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Kinetic and Thermochemical Study of the Antioxidant Activity of Sulfur-Containing Analogues of Vitamin E

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Abstract: Sulfur-containing analogues of vitamin E (thiachromanols), either linked or not to a catechol moiety, were synthesized and their hydrogen-atom donating ability evaluated. The determination of the O–H bond dissociation enthalpy (BDE) of the α-tocopherol analogue **4** by the electron paramagnetic resonance (EPR) equilibration technique provided a value of 78.9 kcal mol^{-1}, that is, approximately 1.8 kcal mol^{-1} higher than that of α-tocopherol. The kinetic rate constants for the reaction with peroxyl radicals (k_{inh}), measured by inhibited autoxidation studies, showed that thiachromanols react 2.5 times slower than the corresponding tocopherols, in agreement with the higher BDE value. This behavior was explained, on the basis of crystallographic analyses and DFT calculations, in terms of a change in the molecular geometry caused by insertion of a sulfur atom into the framework of vitamin E. This behavior implies a greater deviation of the condensed ring from coplanarity with the aromatic ring, thus giving rise to a decrease in the conjugative stabilization of the phenoxyl radical and consequently to an increase in the O–H bond strength. Although less reactive than tocopherols, thiachromanols may, however, act as bimodal antioxidants as a result of the hydroperoxide decomposing ability of the sulfur atom.

Keywords: autoxidation · bond energy · EPR spectroscopy · hydrogen transfer · radical reactions · sulfur heterocycles

Introduction

The autoxidation of unsaturated hydrocarbons and lipids is one of the most studied reactions, both for technological reasons and for its relevance in biological environments, since this process accounts for the damage caused by free radicals to organic and bioorganic systems. This reaction can be inhibited, or at least retarded, by chain-breaking antioxidants capable of trapping free radicals without transforming themselves in reactive intermediates. Phenols, which represent the main antioxidant family, can donate the phenolic hydrogen atom to the chain-carrying peroxyl radicals to form a phenoxyl radical stabilized by resonance, hence they are relatively unreactive toward oxygen and organic materials.[1] The extent to which the autoxidation is retarded by phenols depends upon the rate constant of the inhibition reaction between antioxidant and peroxyl radicals k_{inh} [Eq. (1)].

$$ArOH + ROO^{\cdot} \rightarrow ArO^{\cdot} + ROOH \quad (1)$$

Over recent decades, much work has been done to clarify the basic principles that determine the rates of reaction (1) and to synthesize compounds that show k_{inh} values higher than α-tocopherol,[2] the most effective lipid-soluble antioxidant present in nature. Actually, several of the synthetic

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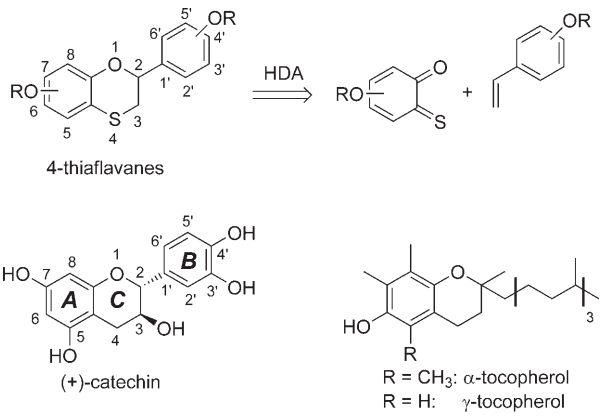
phenols developed show excellent features, such as very high reactivity towards peroxyl radicals[3,4] and improved air stability.[4]

Another active area of research is the study of preventive antioxidants,[5] compounds often containing divalent S, Se, or Te atoms in the molecular skeleton,[6] which inhibit the formation of initiating free radicals by decomposing hydroperoxides by a nonradical process. Chalcogen-substituted phenols have been found to possess both chain-breaking and preventive antioxidant activities.[7]

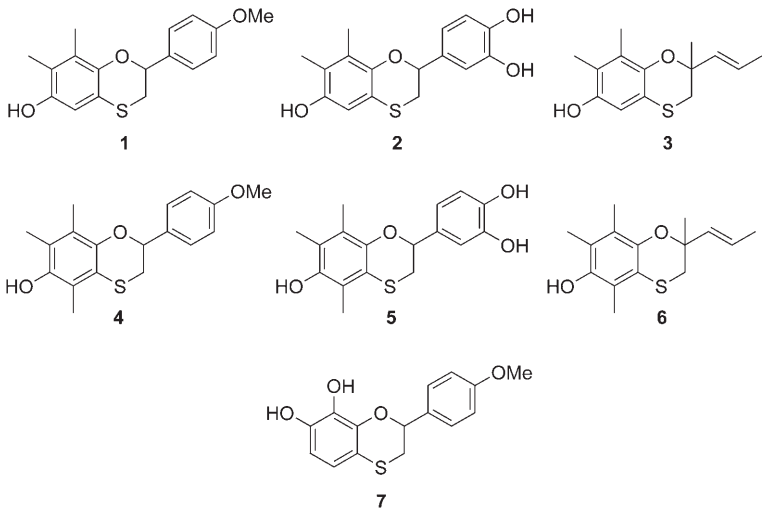
Recently, an original hetero-Diels–Alder approach, consisting of the reaction of electron-poor *ortho*-thioquinones with suitable alkenes (Scheme 1),[8] was used to prepare hydroxylated sulfur-containing heterocycles that resemble natural polyphenols present in plants. The main appeal of this new family of phenolic compounds is that both quinone and alkene moieties can be readily modified to confer the desired attributes to the final products. In previous studies,[9] several sulfur-containing analogues of flavonoids were synthesized and tested for their antiradical activity both in model and biological systems. Herein, the chain-breaking activity of such molecules has been optimized (compounds **1**–**7**) both by substituting the A ring protons with methyl groups or by introducing a catechol moiety into the A or B rings. In the latter case, the cooperative antioxidant effect of the two moieties was investigated.



