Do garlic-derived allyl sulfides scavenge peroxyl radicals?

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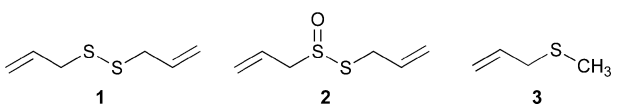
The chain-breaking antioxidant activities of two garlic-derived allyl sulfides, *i.e.* diallyl disulfide (**1**), the main component of steam-distilled garlic oil, and allyl methyl sulfide (**3**) were evaluated by studying the thermally initiated autoxidation of cumene or styrene in their presence. Although the rate of cumene oxidation was reduced by addition of both **1** and **3**, the dependence on the concentration of the two sulfides could not be explained on the basis of the classic antioxidant mechanism as with phenolic antioxidants. The rate of oxidation of styrene, on the other hand, did not show significant changes upon addition of either **1** or **3**. This unusual behaviour was explained in terms of the co-oxidant effect, consisting in the decrease of the autoxidation rate of a substrate forming tertiary peroxyl radicals (*i.e.* cumene) upon addition of little amounts of a second oxidizable substrate giving rise instead to secondary peroxyl radicals. The relevant rate constants for the reaction of ROO· with **1** and **3** were measured as 1.6 and 1.0 M^{-1} s^{-1}, respectively, fully consistent with the H-atom abstraction from substituted sulfides. It is therefore concluded that sulfides **1** and **3** do not scavenge peroxyl radicals and therefore cannot be considered chain-breaking antioxidants.

Introduction

Garlic is widely used as dietary supplement for the treatment of many diseases, being traditionally considered a natural remedy for hypercholesterolemia and atherosclerosis.[1] Since oxidative modification of low density lipoproteins (LDL) is a key step in the development of these cardiovascular diseases, the antioxidant efficacy of garlic constituents has received great attention.[2–5] It has been pointed out that the *in vivo* effects of garlic preparations may derive not only from direct radical scavenging, but also from induction of the endogenous antioxidant defences.[1] On the other hand, the abnormal intake of garlic extracts is correlated with severe toxic effects.[1]

Among the constituents of garlic, polysulfides have attracted attention due to their many biological actions, which span from antibacterial to antitumoral activities.[6] Diallyl disulfide (**1**) is the main component of steam-distilled garlic oil,[1] and this, together with other garlic sulfides, was claimed to protect LDL from oxidation and to spare vitamin E.[7,8] Diallyl disulfide and other polysulfides originate from allicin (**2**), a thiosulfinate produced from its precursor alliin by the enzyme allinase upon chopping fresh garlic.[6]



1 **2** **3**

In order to quantitatively assess the antioxidant behaviour of synthetic and natural compounds, the most important approach consists in studying their reaction with peroxyl radicals (ROO·) in the presence of an oxidizable substrate (LH) under air or oxygen.[9] Chain-breaking antioxidants (AH), such as substituted phenols or aromatic amines, inhibit peroxidation by transferring their phenol or amine H atom to the propagating ROO· radicals (eqn (2)) at a rate faster than that of chain propagation (eqn (1)), thus interrupting or reducing the oxidation of the substrate. The best known example of a phenolic antioxidant is α-tocopherol, the most potent constituent of vitamin E.[10]

$$\mathrm{ROO\bullet + LH} \xrightarrow{k_p} \mathrm{ROOH + L\bullet} \quad (1)$$

$$\mathrm{ROO\bullet + AH} \xrightarrow{k_{inh}} \mathrm{ROOH + A\bullet} \quad (2)$$

The peroxyl radical trapping ability of garlic sulfides was recently investigated by Okajima and coworkers[11] by using this technique. These authors reported that allicin (**2**) actively scavenges ROO· with a k_{inh} value of 2.6×10^3 M^{-1} s^{-1} and 1.6×10^5 M^{-1} s^{-1} at 30 °C depending on the oxidizable substrate, cumene or methyl linoleate (MeLH), respectively.† They proposed that peroxyl radicals abstract a H-atom from allicin forming an alkyl radical centred on the carbon in α position to the reduced S atom,[12] which in turn reacts with another ROO· radical. The computed dissociation enthalpy (BDE) of this allylic C–H bond (85.8 kcal mol^{-1}) obtained by DFT calculation was given to support this hypothesis.[11]

