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PERSPECTIVE

Modulation of the antioxidant activity of phenols by non-covalent interactions

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Non-covalent (H-bonding) interactions, either intramolecular or with the surrounding medium, have a major influence on the activity of natural and synthetic phenolic antioxidants, due to the modulation of their reactivity with radical species, such as peroxyl radicals. Different cases can be distinguished. (i) Intra- or inter-molecular H-bonding involving the reactive –OH moiety will depress the antioxidant activity if the –OH acts as H-bond donor, while the opposite will generally occur if it acts as H-bond acceptor. (ii) Remote intra- and inter-molecular H-bonding, involving a distant –OH group (in polyphenols) or a ring substituent, may increase or decrease the reactivity of an antioxidant toward free radicals, depending on whether the stabilization produced by the H-bond increases or decreases along the reaction coordinate, on proceeding from reactants to the transition state. In this Perspective, the role of non-covalent interactions in the complex chemistry of natural polyphenolic antioxidants is discussed with the aid of literature data on simplified model compounds, aiming at the composition of a clear picture that might guide future research.

Introduction

The majority of chain-breaking antioxidants in nature are phenolic.[1] The antioxidant activity of these compounds stems from their ability to transfer the phenolic hydrogen to lipid peroxyl radicals [eqn (1)] much faster than the chain-propagating H-atom transfer step of lipid peroxidation [eqn (2)].[1]

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$$LOO^{\bullet} + ArOH \xrightarrow{k_{inh}} LOOH + ArO^{\bullet} \quad (1)$$

$$LOO^{\bullet} + LH \rightarrow LOOH + L^{\bullet} \quad (2)$$

Autoxidation of organic oxidizable substrates such as plastics, lubricants, foods, cosmetics, as well as that of other biological molecules like proteins, parallels lipid peroxidation following a superimposable mechanism where alkylperoxyl radicals ($ROO^{\bullet} \equiv LOO^{\bullet}$) are the chain-carrying species.[2] Due to its importance, lipid peroxidation is chosen as the model reaction to evaluate and classify antioxidants: compounds like phenols, able

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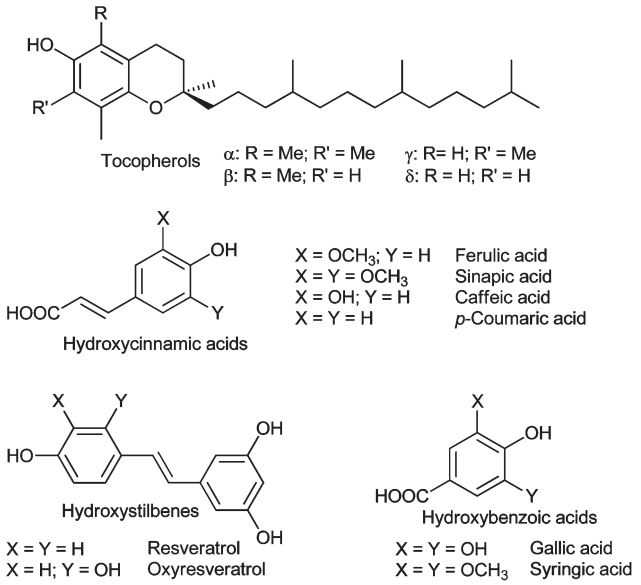
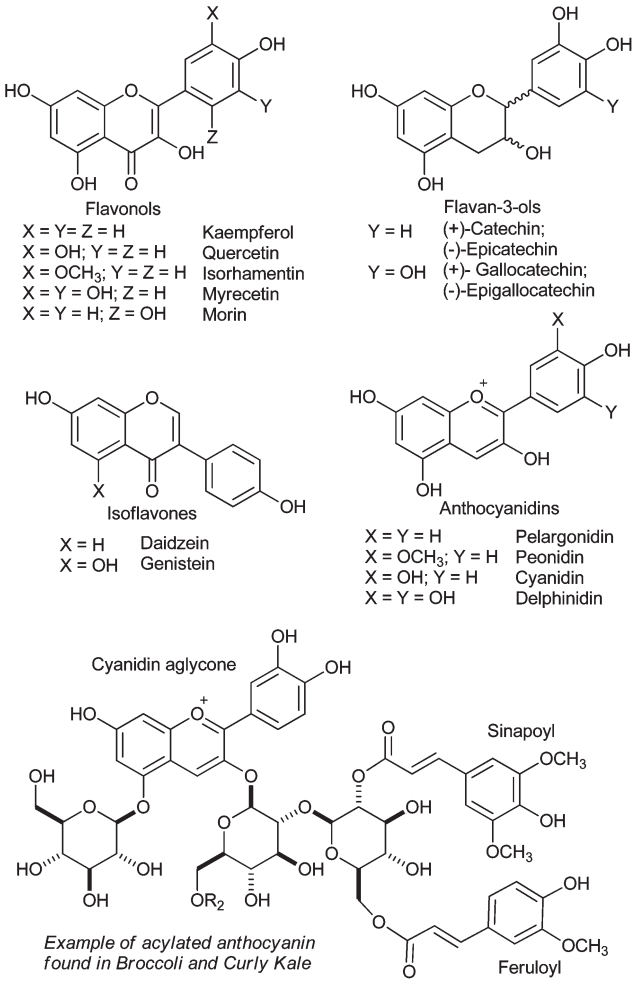


Scheme 1 Examples of monophenolic antioxidants.



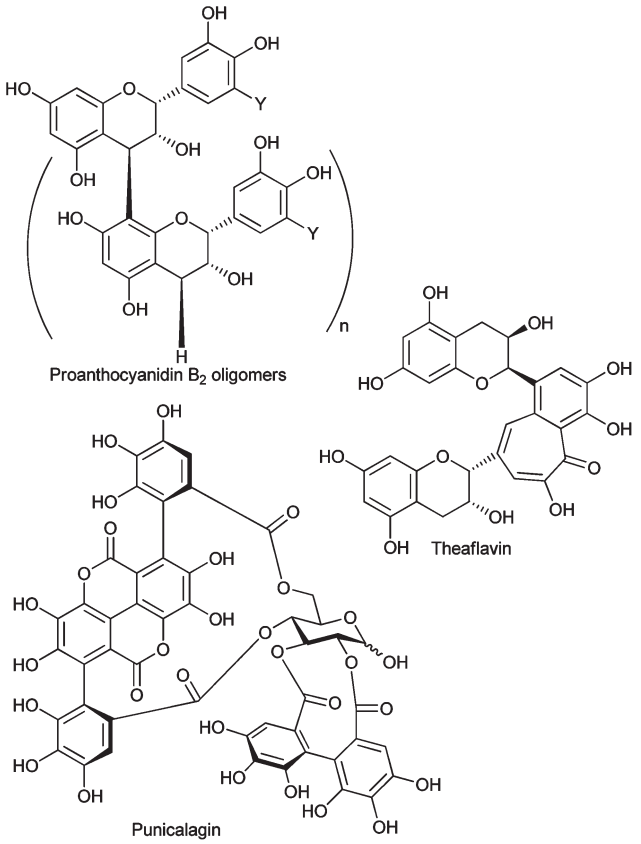
Scheme 2 Flavonoids and example of a polyphenol composed by a flavonoidic nucleus linked to various sugars and phenolic acids (ref. 4).

to impair chain-propagation by reacting with chain-carrying peroxyl radicals, are classified as chain-breaking antioxidants.