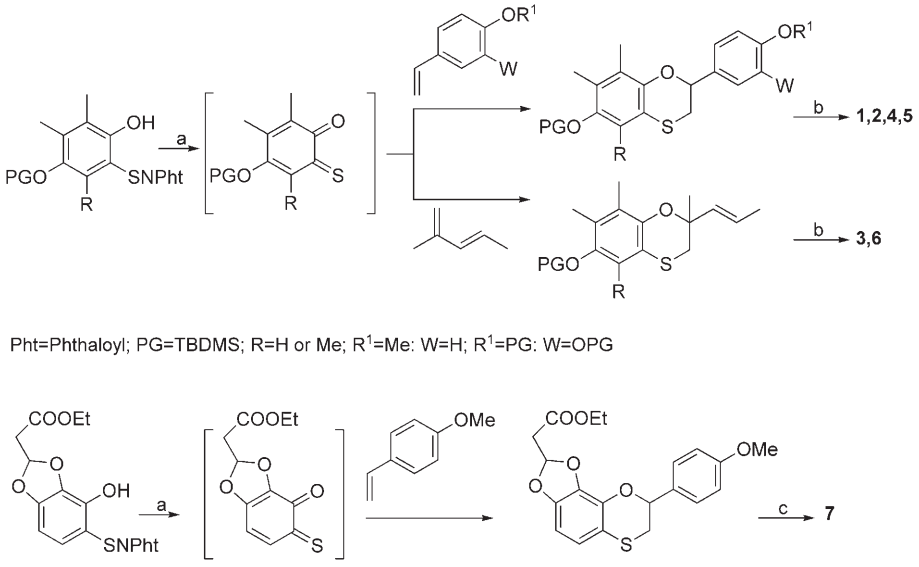
Scheme 1. Access to 4-thiaflavan structure and natural phenolic antioxidants containing the chromane skeleton by using the hetero-Diels–Alder (HDA) reaction.

Results and Discussion

Synthesis: Derivatives **1**–**7** were prepared by using readily available phenols (2,3-dimethylhydroquinone, trimethylhydroquinone, and pyrogallol) as starting materials. These phenols were protected as *tert*-butyldimethylsilyl (TBDMS) ethers[10] or 2-ethylidene acetals[11] by using previously reported procedures (Scheme 2). Monohydroxy arenes were sulfenylated with PhtNSCl to give the *ortho*-hydroxythiophthalimides regiospecifically as suitable precursors for the corresponding *ortho*-thioquinones obtained by reaction with Et_3N in $CHCl_3$ at 60–70 °C.[12] The inverse electron-demand hetero-Diels–Alder reaction of thioquinones with 4-methoxy or 3,4-bis-(*tert*-butyldimethylsilyloxy)styrene and 2-methyl-1,3-pentadiene[13] led to the formation of the expected benzoxathiin cycloadducts, which were transformed into the final 4-thiaflavanes by deprotection of the hydroxy groups. The use of 2-methyl-1,3-pentadiene as a dienophile required the cycloaddition to be performed at 70 °C to drive the reaction exclusively towards the thermodynamic oxathiin cycloadducts **3** and **6.**[13] The pyrrolidine-mediated deprotection, carried out as the final step in the preparation of derivative **7**,[11] required careful deoxygenation of all reagents and solvents to prevent the oxidation of **7** to the corresponding *ortho*-quinone. Preparation of the analogue of **7**, bearing two hydroxy groups in positions 6 and 7 (see Scheme 1), was also tried without success, as the final product was too reactive toward molecular oxygen to be stored and analyzed. As a further endorsement of the validity of the procedure for the preparation of these valuable heterocycles, hydroxy-4-thiaflavanes **1**–**7** were isolated in overall yields of 25–40% and were fully characterized, as reported in the experimental section.



