Reactivity of hydroxynaphthalenes towards peroxyl radicals[†]

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ABSTRACT: In the present work we report data bearing on the reaction of mono- and dihydroxynaphthalenes towards 2,2′-azo-bis(2-amidinopropane) dihydrochloride (AAPH) derived peroxyl radicals. A comparison of the bleaching rates allows establishing their relative reactivity towards peroxyl radicals: 1,2-dihydroxynaphthalene (1,2-DHN) $\gg$ 2,3-dihydroxynaphthalene (2,3-DHN) > 1,3-dihydroxynaphthalene (1,3-DHN) $\geq$ 2,7-dihydroxynaphthalene (2,7-DHN) > 1-hydroxynaphthalene (1-N) $\geq$ 2-hydroxynaphthalene (2-N). Bleaching rates measured under conditions of quantitative trapping of peroxyl radicals allow an estimation of the number of molecules consumed per each radical introduced into the system. The results obtained imply a chain consumption of 1,2-DHN and 2,3-DHN. In fact, a fast autoxidation of 1,2-DHN is observed even in absence of AAPH. The high reactivity of this compound suggests a fast hydrogen abstraction, due to its low O—H bond dissociation energy (BDE), and/or a very fast electron transfer from the deprotonated form. Oxygen radical absorbance capacity (ORAC) indexes of the naphthalene derivatives were evaluated from their effect upon the peroxyl radical promoted bleaching of *c*-phycocyanin (*c*-Pc). ORAC values are strongly influenced by the secondary reactions of the additive and do not correlate with the reactivity of the compound or the number of the naphthalene derivative molecules bleached per each radical. *c*-Pc bleaching rate in presence of an excess of hydroxyl-naphthalene derivative was taken as a measure of the damaging capacity of the corresponding naphthoxyl radicals. The results indicate that this damaging capacity is inversely proportional to the reactivity of the parent compound. In fact, addition of 2-naphthol, the less reactive of the tested compounds, increases the rate of *c*-Pc bleaching promoted by peroxyl radicals. Copyright © 2006 John Wiley & Sons, Ltd.

KEYWORDS: peroxyl radicals; hydroxynaphthalenes; ORAC; c-phycocyanin

INTRODUCTION

The reactivity of phenolic compounds towards free radicals is a matter of current interest that mainly arises from their potential use as antioxidants.[1,2] Furthermore, since phenolic compounds are persistent pollutants, their possible removal from the environment through free radical-mediated processes is also a matter of interest.[3–5] Extensive studies have been carried out in different families of phenolic compounds, such as flavonoids,[6,7] polysubstituted benzene derivatives,[8] and hydroxylated cynnamic acids.[9–11] These studies comprise several free radicals, in particular peroxyl radicals. On the other hand, few studies have been carried out employing other aromatic compounds, such as naphthalene, phenanthrene, or anthracene derivatives. In particular, in a previous work we have shown that 1-naphthol is considerably more reactive than quercetin,[12] a well known antioxidant

and free radical scavenger,[13,14] and Foti *et al.* (2002) have shown that 1,8-dihydroxynaphthalene is a potent H-transfer and antioxidant.[15,16] Recently, Flueraru *et al.*[17] have shown that, regarding oxidative stress induced by peroxyl radicals, cytotoxicity and cytoprotective activity in dihydroxynaphthalenes depends on their tendency to form naphthoquinones. In the present work we report data bearing on the reactivity towards peroxyl radicals of various hydroxynaphthalenes. The compounds considered comprise mono hydroxy derivatives (1- and 2-naphthol) and dihydroxy derivatives (1,2-, 1,3-, 2,3-, and 2,7-dihydroxynaphthalene). Cyclic voltammetry measurements were performed in order to estimate the O—H bond dissociation energy (BDE) in these compounds.