This interpretation is surprising for two reasons: first, the reported k_{inh} values are much larger than typically found for H-atom abstractions from C–H bonds even weaker than that of allicin. For instance, the rate constant for H-atom abstraction from the bisallylic C–H bond of MeLH, characterized by a BDE value of 76.6 kcal $mole^{-1}$,[13] is only 62 M^{-1} s^{-1},[14] *i.e.* more than three orders of magnitude lower than the value reported for allicin when using

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† A possible reason for the very short induction time observed by Okajima and co-workers,[11] when oxidizing cumene or MeLH in the presence of allicine, might be that the oxidizing mixture contains some antioxidant impurities. In our case, in order to overcome this problem, the sulfide was added to the mixture only after the oxidation reaction reached a constant rate.

MeLH as oxidizable substrate. Second, the dependence of the rate constant k_{inh} on the nature of the abstracting peroxyl radicals (which in MeLH is claimed to be 60 times larger than in cumene), seems unusually strong. In addition, it should be emphasized that allicin does not fulfil one of the conditions required by a molecule to behave as a chain-breaking antioxidant,[9] *i.e.* that the radical formed by the inhibitor (A·) has to be unreactive toward molecular oxygen, otherwise the resulting peroxyl radical will propagate the oxidative chain (eqn (3)–(4)).[9] Being, in the case of allicin, A· a carbon centred radical, it is expected to react with oxygen at an almost diffusion controlled rate.[15]

$$A\bullet + O_2 \longrightarrow AOO\bullet \quad (3)$$

$$AOO\bullet + LH \longrightarrow AOOH + L\bullet \quad (4)$$

With the aim to better clarify the chemistry underlying the aerobic oxidation of garlic-derived sulfides, we investigated the reaction of diallyl disulfide (**1**) and allyl methyl sulfide (**3**) with peroxyl radicals in an apolar solvent by studying the autoxidation of cumene and styrene in the presence of these sulfur derivatives.

Results and discussion

Cumene autoxidations

The first set of autoxidations was performed by varying the concentration of **1** and **3** and by keeping constant those of cumene and of the radical initiator (azobisisobutyronitrile, AIBN). Cumene is a convenient oxidizable substrate for studying the chain-breaking activity of inhibitors of moderate efficacy, thanks to the low values of its rate constants for chain propagation k_p and termination $2k_t$. As shown in Fig. 1, the AIBN initiated autoxidation of cumene in chlorobenzene is practically unaffected by the presence of sulfides **1** and **3**, when these are used at the concentrations ($<10^{-4}$ M) normally employed with effective antioxidants. The oxygen consumption is instead retarded at much higher concentrations ($\geq 10^{-3}$ M). The dependence of the rate of oxidation of the mixtures on the concentration of the added sulfide is shown in Fig. 2.

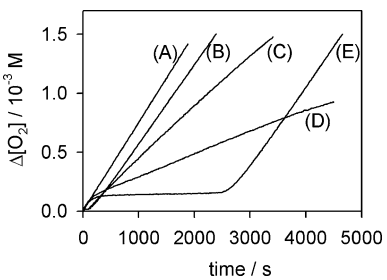


Fig. 1 Oxygen consumption observed during the AIBN (0.05 M) initiated autoxidation of cumene (6.2 M) at 30 °C in chlorobenzene in the presence of increasing amounts of **1**: (A) 0 M; (B) 1.2×10^{-3} M; (C) 6.3×10^{-3} M; (D) 1.7×10^{-2} M. Trace (E) shows also the autoxidation of the same substrate in the presence of the very effective phenolic inhibitor PMHC (6.3×10^{-6} M).

