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Antioxidant Activity of Magnolol and Honokiol: Kinetic and Mechanistic Investigations of their Reaction with Peroxyl Radicals

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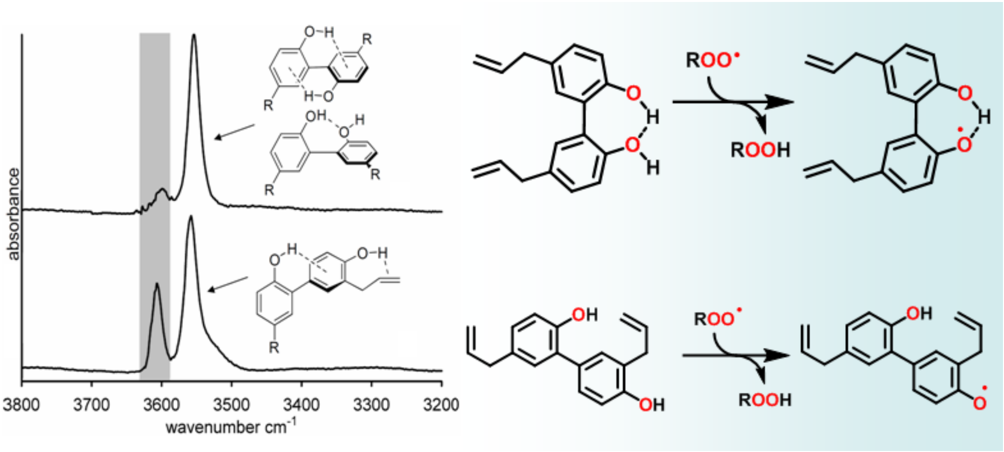
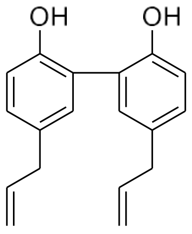


Abstract

Magnolol and honokiol, the bioactive phytochemicals contained in *Magnolia officinalis,* are uncommon antioxidants bearing isomeric bisphenol cores substituted with allyl functions. We have elucidated the chemistry behind their antioxidant activity by experimental and computational methods. In the inhibited autoxidation of cumene and styrene at 303 K, magnolol trapped 4 peroxyl radicals, with k_{inh} of 6.1 x 10^4 $M^{-1}s^{-1}$ in chlorobenzene and 6.0 x 10^3 $M^{-1}s^{-1}$ in acetonitrile, and honokiol trapped 2 peroxyl radicals in chlorobenzene ($k_{\mathrm{inh}} = 3.8 \times 10^4$ $M^{-1}s^{-1}$) and 4 peroxyl radicals in acetonitrile ($k_{\mathrm{inh}} = 9.5 \times 10^3$ $M^{-1}s^{-1}$). Their different behaviour arises from a combination of intramolecular H-bonding among the reactive OH groups (in magnolol) and of the OH groups with the aromatic and allyl π-systems, as confirmed by FT-IR spectroscopy and DFT calculations. Comparison with structurally related 3,3',5,5'-tetramethylbiphenyl-4,4'-diol, 2-allylphenol and 2-allylanisole allowed excluding that the antioxidant behaviour of magnolol and honokiol is due to the allyl groups. The reaction of the allyl group with peroxyl radical (C-H hydrogen abstraction) proceeds with rate constant of 1.1 $M^{-1}s^{-1}$ at 303 K. Magnolol and honokiol do not react with molecular oxygen and produce no superoxide radical under the typical settings of inhibited autoxidations.

Introduction

Magnolol (**1**) and honokiol (**2**) are two bisphenolic neolignans contained in the bark of *Magnolia officinalis*, which is used for treatment of gastrointestinal disorders, anxiety and allergic disease in Chinese and Japanese traditional medicines.[1] More recently, magnolol and honokiol have been the object of intense research because of their promising antitumoral,[2] anti-angiogenic[3] and anxyolitic[4] activities.



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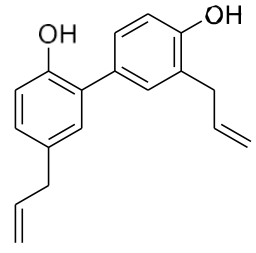

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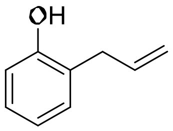
Some studies have evidenced that these compounds possess antioxidant activity, which has been attributed to the presence of two phenolic functions in their structure,[5,6,7] or to the allyl groups.[3] These studies showed that **2** is more effective than **1**, although the reason remains unclear. Zhao and Liu attributed the different reactivity to the formation of a intramolecular H-bond interaction between the 1,1' dihydroxy moieties in **1**, which causes a "hindering" of the H-atoms towards the reaction with radicals.[6] However, phenols in which two OH groups are engaged in intramolecular H-bonds are usually more reactive with radicals than phenols having isolated OH groups, such as in the case of catechol (1,2-dihydroxybenzene).[8] In catechols, the intramolecular H-bond strengthens during the reaction with radicals, stabilizing the transition state for formal H-atom transfer to radical species and making the compound more reactive than isomeric hydroquinones.[8] Magnolol and honokiol have unusual structural motives among phenolic antioxidants in that they feature isomeric bis-phenolic cores bearing allyl substituents. A deep understanding of their chemistry is unfortunately hampered by the fact that all investigations performed so far on **1** and **2** rely on indirect methods, such as on the quenching of coloured persistent radicals, or on the study of lipid oxidation monitored by single-point detection of the TBARS (thiobarbituric reacting species), which can provide only qualitative estimates of the antioxidant action.[9] One of the best quantitative approaches of evaluation of the performance of chain-breaking antioxidants is the inhibited autoxidation method. It mimics the natural autoxidation process under strictly controlled conditions (constant rate of initiation) and monitors the oxygen uptake by the system, allowing to determine the oxidation rate and the absolute rate constants governing the inhibited autoxidation.[9,10] By using this method we were able to measure the rate constants for the reaction of magnolol, honokiol and related phenols with peroxyl radicals in two solvents with different polarities, and to rationalize the results by FT-IR and ESI-MS techniques, combined with computational methods. These studies allowed us to shed light into the complex radical chemistry of these two natural compounds, and to propose a likely explanation for the unusual reactivity order of magnolol and honokiol, which broadens the understanding of structure-reactivity relationships in bis-phenolic compounds.

Results and discussion

1) Kinetics and stoichiometry of the reaction with peroxyl radicals

In order to understand the antioxidant behaviour of magnolol (**1**) and honokiol (**2**), also other structurally related phenols where considered, as reported below. Monophenols **3-5** were chosen to investigate the role of the allyl group. The bisphenol **6** was included to study the role of the linkage position in bis-phenols, while the commercial antioxidant BHT (**7**), and the α-tocopherol analogue 2,2,5,7,8-pentamethyl-6-hydroxychromane (**8**) were used as reference compounds.