Antioxidant activity: The rate constant for the reaction with peroxyl radicals k_{inh} of the above derivatives was determined by studying the inhibition of the thermally initiated autoxidation of a hydrocarbon [Eqs (2)–(7)] under controlled conditions, as described elsewhere.[14] As styrene was chosen as an oxidizable substrate, the propagation step [Eq. (4)] actually consists of the addition of peroxyl radicals to the olefinic double bond of styrene.[15]



Scheme 2. Reagents and conditions: a) Et_3N, $CHCl_3$, 60–70 °C; b) TBAF·3 H_2O, THF, 0 °C; c) pyrrolidine, CH_3CN, RT. PG = protecting group, TBAF = tetra-*n*-butylammonium fluoride.

related tocopherols (see Table 1) and the inspection of Figure 1 a, which reports the oxygen-uptake traces recorded in the presence of the various antioxidants, allows us to make some considerations about the contributions of groups or substituents to the activity of the various antioxidants. The comparison of **1** and **3** with γ-tocopherol and **4** and **6** with α-tocopherol (γ- and α-TOH) shows that the introduction of a sulfur atom in the C ring causes a decrease in the k_{inh} values by a factor of approximately 2.5. The reasons for the decreased antioxidant efficacy of thiaflavanes will be discussed later.

$$\text{Initiator} \xrightarrow{R_i} \text{R}^{\cdot} \quad (2)$$

$$\text{R}^{\cdot} + \text{O}_2 \rightarrow \text{ROO}^{\cdot} \quad (3)$$

$$\text{ROO}^{\cdot} + \text{RH} \xrightarrow{k_p} \text{ROOH} + \text{R}^{\cdot} \quad (4)$$

$$\text{ROO}^{\cdot} + \text{ROO}^{\cdot} \xrightarrow{2k_t} \text{Products} \quad (5)$$

$$\text{ROO}^{\cdot} + \text{ArOH} \xrightarrow{k_{inh}} \text{ROOH} + \text{ArO}^{\cdot} \quad (6)$$

$$\text{ROO}^{\cdot} + \text{ArO}^{\cdot} \rightarrow \text{Products} \quad (7)$$

The number of radicals trapped by each antioxidant molecule n is related to the length of the inhibited period τ through Equation (8), in which R_i is the initiation rate that can be determined by using a reference antioxidant. The values of k_{inh} were calculated by using Equation (9): a plot of $\Delta[O_2]_t$ versus $\ln(1-t/\tau)$ gives a straight line of slope k_p-[styrene]/k_{inh} from which k_{inh} is obtained by using the known k_p value at 30 °C of styrene, that is, 41 $M^{-1}s^{-1}$.[15]

$$R_i = n[\text{ArOH}]/\tau \quad (8)$$

$$-\Delta[O_2] = (k_p/k_{inh})[\text{styrene}]\ln(1-t/\tau) \quad (9)$$

In the case of the bifunctional antioxidants **2** and **5**, the k_{inh} values were obtained by numerical simulation of the oxygen-consumption traces, which also consider the equilibration between phenols and phenoxyl radicals [Eq. (10)] and the radical-radical reactions involving the semiquinone unit from the catechol group[16] (the complete list of equations is reported in the Supporting Information).

$$\text{ArO}^{\cdot} + \text{Ar}'\text{OH} \rightleftharpoons \text{ArOH} + \text{Ar}'\text{O}^{\cdot} \quad (10)$$

The examination of the measured values of the inhibition rate constants k_{inh} for the investigated thiaflavanes and the

Table 1. Kinetic rate constant for the reaction with peroxyl radicals k_{inh}[a] and number of radicals trapped by each antioxidant molecule n.[b]

Phenol	$k_{inh(PhCl)} \times 10^6$ [$M^{-1}s^{-1}$]	$k_{inh(MeCN)} \times 10^6$ [$M^{-1}s^{-1}$]	$k_{inh(MeCN)}/k_{inh(PhCl)}$	n
1	0.51	0.26	0.5	1.7
2	0.43[c]	–		3.6
	0.42[c]			
3	0.48	–		1.7
4	1.3	0.95	0.7	1.8
5	1.0[c]	0.73[c]	0.7[c]	3.6
	0.41[c]	0.18[c]	0.4[c]	
6	1.2	–		1.8
7	0.42	0.26	0.6	1.9
γ-TOH	1.4[d]	–		2[d]
α-TOH	3.2[d]	–		2[d]
Catechol	0.52	0.16	0.3	1.9

[a] Error within ± 10%. [b] Measured in a mixture of styrene/chlorobenzene in the presence of 0.48 M acetonitrile (MeCN). Mean value of three determinations. [c] From numerical simulations. [d] From reference [3].

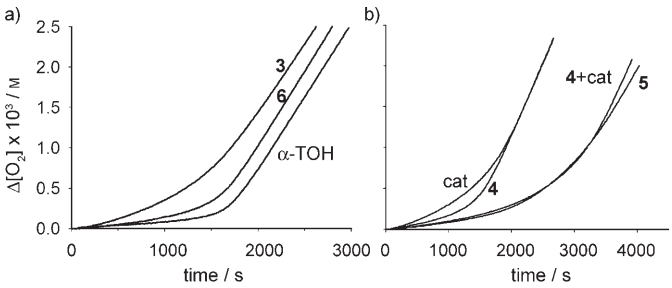


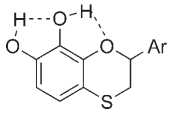
Figure 1. Oxygen-consumption traces observed during the autoxidation of styrene (4.3 M) in chlorobenzene initiated by AMVN (5×10^{-3} M) at 30 °C in the presence of the investigated antioxidants (5×10^{-6} M).