EXPERIMENTAL

Chemicals

AAPH (2,2′-azo-bis(2-amidinopropane) dihydrochloride) was employed as peroxyl radical source. *c*-Phycocyanin (*c*-Pc), Trolox (6-hydroxy-2,5,7,8-tetramethyl chroman-2-carboxylic acid), 1,2-dihydroxynaphthalene (1,2-DHN),

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1,3-dihydroxynaphthalene (1,3-DHN), 2,3-dihydroxy-naphthalene (2,3-DHN), and 2,7-dihydroxynaphthalene (2,7-DHN), 1-naphthol (1-N), 2-naphthol (2-N), and AAPH were purchased from Sigma-Aldrich (St. Louis, MO) and employed as received.

Naphthalene derivatives oxidations

Oxidations were promoted by peroxyl radicals generated in AAPH thermolysis under aerobic conditions. Solutions containing the tested naphthalene derivative and 10 mM AAPH in phosphate buffer, pH 7.0, were incubated at 37 °C in the thermostatized cuvette of a Fluorolog Spex 1681 spectrofluorimeter. Consumption of the naphthalene derivatives was evaluated by the decrease of their fluorescence, measured at the maximum of the emission band.

Autoxidation of 1,2-DHN (50 μM) was evaluated from the decay of its UV absorbance, measured at 232 nm, in 10 mM phosphate buffer, pH 7.0, at 25 °C.

Competitive kinetics experiments

Solutions containing *c*-Pc with or without the tested hydroxynaphthalene in phosphate buffer (10 mM) at pH 7.0, were incubated at 37 °C in the thermostatized cuvette of a Fluorolog Spex 1681 spectrofluorimeter. The reaction was initiated by addition of a small aliquot (50 μL) of AAPH (10 mM final concentration). The consumption of the target molecule (*c*-Pc) was evaluated from the decrease in its fluorescence intensity (excitation: 620 nm; emission 640 nm) quantified by the ratio between the observed fluorescence intensity (F) and that measured at zero reaction time (F^0). Solutions of the corresponding hydroxynaphthalene were prepared immediately before the experiments by dilution of an ethanolic solution with phosphate buffer (EtOH < 1%).

Oxygen radical absorbance capacity (ORAC) determinations

c-Phycocyanin bleaching, elicited by its incubation in presence of AAPH, was estimated from fluorescence kinetics profiles. Integration of the area under the curve (AUC) was performed up to a time such that (F/F^0) reached a value of 0.2. These areas were employed to obtain ORAC values,[18,19] defined by:

$$\text{ORAC} = \frac{(\text{AUC} - \text{AUC}^0)}{(\text{AUC}_{\text{TROLOX}} - \text{AUC}^0)} \left(\frac{[\text{Trolox}]}{[\text{Additive}]} \right) \quad (1)$$

where AUC, AUC^0, and $\text{AUC}_{\text{TROLOX}}$ are the area under the curve in presence of the tested naphthalene derivative, the area under the curve for the control

sample, and the area under the curve in presence of Trolox, respectively.

BDE estimations. Bond dissociation energies of the tested compounds were calculated from their oxidation potentials measured by cyclic voltammetry (scan rate = 100 mV/s) in 100 μM solutions in phosphate buffer (50 mM) with KCl 0.1 M at pH 7.0. The experiments were performed with a Wenking PO753 instrument. A glassy carbon stationary electrode was used as working electrode, and a platinum wire as counter electrode. All potentials were measured against a saturated calomel electrode (SCE) under an atmosphere of pure (dry) Nitrogen. BDE were estimated according to Bordwell *et al.*[20]

$$\text{BDE} = 1.37 \, pK_a + 23.06 \, E_{\text{ox}} + C \quad (2)$$

where C is a constant that takes into account the change in entropy associated to the electrochemical oxidation.

RESULTS

Peroxyl radicals were produced by the thermolysis of AAPH.[21] A 10 mM solution of this compound, heated to 37 °C in air-saturated solutions, produces peroxyl radicals with a rate of 0.75 μM/min.[22] These peroxyl radicals are readily trapped by micromolar concentrations of 1-naphthol and other hydroxylated naphthalene derivatives. Different experiments were carried out in order to establish the relative reactivity of these compounds, the stoichiometry of the process (i.e., the average number of additive molecules removed per each peroxyl radical), and the reactivity of the naphthoxyl radicals produced after the initial interaction between the hydroxynaphthalene and peroxyl radicals:

1 Bleaching of the naphthalene derivatives over a wide range of concentrations, and
2 Protection of *c*-Pc by the additives (competitive experiments).