Most dietary antioxidants found in fruits and fresh vegetables belong to the phenol family. The liposoluble vitamin E, mainly contained in seed oils, is composed by a mixture of variously alkylated chromanol derivatives (Scheme 1), among which α-tocopherol has the largest reactivity toward peroxyl radicals.[1a] Simple phenolic acids (*e.g.* benzoic or cinnamic derivatives) can be found in food either as such (Scheme 1), or esterified to sugar units, or as acylating moieties connected to flavonoids (Scheme 2).[3] These, in turn, are polyphenolic compounds comprising a 15-carbons core, the aglycone, often glycosylated with one to several sugar units.[3] Flavonoids are classified according to the aglycone structure and main structures of dietary interest are illustrated in Scheme 2. Oligomerization of flavonoids, hydroxystilbenes, cinnamic and gallic acids leads to the formation of a wide array of polyphenolic derivatives, recently reviewed by Crozier *et al.* and Quideau *et al.*,[3] such as the proanthocyanidins and ellagitannins depicted in Scheme 3. In all these derivatives, interaction involving the many OH groups *via* H-bond formation is a common feature, which is expected to distinctively influence the antioxidant activity. Indeed, in the case of polyphenols, H-bonding, either intramolecular or to the solvent, can involve either the "active" OH function or a remote OH group, producing different outcomes on the rate of reaction with peroxyl or other radical species. Rationalization of the antioxidant activity of polyphenols in real systems is further complicated by the ease with which they bind to biological molecules such as proteins, membrane lipids, polysaccharides and DNA.[3b]

The rate constant for eqn (1) (k_{inh}) is the key element in evaluating the performance of phenols as antioxidants. It generally depends on the bond dissociation enthalpy (BDE) of the phenolic O–H bond,[5] and on the steric crowding around this group,[5] as illustrated in Fig. 1. Electron-donating (ED) groups lower the BDE(O–H) while electron-withdrawing (EW) groups have the opposite effect; this mainly depends on their ability to stabilize the phenoxyl radical ArO$^{\cdot}$ formed after H-atom abstraction.[5] Therefore, on a qualitative ground, the pattern of substituents in the phenolic ring produces a similar influence on the reactivity of phenols with any H-atom abstracting radical species. As shown in Fig. 1, this holds for transient peroxyl[1,6,7] or persistent 2,2-diphenyl-1-picrylhydrazyl (DPPH$^{\cdot}$) radicals,[8] and it has been demonstrated also for the reaction with alkyl radicals (R$^{\cdot}$).[9] The solvent, on the other hand, has a profound influence on the absolute reactivity of phenols with H-abstracting radicals [eqn (1)], due to the occurrence of hydrogen-bonding interactions with the polar and relatively acidic phenolic –OH. Conversely, it has very limited (if any) influence on the rate of oxidative chain-propagation [eqn (2)]. Therefore the reaction medium is able to influence the competition between ArOH and LH for peroxyl radicals,[10] because of the major differences between ArOH and LH in terms of polarity and acidity. In general, solvents or ligands able to bind or to deprotonate ArOH have a deep influence on the overall antioxidant activity.[10]

In this Perspective, we summarize and discuss some recent results obtained with simplified models, which can help to understand the effects of intra- and inter-molecular H-bonds on the radical chemistry of natural phenolic and polyphenolic antioxidants.



Scheme 3 Examples of polyphenols. From top: condensed tannins, theatannins and hydrolysable tannins.

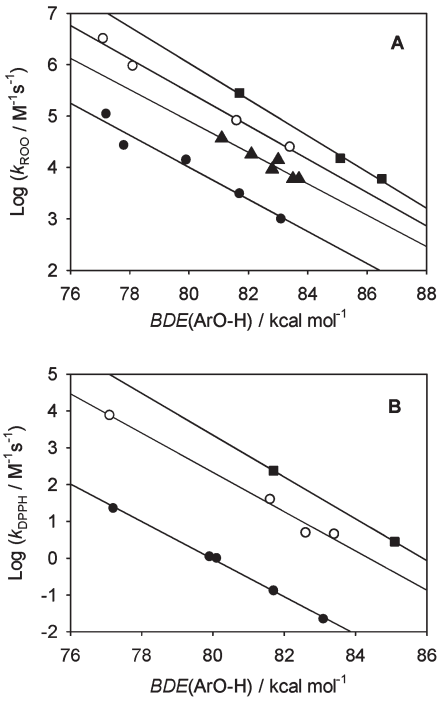


Fig. 1 Relationship between the bond dissociation enthalpy (BDE) of the phenolic O–H and the logarithm of the rate constant for the reaction with peroxyl radicals in PhCl (A) or with DPPH$^{\cdot}$ in heptane (B), for *ortho* and *para* substituted phenols: (■) no substituents in *ortho* positions; (○) *ortho*-di-methyl phenols; (●) *ortho*-di-*tert*-butyl phenols, (▲) *ortho*-methoxy phenols. Data from ref. 1, 6–8.

1 Intramolecular hydrogen bonds†

1.1 Reactive OH group as H-bond donor

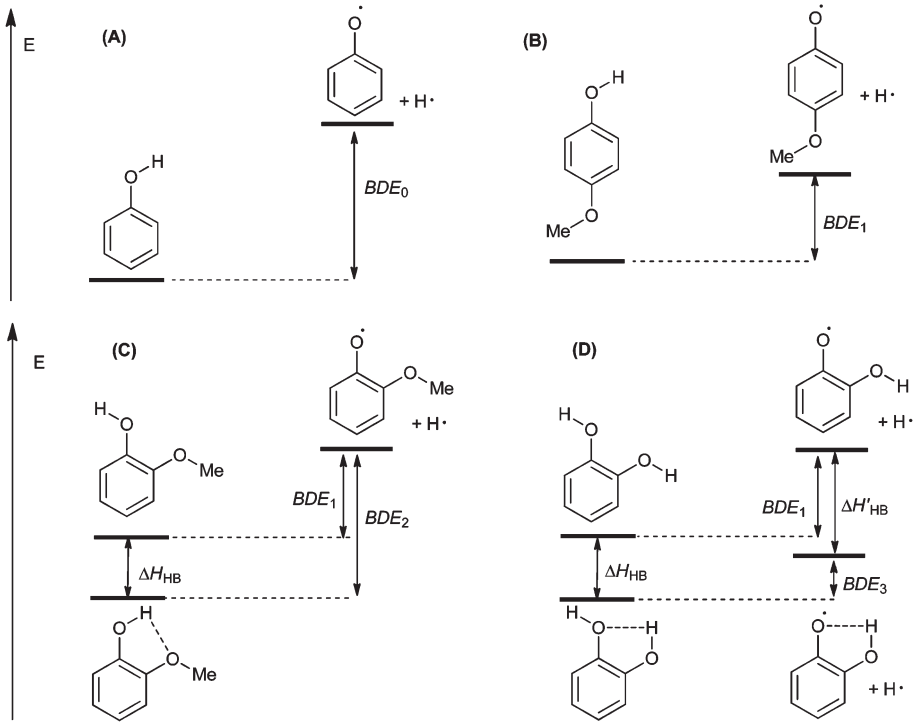
Strong intramolecular H-bonds. The presence of intra-molecular H-bonds is a common feature in polyphenols, as –OH groups can be in contact with carboxyl, methoxyl or other –OH groups. Reactions of H-atom abstraction from model phenols bearing *ortho* –OR,[6] –SR,[11] –SeR[12] and –TeR[12] groups (where R is an open alkyl chain or it is cyclised onto the phenol ring) have been thoroughly investigated in recent years and can be summarized as follows. The presence of electron-donating groups on the aromatic ring normally lowers the phenolic BDE (O–H), mainly because they stabilize the phenoxyl radical. However, when such groups are in the *ortho* position, they also stabilize the parent phenol by accepting an intramolecular H-bond (see Scheme 4). Stabilization of the parent phenol by *ortho* –XMe groups can be as large as the stabilization of the corresponding phenoxyl radical, thereby partly or entirely counteracting the expected decrease in BDE(O–H) with respect of the unsubstituted phenol. A quantification of such effects in the case of *ortho*-chalcogen phenols is summarized in Table 1, in which it can be seen that, despite the fact that *ortho* or *para* –XMe groups are expected to have similar electronic properties and are calculated to stabilize the phenoxyl radical to almost the same extent, experimental BDE(O–H) in *ortho* –XMe phenols, are always larger than in the *para*-substituted counterparts. The only exception is the –OH substituent, which will be discussed later on. In Table 1, the effects of substituents are dissected in terms of stabilization of the parent phenols (MSE) and of the corresponding phenoxyl radicals (RSE). In the case of *para* –XMe groups, MSE increases from −2 to 0.5 kcal mol^{-1} on passing from –OMe to –TeMe, whereas RSE has a rather constant value of about 3–4 kcal mol^{-1}, indicating that these substituents have a relatively small perturbing effect on the parent phenol, while they significantly stabilize the phenoxyl radical through electron donation.‡