The comparison of **1** with **4** and of **3** with **6** shows instead that the antioxidant activity of phenols increases with increasing the number of methyl substituents on the aromatic

A ring. This behavior, common to other antioxidants, has been explained in terms of a favorable balance between the electron-donating effect of methyl groups that decrease the BDE(O–H) and the steric crowding around the phenolic OH group.[17] It can be seen that the ratio between the reactivity of **1** and **4**, or of **3** and **6**, is almost identical to that between those of γ- and α-tocopherol (≈ 0.4).[3]

Other noteworthy features are that compounds **2** and **5**, containing both thiachromanol and catechol groups, show oxygen-uptake traces characterized by longer inhibition periods and lower slopes (see Figure 1b for **5**). This behavior is indicative of enhanced antioxidant activity with respect to the other thiaflavanes and is in line with the simultaneous presence of two groups capable of inhibiting the autoxidation of styrene. To obtain the experimental values of k_{inh}, the oxygen-decay curves were simulated by numerical solution of the simultaneous differential equations for a model system containing two reactive sites with different inhibition rate constants. The two k_{inh} values determined for **2** (Table 1) are quite similar to those determined in separate experiments for **1** and catechol, whereas those for **5** resemble those of **4** and catechol, thus suggesting that the thiachromanol and catechol moieties behave independently. The absence of synergistic effects was also confirmed by studying equimolar mixtures of catechol with **1** or **4** (Figure 1b), which gave inhibition traces practically superimposable to those obtained in the presence of **2** or **5**.

Another approach adopted to improve the antioxidant properties of thiaflavanes was to introduce two adjacent hydroxy groups into the A ring. As already mentioned, the isomer with the OH substituents at positions 6 and 7 was not stable in air, whereas the 7- and 8-disubstituted compound **7** ($k_{inh} = 4.2 \times 10^5$ M^{-1} s^{-1}) was characterized by an antioxidant activity slightly lower than that of catechol ($k_{inh} = 5.2 \times 10^5$ M^{-1} s^{-1}), despite the presence of the electron-donating atoms oxygen and sulfur in the condensed C ring. An explanation of the relatively low reactivity of **7** toward peroxyl radicals can be given in terms of the adopted structure as inferred by its IR spectrum, which shows a single absorption at 3563 cm^{-1} in the O–H stretching region. This frequency value is characteristic of intramolecularly hydrogen-bonded OH groups similar to that of *ortho*-methoxyphenol (3557 cm^{-1}). The absence of an absorption as a result of a free OH group (for comparison, the two OH stretching vibrations of catechol are observed at 3618 and 3556 cm^{-1} for the free and hydrogen-bonded OH groups, respectively) indicates that both hydroxy protons form intramolecular hydrogen bonds with the adjacent oxygen atoms (Scheme 3) and that their vibrational stretching frequencies are too close to be resolved. As intramolecularly hydrogen-bonded hydroxy groups are known to be characterized by low reactivity toward attacking radicals,[18] the behavior of **7** is entirely expected.



Scheme 3. Intramolecular hydrogen bonds in thiachromanol **7**.

Additional evidence that supports this interpretation was obtained by studying solvent effects on k_{inh} caused by complexation of the investigated thiaflavanes by a hydrogen-bond-accepting solvent, such as acetonitrile (MeCN).[19] Actually, antioxidants that behave as good inhibitors in polar solvents may give poor performances in polar environments, as the formation of hydrogen bonds between the solvent and phenolic OH group decreases the reactivity toward free radicals.[20] The data in Table 1 show that the decrease in k_{inh} values observed in the presence of a small amount of MeCN depends on the phenol structure since the ratio $k_{inh(MeCN)}/k_{inh(PhCl)}$ decreases in the order **4** > **7** > **1** > catechol. The smaller solvent effect observed in **4** can be explained in terms of the higher steric hindrance around the phenolic OH group in **4**, which decreases its interaction with the solvent with respect to the less-crowded compound **1**.[19] Catechol shows the larger solvent effect, as the OH group, not involved in intramolecular hydrogen bonding, is more acidic than the hydroxy groups of simple phenols.[20] Derivative **7**, on the other hand, shows a decrease in k_{inh} values much smaller than catechol upon addition of MeCN, thus indicating that neither hydroxy group is readily solvated as a result of the presence of two intramolecular hydrogen bonds.[18]

Notably, for the bifunctional thiaflavane **5** the variations of the k_{inh} values with the solvent measured for the two antioxidant moieties are similar to those observed in **4** and catechol.