In the first type of experiments, simple kinetic considerations allow to predict that:

1 The initial naphthalene derivative bleaching rate, measured at low substrate concentration, must be a first order process. The consumption rate evaluated under these conditions is a measure of the compound reactivity;
2 Zero order kinetics should hold at high substrate concentrations. The substrate concentration required to reach these conditions is inversely proportional to the reactivity of the compound towards peroxyl radicals increases; and
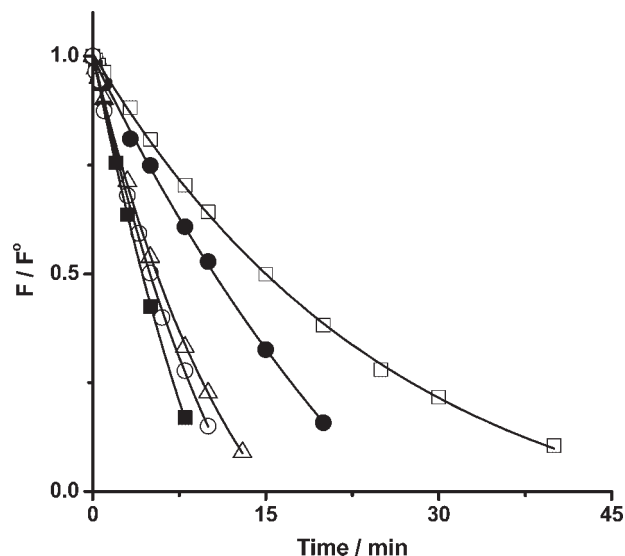3 The rate of substrate consumption in the zero order kinetics range allows an evaluation of the number of

Figure 1. Consumption of the hydroxylated naphthalenes (5 µM) elicited by AAPH (10 mM) derived peroxyl radicals. The data are given as (F/F^0) values as a function of time (F is the naphthalene derivative fluorescence at the time considered, and F^0 is the fluorescence at zero reaction time). Data obtained for: 2,3-DHN (■); 2,7-DHN (○); 1,3-DHN (△); 1-N (●); and 2-N (□). Temperature: 37 °C
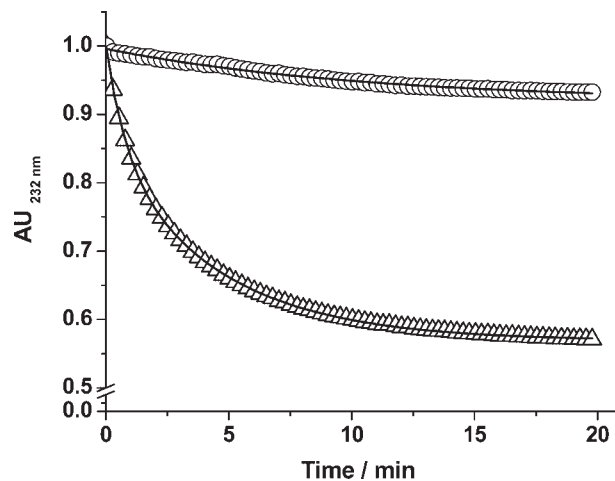

Figure 2. Autoxidation of 1,2-DHN evaluated by the absorbance decay, measured at 232 nm. Data obtained in air-saturated solution (△) or nitrogen-saturated solution (○). Phosphate buffer (10 mM, pH 7.0). Temperature: 25 °C

molecules of the free radical scavenger bleached per radical introduced into the system (n).

In competitive experiments, the protection afforded to a target molecule, such as c-Pc, would be related to the reactivity of the naphthalene derivative, but can be influenced by the secondary reactions of the free radical scavenger derived radicals.

