) ppm; ^{13}C NMR (100 MHz, $(CD_3)_2CO$) δ 13.2, 14.1, 15.7, 54.8, 61.4, 128.5, 149.4, 163.8 ppm; HRMS (ESI) calcd for $C_8H_{14}O_3S$ m/z 190.0661, found 190.0664.

Thiosulfinate-inhibited autoxidations of methyl linoleate

The oxidation of MeLin (91 mM) was initiated by AIBN (40 mM) in the presence of the thiosulfinate (50 μM) at 37 °C in chlorobenzene. The MeLin was added to the appropriate amount of solvent followed by the thiosulfinate. AIBN was always added last when the reaction temperature reached 37 °C. To observe the effect of co-solvents on the rate of autoxidation acetonitrile or hexafluoroisopropanol were added to final concentrations of 1 M and 0.15 M, respectively, prior to the addition of thiosulfinate. Aliquots were withdrawn from the reaction mixtures at regular intervals, immediately quenched by excess of BHT (0.1 M in hexanes) followed by the addition of an internal standard (10 mM benzyl alcohol in chlorobenzene), and the samples analyzed as their hydroperoxides by HPLC (1% *i*-PrOH in hexanes, 1 mL min^{-1}, Sun-Fire Silica, 5 μm, 4.6 × 250 mm column, UV detection at 234 nm).

Decomposition of thiosulfinates

Thiosulfinate in chlorobenzene (50 μM) was added to a 1 mL HPLC autosampler vial with a 1 mL reaction mixture of internal standard (benzyl alcohol, 1 mM) in chlorobenzene and was placed in an autosampler pre-heated to 37 °C for two hours. The samples were analyzed in twenty-minute intervals by HPLC (1% *i*-PrOH in hexanes, 1 mL min^{-1}, Sun-Fire Silica, 5 μm 4.6 × 150 mm column). For BPT and allicin, UV detection at 234 nm was used and for PPT, UV detection at 262 nm was used. To determine the decomposition rate of thiosulfinate in the presence of acetonitrile or hexafluoroisopropanol, the final concentration of 1 M acetonitrile or 0.15 M hexafluoroisopropanol was added to the initial reaction mixture prior to the addition of thiosulfinate. The experiments were also carried out in the presence of 0.1 M ethyl propiolate.

Peroxyl radical clock experiments to determine effective inhibition rate constants of thiosulfinates

Stock solutions of MeLin (1.0 M), MeOAMVN (0.1 M), and the thiosulfinates were prepared in chlorobenzene. Samples were assembled in 1 mL HPLC autosampler vials with a total reaction volume of 100 μL. Solutions were prepared in the following order to avoid premature oxidation: thiosulfinate (2.50 mM–0.225 M), MeLin (0.10 M) and then MeOAMVN (0.01 M), and diluted to 100 μL with chlorobenzene. The sealed samples were then heated to 37 °C for 1 h. After 1 h, the oxidation was stopped by the addition of BHT (50 μL of 1.0 M solution in hexanes), followed by reduction of the hydroperoxides to alcohols by triphenylphosphine (50 μL of 1 M solution in chlorobenzene). The samples were then diluted to 1 mL with HPLC grade hexanes and analyzed by HPLC (1.5% *i*-PrOH in hexanes, 1 mL min^{-1}, 30 min, Sun-Fire Silica, 5 μm 4.6 × 250 mm column, UV detection at 234 nm). The ratio of products (Z,E : E,E) was plotted *versus* thiosulfinate concentration to derive k_H.[28]

Theoretical calculations

All calculations were carried out with the complete basis set approach of Petersson and co-workers at the CBS-QB3 level.[29] Briefly, this involves geometry optimizations and vibrational frequency calculations using density functional theory at the B3LYP level, followed by an extrapolation of the CCSD(T) energy to a complete basis using successive MP2 calculations of increasing basis set size. The calculations were performed using the Gaussian 09 suite of programs and results visualized using GaussView 4.0.[50]

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