O–H bond dissociation enthalpy (BDE): To obtain a thermochemical estimate of the antioxidant activity of thiachromanols and of the effect of the heterocyclic sulfur atom in the C ring, we determined the BDE of the O–H bond in **4**, which is structurally related to α-tocopherol. The BDE was determined by using the electron paramagnetic resonance (EPR) radical equilibration technique,[17] which measures the equilibrium constant K_e of two equilibrating phenols and the corresponding phenoxyl radicals [Eq. (10)]. This measurement was made by determining the relative concentrations of the phenoxyl radicals generated by photolyzing a mixture of **4** (ArOH) and 2,6-di-*tert*-butyl-4-methoxyphenol (BHA). The latter is used as reference phenol (Ar′OH) and is characterized by a BDE(O–H) value of 77.2 kcal mol^{-1} (recently revised).[17,21]

$$\mathrm{BDE(ArO{-}H)} \approx \mathrm{BDE(Ar'O{-}H)} - RT\ln(K_e) \qquad (11)$$

The phenoxyl radical from thiachromanol **4**, produced photolytically in deoxygenated benzene, was highly persistent and showed an EPR spectrum interpretable, by similarity with the related radical from α-tocopherol,[3] in terms of the proton hyperfine splitting constants reported in Table 2. Figure 2 shows the full EPR spectrum observed on photolysis of a mixture of **4** and BHA together with its computer simulation; the insert also shows part of the high-resolution spectrum of the radical from **4**.

The measurements were repeated under different light intensities to check the constancy of K_e and, therefore, the po-

Table 2. EPR spectral parameters[a,b] of the phenoxyl radicals from **4** and BHA and the BDE(O–H) values of the two parent phenols.

Phenol	$a^{H}(CH_3)$	a^{H}_{other}	g	BDE [kcal mol^{-1}]
4	5.94 (5-Me)	2.61 (1H)	2.0047	78.9 ± 0.3
	5.17 (7-Me)	0.38 (1H)		
	1.29 (8-Me)	0.19 (1H)		
BHA[c]	–	0.93 (2H)	2.00475	77.2
		1.53 (OMe)		

[a] In benzene/di-*tert*-butyl peroxide (9:1, v/v) at room temperature. [b] Hyperfine splitting constants are given in Gauss; peak-to-peak line width = 0.12 G. [c] Data for the radical from BHA is taken from reference [17].

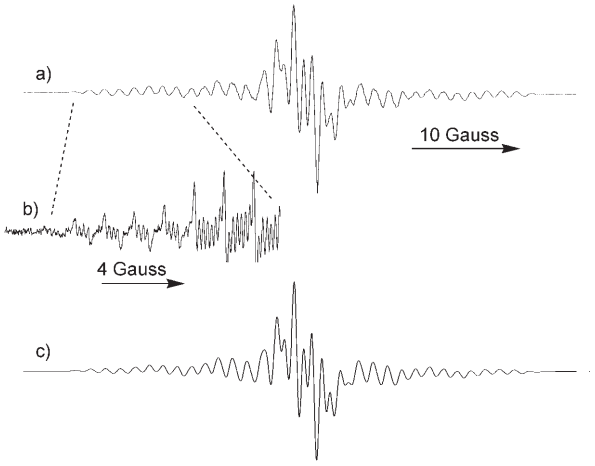


Figure 2. a) Experimental EPR spectrum obtained by photolysis of a mixture of **4** (7.5×10^{-3} M) and BHA (2.1×10^{-4} M) in degassed benzene containing 10% di-*tert*-butyl peroxide at room temperature and c) the computer simulated spectrum. b) The expansion of the first part of the of the high-resolution EPR spectrum of the phenoxyl radical from **4**.

sition of the equilibrium. The BDE(O–H) for **4** was calculated by making the reasonable assumption that the entropic term can be neglected[17] and by using Equation (11). The BDE(O–H) of **4** was found to be 78.9 kcal mol^{-1}, that is, 1.8 kcal mol^{-1} larger than that of α-tocopherol. This difference, which indicates a stronger O–H bond in **4**, is consistent with its weaker antioxidant activity and is in line with the kinetic results. Actually, the inhibition rate constant and the BDE value obtained for thiaflavane **4** lie on the correlation line between the log(k_{inh}) and the BDE(O–H) values of other *ortho*-dimethylphenols (Figure 3).[17]

Computational studies: The energies and relevant geometries of **4**, **8**, and the corresponding phenoxyl radicals were calculated at the B3LYP/6-31+G(2d,p)//B3LYP/6–31G(d) level to explain the effect of introducing a heterocyclic sulfur atom in the condensed ring of the chromanol structure.[22,13b] The gas-phase differences ΔBDE(O–H) between the BDE values of the investigated antioxidants ArOH and that of the unsubstituted phenol (PhOH) were calculated by using the isodesmic approach shown in [Eq. (12)].[23]