The initial slope (IS) of a R_i *versus* substrate concentration plot (Fig. 3) is given by

$$IS = k[\text{ROO}^\cdot]_{ss} \quad (4)$$

where k is the weighted average of the specific rate constants of process (5) and/or (6).

$$\text{ROO}^\cdot + \text{Naph} - \text{OH} \rightarrow \text{ROOH} + \text{Naph} - \text{O}^\cdot \quad (5)$$

$$\text{ROO}^\cdot + \text{Naph} - \text{O}^- \rightarrow \text{ROO}^- + \text{Naph} - \text{O}^\cdot \quad (6)$$

In fact:

$$k = f_{\text{Naph-OH}}k_5 + f_{\text{Naph-O}^-}k_6 \quad (7)$$

Bleaching of the hydroxylated naphthalene derivatives by peroxyl radicals

Peroxyl radicals readily bleach all tested hydroxy-naphthalenes. Typical data are shown in Figure 1. These data show that incubation with AAPH readily promotes the naphthalene derivatives consumption. Even more, 1,2-DHN is readily consumed in absence of the free radical source (Fig. 2).

The initial rate of a given naphthalene derivative consumption (R_i) increases with the substrate concentration, reaching a plateau at high concentrations. Typical data are shown in Figure 3. A double reciprocal plot of these data allows an evaluation of n from the ordinate:

$$n = (R_{\text{Rad}} \times \text{Ordinate})^{-1} \quad (3)$$

where R_{Rad} is the rate of radical production (0.75 µM/min). The values of n obtained by this procedure are collected in Table 1.
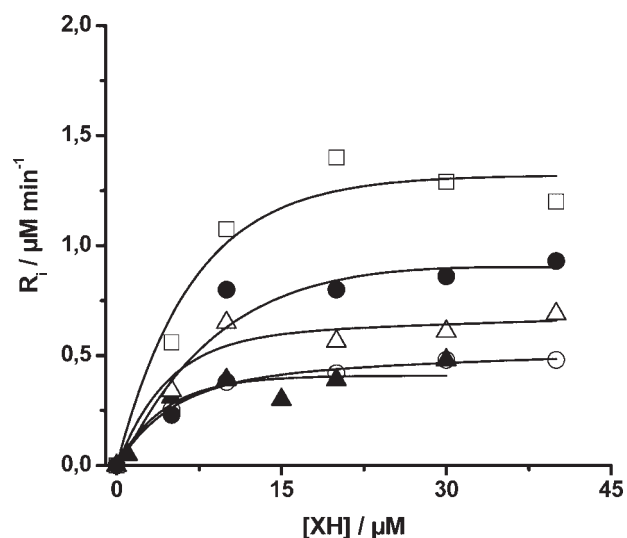

Figure 3. Initial rate of the naphthalene derivative consumption elicited by 10 mM AAPH as a function of the tested compound concentration. 2,3-DHN (□); 2,7-DHN (●); 1,3-DHN (△); 2-N (○); and 1-N (▲). Temperature: 37 °C

Table 1. Values of the number of hydroxynaphthalene molecules removed per each peroxyl radical introduced into the system (n) and relative rates of reaction with peroxyl radicals (k)

Compound	n	k^{a} (Arbitrary units)
1-N	0.6	7.5
2-N	0.6	6.8
1,3-DHN	0.9	10.9
2,7-DHN	1.1	9.8
2,3-DHN	1.7	16.6
1,2-DHN	5.0[c]	51[d]
Caffeic acid[b]	0.7	1.8

[a] k values obtained from the initial slope of plots such as those shown in Figure 3.
[b] Data from Ref. 28
[c] Obtained from the induction time elicited in the AAPH promoted bleaching of c-Pc.
[d] Obtained from the slope of c-Pc bleaching in the inhibited region, and employing 1,3-DHN as reference compound.

where $f_{\text{Naph—OH}}$ and $f_{\text{Naph—O}^-}$ are the fraction of the naphthalene derivative fully protonated or deprotonated at the working pH, respectively. IS values, which under the present experimental conditions are proportional to k, are collected in Table 1.