In the case of *ortho* –XMe groups, the MSE is larger than in *para*-substituted analogues by about 3 kcal mol^{-1}, because of the formation of an intramolecular H-bond between the phenolic –OH and the –XMe groups. On the other hand, the RSE increases on moving from –OMe to –TeMe, which can be explained in terms of reduced repulsion between the –O$^{\cdot}$ and the –XMe group, as the size of the chalcogen X increases.[12]

This picture is not limited to *ortho* –XR phenols: in general intramolecular H-bond donation from the reactive –OH function to a neighbouring hydrogen-bond acceptor (HBA) will hamper

† Unless otherwise stated, k_{inh} values were measured at 30 °C in PhCl/styrene 50 : 50, and k_{DPPH} were obtained at room temperature in dioxane.

‡ Alkyl chalcogens heavier than oxygen behave as EW substituents in the presence of ED groups (such as the phenolic OH), or as ED substituents in electron poor aromatic rings (such as in phenoxyl radicals).[12]



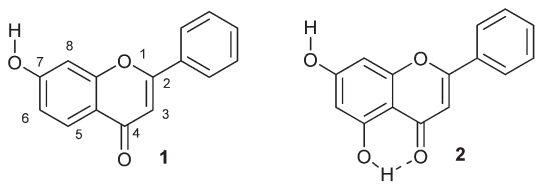
Scheme 4 Energy diagram for O–H bond dissociation in phenols bearing *ortho* or *para* –OMe and –OH groups, showing the effect of intramolecular H-bonding. (A) Unsubstituted phenol. (B) A *para* methoxy (or hydroxyl) group reduces the BDE ($BDE_1 < BDE_0$) because its ED effect destabilizes the phenol and stabilizes the radical. (C) An *ortho* methoxy group, besides the electronic effect which is similar to the case B, has an additional stabilizing effect on the phenol because it can accept an intramolecular H-bond ($BDE_2 > BDE_1$). (D) An *ortho* hydroxyl group stabilizes both the phenol and (to a larger extent) the radical ($BDE_3 < BDE_1$) by donating an intramolecular H-bond. The BDE(OH) and ΔH_{HB} energies are not in scale.

Table 1 Experimental (solvent benzene) and calculated (gas phase) contributions of the substituents to the phenolic BDE(O–H), to the molecule stabilization energy (MSE) and to the radical stabilization energy (RSE) (kcal mol^{-1})

X	ΔBDE_{exp}[a]	ΔBDE_{calc}[a,b]	MSE_{calc}[c]	RSE_{calc}[d]
ortho-OMe	−1.8[e]	−0.9	1.4	2.3
ortho-OH	−6.0[f]	−6.8	0.7	7.5
ortho-SMe	+0.3[g]	−0.3	2.5	2.8
ortho-SeMe	−0.5	−0.8	3.4	4.2
ortho-TeMe	−1.4	−2.5	3.3	5.8
para-OMe	−5.0[h]	−6.1	−1.8	4.3
para-OH	−5.2[i]	−5.8	−2.0	3.8
para-SMe	−3.7[g]	−4.3	−0.6	3.7
para-SeMe	−3.4	−3.5	0.4	3.9
para-TeMe	−3.1	−2.9	0.5	3.4

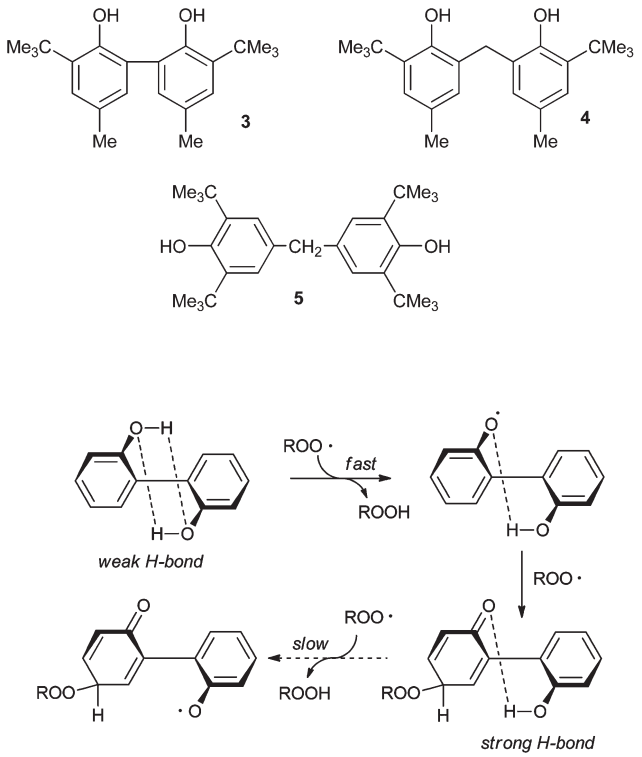
[a] Respect to phenol, BDE = 86.7 kcal mol^{-1} (ref. 7). Unless otherwise stated, data are from ref. 12. The ΔBDE, RSE and MSE for the OMe and OH substituents are from ref. 13 and 14. [b] ΔBDE = MSE − RSE. [c] MSE: X–ArOH + C_6H_6 → ArOH + C_6H_5–X. [d] RSE: X–ArO· + C_6H_6 → ArO· + C_6H_5–X. [e] Data from ref. 6. [f] Data from ref. 15 and 7*b*. [g] Data from ref. 11. [h] Data from ref. 7. [i] Data from ref. 16.

the reactivity of phenols, or that of one specific –OH group in polyphenols. This principle holds, for instance, with common flavonoids and isoflavonoids: the phenolic –OH groups neighbouring HBA substituents, such as C=O groups, undergo strong intramolecular H-bond, particularly when aided by an optimized geometric arrangement achieving a six-membered ring. This completely hampers their reactivity toward peroxyl and other radicals.[17] The flavonoids 7-hydroxyflavone (**1**) and 5,7-dihydroxyflavone (chrysin, **2**) have the same reactivity toward the DPPH· radical in dioxane (k_{DPPH} = 0.003 M^{-1} s^{-1}), indicating that the 5-OH group does not participate in radical quenching.[18]