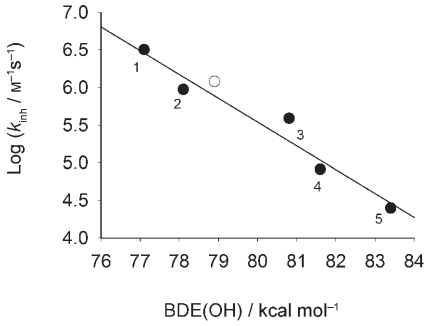
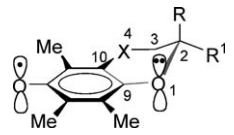


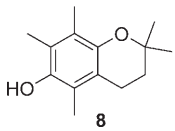
Figure 3. Logarithm of the rate constant for the reaction with peroxyl radicals against the BDE(O–H) of **4** (○) and other substituted 2,6-dimethylphenols (●).[3,17,21] 1) α-TOH, 2) 2,6-dimethyl-4-methoxyphenol, 3) 2,3,5,6-tetramethyl-4-methoxyphenol, 4) 2,4,6-trimethylphenol, 5) 2,6-dimethylphenol.

$$\Delta BDE(ArO{-}H) = E(ArO^{\cdot}) - E(ArOH) - [E(PhO^{\cdot}) - E(PhOH)] \quad (12)$$

The dihedral angles ϑ formed by atoms 10-9-1-2 (see Scheme 4) in both the parent phenols and phenoxyl radicals were determined for **4** and **8**, as indicators of the extent of the conjugation between the heterocyclic oxygen atom and the phenolic aromatic ring.



Scheme 4. Geometry of **4** (X = S; R = H; R^1 = Ar) and **8** (X = CH_2; R = R^1 = CH_3).



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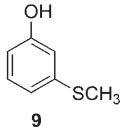

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The computed results indicate that thiachromanol **4** possesses a slightly higher bond-dissociation enthalpy value than **8** (Table 3). The calculated BDE(O–H) difference (+1.9 kcal mol^{-1}) is in good agreement with the experimental value obtained by the EPR equilibration technique (+1.8 kcal mol^{-1}). The effect of a sulfur atom *meta* to the

Table 3. ΔBDE(O–H) values between **4**, **8**, **9**, and phenol and the dihedral angles ϑ defined by atoms 10-9-1-2.[a]

Phenol	ΔBDE [kcal mol^{-1}][b]	ϑ(ArOH) [°]	ϑ(ArO$^{\cdot}$) [°]
4	−9.7	−29 (−30[c])	−17
8	−11.6	−17[d]	−12
9	−0.70	–	–

[a] Calculated at the B3LYP/6-31+G(2d,p)//B3LYP/6–31G(d) level by using the DFT method [b] Values include thermal correction to enthalpy (298 K). [c] Experimental value measured by X-ray crystallographic analyses. [d] Experimental values of −15.9 and −19° were determined for each of the two molecules of **8** contained in the same unit crystallographic cell.[3]

phenolic OH group was also estimated by repeating these calculations on *meta*-thiometylphenol (**9**).

Both kinetic and thermochemical studies agree that thiachromanols are less reactive toward peroxyl radicals than the corresponding chromanols. The reason might depend on electronic effects, as a result of the substitution of a sulfur atom for a CH_2 group, or a change in the preferred conformation of the C ring. Disappointingly, to the best of our knowledge there are no reported data on the experimental effects of *meta*-alkylthio-substituents on the BDE of phenols. This effect can be approximately estimated by considering the effect of alkoxy substituents, which are expected to behave similarly to SR groups.[24] There are two reports on the effect of a *meta*-OMe group: Bordwell and Cheng[25] used an electrochemical method in dimethyl sulfoxide (DMSO) and Lucarini et al.[17] used the EPR equilibration technique in benzene. The values are positive ($+0.4\ kcal\,mol^{-1}$) and negative (-0.5 to $-0.8\ kcal\,mol^{-1}$), respectively, the latter value being presumably more reliable as DMSO gives rise to strong interactions with the investigated compound. However, the two reports agree on the conclusion that the effect of a *meta*-OMe group is small and comparable to the uncertainties of these measurements. Therefore, as a SR substituent is less perturbing than a OR group, because of the lower overlap between the sulfur 3p orbital and the π system of the aromatic ring,[25] the contribution to the BDE(O–H) of a *meta*-SMe group is expected to be smaller than that of a *meta*-OCH_3 group. This conclusion is substantiated by DFT calculations performed on the model phenol **9**, and these calculations also suggest that the introduction of a SCH_3 substituent at the *meta* position to the hydroxy group has a small negative effect on the BDE(O–H).