The fast autoxidation of 1,2-DHN precludes a meaningful evaluation of k and n by the previously described procedures. For this compound, these parameters were estimated from data related to the protection afforded to c-Pc (see following).

c-Phycocyanin bleaching by peroxyl radicals: protection by hydroxynaphthalenes

The bleaching rate of c-Pc by peroxyl radicals can be modified by the presence of compounds able to react with the primary radicals. Phycobiliproteins, such as c-Pc and phycoerythrine,[18,23] have been employed in order to evaluate the free radical scavenging capacity of several compounds.[18,19] The presence of the additive modifies the initial rate of c-Pc bleaching and the time required to bleach totally (or in a given percentage) the target molecule (ORAC type methodologies).[18,19,24] We have employed both approaches to estimate the protective capacity of the hydroxylated naphthalenes considered in the present work.

The results given in Figure 4 show that c-Pc consumption profiles are extremely dependent of the employed naphthalene derivative. A comparison between data obtained employing 1,2-DHN and 1,3-DHN shows that the effect elicited by 1,3-DHN lasts for a longer time, while the initial protection is more marked with the former compound. This would indicate that 1,2-DHN is more reactive but it is readily consumed, leading to an ill-defined induction time. This conclusion is further supported by the fact that the induction time increases
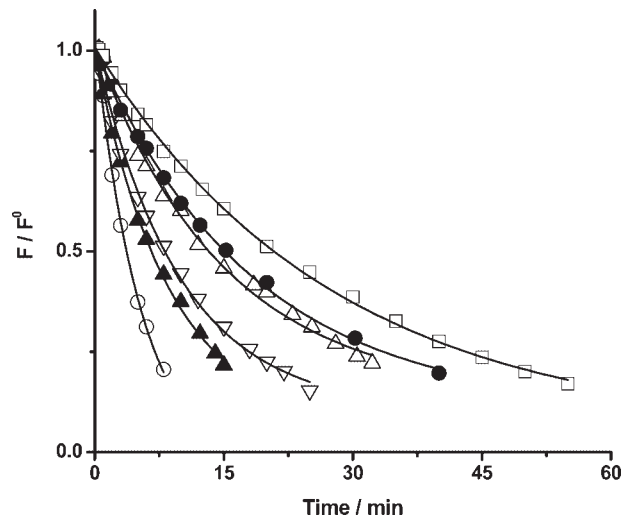

Figure 4. Bleaching of c-Phycocyanin (0.01 mg/mL) elicited by AAPH-derived peroxyl radicals (10 mM) in presence of different 2,3-DHN concentrations. Control (○); 2,3-DHN: 5 μM (▲); 10 μM (▽); 30 μM (△); 50 μM (●); and 100 μM (□). The reaction was followed by the decrease in c-Pc fluorescence intensity (excitation at 620 nm, emission at 640 nm) at 37 °C

when the concentration of 1,2-DHN increases, in a concentration-dependent way (data not shown). However, the data imply that nearly five 1,2-DHN molecules are consumed by each peroxyl radical. This value has been included in Table 1.

Under conditions of almost total trapping of the radicals by the target molecule, it can be expected a simple Stern–Volmer type plot for the protection elicited by an 'ideal' free radical scavenger.[23] Figure 5 shows the change in initial rate of c-Pc bleaching as a function of the additive concentration. The data given show a noticeable
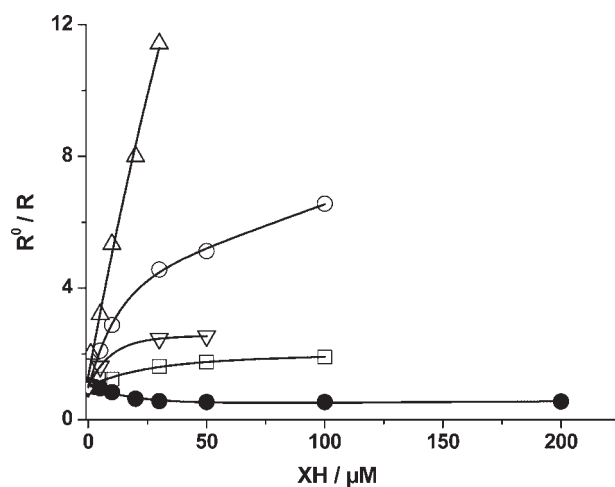