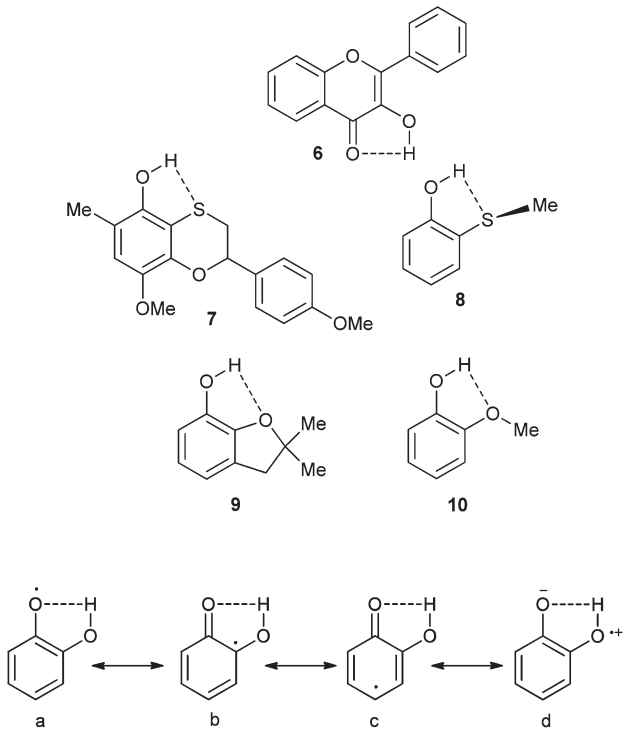


The concept of "neighbouring HBA group" extends also to interactions occurring through space, such as in the case of anthocyanins, proantocyanidins and ellagitannins (Schemes 2 and 3). To model this behaviour it is convenient to discuss the reactivity observed with bisphenols **3**–**5**. Although *ortho*-bisphenols **3** and **4** have two equivalent phenolic moieties, each being potentially able to quench/trap two peroxyl radicals, inhibited autoxidation experiments indicated that they efficiently trap only two peroxyl radicals, instead of four as expected from the isolated phenols and as observed with *para*-bisphenol **5**.[19] After analysis of the reaction products, this peculiar behaviour was explained by considering that one of the phenolic groups reacts with a peroxyl radical to form the corresponding phenoxyl radical, which traps a second peroxyl radical to yield the corresponding ketone. The latter, being a good HBA moiety, blocks the reactivity of the second hydroxyl group through the formation of a strong H-bond, as illustrated in Scheme 5.[19]

Weak intramolecular H-bonds. The reactivity of the H-bonded –OH functional groups may not be completely



Scheme 5 Reaction of ortho-bisphenols with peroxyl radicals. After the oxidation of one phenolic moiety, the reactivity of the second –OH group is hampered by the formation of a strong intramolecular H-bond.



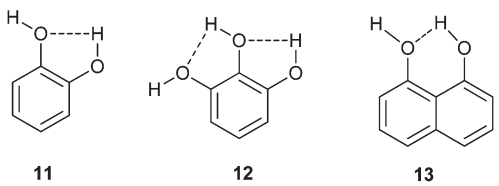
Scheme 6 Some resonance structures in the phenoxyl radical from catechol without (a–c) and with (d) charge separation.

hampered when H-bonding is weakened by some geometrical constrain. The enolic –OH group in the 3 position in flavones (see compound **6**) forms a relatively weak hydrogen bond with the neighbouring carboxyl group for geometrical reasons;[20] thus it is still reactive toward the DPPH· radical in dioxane (k_{DPPH} for **6** is 0.061 M^{-1} s^{-1}).[18] Another interesting example was found in our laboratory when studying heterocyclic thiaflavanes such as compound **7**. In **7** the strength of the intramolecular H-bond is significantly lower than in the simple *ortho* –SMe phenol **8**, because the –SR substituent is constrained to adopt a planar geometry by the presence of a cycle. In fact, chalcogen atoms heavier than oxygen require an orthogonal orientation of the alkyl group to accept a H-bond.[21] By exploiting this feature, we have designed and prepared the thiaflavane **7**, which showed the same BDE(O–H) (77.2 kcal mol^{-1}) and k_{inh} (3.4×10^6 M^{-1} s^{-1}) of α-tocopherol, the most active natural lipophilic antioxidant.[22]

Inclusion of the *ortho* –OR group in a penta-atomic ring in compound **9** causes a weakening of the intramolecular H-bond with respect to compound **10**. Its k_{inh} was determined as 2.4×10^4 M^{-1} s^{-1}, which is about 5-folds larger than that of **10**.[6]

1.2 Reactive OH group as H-bond acceptor

Catechol (**11**)[15] and pyrogallol (**12**)[23] – the most common structural motives in natural polyphenols – as well as synthetic naphthalene diols (*e.g.* **13**)[24] are chain-breaking antioxidants characterized by the presence of phenolic –OH groups acting both as H-bond donors (HBD) and acceptors (HBA).

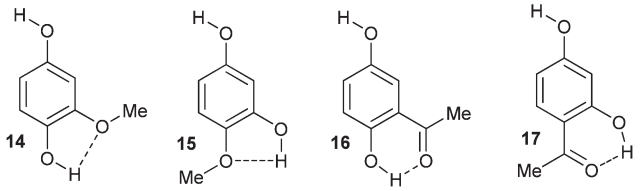


In such compounds, the intramolecular H-bond is not broken during the reaction with peroxyl radicals, so that the *ortho* –OH group stabilizes both the phenol and, to larger extent, the phenoxyl radical (as a consequence of electron polarization in the radical, *vide infra*). Therefore, catechol has a BDE(O–H) lower than 2-methoxyphenol (**10**) and than hydroquinone (1,4-dihydroxybenzene, see Table 1 and Scheme 4): its reactivity with peroxyl radicals is accordingly larger. The k_{inh} in PhCl at 30 °C for **11** is 5.5×10^5 M^{-1} s^{-1}, while that of **10** is 4.7×10^3 M^{-1} s^{-1} and that of hydroquinone is 1.8×10^5 M^{-1} s^{-1} (after statistical correction).[6,25]

The –O· group formed after H-atom abstraction has significant EW character, hence the H-bond donating ability of the bound –OH is increased,[26] while the –O· group itself is a much better H-bond acceptor than the hydroxyl group,[27] overall contributing to the larger stabilization of the phenoxyl radical compared to the phenol. The electron polarization in phenoxyl radicals due to the EW character of the –O· group is easily visualized by taking into consideration the resonance structure with charge separation (d) illustrated in Scheme 6.

1.3 Remote H-bond effects

The BDE(O–H) of phenols and their reactivity toward free radicals can be modulated by intramolecular H-bonds not involving the "reactive" –OH group. The BDE of the "free" –OH group in compound **14** is about 2.5 kcal mol^{-1} lower than that of its isomer **15**,[28] despite the fact that the intrinsic BDE-lowering effect of methoxyl and hydroxyl substituents distant from the reactive –OH is about the same, as can be seen by comparing 4-methoxy and 4-hydroxy phenols in Table 1.

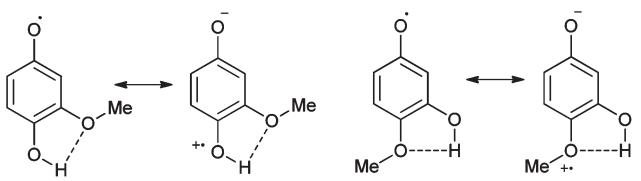


The lower BDE(O–H) of compound **14** stems from the very different electronic properties of –OH and –O$^{\cdot}$ groups, the former being a good ED and the latter a good EW group. Resonance structures with charge separation shown in Scheme 7 indicate that, in the phenoxyl radical originated from **14**, the remote –OH group is more electron deficient and thus a stronger HBD than it is in the parent phenol **14**.