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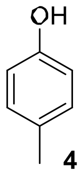

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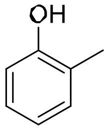

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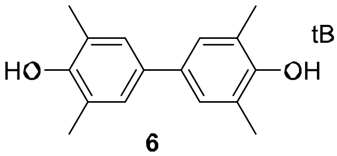

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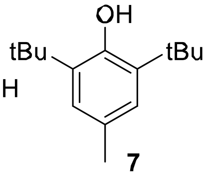

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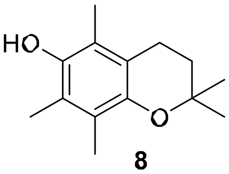

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The antioxidant activity of **1-8** was evaluated by measuring the rate constant (k_{inh}) for the reaction with peroxyl radicals (ROO•), that are responsible for oxidative chain propagation in many natural and man-made materials.[10,11] The values of k_{inh} (equation 5, where AH is an antioxidant) were determined by studying the inhibition of the thermally initiated autoxidation of cumene or styrene (RH) (Eqs. 1-6) under controlled conditions, using chlorobenzene or acetonitrile as the solvent.[10-12]

$$\text{Initiator} \xrightarrow{R_i} R^{\bullet} \qquad (1)$$

$$R^{\bullet} + O_2 \longrightarrow ROO^{\bullet} \qquad (2)$$

$$ROO^{\bullet} + RH \xrightarrow{k_p} R^{\bullet} + ROOH \qquad (3)$$

$$2\,ROO^{\bullet} \xrightarrow{k_t} \text{non radical products} \qquad (4)$$

$$ROO^{\bullet} + AH \xrightarrow{k_{inh}} ROOH + A^{\bullet} \qquad (5)$$

$$A^{\bullet} + ROO^{\bullet} \longrightarrow \text{non radical products} \qquad (6)$$

The reactions were performed at 303 K using 2,2'-azobisisobutyronitrile (AIBN) as initiator, and were followed by monitoring the oxygen consumption in an oxygen-uptake apparatus based on a differential pressure transducer.[10-12] In the presence of good antioxidants, oxidation of the substrate and oxygen consumption are much slower than in their absence, and a clear inhibition period is observed, as shown in Figures 1 and in Figure 2.

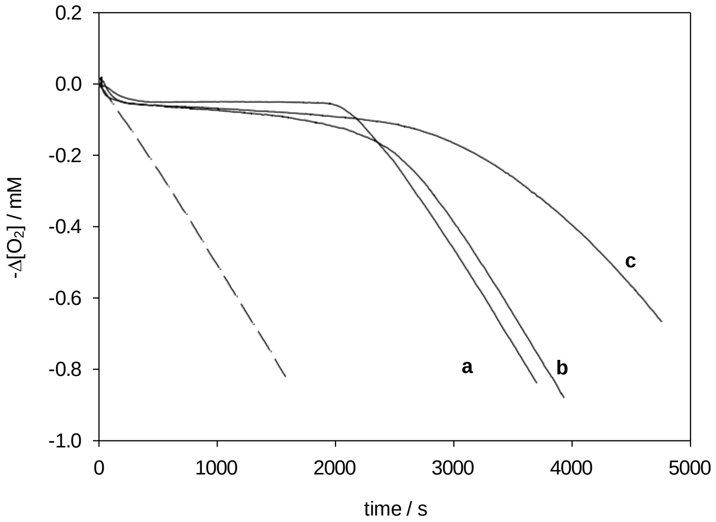


Figure 1. Oxygen consumption traces measured during the autoxidation of cumene (3.5 M) in chlorobenzene initiated by AIBN (0.05 M) in the absence (dashed line) and in the presence of 5.0 × 10^{-6} M of antioxidants: a) **8**; b) **2**; c) **1**.

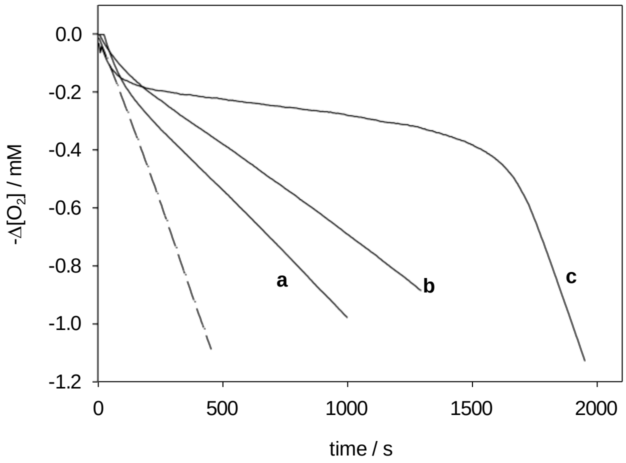


Figure 2. Oxygen consumption traces measured during the autoxidation of styrene (4.3 M) in chlorobenzene initiated by AIBN (0.05 M) in the absence (dashed line) and in the presence of: a) **2** (1.5 × 10^{-5} M); b) **1** (1.5 × 10^{-5} M); c) **8** (5.0 × 10^{-6} M).

The rate constant for the reaction between ROO• radicals and **1-8** could be obtained from the rate of O_2 consumption during the inhibition from the known constants k_p and $2k_t$ for cumene (and styrene) chain propagation and termination respectively, as detailed in the experimental section. The number of radicals trapped by each antioxidant molecule (n) was obtained from the length of the inhibition period, by comparison with the reference antioxidant **8**, for which $n = 2$.[11] The values of k_{inh} and n, determined in chlorobenzene and acetonitrile are reported in Tables 1 and 2 respectively.

Table 1. Rate constants for the reaction with peroxyl radicals in chlorobenzene at 303K and number of trapped radicals (*n*).[a]

Phenol		**k_{inh} / $M^{-1}s^{-1}$**	***n***
1	1st OH	$(6.1\pm0.5)\times10^4$	2.0±0.4
	2nd OH	$(4.3\pm0.8)\times10^3$	1.7±0.4
2		$(3.8\pm0.4)\times10^4$	2.2±0.1
3		$(2.4\pm0.4)\times10^4$	2.0±0.1
4		$(2.3\pm0.2)\times10^4$	1.9±0.1
5		$(1.6\pm0.2)\times10^4$	2.1±0.2
6		$(3.9\pm0.1)\times10^5$ [b]	1.9±0.1 [b]
7		1.0×10^4 [c]	2 [c]
8		3.2×10^6 [d]	2 [d]