Figure 5. Protection of c-Pc elicited by different naphthalene derivatives. The data are plotted as the ratio between the initial rate of c-Pc consumption in absence (R^0) and presence (R) of the additive. 1,3-DHN (△); 2,3-DHN (○); 2,7-DHN (▽); 1-N (□); 2-N (●). Temperature:37 °C

Table 2. Efficiency of c-Pc protection, measured at 'infinite' substrate concentration, and ORAC values

Compound	$(R^0/R)_\infty$	ORAC[a]
1-N	2.0	0.06
2-N	<1.0 (Pro-oxidant)	−0.07
1,2-DHN	≫30	0.37
1-3-DHN	32.2	1.8
2,3-DHN	6.9	0.67
2,7-DHN	2.9	0.53

[a] Values determined at 5 μM additive concentration.

Table 3. Experimentally determined oxidation potentials and pK_a, and BDE values calculated according to Eqn (2)

Compound	E_{ox}^{ENH}/V	pK_a[a]	BDE(O–H)$_{exp}$/(kcal · mol^{-1})
1-N	0.475	9.34	79,1
2-N	0.627	9.51	82,9
1,2-DHN	0.330	6.16	71,4 (70)[b]
1,3-DHN	0.554	7.22	78,1
2,3-DHN	0.490	8.68	78,6 (79)[b]
2,7-DHN	0.625	9.42	82,7

[a] Taken from Ref. [31].
[b] Data from Ref. [17].

downward curvature and, for 2-N, an enhanced rate of c-Pc bleaching. This behavior has been explained in terms of a sequence of reactions, such as Eqns 8 and 9:

$$ROO^. + XOH \rightarrow ROOH + XO^. \quad (8a)$$

$$ROO^. + XO^- \rightarrow ROO^- + XO^. \quad (8b)$$

$$XO^. + c - Pc \rightarrow bleaching \quad (9)$$

The R^0/R value obtained at 'infinite' substrate concentration is a measure of the efficiency of reaction (9), that is, of the capacity of the hydroxynaphthalene-derived radical to bleach the fluorescence of the target molecule. The values obtained are collected in Table 2.

If secondary reactions of the hydroxynaphthalene-derived radicals are disregarded, it must hold that, for a given compound, the value of k is related to that of a reference compound (k_{ref}) through

$$\frac{k}{k_{REF}} = \frac{[(R^0/R) - 1]}{[(R^0/R)_{REF} - 1]} \quad (10)$$

when R^0/R values of the tested and reference compounds are evaluated at the same concentration. Applying this procedure to the consumption rate of c-Pc in presence of 20 μM 1,2-DHN (in the inhibited region) and 1,3-DHN (employed as reference due to its Stern–Volmer like behavior) (Fig. 5), it can be obtained a rough estimation of the value of k (or IS) for the former compound. The value obtained by this procedure has been included in Table 1.

The strong downward curvature of the R^0/R plots of Figure 5 and the values smaller than one measured for 2-N precludes a meaningful evaluation of their relative reactivities towards the peroxyl radicals. On the other hand, the data given in Figure 4 allow an evaluation of the ORAC index for the compounds considered. This index, which is widely considered as a measure of the compound antioxidant activity, has the advantage that it is operational and can be applied irrespective of the protection kinetics. The values obtained at a single additive concentration (5 μM) are given in Table 2.

BDE estimations

The expression of k given by Eqn (7) shows that its value must depend on $k_{Naph—OH}$ (determined by the phenol BDE), $k_{Naph—O^-}$ (determined by the naphthoate oxidation potential) and the values of f (determined by the hydroxynaphthalene pK_a). Values of pK_a and BDE estimated according to Eqn (2)[20] are included in Table 3. These values fairly agree with those obtained by theoretical calculations.[17]

DISCUSSION

The data obtained in the present work allow an estimation of the number of molecules consumed by each free radical introduced into the system (n), the relative reactivity of the compound towards peroxyl radicals (IS), the capacity of the antioxidant-derived radicals to damage c-Pc under our working conditions (measured by $(R^0/R)_\infty$), and the ORAC index of the tested compounds, employing c-Pc as target molecule and Trolox as reference compound.