Similarly, in the phenoxyl radical from **15** the remote –OMe group is also electron deficient, but since it acts as H-bond acceptor (HBA), in this case this will result in a weaker H-bond compared to that occurring in the parent phenol **15**. In other words, the occurrence of a distant intramolecular H-bond will decrease the BDE(OH) in compound **14** and increase it in compound **15**. H-atom abstraction by MeOO$^{\cdot}$ radicals was calculated to have activation enthalpy (E_a) of 2.1 and 3.8 kcal mol^{-1} respectively for **14** and **15**.[28] Remote H-bond effects are relevant also in polyphenols having EW substituents, such **16** and **17**. The BDE(O–H) of the "free" –OH of **16** and **17** is, respectively, 1.6 kcal mol^{-1} lower and 3.7 kcal mol^{-1} larger than expected from the, so called, "additive rule",[7,28] *i.e.* by summing the intrinsic individual contributions of substituents (ΔBDEs) on the BDE(O–H) of phenol itself.[5]

In general, remote H-bonding can either enhance or depress the antioxidant activity of phenols – corresponding respectively to decreasing or increasing the BDE(OH) – by modulating the ED or EW character of substituents in conjugated position (commonly in *para*) with respect of the reactive (or most reactive) –OH function.

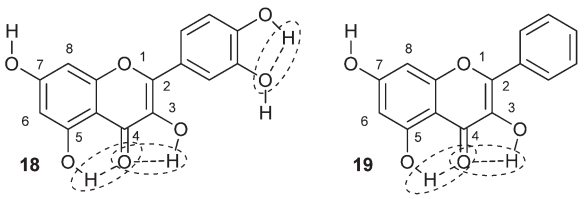
Consideration of these effects can help rationalizing the complex reactivity of natural flavonoids, where remote H-bonding and H-bonding to neighbouring (HBA or HBD) groups often co-exist in the same molecule. As noted above,



Scheme 7 Relevant resonance structures explaining the origin of remote H-bond effects.

7-hydroxyflavone (**1**) and chrysin (**2**) have identical reactivity toward DPPH$^{\cdot}$ radicals in dioxane. Indeed, in chrysin the –OH group in the 5-position is blocked by H-bonding to the neighbouring carbonyl, therefore, like in compound **17**, the "reactive" moiety is the –OH in position 7. In both compounds **1** and **2** the reactivity of –OH in 7 is somewhat depressed by the EW character of the *para* carbonyl group, however in compound **2** (but not in **1**) it should also be enhanced back by the weak ED character of the –OH in *meta*.[7] The fact that the two flavonoids **1** and **2** have the same reactivity[18] may actually be ascribed to the remote H-bonding of the meta –OH to the carbonyl group in compound **2**.

The complex interplay among non-covalent interactions in quercetin (**18**) may be used to summarize some of these concepts. The reactivity of the 7-OH is depressed if compared to the same OH group in compound **1**, because of the remote H-bond effect exerted (mainly) by the 5-OH. On the other hand, the 3-OH in quercetin is expected to be more reactive than in compound **6**, because the carbonyl, which is already accepting a H-bond from the 5-OH, is a relatively weaker HBA group (compared to the carbonyl in **6**). The k_{DPPH} in dioxane for quercetin analogue **19** is in fact 0.27 M^{-1} s^{-1}, *i.e.* about 4-folds larger than that of **6**. However, we suggest that the renown antioxidant behaviour of quercetin stems mainly from the catechol moiety,[24] whose reactivity is enhanced by H-bonding interaction between the two neighbouring hydroxyl groups (see also Scheme 4): in fact, k_{DPPH} for **18** in dioxane is 3.0 M^{-1} s^{-1}, *i.e.* one order of magnitude larger than that of **19**.[18]

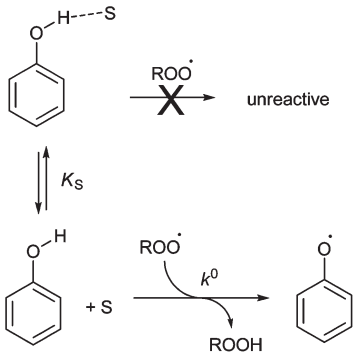


2. Intermolecular hydrogen bonds

The binding of polyphenols to proteins is at the basis of their historical use as tanning agents for the conversion of animal skins into leather.[3] Since the first suggestion that the binding of tea or chocolate flavonoids to milk proteins may lower their bioavailability and antioxidant activity *in vivo*,[29] several studies have been devoted to understanding the antioxidant activity of polyphenols in the presence of various biological molecules. However, contradictory results have been reported on different polyphenols or among the different methods used to evaluate the antioxidant activity, in particular when switching from assays employing artificial coloured radicals to tests based on inhibited peroxidation.[30] For a deeper understanding of these aspects, it is important to step back and rationalize the influence on the reactivity of simpler phenols toward peroxyl (and other) radicals of non-covalent interactions with a simpler ligand, which may be the solvent itself.

2.1 Reactive –OH group as H-bond donor: the "classical" kinetic solvent effect (KSE)

The first case of antioxidant activity affected by non-covalent interactions is probably that described by Howard and Ingold in



Scheme 8 Decrease of the antioxidant activity of phenols caused by H-bond donation by the phenolic OH group to a H-bond accepting solvent.

1964. When studying the autoxidation of styrene inhibited by various phenols, they realized that the inhibition decreased in the presence of polar solvents, the effect being more pronounced with unhindered than with 2,6-di-*tert*-butyl-substituted phenols, and with acidic more than with non-acidic phenols.[31] These effects were explained as being due to H-bond formation between the phenolic –OH group of the antioxidant and the solvent (acting as HBA). As shown in Scheme 8, only the fraction of phenol not H-bonded to a solvent molecule was suggested to be able to react with ROO$^{\cdot}$ radicals. While these important observations were somewhat overlooked at that time and radical reactions were generally regarded as being negligibly affected by the solvent (due to the lower polar character of transition states and intermediates as compared to heterolytic reactions), independent confirmation of solvent effects and their major role in the chemistry of antioxidants came some 30 years later.

Influence of the solvent on the inhibition of peroxidation is indeed a specific (albeit very relevant) case of a more general "kinetic solvent effect" (KSE) that holds for any formal H-atom transfer reaction from polar X-H groups to almost any radical,[32] regardless of its absolute reactivity (as long as the reaction has an activation barrier, *i.e.* it is not diffusion controlled), in a wide variety of solvents (exceptions will be described below). Quantitative treatment of KSE allowed to predict the rate constant k^S for the reaction between a phenol and a radical in virtually any solvent *S*, provided that the rate for the same reaction is known at least in one solvent, possibly in an apolar medium (*i.e.* alkanes) unable to establish H-bonding with the phenol. According to eqn (3),[33] if k^0 is the rate constant in a non H-bonding solvent, the rate constant in a different solvent (k^S) is a function of Abraham's thermodynamically-based solute parameters, α_2^H and β_2^H, which refer, respectively, to the relative HBD ability of the phenol and HBA ability of the solvent, or in general to the H-bonding abilities of two interacting species (the range for both the α_2^H and β_2^H scales is 0 to 1).[34]

$$\text{Log}\ (k^S/\text{M}^{-1}\ \text{s}^{-1}) = \text{Log}\ (k^0/\text{M}^{-1}\ \text{s}^{-1}) - 8.3\ \alpha_2^H\ \beta_2^H \quad (3)$$

Interestingly, Abraham α_2^H and β_2^H parameters refer to 1 : 1 H-bonding events in diluted solutions, so eqn (3) implies that KSE does not depend on the dielectric constant of *S*, but only on its ability to form 1 : 1 complexes with the phenolic –OH group.[32] As hundreds of α_2^H and β_2^H values are reported in the literature, in practice eqn (3) allows us to calculate k^S in a wide range of conditions from a single kinetic measurement. A selected list of such parameters for the most common solvents and inhibitors encountered in the chemistry of antioxidants is reported in Table 2 and 3.