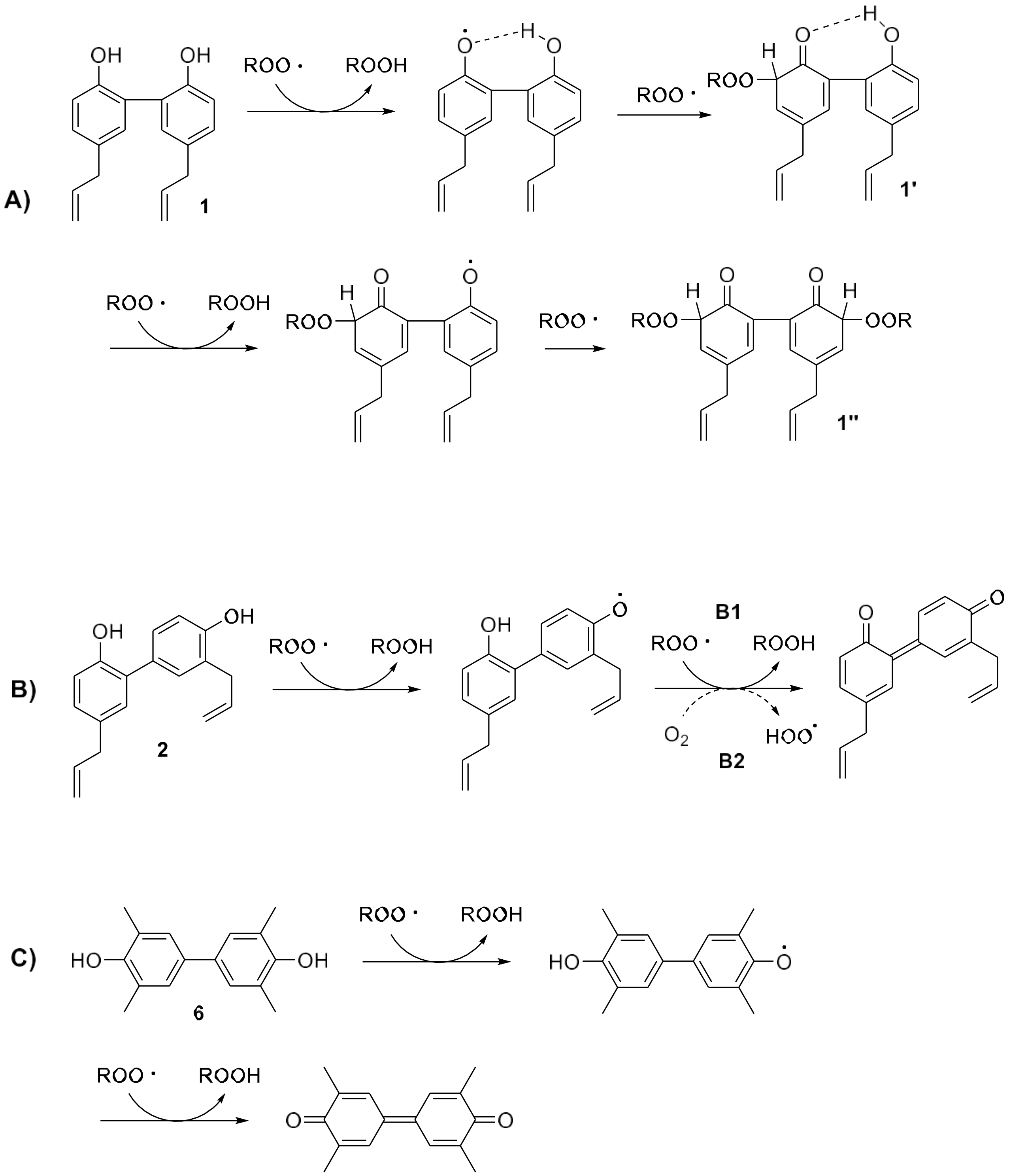
a) From cumene autoxidation studies unless otherwise noted.

b) Measured in styrene. In cumene, the *n* value was 2.6±0.2.

c) From reference 12.

d) From reference 11.

The results reported in Table 1 show that k_{inh} values of **1** and **2** are within the same order of magnitude as those of related alkyl-substituted phenols **3-5**, whereas they are about 100-fold smaller than that of the α-tocopherol analogue **8**. Magnolol (**1**) in chlorobenzene is more reactive than simple phenols **3-5** and than **2**. The inhibition given by magnolol is composed by two parts (see Figure 1), a strong inhibition (lasting ~2000 s in Figure 1) corresponding to the trapping of two ROO• radicals, and a weaker retardation of oxidation which approximately corresponds to the trapping of two additional radicals. This peculiar behaviour can be interpreted on the basis of our previous studies on the antioxidant activity of *ortho*-bisphenols, as reported in Scheme 1A.[8,13] After the trapping of the first two ROO• radicals, one of the two phenolic rings of **1** is converted into a cyclohexadienone, which engages a strong H-bond interaction with the OH group of the second phenolic rings. This interaction reduces the reactivity of the second OH group by about 10-folds.



Scheme 1. Mechanism for the trapping of peroxyl radicals by magnolol (**1**), honokiol (**2**) and **6** in chlorobenzene.

Honokiol (**2**) is about twice as reactive as simple phenols **3-5,** conceivably for statistical reasons, because the two phenolic OH groups of **2** are expected to have similar reactivities. The allyl substituent has similar effect on phenol's reactivity as compared to the simpler methyl group (see compounds **3-5** in Table 1). Compound **2** traps two ROO• radicals in PhCl, suggesting that the phenoxyl radical from **2** reacts with a second ROO• radical by formal H-atom transfer from a OH group (Scheme 1 path B1). Alternatively, the phenoxyl radical from **2** may transfer the H-atom of the remaining OH group to O_2 to afford a hydroperoxyl radical (HOO•; see Scheme 1 path B2), similarly to what was previously observed in the case of 4-hydroxyphenoxyl (semiquinone) radicals[16] (see Scheme 1B, dashed arrow). The overall effect of HOO• formation depends on the nature of the oxidizable substrate: in cumene, which has low k_p and k_t values (0.32 and 2.3×10^4 $M^{-1}s^{-1}$ respectively),[12] HOO• radicals increase the rate of chain-termination by quenching cumylperoxyl radicals by the reaction ROO• + HOO• → ROOH + O_2, whose rate constant is in the range 10^8 - 10^9 $M^{-1}s^{-1}$ [14], thereby causing inhibition of the oxidation.[15] On the other hand, in styrene, having large k_p and k_t values (4.1 and 2.1×10^7 $M^{-1}s^{-1}$ respectively),[11] propagation of the oxidative chain prevails, as it was observed by us in the case of 2,5-di-tert-butylhydroquinone[16] and by others in the case of alkylated hydroquinones.[17] In all these cases, low stoichiometries for radical trapping are observed, with n ranging from 0.2 to 1.5 using styrene as the oxidizable substrate and PhCl as the solvent.[16,17]

Bisphenol **6** has a relatively large k_{inh} value, because of the presence of two methyl groups in *ortho* position, which lower the bond dissociation enthalpy of the reactive OH. The fact that **6** has a stoichiometric coefficient of 1.9 in the inhibited autoxidation of styrene indicates that the phenoxyl radical from **6** transfers the second O-H atom to a second peroxyl radical, as shown in Scheme 1C.

In order to study the effect of solvent polarity on the reactions of **1** and **2** with ROO• radicals, inhibited autoxidation studies were performed by using acetonitrile as the solvent. The results collected in Table 2 show that the k_{inh} values decrease for all phenols, as expected from the well known kinetic solvent effect (KSE) that occurs in the case of H-atom abstraction from polar X-H bonds.[18] The decrease is more evident for **1** than for **2**, as the KSE is 10 for magnolol and 4 for honokiol. The KSE is the cause of the inversion of the relative reactivities of **1** and **2**. Interestingly, in acetonitrile the total number of radicals trapped by **2** and **6** approaches $n = 4$ (hence they behave similarly to magnolol **1**) conceivably because the second OH group in the phenoxyl radicals from **2** and **6** is H-bonded to the solvent and thus it is less available to being transferred to a second ROO• radical, as shown for **2** in Scheme 2. As a consequence, the phenoxyl radical decays preferably by addition of a second ROO• radical to the aromatic ring. The intact second phenolic ring is then available to trap two additional peroxyl radicals, similarly to monophenolic compounds.

