Reaction of superoxide with phenoxyl-type radicals

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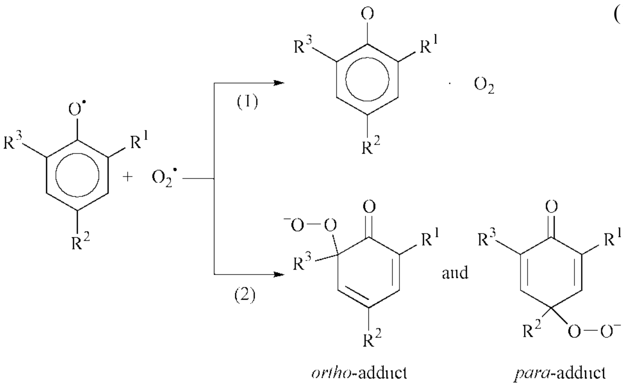
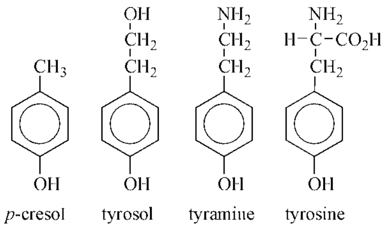
Radiolytically generated phenoxyl radicals derived from the structurally similar phenols cresol, tyrosine, tyramine and tyrosol were reacted with $O_2^{\bullet-}$ [$k = (1–4.5) \times 10^9$ dm^3 mol^{-1} s^{-1}, by pulse radiolysis] and the consumption of the phenol determined. Although the reduction potentials of these phenoxyl radicals are very close, ranging between +0.64 and +0.68 V, the yields of phenol consumption vary considerably [between 8% (tyrosol) and 90% (tyrosine)]. This indicates that electron transfer with restitution of the phenol does not necessarily occur to a major extent, although this reaction is thermodynamically favoured (reduction potential of $O_2^{\bullet-}$, −0.33 V). The reactivity of phenoxyl radicals from phenols that are structurally different from the above has also been studied. In the case of the reaction of $O_2^{\bullet-}$ with 2,4,6-trimethylphenoxyl, hydroperoxides are formed which revert to the phenol by eliminating dioxygen (perhaps in the singlet state) in a slow reaction on a time-scale of many minutes. The rate of this reaction increases with increasing pH and increasing temperature. From these data it is calculated that the hydroperoxides from 2,4,6-trimethylphenol have a pK_a of 11.3 and that the reaction requires an activation energy of 105 kJ mol^{-1} (frequency factor, 3.5×10^{14} s^{-1}). In competition with the elimination of dioxygen, depending on the presence of suitable substituents, such hydroperoxides can undergo other reactions. In the case of tyrosine, there is a cyclization reaction followed by the elimination of hydrogen peroxide. Phenoxyl radicals that carry hydrogen in the *ortho* or *para*-position (*e.g.* such as those derived from 4-methylphenol, 2,6-dimethylphenol or 2-methoxy-4-methylphenol) give rise to hydroperoxides which can eliminate water to produce the corresponding quinones. These subsequently suffer stepwise reduction by $O_2^{\bullet-}$ to the corresponding catechols or hydroquinones.

Apart from being of academic interest, the question as to the chemical consequence of the reaction of phenoxyl radicals with $O_2^{\bullet-}$ carries additional weight since a representative of this species, the tyrosyl radical, is involved in various enzymatic redox processes.[1,2] There is a likelihood that superoxide because of its ubiquitous presence in the living cell will react with tyrosyl. It was therefore of importance to learn whether, as has in the past been generally assumed, this reaction is nondestructive in the sense that it leads exclusively to the reduction of the phenoxyl function to the phenolic function,[3–5] or rather leads irreversibly to the formation of oxidation products and thereby to the destruction of the phenolic moiety. Indeed, the former assumption was recently shown to be incorrect when it became apparent that the tyrosine-derived phenoxyl radical essentially reacts only by addition [*cf.* reaction (2)].[6] Moreover, the reaction of $O_2^{\bullet-}$ with a number of differently substituted phenoxyl radicals has shown a considerable variation in the consumption of the parent phenol.[7] It has been suggested that this variation in phenol consumption might be explained on the basis of the Marcus theory of electron transfer which might govern the branching ratio between reactions (1) and (2).[7] In principle, there exists a considerable driving force in favour of the electron transfer [reaction (1); note that dioxygen can be released in its triplet ground state in accordance with the spin conservation rule], as the difference between the reduction potential of the superoxide radical ($O_2^{\bullet-}$; −0.33 V) and those of several substituted phenoxyl radicals varies between +0.19 and +1.45 V, *cf.* refs 7 and 8 and Table 1 below.