The KSE is independent of the abstracting radical (as can be seen from eqn (3)), therefore, according to eqn (4), the ratio

Table 2 Abraham α_2^H and β_2^H parameters for common solvents and biologically relevant functional groups

	α_2^H [a]	β_2^H [a]
Alkanes	0	0
Carbon tetrachloride	0[b]	0[b]
Benzene	—	0.14
Chlorobenzene	—	0.09
Diethylether	—	0.45
Dioxane	—	0.47
Tetrahydrofuran	—	0.51
Water	0.35	0.38
Methanol	0.36	0.41
Ethanol	0.33	0.44
tert-Butanol	0.32	0.49
Hexafluoropropan-2-ol	0.77	—
Acetonitrile	—	0.44
Acetone	—	0.50
Dimethylsulfoxide	—	0.78
Pyridine	—	0.62
Dimethylformamide	—	0.66
Propylamine	—	0.70
Butyric acid	0.54	0.42
Ethyl acetate	—	0.45

[a] Unless otherwise stated, data are from ref. 34. Dashes indicate that the relevant α_2^H or β_2^H values are not reported. [b] The values for CCl_4 are assumed to be zero since it is the reference solvent for the Abraham scale.

Table 3 Abraham α_2^H and β_2^H parameters for selected molecules with antioxidant action

	α_2^H [a]	β_2^H [a]
Phenol	0.60	0.22
4-Methoxyphenol	0.57	—
4-Methylphenol	0.57	0.24
4-Acetylphenol	0.72	—
α-Tocopherol	0.37[b]	—
2,6-*t*Bu-4-Me-phenol	0.22[c]	—
2-OMe phenol	0.26[b]	—
Catechol	0.73[d]	—
5-Pyrimidinol	0.8[e]	—
2,4,6-Me_3-5-pyrimidinol	0.7[e]	—
2-Me_2N-5-pyrimidinol	0.55[f]	—
6-Me_2N-3-pyridinol	0.50[f]	—
Phenylamine	0.26[b]	—
Diphenylamine	0.32	—
$(PhCH_2)_2NOH$	0.45	—
TEMPOH[g]	0.39[c]	—
Trpt-SOH[h]	0.7[i]	—

[a] See note *a* in Table 2. [b] Data from ref. 33. [c] Data from ref. 35. [d] Data from ref. 24. [e] Estimated from eqn (6) and kinetic data in ref. 36. [f] Estimated from eqn (6) and kinetic data from ref. 37. [g] 2,2′-6,6′-Tetramethylpiperidine-1-ol. [h] trpt = triptycene. [i] Value estimated by comparing the k_{inh} decrease in autoxidation studies from ref. 38 to that of catechol.

$k^{S1}_{(AH/X^{\cdot})}/k^{S2}_{(AH/X^{\cdot})}$ for the reaction between and antioxidant AH and the radical $X^{\cdot}$ in two different solvents S1 and S2 is expected to be identical to the relative rate of reaction of the same antioxidant in the same solvents, with a different radical species $Y^{\cdot}$.

$$KSE = k^{S1}_{(AH/X^{\cdot})}/k^{S2}_{(AH/X^{\cdot})} = k^{S1}_{(AH/Y^{\cdot})}/k^{S2}_{(AH/Y^{\cdot})} \quad (4)$$

By combining eqn (4) with previous eqn (3) we get, for any abstracting radical, eqn (5) and (6) which allow one to obtain the decrease in the rate constant, and the α_2^H value for the antioxidant, respectively.

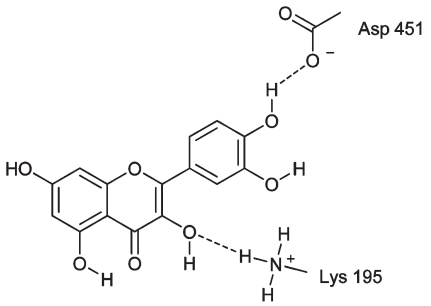
$$\text{Log } (k^{S1}/k^{S2}) = 8.3\ \alpha_2^H\ (\beta_2^{H\,S2} - \beta_2^{H\,S1}) \quad (5)$$

$$\alpha_2^H = [\text{Log } (k^{S1}/k^{S2})]/8.3(\beta_2^{H\,S2} - \beta_2^{H\,S1}) \quad (6)$$

The latter equation may be used to identify the reactive moiety in polyfunctional antioxidants,[39] as structurally different phenols or phenolic –OH groups possess characteristic α_2^H values. For instance, on the basis of the data reported in Table 3, *ortho*-di-methylphenols and *ortho*-methoxyphenols are expected to show a smaller KSE than catechols. As can be guessed from the logarithmic form of eqn (3), the absolute magnitude of the KSE can be very large for good H-bond donors like most natural polyphenols, with k_{inh} ranging over orders of magnitude for the same antioxidant in different media: this point should not be overlooked when comparing the antioxidant performance of natural or synthetic compounds recorded under different settings.

KSE theory is based on the experimental finding that abstracting radicals are not significantly complexed by the solvent (or that complexation doesn't affect their intrinsic reactivity),[32] and that alternative reaction mechanisms are not occurring. The $DPPH^{\cdot}$ radical, which is widely used to estimate the antioxidant activity, was found to follow the predicted behaviour only in non-alcoholic media (*vide infra*).[8] Indeed, its reactivity with 1,4-cyclohexadiene (unable to donate H-bonds, $\alpha_2^H \sim 0$) was unaffected by the solvent,[40] while the rate constant for its reaction with α-tocopherol ($\alpha_2^H = 0.37$, see Table 2) spanned over two orders of magnitude, nicely matching the KSE recorded for reaction of α-tocopherol with peroxyl radicals, with alkoxyl radicals or with alkyl radicals in the same set of solvents, as predicted by eqn (4) and despite the fact that the absolute value of those rate constants, on moving from $DPPH^{\cdot}$ to alkoxyl radicals, differs by 10 orders of magnitude.[41]

Concerning peroxyl radicals, if we exclude the very strong H-bond donating fluorinated alcohols,[42] their intrinsic reactivity also seems to be not significantly influenced by the solvent. For instance, the rate constant for the reaction between cumylperoxyl radicals and cumene is about the same in isooctane, benzene, acetonitrile, *tert*-butanol or pyridine ($k_p = 0.82 \pm 0.10\ M^{-1}\ s^{-1}$ at 50 °C).[43] Nonetheless their reaction with antioxidants is progressively hampered as the HBA ability (*i.e.* the β_2^H value) of the solvent increases (*vide supra*).[31,41,44] For instance, quercetin and (−)-epicatechin in the moderately hydrogen-bonding solvent chlorobenzene had k_{inh} values at 50 °C of $4.3 \times 10^5\ M^{-1}\ s^{-1}$ and $4.2 \times 10^5\ M^{-1}\ s^{-1}$ respectively, nearly identical to that of catechol. In the protic solvent *tert*-butanol, the values were $2.1 \times 10^4\ M^{-1}\ s^{-1}$ and $1.7 \times 10^4\ M^{-1}\ s^{-1}$, respectively, with a KSE of about 20.[45] Under identical conditions, the reactivity of α-tocopherol decreased only by a factor of 4. Large KSE on k_{inh} were also measured for catechol itself.[46]§



Scheme 9 Proposed binding mode of quercetin to human serum albumin (ref. 47 and 48).