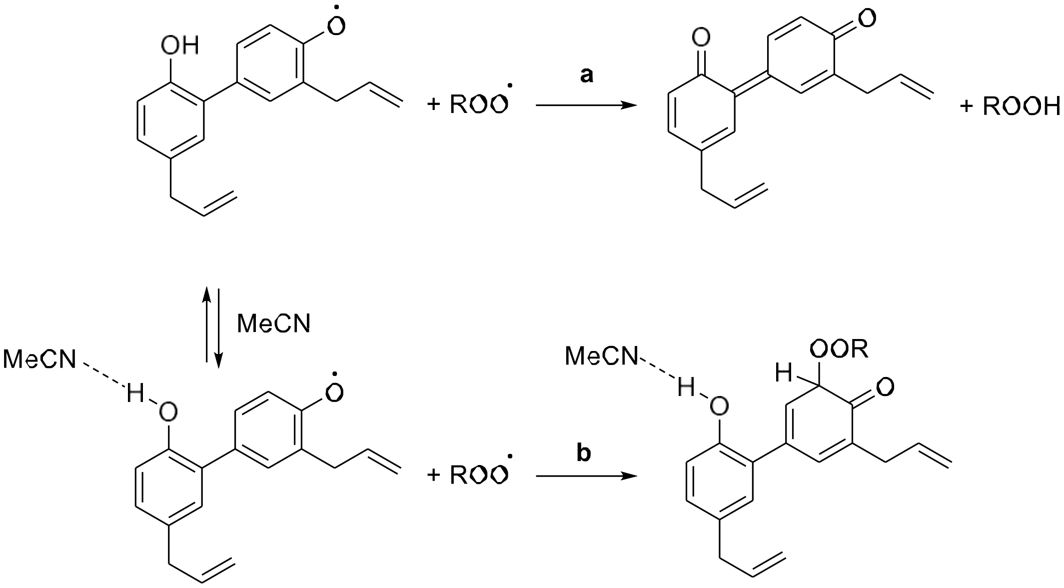
Table 2. Rate constants for the reaction with peroxyl radicals in acetonitrile at 303K, number of trapped radicals (*n*), and kinetic solvent effect (KSE)

inhibitor		***k*inh**	***n***	**KSE [a]**
1	1st OH	$(6.0\pm0.7) \times 10^3$	2.1 ± 0.2	10.1
	2nd OH	$(1.1\pm0.2) \times 10^3$	2 [b]	3.9
2		$(9.5\pm1.5) \times 10^3$	3.7 ± 0.3	4.0
6		$(5.4\pm0.2) \times 10^4$	3.5 ± 0.2	7.2
7		$(4.9\pm0.4) \times 10^3$	2.0 ± 0.1	2.0
8		6.8×10^5 [c]	2 [c]	4.7

a) Defined as KSE = k_{inh}(PhCl) / k_{inh} (MeCN).

b) The stoichiometric coefficient could not be measured because the k_{inh} value is too low, so it was assumed equal to 2.

c) From reference 19.



Scheme 2. Change in the stoichiometric coefficient in acetonitrile (compared to chlorobenzene) due to the H-bonding between the intermediate phenoxyl radical of **2** and the solvent.

To gain further mechanistic insight for the proposed mode of decay of the phenoxyl radical in acetonitrile, the reaction of **1** and **2** with peroxyl radicals from AIBN was followed by electrospray ionization mass spectroscopy (ESI-MS). Compounds **1** and **2** (5 μM) were incubated with AIBN (5 mM) at 333 K in MeCN, and aliquots of the reaction mixture were cooled and analyzed after 1:1 dilution with methanol. In negative ion mode, the peaks relative to the starting **1** and **2** could be detected (*m/z* = 265, Figure 3A). Their intensity decreased during the reaction course. In positive ion mode, magnolol showed a peak at *m/z* = 388 which increased during the first 30 min and then decreased, and a smaller one at *m/z* = 487 (Figure 3B). These two peaks have m/z values that are consistent with the sodium salts of the products **1'** and **1''** (Scheme 1A), formed by reaction of magnolol with the peroxyl radicals from AIBN (ROO• = $(CH_3)_2C(CN)OO\bullet$). Instead, in the case of honokiol only the growing signal at *m/z* 283 was observed in positive ion mode, corresponding to $(M+O+H)^+$ possibly due to fragmentation of the peroxyl radical adducts because of lower stability under the ionization conditions (see supporting information).

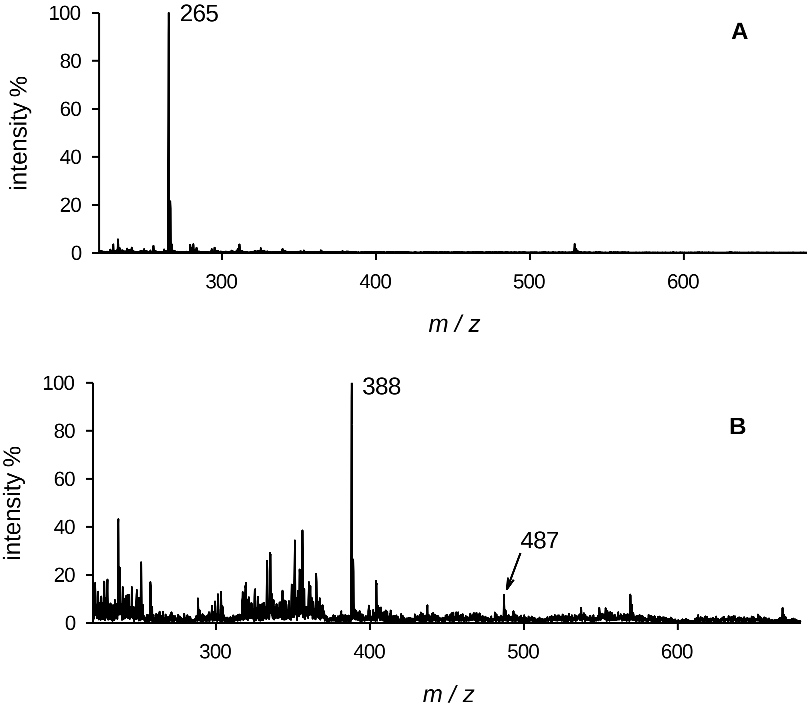


Figure 3. ESI-MS spectra of the reaction between **1** and AIBN after 30 min of reaction. A: negative ion mode; B: positive ion mode.

Since it was previously suggested by Fried and Arbiser[3] that the allyl moiety is responsible for the antioxidant activity of **1** and **2**, at variance with our current findings, we set to evaluate any contribution of the allyl moiety in inhibiting the autoxidation of cumene. In order to distinguish its contribution from that of the phenolic function we used 4-allylanisole (**9**) as model compound, since it lacks the phenolic OH (see Scheme 3). In the presence of **9**, the rate of oxygen consumption during the autoxidation of cumene is slightly slowed down, as shown in Figure 4. It should be noted, however, that the concentrations of **9** required to observe this modest effect are much larger (by 100-10000 fold) than those previously used for **1**-**8** and typically needed for antioxidants.