Values of *n*

The n values given in Table 1 measure the number of additive molecules consumed per radical introduced into the system at high concentrations of the additive. Values of n considerably smaller than one, as obtained for 1-N and 2-N, can be explained in terms of a mechanism comprising process
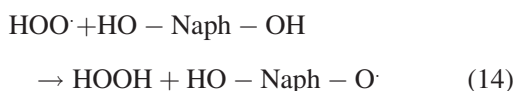
$$Naph - O^. + ROO^. \rightarrow adduct \quad (11)$$
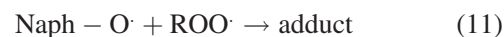
as the main radical–radical reaction. This process leads to $n = 2$ and most probably involves an addition of the peroxyl radical to the aromatic ring, as frequently found in the reactions of phenoxyl radicals.[25]

The values of n determined for the dihydroxynaphthalenes are larger than those obtained for the mono substituted compounds. The values close to one obtained employing 2,7-DHN and 1,3-DHN, compounds that can

not give the corresponding naphthones, suggest that under the employed conditions the main radical–radical process can be represented by

$$\text{Naph} - \text{O}^{\cdot} + \text{Naph} - \text{O}^{\cdot} \rightarrow \text{non radical products} \quad (12)$$
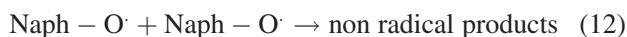
1,2-DHN and 2,3-DHN, compounds that can lead to the corresponding naphthones,[17] render *n* values considerably larger than one. This high consumption can be ascribed to the ocurrence of a chain reaction

$$\text{HO} - \text{Naph} - \text{O}^{\cdot} + \text{O}_2 \rightarrow \text{HOO}^{\cdot} + \text{O} = \text{Naph} = \text{O} \quad (13)$$

$$\text{HOO}^{\cdot} + \text{HO} - \text{Naph} - \text{OH}$$

$$\rightarrow \text{HOOH} + \text{HO} - \text{Naph} - \text{O}^{\cdot} \quad (14)$$

This reaction scheme is similar to that previously proposed by Munday and Chichirau *et al.*[26,27] The chain reaction could be carried out by the neutral species, reacting by hydrogen transfer (as represented in Eqns (13) and (14)) and/or by the deprotonated radicals, reacting by electron transfer.[27] This chain process could be particularly relevant for 1,2-DHN, as suggested by its fast autoxidation. This process would befavored by the relative weakness of the O—H bonds (Table 3 and Ref. 17). Furthermore, in agreement with the occurrence of this chain reaction, both hydrogen peroxide (evaluated by Fox's method) and 1,2-naphthoquinone (detected by its absorption spectra) were produced along the autoxidation (data not shown).

Reactivity of naphthalene derivatives towards peroxyl radicals

The data collected in Table 1 show that hydroxynaphthalenes readily react with peroxyl radicals. In fact, all the compounds considered are more reactive than caffeic acid, a widely recognized potent antioxidant.[28]

IS values collected in Table 1 imply that, regarding their reactivity towards peroxyl radicals, hydroxynaphthalenes follow the order

$$1, 2 - \text{DHN} \gg 2, 3 - \text{DHN} > 1, 3 - \text{DHN}$$

$$\geq 2, 7 - \text{DHN} > 1 - \text{N} \geq 2 - \text{N}$$
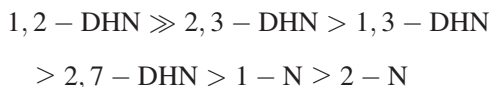
However, the only compound that is considerably more reactive than the others is 1,2-DHN. In fact, if it is considered that 2,3-DHN, 1,3-DHN, and 2,7-DHN posses two reactive centers, the difference in reactivity (per HO group) between all the other compounds would amount to less than a factor two. In order to rationalize these results, we have considered the pK_a and BDE of the hydroxynaphthalene derivatives employed in the present work (Table 3). The data collected in this Table show that 1,2-DHN presents a considerably lower pK_a and BDE energy than the other compounds (Table 3). The higher reactivity of 1,2-DHN can then be explained either in terms of a

weaker H—O bond and/or the predominance of a deprotonated HO group at the working pH. This could explain its large *k* value if electron transfer were a faster process than hydrogen transfer (i.e., if $k_{\text{Naph}-\text{O}^-} \gg k_{\text{Naph}-\text{OH}}$).[29]