A more reasonable explanation for the increase in BDE observed on passing from chromanols to thiachromanols can be given instead by considering the change in the ϑ angle induced by replacing the sulfur with the carbon atom in the C ring. Actually, it is well known that the presence of the condensed, saturated ring in chromanols is essential for their good antioxidant activity, as it forces the heterocyclic oxygen atom to conjugate better with the aromatic ring.[3] The introduction of the sulfur atom into chromanol affects the geometries of both phenol and the phenoxyl radical, as the computed ϑ values are larger by 12 and 5°, respectively. This difference implies that the preferred geometry of thiachromanol **4** deviates appreciably from planarity, so that the conjugation between the aromatic ring and lone pair on the oxygen atom should be considerably decreased with respect to α-TOH in both phenol and the phenoxyl radical. To confirm experimentally the significant differences between the condensed-ring geometries predicted by calculations on tocopherol and **4**, a determination of the structure of the latter compound was made by X-ray crystallographic studies (see the Experimental Section).[26] The more relevant result concerns the value of the ϑ angle, which was found to be −30° and is, therefore, very close to that predicted computationally.

The larger distortion of the condensed ring of thiaflavanes with respect to chromanols is responsible for the larger BDE value observed in the former compounds.[3] The reason depends on the different effects that departure from coplanarity has in the phenoxyl radical and in the parent phenol. Actually, the decrease in conjugative stabilization as a result of the decreased overlap between the π orbitals of the aromatic ring and the $2p_z$ orbital of the heterocyclic oxygen atom is higher in the phenoxyl radical than in the parent phenol. Thus, little destabilization of the phenol will occur upon deviation from planarity, whereas a much larger effect will be experienced by the corresponding phenoxyl radical in which delocalization of the unpaired electron in the *para*-alkoxy substituent is a very important stabilizing factor. Experimental evidence of this different behavior is provided by the barrier to internal rotation of the methoxy group, which in the 4-methoxyphenoxyl radical ranges from 8.3 to $10.4\ kcal\,mol^{-1}$ depending on the solvent,[23] whereas in anisole it does not exceed 2–3 $kcal\,mol^{-1}$.[27] Thus, increased distortion of the C ring, even if small, can substantially decrease the stability of the phenoxyl radical with a consequent increase in the BDE(O–H) value.

Reactivity toward hydroperoxides: The lower efficacy of thiaflavanes as chain-breaking antioxidants with respect to tocopherols might be compensated by the presence of a bivalent sulfur atom in the molecular skeleton. This sulfur atom can confer the properties of preventive antioxidants, capable of decomposing hydroperoxides to give nonradical products, to these derivatives. In the case of sulfides, the products of this reaction are known to be sulfoxides and the alcohol from the peroxide. Thus, we treated **4** (0.32 mmol) in methanol with *t*ButOOH (3.2 mmol) at room temperature for 68 h. Analysis of the reaction mixture by ^1H NMR spectroscopy allowed the identification of the residual **4** and *trans*-sulfoxide **10** in a 10:1 ratio. The rate constant for the bimolecular reaction between **4** and *tert*-ButOOH ($3\times10^{-3}\ M^{-1}s^{-1}$), estimated from these data, is not far from that reported for the reaction of other sulfides with hydroperoxides. For example, the rate constants for the reactions of dibenzylsulfide and diphenylsulfide with cumylhydroperoxide are 1.3×10^{-3} and $1.0\times10^{-2}\ M^{-1}s^{-1}$ (at 353 and 423 K), respectively.[5]

It must be therefore concluded that thiaflavanes are able to decompose the peroxides formed during the oxidation of hydrocarbons, that is, they may also behave as preventive antioxidants.

Conclusion

It has been shown herein that the introduction of methyl groups in the thiachromanol A ring affords tocopherol-like phenols with a chain-breaking activity almost as good as that of the related tocopherols. Furthermore, the presence of a sulfur atom in these molecules represents an important property that is typical of preventive antioxidants. The reason why thiachromanols are characterized by a lower re-

activity toward free radicals than their natural counterparts was explained in terms of greater nonplanarity of the molecular skeleton, which causes a decrease in the conjugative stabilization of the phenoxyl radicals. The ready insertion of other functional groups into these compounds is an important feature that has been exploited to introduce supplementary antioxidant functions, such as the catechol group on the B ring. Although in homogeneous solutions no advantages can be obtained with respect to equimolecular mixtures of the parent derivatives, it can not be excluded that in more complex systems, such as low-density lipoproteins and liposomes, in which diffusion of inhibitors among particles becomes the rate-determining step,[28] the covalent link between the two antioxidant moieties may be relevant to the overall inhibiting activity.

Experimental Section

Materials: The protection of 2,3,5-trimethylhydroquinone[10] and pyrogallol[11] was carried out following previously reported procedures. The sulfenylation of protected phenols with phthalimidesulfenyl chloride[8,9] and the cycloaddition with styrenes[8,9] and 1,3-dienes[13] were performed as reported elsewhere. The experimental data are available in the Supporting Information. All other compounds used are commercially available. Solvents of the highest-purity grade were used as received. Styrene was percolated on alumina before each experiment to remove traces of inhibitor.