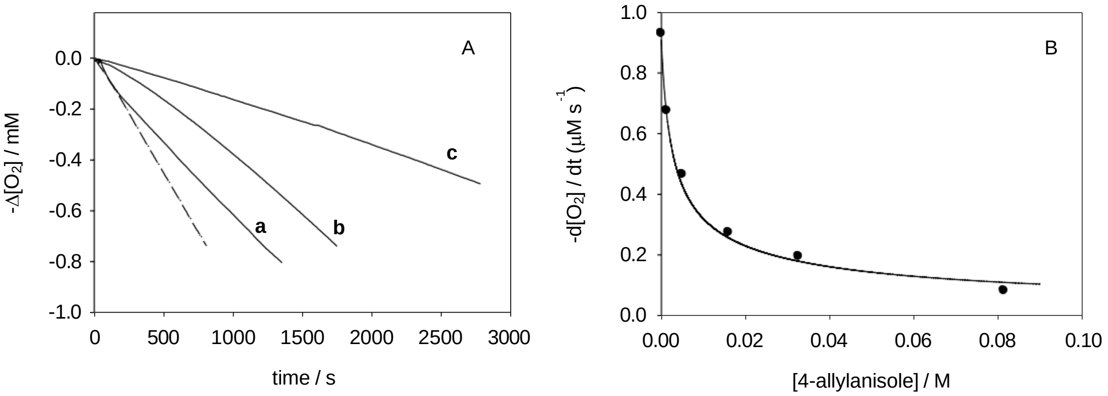
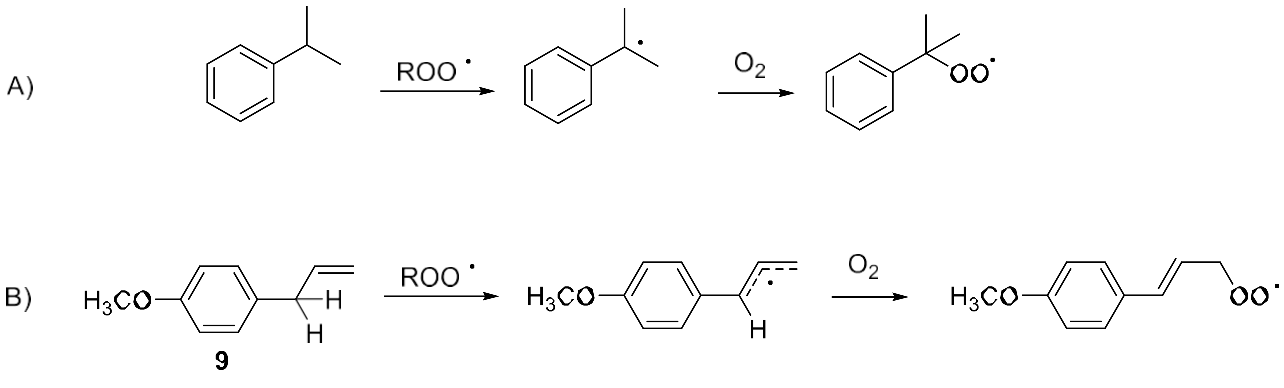


Figure 4. A) Oxygen consumption traces measured during the autoxidation of neat cumene (7.1 M) initiated by AIBN (0.05 M) at 303 K in the absence (dashed line) and in the presence of **9**: a) 0.7 mM, b) 5 mM, c) 32 mM. B) Relationship between the O_2 consumption rate and the concentration of **9**, and the fitting on the basis of the co-oxidation model.

The apparent antioxidant activity observed at large concentrations of **9** can be explained as deriving from the co-oxidation of **9** with cumene. This phenomenon can typically be observed in case an oxidizable substrate having a low k_t (see Equation 4) is mixed to another substrate having a larger k_t.[20,21] Cumene generates tertiary peroxyl radicals that have a relatively low k_t (2.3×10^4 $M^{-1}s^{-1}$)[11] compared to primary peroxyl radicals (10^7-10^8 $M^{-1}s^{-1}$)[22] that are formed during the oxidation of **9** (Scheme 3B).[23]



Scheme 3. Formation of tertiary peroxyl radicals in the case of cumene (A) and of primary peroxyl radicals in the case of 4-allylanisol **9** (B).

From the numerical fitting of the O_2 consumption rates reported in Figure 4B, by using the co-oxidation model,[20,21] the rate constant for the reaction of cumylperoxyl radicals and 4-allylanisol can be obtained as 1.1 $M^{-1}s^{-1}$. This value is very similar to that reported for the reaction between ^tBuOO• radicals and allylbenzene, 1.5 $M^{-1}s^{-1}$ at 30°C,[22] and thus it can be attributed to the H-atom abstraction from the allylic position. The above experiments show that in allyl-substituted phenols ROO• radicals react selectively with the OH group, since the reaction is at least 1000-fold faster than H-atom abstraction from the C-H bonds. This clearly rules out any contribution from chemistry of the allyl moiety in the antioxidant activity of **1** and **2**,

2) About the reactivity order of magnolol and honokiol

From the values reported in Table 1 it is evident that the k_{inh} value in PhCl of 2-allylphenol (**3**), is about the same as 2-methylphenol (**5**), while that of **2** is slightly larger than those of **3** and **5**, as expected from the presence of a second OH group. FT-IR spectra reported in Figure 5 evidenced the presence of weak intramolecular H-bonds from the phenolic OH acting as donors to the π systems of the aryl and of the allyl groups. However, significant signals from the not H-bonded OH are present at 3610 cm^{-1} which indicate that in compounds **2** and **3** these interactions are weak, thus they influence only marginally the reactivity of the phenols.

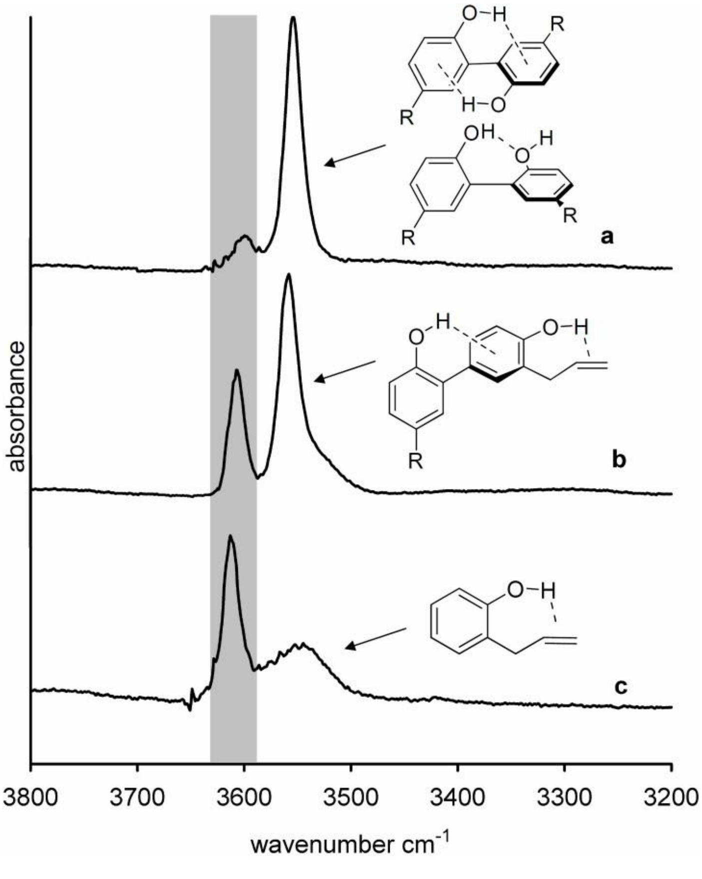


Figure 5. Infrared spectrum in CCl_4 of a 5 mM solution of (a) **1**; (b) **2**; (c) **3**. The stretching frequency of the not H-bonded OH is shaded.