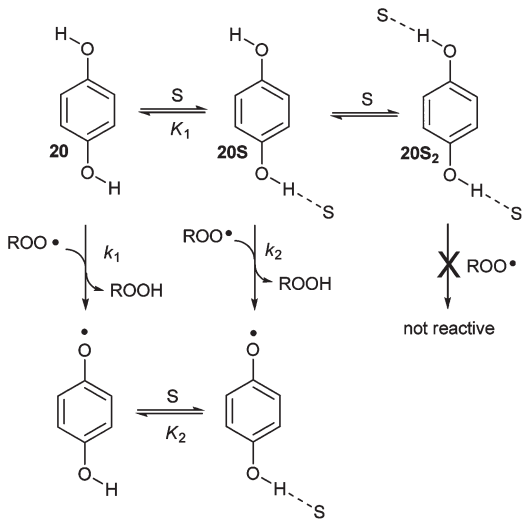
The decrease in antioxidant activity observed when polyphenols are non-covalently bound to biomolecules can be explained on a similar basis. Naringenin and hesperetin, together with their glucosides, are the main polyphenol-type (flavanone) antioxidants found in citrus fruits and juices. It has recently been shown that they undergo non-covalent interaction with human serum albumin with binding constants as high as $3–9 \times 10^4\ M^{-1}$, main through H-bonding to histidine, lysine and aspartate residues, with consequences in their plasma half-life and tissue distribution.[47] Quercetin has been proposed to bind non-covalently to albumin, by forming H-bonds as shown in Scheme 9.[47,48]

Similar non-covalent interactions of several flavonoid components of green and black tea with α-, β- and κ-casein and albumin masked (*i.e.* decreased) their antioxidant activity as assessed by their ability to react with the 2,2′-azinobis(3-ethylbenzo-thiazoline-6-sulfonic acid) radical ($ABTS^{\cdot}$).[30c] Using the same approach, Riedl and Hagerman similarly found that bovine serum albumin (BSA) and calf-skin gelatine decreased the antioxidant activity of an oligomeric procyanidin, PC (a tannin comprising 17 flavonoid units), isolated from *Sorghum bicolor*.[30b] Interestingly the authors reported that PC-protein non-covalent interaction caused temporary precipitation of the complex and that oxidation of the PC-gelatine complex resulted in covalent binding of the two units.[30b]

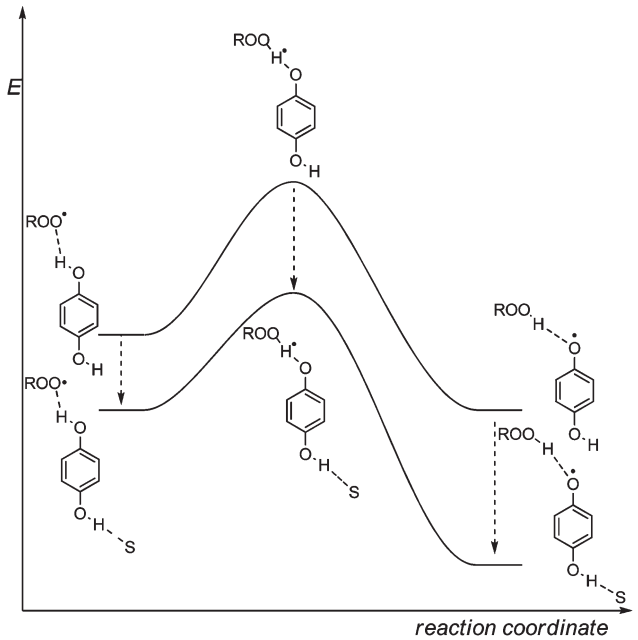
2.2 Remote kinetic solvent effect (RKSE)

An interesting case of KSE occurs in phenols in which the solvent interacts with a functional group distant from the reactive –OH. The reactivity of 2,5-di-*tert*-amylhydroquinone (**20**) with $ROO^{\cdot}$ or $DPPH^{\cdot}$ radicals in CCl_4 solution was increased by small additions of H-bond accepting (HBA) solvents, such as MeCN or DMSO, then it decreased at larger concentration of the HBA solvents.[49] After fitting the kinetic results to the reaction Scheme 10, it was evident that the mono-H-bonded species **20S**

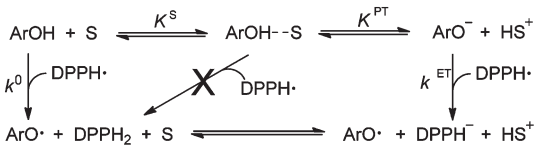
§ When the reactivity of antioxidants with peroxyl radicals in a HBA solvent is measured by the inhibited autoxidation method, the observed KSEs, although often quite large, are however smaller than expected on the basis of eqn (3), due to the fact that the solution contains as much as 50% v/v of the oxidizable substrate, generally a moderately HBA hydrocarbon; hence the solvent is in fact a co-solvent.



Scheme 10 Equilibria underpinning remote kinetic solvent effect (RKSE). The mono-solvated specie **20S** reacts faster than **20**, while **20S$_2$** is unreactive. Alkyl groups of 2,5-di-*tert*-amylhydroquinone have been omitted for clarity.



Scheme 11 Energy diagram for H-atom abstraction from a hydroquinone donating a H-bond to a solvent molecules (S).



Scheme 12 Sequential proton loss electron transfer (SPLET) mechanism for the formal H-atom transfer from phenols to the DPPH$^{\cdot}$ radical.

reacted 5- to 10-fold faster than **20**, while, as expected, **20S$_2$** was unreactive toward H-atom abstraction. The kinetic acceleration (k_2/k_1) was larger with DMSO than with MeCN. From the k_2/k_1 ratio, the BDE of the "free" –OH in **20S** was found to be lower than that of the –OH groups in **20** by −1.0 and −2.0 kcal mol^{-1} after H-bonding to MeCN and DMSO, respectively.[49] The reason for the lower BDE(O–H) of **20S** compared to **20** is the fact that the semiquinone radical forms stronger H-bonds with the solvent molecules than the parent hydroquinone, because α_2^H of phenols increases with the electron-withdrawing ability of ring substituents. As previously discussed, while the –OH group is an electron donor, the –O$^{\cdot}$ group is a quite strong electron attractor, comparable to a –NO$_2$ group.[14] The observed acceleration $k_2 > k_1$ implies that this preferential stabilization is transmitted to the transition state for H-atom abstraction. The overall picture of the RKSE is given in Scheme 11.

The RKSE can be also predicted on the basis of the α_2^H and β_2^H parameters of the involved species. Equilibrium constants, K_{HB}, for the formation of intermolecular H-bonds at 25 °C in CCl_4 can be calculated from eqn (7).[34c] Converting eqn (7) into free energies for H-bond formation, ΔG_{HB}, yields eqn (8).

$$\text{Log}\ (K_{HB}\ \text{M}^{-1}) = 7.354\ \alpha_2^H\ \beta_2^H - 1.094 \qquad (7)$$

$$\Delta G_{HB}/\text{kcal mol}^{-1} = -10.03\ \alpha_2^H\ \beta_2^H + 1.492 \qquad (8)$$

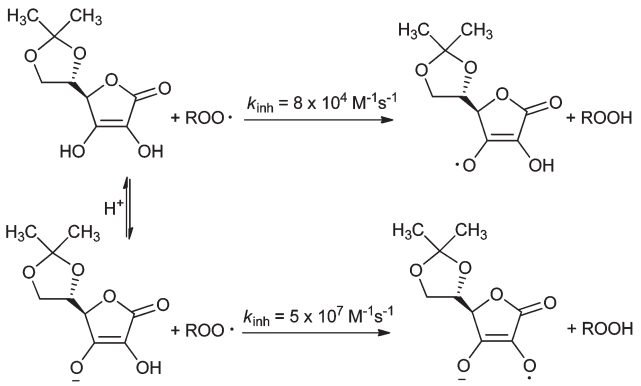
From the α_2^H of the alkylated semiquinone (~0.80),[50] the free energy of H-bonding ΔG_{HB} between the semiquinone radical and the solvent can be calculated as −2.0 and −4.8 kcal mol^{-1} respectively for MeCN and DMSO. When combining these numbers with the ΔG_{HB} for H-bonding of the parent hydroquinone (α_2^H = 0.57),[49] calculated by eqn (8) as −1.0 and −3.0 kcal mol^{-1} for MeCN and DMSO, respectively, the preferential stabilization of the radical with respect of that of the hydroquinone can be estimated as −1.0 and −1.8 kcal mol^{-1} for MeCN and DMSO.[51]¶ In general, RKSE may increase or decrease the reactivity of an antioxidant toward free radicals, depending on whether the strength of the intermolecular H-bond increases or decreases along the reaction coordinate (see for instance the case of remote intra-molecular hydrogen bonds reported in section 1.3).