For 1-N, 2-N, 2,3-DHN, and 2,7-DHN, their pK_a are considerably higher than the working pH. This implies that $f_{\text{Naph}-\text{OH}} \approx 1$ and the experimentally determined value of *k* should be equated to $k_{\text{Naph}-\text{OH}}$, provided that $k_{\text{Naph}-\text{O}^-}$ is not considerably larger than $k_{\text{Naph}-\text{OH}}$. To test this possibility we have measured the rate of 1-N consumption at pHs 6.0 and 8.0. The results obtained show only a minor increase in the rate of the process at pH 8.0. This would indicate that, at pH 7.0, the value of *k* can be safely equated to $k_{\text{Naph}-\text{OH}}$.

If it is assumed that for the compounds of pK_a higher than 8.0 the experimentally determined value of *k* equals $k_{\text{Naph}-\text{OH}}$, the data obtained can imply that its value is nearly the same for all the compounds, in spite of more than 4 kcal of difference in their BDEs. This low sensitivity of the hydrogen transfer process to the O—H BDE would require that the activated complex be near the reactives, as expected in relatively exothermic processes.

Capacity of the naphthalene-derived radicals to bleach *c*-Pc

The value of R^0/R obtained for the rate of *c*-Pc bleaching in the presence of a large excess of additive can be considered as a measured of the fraction of naphthalene-derived radicals that react with *c*-Pc. The data given in Table 2 indicate that this fraction follows the order

$$1, 2 - \text{DHN} < 1, 3 - \text{DHN} < 2, 3 - \text{DHN}$$

$$< 2, 7 - \text{DHN} < 1 - \text{N} < 2 - \text{N}$$
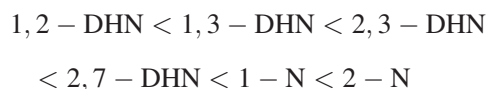
This sequence of radical reactivities is nearly inverse to the reactivity of the parent compounds. This would be compatible with the naïve assumption that the most stable radical (that more easily produced) is the less reactive. Any way, these data emphasize the role of the reactions of the antioxidant-derived radicals in the bleaching of the target molecule. 2-N derived radicals are an extreme example of the importance of these reactions. In fact, when all the initial peroxyl radicals are transformed in 2-naphthoxyl radicals, the rate of *c*-Pc consumption increases. This is due to the fact that, at the low *c*-Pc concentrations employed in the present work, only a fraction of the peroxyl radicals are trapped by *c*-Pc. The presence of 2-N would transform these peroxyl radicals in naphthoxyl radicals. If, due to its low rates of radical–radical reactions, a large fraction of the 2-N derived radicals react with *c*-Pc, the presence of the 'antioxidant' would increase the rate of *c*-Pc bleaching. Similar results

have been already reported employing pyranine as free radical scavenger.[30]

ORAC evaluation

The ORAC index obtained according to Eqn (1) is a measure of the efficiency (relative to Trolox) of the tested compound in preventing the free radical-mediated c-Pc consumption, and is determined by the reactivity of the additive, the number of additive molecules consumed per each peroxyl radical, and the secondary reactions of the additive-derived radicals. As such, interpretation of the values given in Table 2 is not straightforward. It is interesting to note that 1,3-DHN has the largest ORAC, while other data suggest a considerably higher reactivity for 1,2-DHN. This can be related to the chain consumption of 1,2-DHN and emphasize the care that must be taken in relating ORAC values to the reactivity of the tested additive.

Two other aspects of the ORAC values given in Table 2 deserve consideration. In the first place, the negative value of 2-N ORAC, a consequence of the faster rate of c-Pc consumption in presence of this additive. The second aspect is that, if 1,2-DHN is excluded due to its chain consumption, there is an inverse relationship between ORAC and $(R^0/R)_\infty$ values. This expected result emphasizes the role of antioxidant-derived radicals in determining the ORAC value.

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