In compound **1**, however, the peak attributed to the not H-bonded OH is much smaller than in **2**, indicating a smaller concentration of "free" OH groups in CCl_4. In the framework of the KSE theory, this would imply that **1** is less reactive than **2** in apolar solvents such as PhCl. However, since this observation is in contrast with the experimental k_{inh} order, this point was further investigated by theoretical calculations.

The geometries and the enthalpies of **1** and of its phenoxyl radical were computed at the B3LYP/6-31+g(d,p) level in the gas phase and the most stable conformations are reported in Figure 6. To speed up calculations, the allyl group was truncated to a methyl group because in the previous section it was shown that they have similar effects on the k_{inh} of phenols. The most stable conformation for **1** is that in which both OH groups point toward the aromatic rings, with a nearly perpendicular arrangement of the aromatic rings (structure **A**). In conformation **B,** characterized by a smaller dihedral angle between the aromatic rings (64°), a phenolic OH group donates a H-bond to the oxygen of the second OH group, and it is less stable than **A** by 1.5 kcal/mol. Conformation **C** in which both OH groups point away from the aromatic rings is less stable than **A** by 5.2 kcal/mol.

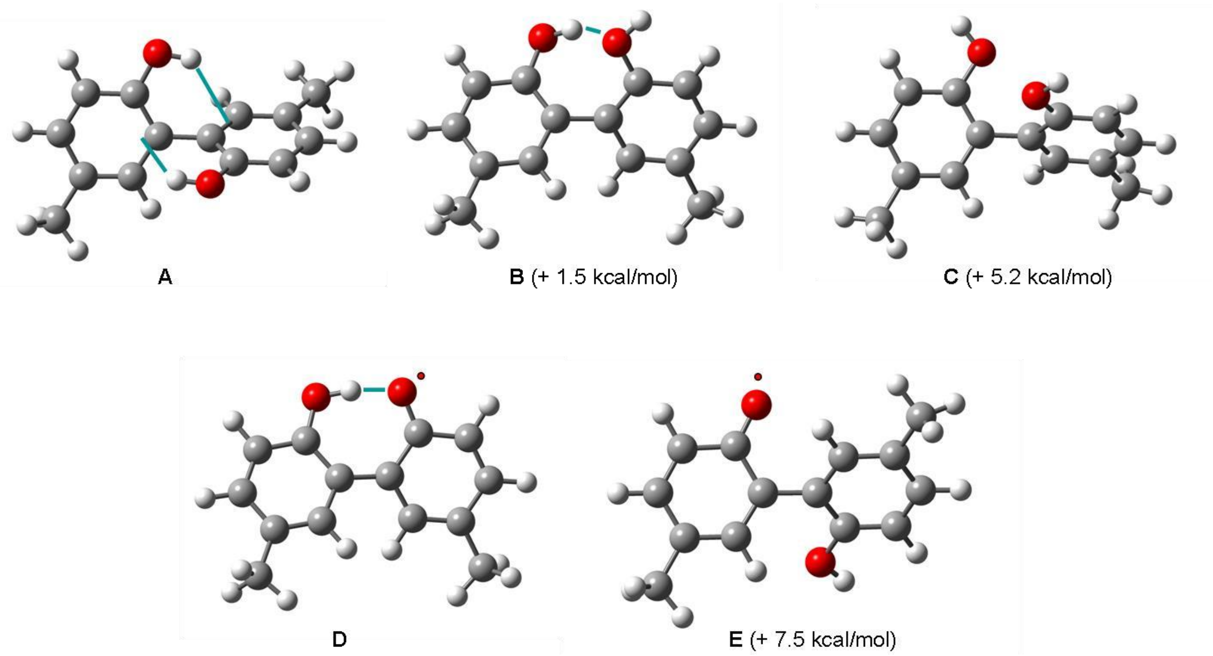
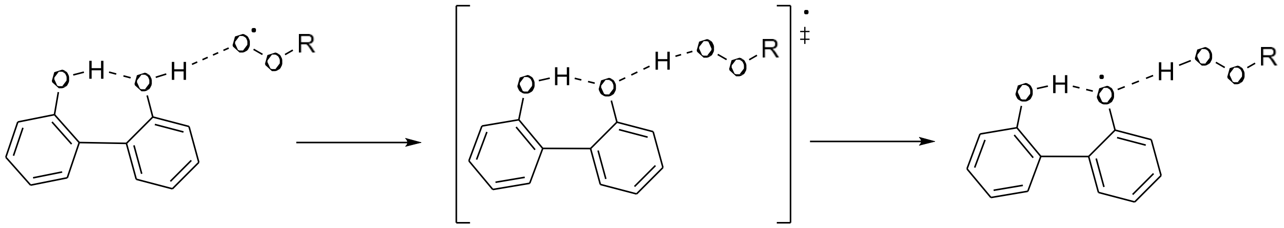


Figure 6. Most stable conformations of **1** (**A**-**C**) and of its phenoxyl radical (**D**, **E**) computed at the B3LYP/6-31+g(d,p) level in the gas phase. The relative enthalpy respect the most stable conformation is indicated.

On the basis of these results, the FT-IR spectrum of **1** in CCl_4 can be rationalized by considering that the small peak of the free OH (Figure 5a) is due to those molecules of **1** adopting conformation **B**, which could be estimated on the basis of the peak area as 2.9 %. When considering the phenoxyl radical of **1**, the most stable conformation is **D**, in which the OH group donates a strong H-bond to the phenoxyl oxygen and the two aromatic rings are nearly coplanar (Ar-Ar dihedral angle = 32°). The conformation **E** in which there is no H-bond is less stable by 7.5 kcal/mol than **D**. When considering the reaction with ROO• radicals, in conformation **B** the H-bond is conserved throughout the proton-coupled electron-transfer[24] (formal H-atom transfer) to the peroxyl radical so that it can stabilize the transition state and lead to the phenoxyl radical **D** with minimal geometry changes (Scheme 4). Moreover, since the -O• is a stronger H-bond acceptor than –OH,[8] the strength of the intramolecular H-bond in conformer **B** increases along the reaction coordinate, causing a decrease of the *BDE* of the "free" O-H group.[8]



Scheme 4. Role of H-bonding in formal H-atom transfer (HAT or PCET) from bisphenol