This experimental behaviour cannot be ascribed to a classical chain-breaking antioxidant action as, for instance, that observed in the presence of 2,2,5,7,8-pentamethyl-6-chromanol (PMHC), which strongly inhibits cumene autoxidation at concentrations

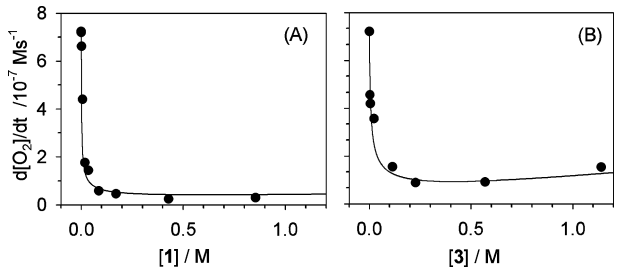
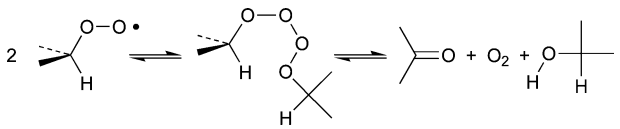


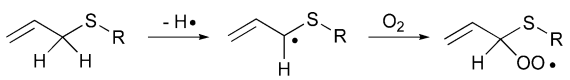
Fig. 2 Oxygen consumption rate observed during the AIBN (0.05 M) initiated autoxidation of cumene (6.2 M) at 30 °C in chlorobenzene in the presence of increasing amounts of either **1** (A) or **3** (B). Experimental points were fitted considering the co-oxidation of cumene and the added sulfides by eqn (15).

as low as 6×10^{-6} M (see Fig. 1). It is instead similar to what was observed by Russell when oxidizing a moderately oxidizable hydrocarbon such as cumene in the presence of the more reactive substrate (co-oxidant) tetralin.[16] Russell explained the retarded oxidation of the mixture by proposing that some secondary peroxyl radicals from tetralin are formed in solution containing mostly cumene. These secondary peroxyl radicals undergo bimolecular termination and cross-termination (according to the reaction depicted in Scheme 1) much more readily than tertiary cumylperoxy radicals, so that the rate of oxidation for the mixture is lower than for pure cumene because of the small overall concentration of peroxyl radicals.



Scheme 1

The addition to cumene of little amounts of substrates **1** and **3** that produce fast terminating secondary peroxyl radicals (see Scheme 2) is also expected to retard the oxidation of the mixture, provided that the co-oxidants are more reactive than cumene with peroxyl radicals. In this case, the results of Fig. 1 and 2 can be interpreted in terms of the co-oxidant effect. A quantitative justification of this interpretation was obtained as follows.



Scheme 2

The overall reaction scheme can be described as shown in eqn (5)–(14), where RH and PH are the oxidizable substrates undergoing co-oxidation, *i.e.* cumene and the sulfides **1** or **3**, respectively, and R·, P·, ROO· and POO· the corresponding alkyl and peroxyl radicals.[16]

$$\text{Initiator} \xrightarrow{R_i} \text{production of free radicals} \quad (5)$$

$$R\bullet + O_2 \longrightarrow ROO\bullet \quad (6)$$

$$P\bullet + O_2 \longrightarrow POO\bullet \quad (7)$$

$$ROO\bullet + RH \longrightarrow ROOH + R\bullet \quad (8)$$

$$ROO\bullet + PH \longrightarrow ROOH + P\bullet \quad (9)$$

$$POO\bullet + PH \longrightarrow POOH + P\bullet \quad (10)$$

$$POO\bullet + RH \longrightarrow POOH + R\bullet \quad (11)$$

$$ROO\bullet + ROO\bullet \longrightarrow \text{inactive products} \quad (12)$$

$$ROO\bullet + POO\bullet \longrightarrow \text{inactive products} \quad (13)$$

$$POO\bullet + POO\bullet \longrightarrow \text{inactive products} \quad (14)$$

By assuming the usual steady state approximation, the rate of oxidation can be derived as in eqn (15).[17]