2.3 Other solvent effects

Acid-base catalysis. Deviations from the behaviour expected from the "classical" KSE model illustrated in Scheme 8 were observed when reacting phenols with DPPH$^{\cdot}$ in alcoholic solvents. Larger than expected rate constants in alcohols have been rationalized as arising from a substantially different reaction mechanism, consisting in a two-steps sequential proton-loss electron-transfer (SPLET), illustrated in Scheme 12 and recently reviewed.[10]

Acidic H-bonded phenols, although unable to transfer the H-atom to DPPH$^{\cdot}$, could transfer the proton to the solvent itself and then undergo a very fast electron transfer to the electron

¶For H-bonds of moderate strength, the ΔS_{HB} is aproximately constant, −11 cal mol^{-1} K^{-1}.[51] The difference between ΔG_{HB} in the radical and in the parent phenol can therefore be used to estimate variations in phenolic BDE.



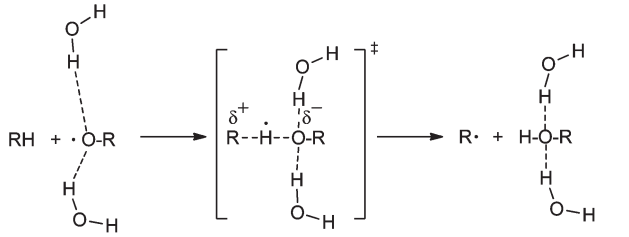
Scheme 13 Rate constants in MeCN for the reaction of peroxyl radicals with an ascorbic acid derivative in its neutral and anionic form (data from ref. 53).

deficient DPPH· radical, resulting in acceleration rather than impairment of the reaction.[10] As an example, in methanol the flavonoids morin and quercetin were reported to react with DPPH· with rate constants about 1000-fold higher than in dioxane (having similar HBA ability), thanks to the SPLET mechanism.[18]

However, it is unclear whether such mechanism plays a role also in the reaction of antioxidants with peroxyl radicals. When tested in *tert*-butanol, the reactivity of quercetin with peroxyl radicals was much lower than in apolar solvents, as expected from the classical KSE (*vide supra*).[45] Accelerations of the reactions with ROO· in the presence of bases have been clearly established only in the case of poly-hydroxylated antioxidants (caffeic acid,[52] ascorbic acid,[53] 5-hydroxy-6-methyluracil[54]), however, in these cases ROO· can abstract a H-atom from the electron rich anion of the antioxidant, as recently proven to occur in the case of ascorbic acid derivatives (Scheme 13).

Quite surprisingly, and on the contrary to what was observed with DPPH· radicals, we found that the reaction between ROO· radicals and some phenolic and non-phenolic antioxidants is significantly accelerated by the addition of weak organic acids in MeCN.[53,55] This was attributed to a sequential reaction mechanism consisting in a fast electron transfer from the solvent-H-bonded antioxidant to the electron-poor protonated peroxyl radical (cation).[55] Further investigation is ongoing in our group to clarify the role and mechanism of acid and basic catalysis (equivalent to that occurring in the SPLET mechanism) on the chain-breaking antioxidant behaviour of phenols and polyphenols.

Solvent effects on the H-atom abstracting radical. We have previously stated that alkoxyl radicals, such as cumyloxyl, behave as depicted by the classical KSE model (Scheme 8 and eqn (3)), *i.e.* they do not change their intrinsic reactivity upon interaction with different solvents, and any KSE observed in their H-abstraction from a substrate depends on H-bonding of the solvent with the substrate itself. As a consequence they experience no KSE when reacting with a hydrocarbon (that cannot act as HBD toward the solvent).[32] This experimental observation has recently been challenged by the finding that C–H hydrogen-abstraction by akoxyl radicals from 1,4-



Scheme 14 Acceleration of the reaction of alkoxyl (and hydroxyl) radicals by H-bond formation with a H-bond donating solvent.

cyclohexadiene (CHD), tetrahydrofuran, (THF) and other substrates would be sensitive to the polarity of the solvent.[56] El-Sheshtawy *et al.* reported that the rate constant for reaction of cumyloxyl radicals with those hydrocarbons *decreases* by *ca.* 5% to *ca.* 25% on passing from less polar ethyl acetate to more polar acetonitrile, despite the fact that the two solvents have the same HBA ability, and attributed it to preferential stabilization of the polar alkoxyl radical as compared to the transition state (TS), resulting in higher activation barrier.[56] Interestingly, however, Bietti and co-workers came to almost opposite conclusions on studying the same reactions in a broader range of solvents.[57] They found that the rate of the reaction between cumyloxyl (or benzyloxyl) radicals and 1,4-cyclohexadiene or cyclohexane was identical within experimental error in solvents of largely different polarity (from isooctane to acetonitrile), while the rate constant *increased* slightly (by a factor or 2 or less) but significantly in alcohols (*tert*-butanol or methanol), and the acceleration became more meaningful (a factor of 3.5–4) in the strong HBD solvent 2,2,2-trifluoroethanol.[57] The higher reaction rate in HBD (alcoholic) solvents was attributed to hydrogen bonding from the solvent to the oxygen of the alkoxyl radical, since such a H-bonding interaction would become progressively stronger as the reaction progresses, thereby stabilizing the TS more than the reactants.

Due to their much higher reactivity and lack of selectivity, the reaction with hydroxyl radicals is normally of limited interest in the chemistry of antioxidants. However it is interesting to note that a qualitatively similar KSE was recently documented on C–H atom-abstraction by hydroxyl radicals. Tanko and co-workers showed that the rate of reaction of hydroxyl radicals with a variety of hydrocarbons was larger by as much as 2 orders of magnitude in water than in polar aprotic solvents like acetonitrile, and attributed this massively enhanced reactivity to stabilization of the dipolar transition state by H-bonding, as illustrated in Scheme 14.[58]

In summary, deviations from the classical KSE model, due to modulation of the reactivity of the abstracting radical, can be expected in protic solvents due to H-bond donation to the (δ^-) O–X moiety in the TS. However, with the exception of hydroxyl radicals (X = H), such deviations might be relevant only in the presence of strong HBD solvents/chemicals like 2,2,2-trifluoroethanol.

Conclusions

Natural polyphenolic antioxidants are still attracting the attention of many researchers because of their importance in human

health and because of the large dietary exposure of population to these nearly ubiquitous phytochemicals. While for mono-phenolic antioxidants structure–activity relationships have been established within a robust framework of kinetic and thermodynamic studies, less is known about natural polyphenolics, due to their more complex multifunctional structure and the major role that non-covalent interactions play on the their reactivity, as accounted for in this perspective. As we have outlined, their antioxidant behaviour cannot be fully rationalized unless interactions with the surrounding medium are carefully considered. This is especially true for complex biological environments, where, beside water itself, a multitude of H-bonding ligands are available to modulate antioxidants' reactivity.

Acknowledgements

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