On the other hand, H-atom abstraction from conformation **A** is not assisted by any intramolecular H-bond, as it would require substantial reorganization of the molecular geometry. Conformation **A** is expected to have a reactivity similar to that of other alkylphenols **2-5**. Conformation **B** is therefore expected to be significantly more reactive than **A**. To quantify this effect, the *BDE* of the free OH group in conformation **B** was calculated by using an isodesmic approach by employing the experimental *BDE* of unsubstituted phenol as reference (86.7 kcal/mol).[8] By using this procedure, the *BDE* of the free OH group in conformation **B** was calculated as 78.4 kcal/mol. The reactivity of conformation **B** could be in turn estimated because, in phenols having the same pattern of substituents in *ortho* position to the reactive OH, there a is linear relationship between the *BDE* of the phenolic groups and the logarithm of k_{inh}, as indicated by equation 7, where the parameter q depends on the *ortho* substituents (k_{inh} values in PhCl at 303 K, *BDE* values in benzene).[25]

$$\mathrm{Log}\ k_{\mathrm{inh}} = 0.34 \times BDE(\mathrm{OH}) + q \qquad (7)$$

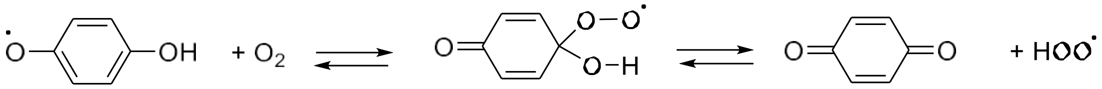
As the reactive OH group in conformation **B** points toward a C-H group, it can be compared to the unhindered phenol **4,** which has a *BDE* value of 85.1 kcal/mol.[18,26] The k_{inh} of conformation **B** can be therefore estimated as $4.3 \times 10^{6}\ \mathrm{M^{-1}s^{-1}}$, and considering that from FT-IR spectra its concentration is 2.9 %, it is estimated to contribute to k_{inh} by ~ $9.9 \times 10^{4}\ \mathrm{M^{-1}s^{-1}}$. Considering the simplifications adopted, and the errors expected in BDE calculations that tend to overestimate H-bonding to phenoxyl radicals,[27] this value that is in reasonable agreement with the experimental k_{inh} of magnolol in PhCl (6.1×10^4 $M^{-1}s^{-1}$).

The significant role of conformation **B** in the antioxidant activity of **1** also explains the large KSE on passing from chlorobenzene to acetonitrile. The "free" OH group is expected to be a stronger H-bond donor if compared to simple monophenols, because the intramolecular H-bond makes the H-atom more positive, similarly to what observed in catechols.[28] Therefore, because of the presence of conformation **B**, magnolol forms stronger H-bonds with MeCN, and it experiences a more marked reactivity decrease than honokiol in acetonitrile.

It had previously been suggested that magnolol is less reactive that honokiol due to the occurrence of intramolecular H-bonds between the reactive OH moieties.[6] We have instead found that the order of reactivity depends on the solvent and have clarified the complex role of intramolecular / intermolecular interactions in fine-tuning the reactivity of magnolol and honokiol.

3) Do bisphenols generate superoxide?

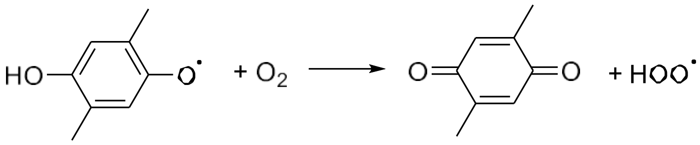
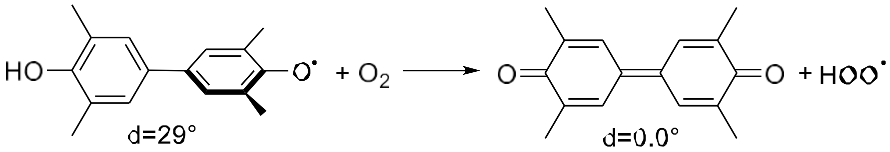
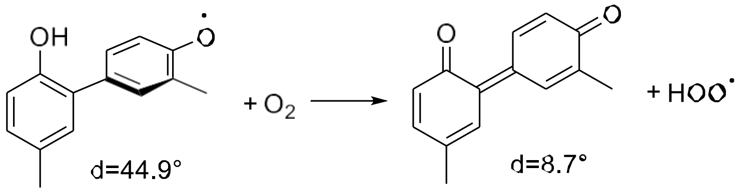
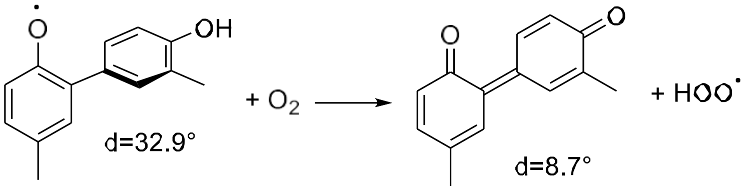
Polyphenols that have two OH groups in conjugated positions may, in principle, generate protonated superoxide from molecular oxygen via the reaction reported in Scheme 5, which consists in the formal H-atom transfer from the semiquinone to O_2, and whose actual mechanism has been shown to follow an addition-elimination pathway.[16]

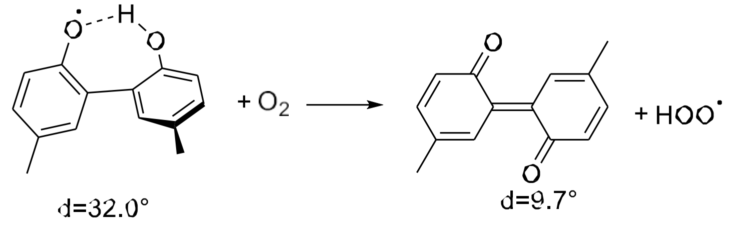
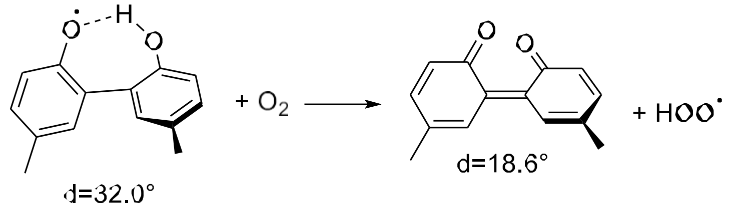


Scheme 5. Mechanism for the production of hydroperoxyl radical by semiquinone radicals.

To a first approximation, the generation of HOO• from semiquinones depends on the *BDE* of the phenolic O-H bond in the semiquinone: the weaker the O-H bond, the easier the H-atom transfer to O_2. To assess the ability of the various biphenyls to produce protonated superoxide, the enthalpy variation for the reaction between the semiquinone radicals and O_2 were calculated by DFT methods, as reported in Table 3.[29] The 2,5-dimethyl-1,4-semiquinone (entry 1) was included as simplified analogue of 2,5-di-*tert*-butyl-1,4-semiquinone, that is known to react with O_2 to form HOO•.[16] We previously observed that this reaction causes a marked decrease of the stoichiometric coefficient of the parent 2,5-di-*tert*-butyl-1,4-hydroquinone when used as inhibitor of the autoxidation of styrene in chlorobenzene.[16] Although the reaction is endothermic, the relatively large concentration of O_2 that is present in air-equilibrated solutions shifts to the right the equilibrium reported in Scheme 5 and causes the observed stoichiometry decrease. Phenoxyl radical from **6** has a ΔH value 10 kcal/mol larger than 2,5-dimethylsemiquinone (entry 2) and accordingly this radical is not found to react with O_2, as it is witnessed by the stoichiometric factor of 2 recorded for its parent bisphenol when used to inhibit the autoxidation of styrene in PhCl (see Table 1). It can be therefore concluded that the radicals from honokiol (entries 3a-b) and magnolol (entries 4a-b), having ΔH values larger than that of the radical from **2**, don't react with O_2 either, under the conditions considered in the present work. It should be pointed out that the observed lack of reaction of the semiquinone radicals from honokiol and magnolol with O_2 under our autoxidation conditions does not imply that the reaction is not feasible; however it is sufficiently slow to be outcompeted by other faster processes, like the reaction of the semiquinone with peroxyl radicals to block oxidative chain propagation.