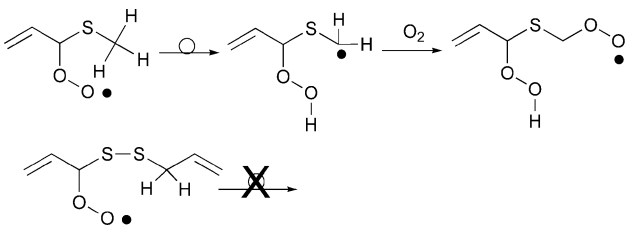
$$\frac{-d[O]_2}{dt} = \frac{\left\{k_8k_{11}[RH]^2+2k_9k_{11}[RH][PH]+k_9k_{10}[PH]^2\ R_i^{1/2}\right\}}{\left\{k_{12}k_{11}^2[RH]^2+k_{13}k_9k_{11}[RH][PH]+k_{14}k_9^2[PH]^2\right\}^{1/2}} \quad (15)$$

Here the propagation and the termination rate constants of cumylperoxyl radicals, k_8 and k_{12}, are known (0.32 M^{-1} s^{-1} and 4.6 × 10^4 M^{-1} s^{-1}, respectively).[18] The other rate constants were obtained by simulating the plots of Fig. 2, reporting the dependence of the oxygen consumption rates on the sulfide concentrations, by keeping in mind that the rate constants of the termination reactions involving POO· radicals (k_{13} and k_{14}) are expected to be 2–3 orders of magnitude greater than k_{12}.[17] Eqn (15) was simplified by assuming that the kinetics of H-atom abstraction from cumene (RH) or sulfides (PH) is independent from the peroxyl radical nature ($k_8 = k_{11}$ and $k_9 = k_{10}$) and by considering the rate constant for cross-termination between ROO· and POO·, k_{13}, equal to k_{14}.

The rate constants obtained with this procedure, reported in Table 1, show that **1** and **3** react with peroxyl radicals (k_9) only 3–5 times faster than cumene. Since the inhibiting effect is observed for sulfide concentrations of about 1 mM, the fraction of ROO· radicals that react with cumene is ≈1000 times larger than the fraction that react with the sulfides. However, the small amount of POO· radicals formed is compensated by the large k_{13}(or k_{14})/k_{12} ratio.

It should be emphasized that, although the propagation step of the oxidation of the allyl sulfides **1** and **3** (eqn (9) and (10)) was assumed to be a H-atom transfer to peroxyl radicals, the kinetic scheme does not change if addition of peroxyl radicals to the double bond takes place.[20] On thermodynamic grounds, the latter reaction seems, however, unlikely since the small enthalpy decrease associated with addition of ROO· to the double bond (−3.8 against −0.4 kcal mol^{-1} for H-atom transfer)[13,21] is largely counterbalanced by the entropic term (*ca.* −35 e.u. corresponding at room temperature to 10.5 kcal mol^{-1}). H-atom transfer should therefore be preferred over addition by a free energy of *ca.* 7 kcal mol^{-1}.[13,21]

In the case of compound **3**, a good fitting of the data of Fig. 2b could not be obtained if the rate constants, k_9 and k_{10}, are kept equal. Better agreement with the experimental results was instead achieved for a k_{10} value several times larger than k_9; this means that the labile H-atom of sulfide **3** reacts with its own peroxyl radical faster than with the cumylperoxyl radical. Although different reactivity of the peroxyl radicals POO· and ROO· has been observed in other cases and has been interpreted in terms of steric crowding,[22] it seems better to explain the difference between **1** and **3** by considering that, once formed, the peroxyl radical from **3** can undergo a second intramolecular H-transfer through a six membered transition state (Scheme 3).[23] In the case of **1**, instead, where the second hydrogen abstraction could only take place through an energetically unfavourable seven membered transition state, this reaction, ultimately affording a di-peroxide, does not occur. If this is the case, the oxidizability of compound **3** will be larger and will thus explain the increase of the oxidation rate observed at higher concentrations of **3** (see Fig. 2b). Support to this suggestion comes from the $k_{10}/(k_{14})^{1/2}$ ratio measured in the autoxidation of **3** (see below), which is seven times larger the corresponding value obtained in the autoxidation of the disulfide **1** (see Table 1). Unfortunately we could not reveal the expected oxidation products by ESI-MS analysis of the crude reaction mixture, following a procedure reported earlier.[24] Actually, it is known that, unlike hydrocarbons, organic sulfides give little or no hydroperoxides upon autoxidation, while the principal reaction products appear to be sulfoxides, water and compounds resulting from the decomposition of the initial hydroperoxides.[19]