Oxidation reactions of sulfoxides with *t*ButOOH: A solution of *t*ButOOH in hexane (5 M, 640 μL, 3.2 mmol) was added to a solution of cycloadduct **4** (100 mg, 0.32 mmol) in MeOH (5 mL). The reaction mixture was stirred at room temperature for 68 h, diluted with CH_2Cl_2 (80 mL) and washed with a solution of $Na_2S_2O_3$ (10%, 2×20 mL) and brine (30 mL). The organic layer was dried over anhydrous Na_2SO_4 and concentrated in vacuo. ^{1}H NMR spectroscopic analysis of the crude mixture allowed the identification of the residual cycloadduct **4** and the *trans*-sulfoxide **10** in a 10:1 ratio.

Kinetic measurements: The rate constants for the reaction of the title compounds with peroxyl radicals were measured by following the autoxidation of styrene at 30 °C with 2,2′-azobis(2,4-dimethylvaleronitrile) (AMVN; 5×10^{-3} M) as the initiator. The reaction was performed in 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 styrene in chlorobenzene (4.3 M) containing the antioxidant was equilibrated with the reference solution containing an excess of α-tocopherol (1×10^{-3} M) in the same solvent. After equilibration, a concentrated solution of AMVN in chlorobenzene was injected in both the reference and sample flasks, and the oxygen consumption in the sample was measured, after calibration of the apparatus, from the differential pressure recorded over time between the two channels. This instrumental setting allowed us to have the N_2 production and oxygen consumption derived from the azo-initiator decomposition already subtracted from the measured reaction rates. Initiation rates R_i were determined for each condition in preliminary experiments by using the inhibitor method[15] with α-tocopherol as the reference antioxidant: $R_i=2[\alpha\text{-TOH}]/\tau$. The length of the induction period τ was determined using an integration procedure suggested by Roginsky et al.[29] Numerical simulation of the oxygen-consumption traces were performed by using the software Gepasi, version 3.30,[30] freely available on the Internet at http://www.gepasi.org, as described in the Supporting Information.

EPR and thermochemical measurements: Deoxygenated solutions of **4** and di-*tert*-butyl peroxide (10% v/v) in benzene were sealed under nitrogen in a suprasil quartz EPR tube. The sample was inserted in the cavity of an EPR spectrometer, and photolyzed with the unfiltered light from a 500-W high-pressure mercury lamp at room temperature. The EPR spectra were recorded on a spectrometer equipped with a microwave frequency counter for the determination of the *g* factors, which were corrected with respect to the perylene radical cation in concentrated H_2SO_4 ($g=2.00258$). The BDE value of **4** was determined by studying mixtures of BHA and **4**. The molar ratio of the two equilibrating radicals, obtained from the EPR spectra, was used to calculate the equilibrium constant K_e. Different concentration ratios of the starting phenols were used to check if the equilibrium had been reached. The spectra were recorded a few seconds after beginning the irradiation to avoid significant consumption of the phenols during the course of the experiment. Relative radical concentrations were determined by comparison of the digitized experimental spectra with computer-simulated spectra, as previously described.[17]

IR measurements: FT-IR spectra of **7** were measured in dilute solutions (0.01–0.05 M) of tetrachloromethane in a sealed KBr cell with a 0.5-mm optical path.

Computational details: The 2*R* configuration of **4** was arbitrarily chosen for all calculations. A Monte Carlo conformational analysis was performed by means of MacroModel9[31] to investigate the relative minima adopted by the C ring of the chromane skeleton in both molecules. The analysis was done under vacuum employing 5000 MC steps per molecule and using an energy window of 50 kJ mol^{-1}. The MMFF94* force-field[32] was used for the geometry optimization, which was performed by means of a conjugate gradient algorithm[33] with a derivative convergence criterion of 0.05 kJ Å mol^{-1}. The local minima found by the conformational analysis were used as starting structures for the following quantum mechanical calculations. Geometry optimization was calculated with the B3LYP hybrid functional,[34] with a 6–31G(d) basis set, and single point energies at the level 6-31+G(2d,p) using the Gaussian03 program suite.[35] Unrestricted wave functions were used for radical species. Geometry optimizations were carried out in the gas phase by using the Berny algorithm along with the default convergence criteria,[36] whereas frequency calculations at the reference temperature of 298.15 K were performed at the same level of theory to both characterize stationary points and calculate their thermodynamic properties. The zero-point vibrational energies (ZPVE) were corrected using a scale factor of 0.9806.

X-ray crystallographic analyses: RX analysis was carried out with a Goniometer Oxford Diffraction KM4 Xcalibur2 at room temperature. Graphite-monochromated $Mo_{K\alpha}$ radiation (40 mA/−40 KV) and a KM4 CCD/SAPPHIRE detector were used for cell-parameter determination and data collection. The integrated intensities were measured using the ω scan mode and corrected for Lorentz and polarization effects.[37] The substantial redundancy in the data allows empirical absorption corrections to be applied using multiple measurements of symmetry-equivalent reflections. The structure was solved by direct methods of SIR97[38] and refined using the full-matrix least squares on F^2 provided by SHELXL97.[39] The non-hydrogen atoms were refined anisotropically, the methyl hydrogen atoms were assigned in calculated positions, and the other hydrogen atoms were found in the Fourier synthesis (all of them were refined as isotropic).

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