Table 3. Calculated bond dissociation enthalpy of the O-H bond of semiquinones, enthalpy variation for the reaction of semiquinones with O_2 to form HOO•, and dihedral angles between the two aromatic rings.

#	**Reaction** [a]	***BDE***$_{\text{(O-H)}}$ **(kcal/mol)**	**Δ*H*** [b] **(kcal/mol)**	**Reaction with O_2** [c]
1		60.8	+ 11.6	yes
2		71.1	+ 21.9	no
3a		76.7	+27.5	no
3b		76.9	+27.7	no

4a		86.1	+36.9	no
4b		108	+58.8	no

a) B3LYP/6-31+g(d,p) level; b) Calculated from the BDE(O-H) of the semiquinones and the known BDE(H-OO•) = 49.2 kcal/mol in the gas phase;[29] c) experimentally assessed by the reduction of the stoichiometric coefficient for the corresponding antioxidant during the autoxidation of styrene in chlorobenzene at 30 °C.[16,17]

Table 3 also shows that the *BDE*(O-H) values, for the various bisphenoxyl radicals, vary considerably depending of the linkage position, and they increase with the order *para-para* < *ortho-para* < *ortho-ortho*. The differences among the BDE values mainly depend on the steric crowding occurring among the substituents in *ortho* position to the aryl-aryl linkage in bisphenols, which hampers the bisquinones from adopting the preferred planar geometry. The calculated dihedral angles between the two rings, reported in Table 3, provide a quantitative estimate of this steric repulsion. The *BDE* values reported in Table 3 provide also a rationale for the different stoichiometries of **1**, **2** and **3** in the autoxidation experiments in chlorobenzene, reported in Table 1. In the case of **2** and **3**, the pathway **a** in Scheme 2 dominates, giving a stoichiometric coefficient of 2. The *BDE* values of ~76.8 and 71.1 kcal/mol calculated for the radical of **2** and **3** are apparently low enough to make feasible a fast reaction of the OH group with a second ROO• radical. For comparison, the *BDE* for CH_3CH_2OO-H is 84.8 ± 2.2 kcal/mol in the gas phase,[30] and the rate of reaction of **8**, having a *BDE* (O-H) of 77.1 kcal/mol,[18,26] with alkylperoxyl radicals is $k_{\mathrm{inh}} = 3.2\times10^{6}$ $M^{-1}s^{-1}$ in PhCl.[10] This reaction has to compete with the addition of ROO• to the aromatic ring, a reaction that has a rate constant in the range 10^{7}-10^{8} $M^{-1}s^{-1}$ (Scheme 2 path **b**).[31] In the case of the radical from **1** (Table 3, entries 4a-b) the *BDE* of the OH bond (86.1 or 108 kcal/mol) is too high for a fast H-atom transfer to the ROO• radical, so only the addition to the ring occurs.

Therefore, although the actual stoichiometric factor ranges from two to four for *ortho-para* bisphenol **2** (honokiol) and *para-para*-bisphenol **6** on changing the solvent from PhCl to MeCN, no relevant formation of superoxide radicals is to be expected under the typical settings for inhibited autoxidations, at variance with 1,4-hydroquinones.

Conclusions

Magnolol and honokiol, the bioactive phytochemicals contained in *M. officinalis,* are uncommon antioxidants bearing isomeric bisphenol cores substituted with allyl functions. We have elucidated their complex redox chemistry clarifying the influence of intramolecular and intermolecular interactions in fine-tuning their chain-breaking antioxidant behaviour and in preventing any generation of superoxide radical by reaction with molecular oxygen. While there is extremely high current interest in their biological and pharmacological properties, the lack of detailed mechanistic and kinetic data concerning their antioxidant activity has so far prevented a clear understanding of its role in the purported therapeutic potential. Hopefully the data presented herein will aid future investigation in the area, including the rational design of novel bioactive structures.

Experimental section

Materials. All compounds used in the present investigation were commercially available. Solvents of the highest purity grade were used as received. Cumene and styrene were twice percolated on alumina column before use. 2,2'-Azobisisobutyronitrile (AIBN) was recrystallized from methanol.

Autoxidation Experiments. Autoxidation experiments were performed in a two-channel oxygen uptake apparatus, based on a Validyne DP 15 differential pressure transducer built in our laboratory.[9,10] In a typical experiment, an air-saturated solution of either styrene or cumene containing AIBN was equilibrated with an identical reference solution containing also excess of 2,2,5,7,8-pentamethyl-6-hydroxychromane **8** (25 mM). After equilibration, and when a constant O_2 consumption was reached, a concentrated solution of the antioxidant (final concentration 5-15 μM) was injected in the sample flask. The oxygen consumption in the sample was measured, after calibration of the apparatus, from the differential pressure recorded with time between the two channels. Initiation rates, R_i, were determined for each conditions in preliminary experiments by the inhibitor method using **8** as reference antioxidant: $R_\mathrm{i} = 2\,[\mathbf{8}] / \tau$, where τ is the length of the induction period.

The inhibition rate constants were determined by using the kinetic equations previously reported[10,11] from the known k_p and $2k_\mathrm{t}$ of styrene and cumene (see text).[11,32]

FT-IR Measurements. The liquid phase FT-IR spectra were measured in a sealed KBr cell with 0.5 mm optical path. Solutions were prepared in CCl_4 in the concentration range 2-10 mM to avoid dimerization.

ESI-MS Analysis. A 5 μM solution of **1** or **2** in acetonitrile was stirred under air at 333 K in the presence of AIBN 5 mM. Reaction time was chosen on the basis of inhibited autoxidation experiments so as to correspond approximately to the second half of the inhibited period. Aliquots of the reaction mixture were cooled, diluted 1:1 with MeOH, and analyzed by mass spectrometry using electrospray ionization (ESI) by direct liquid injection at flow rate of 15 μL/min. Spectra were recorded by using the following instrumental settings: positive or negative ions; desolvation gas (N_2), 250 L/h; cone gas (skimmer), 22 L/h; desolvation temperature, 100 °C; capillary voltage, 3.0 kV; cone voltage, 10-40 V; hexapole extractor, 3 V; RF lens, 0.3 V.[33]

Theoretical calculations. Geometry optimization and frequencies were computed in the gas phase at the B3LYP/6-31+g(d,p) level using Gaussian09 rev. D0.1,[34] and stationary points were confirmed by checking the absence of imaginary frequencies. Frequencies were scaled by 0.9806.[35] BDE values were obtained from the sum of electronic and thermal enthalpies by using the isodesmic approach, which consists of calculating the ΔBDE(OH) between the investigated compounds and phenol, and by adding this value to the known experimental BDE(OH) of phenol in benzene (86.7 kcal/mol).[26] The change for the H-bond formation in the gas-phase was calculated from the differences between the enthalpy of the products and those of the reactants.

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Supporting Information

ESI-MS spectra, and cartesian coordinates for calculated minimized structures (16 pages). This material is available free of charge via the Internet at http://pubs.acs.org.

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