Scheme 3

Styrene autoxidations

The co-oxidation experiments were also repeated in chlorobenzene using styrene as the main oxidizable substrate; in this case, no

Table 1 Propagation (k_9, k_{10}) and termination ($k_{14} = k_{13}$) rate constants at 30 °C of diallyl disulfide **1** and allyl sulfide **3** obtained by co-oxidation studies with cumene.[a]Oxidizability, $k_{10}/(k_{14})^{1/2}$, determined from the AIBN initiated autoxidation of the two sulfides at 30 °C

Substrate	k_9 /M^{-1} s^{-1}	k_{10} /M^{-1} s^{-1}	k_{14} /10^7 M^{-1} s^{-1}	$k_{10}/(k_{14})^{1/2}$ /10^{-4} $M^{-1/2}$ $s^{-1/2}$
1	1.6 ± 0.8[b]	1.6 ± 0.8[b]	9 ± 6	1.5 ± 0.5
3	1.0 ± 0.6	8 ± 5	5 ± 3	10 ± 2
Tetrahydrothiopyran[c]		1.5	3	2.7
Tetrahydrothiophene[c]		6.4	3	12
Benzyl phenyl sulfide[c]		9.5	5.7	13

[a] The data reported are not corrected for the number of abstractable H atoms. [b] k_9 and k_{10} were assumed to be equal, see text. [c] From ref. 19.

reduction of oxidation rate was detected upon addition of either sulfide **1** or **3** (see Fig. 3) at concentrations up to 1 M. This behaviour provides additional evidence that the two sulfides do not act as antioxidants. Actually, the absence of any retarding effect can be easily explained by considering that the peroxyl radicals from styrene are secondary radicals, already characterized by a high value of the termination rate constant k_{12} (4.2×10^7 M^{-1} s^{-1}).[22] Thus the peroxyl radicals from the added sulfides do not decrease the overall stationary concentration of ROO· radicals, and the rate of oxidation is expected to remain the same both in the absence and in the presence of co-oxidant.

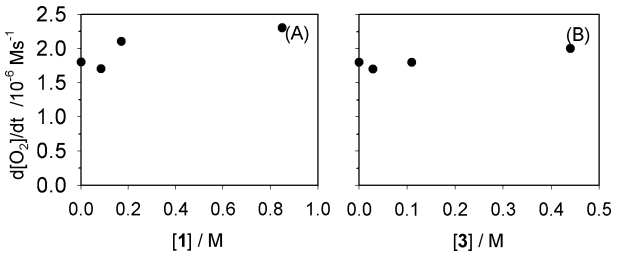


Fig. 3 Oxygen consumption rate observed during the AIBN (0.05 M) initiated autoxidation of styrene (4.3 M) in chlorobenzene at 30 °C in the presence of increasing amounts of either **1** (A) or **2** (B).

Autoxidation of allyl sulfides

In order to check the reliability of the k_{10} and k_{14} values, we also studied the AIBN initiated autoxidation of the sulfides in chlorobenzene in the absence of other oxidizable substrates. At low conversions, the rate of oxygen consumption is given by eqn (16), that provides the oxidizability[22] of the sulfides **1** and **3**, *i.e.* the value of $k_{10}/(k_{14})^{1/2}$ (k_{14} is usually indicated as $2k_t$).

$$\frac{-d[O_2]}{dt} = k_{10}k_{14}^{-1/2}[\text{sulfide}]R_i^{1/2} \quad (16)$$

The good agreement between the oxidizabilities obtained by this procedure, reported in Table 1, and those calculated by using the k_{10} and k_{14} values from the co-oxidation kinetics (1.7×10^{-4} and 1.1×10^{-3} $M^{-1/2}$ $s^{-1/2}$ for **1** and **3** respectively), demonstrates that the results of the co-oxidation experiments have been interpreted correctly. The data obtained in the present work are also in reasonable agreement with those measured for other organic sulfides by Howard and Korcek (see Table 1) using the rotating sector method.[19]

Chain-breaking activity of allicin

On the basis of the present results, some considerations on the claimed chain-breaking antioxidant activity of allicin (**2**) can be made. As far as the reaction with peroxyl radicals is concerned, it seems dubious that **2** can react with them 10^3–10^5 times faster than **1** or **3**. The values reported by Okajima and coworkers[11] for **2** perhaps might be justified if the reactive site was the CH_2 adjacent to the SO group rather than to the S atom, although this would be in contrast with what was reported in a previous paper by the same authors.[12] However, literature data show that the strengths of CH_3SCH_2–H and CH_3SOCH_2–H bonds are approximately the same (93.7 and 94 kcal mol^{-1}, respectively),[13] so that little difference is expected between the reactivities of the two allylic C–H bonds in allicin.

Additional evidence against the alleged antioxidant activity of allicin is provided by the observation that compounds **1** and **3** take part in the oxidative chain reaction so that the allyl radicals resulting from H-atom abstraction must undergo fast reaction with molecular oxygen to give the chain-propagating peroxyl radicals. Allicin should behave similarly to **1** and **3**, although, as suggested by Pratt *et al.* for several EW–C· radicals,[25] the possibility that the presence of an electron withdrawing (EW) S=O group in allicin may reduce the reactivity toward O_2 of the nearby alkyl radical, cannot be rejected beyond any doubt.

Conclusions

The two allyl sulfides examined in the present work were found to undergo an autoxidation reaction in the presence of radical initiators. The rate constants for their reaction with the related chain propagating peroxyl radicals are similar to those reported in the literature for other substituted sulfides[19] and are 4–6 orders of magnitude smaller than those typical of phenolic antioxidants.[10] Therefore, it can be concluded that sulfides **1** and **3** (and almost certainly allicin) do not act as chain-breaking antioxidants.

Indeed, a deeper knowledge of the chemistry of this important class of natural products may help to rationalize their interesting biological actions.[6]

Experimental

Materials

Solvents and reagents were of the highest grade commercially available. Cumene was purified on a silica column before use, styrene on an alumina column to remove the inhibitor. Diallyl disulfide **1** was purified by fractional distillation. AIBN was recrystallized from methanol. Allyl methyl sulfide **3** and 2,2,5,7,8-pentamethyl-6-chromanol (PMHC) were used as received.

Autoxidation procedure

Experiments were carried out by following the autoxidation of cumene (6.2 M) or styrene (4.3 M) in chlorobenzene at 30 °C using as an initiator AIBN (0.05 M) in the presence of variable amounts of **1** or **3**. The reaction was monitored with an oxygen uptake apparatus built in our laboratory and based on a differential pressure transducer. The entire apparatus was immersed in a thermostatted bath, which ensured a constant temperature within ±0.1 °C. In a typical experiment, an air-saturated solution of cumene in chlorobenzene containing AIBN was equilibrated with a reference solution of the same composition also containing an excess of PMHC (1×10^{-4} M). When constant oxygen consumption was reached, a small amount of sulfide **1** or **3** in a concentrated chlorobenzene solution was added to the sample and the differential pressure between the two channels was recorded as function of time. This instrumental setting allowed us to have N_2 production and the oxygen uptake due to the azo-initiator decomposition already subtracted from the measured reaction rates.

The autoxidation of **1** or **3** was carried out using either of the two sulfur compounds as an oxidizable substrate instead of cumene.

Initiation rates, R_i, were determined for each condition in preliminary experiments using PMHC as reference antioxidant.

Acknowledgements

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