(1)



(2)

However, as already seen above, in the case of the tyrosyl radical we had observed that restitution was of little importance, if it occurred at all.[6] If the competition between reactions (1) and (2) was mainly governed by the Marcus theory, one would expect that the tyrosine[6] and *p*-cresol[7] derived phenoxyl radicals that are structurally similar (alkyl substituent in the *para* position) and have practically the same reduction potential, should also show very similar degrees of phenol restitution which is, however, not the case (*cf.* Table 1). This apparent contradiction has led us to extend the study to further examples, among them tyrosol and tyramine whose oxidation

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Table 1 Phenol consumption in radiolysis (relative to the maximum possible, *i.e.* $G(\text{phenoxyl}) = G(^{\bullet}N_3) \approx G(^{\bullet}OH) \approx 2.9 \times 10^{-7}$ mol J^{-1}), in the presence of dioxygen ($G(O_2^{\bullet -}) = G(e_{aq}^{-}) + G(H^{\bullet}) \approx 3.5 \times 10^{-7}$ mol J^{-1}). Here and elsewhere Ph stands for the group $C_6H_nX_{5-n}$, where X is any substituent

Radical	$E(PhO^{\bullet}/PhO^{-})$/V	Consumption (%)
p-CH_3-$PhO^{\bullet}$	0.68[a]	17,[a] ≈30[b]
p-H_2N-$CH(CO_2H)$-$CH_2PhO^{\bullet}$	0.64[c]	90[d]
p-H_2N-CH_2-CH_2-$PhO^{\bullet}$	[e]	34[d]
p-HO-CH_2-CH_2-$PhO^{\bullet}$	[e]	8[d]
p-CH_3-m-CH_3O-$PhO^{\bullet}$	0.71[f]	93[b]
2,4,6-$(CH_3)_3$-$PhO^{\bullet}$	0.49[a]	60,[a] 93[b]

[a] Data from ref. 7. [b] Estimated on the basis of the formation of hydroperoxides after pulse irradiation at ice-bath temperature (see text). [c] From ref. 9. [d] γ-Radiolysis at room temperature and subsequent determination of the phenol by HPLC. [e] Assumed to be similar to those of cresol and tyrosine, *i.e.* 0.64–0.68 V. [f] Estimated on the basis of the data given in ref. 10, Fig. 3, and ref. 11, p. 241.

potential is expected to closely resemble that of tyrosine, and 2,4,6-trimethylphenol.

It will be shown below that the discrepancy between the experimental result and the expectation based on the Marcus theory remains unresolved; rather, the explanation must be that in these systems, hydroperoxidic $O_2^{\bullet -}$–phenoxyl radical recombination products are predominantly formed which eliminate dioxygen (in the singlet state if the principle of spin conservation is obeyed) and thus restitute the parent phenol, in competition with other reactions, over a period longer than pulse-radiolysis timescale. Any contribution to the restitution of phenol *via* the decomposition of a peroxidic intermediate (see below) that occurs within the time needed to carry out the product analysis cannot be distinguished from restitution by electron-transfer [reaction (1)].

Experimental

All chemicals used were of the highest purity commercially available and were used without further purification. Solutions were made up in Milli-Q-filtered (Millipore) water. γ-Irradiations were carried out in a ^{60}Co-γ-source (Nuclear Engineering Ltd.) at dose rates ranging from 0.085 to 0.39 Gy s^{-1}. Pulse-radiolytic kinetic measurements were done using optical detection. The set-up has been described before.[12]

The consumption of tyrosine, tyramine, and tyrosol was determined directly by HPLC on a 25 cm Nucleosil-7-C_{18} column (Merck), using water containing 0.5% acetic acid (for tyrosine and tyramine), or 20% methanol in water containing 0.5% acetic acid (for tyrosol), as eluents. The consumption of *p*-cresol, 2,4,6-trimethylphenol, and 2-methoxy-4-methylphenol was estimated on the basis of *G*(organic hydroperoxides), formed upon pulse radiolysis of O_2-saturated aqueous solutions (pH 11) of the phenols (10^{-3} mol dm^{-3}) containing NaN_3 (2×10^{-2} mol dm^{-3}) at ice-bath temperature. This approach is feasible in those cases where the lifetime of these hydroperoxides is much longer than the time needed to complete the experiment. It must be borne in mind that under the present experimental conditions, a portion of the phenoxyl radicals is irretrievably lost owing to self-recombination. It can be estimated (see below) that when the irradiation is done with the electron pulse, the lower limit of *G*(phenol consumption) should be set equal to *G*(organic hydroperoxides) multiplied by a factor of 1.25. The determination of the hydroperoxides was by HPLC coupled with post-column derivatization with molybdate-activated iodide.[13]

It turns out that there is a process that reconverts the 2,4,6-trimethylphenol-derived hydroperoxide(s) to 2,4,6-trimethylphenol (see below). This reaction was to be studied in some detail. For this reason, O_2-saturated aqueous solutions of 2,4,6-trimethylphenol (10^{-3} mol dm^{-3}; at these concentrations, all of the e_{aq}^{-} react with O_2 and not with the phenol) containing NaN_3 (2×10^{-2} mol dm^{-3}) were pulse-irradiated to about 2 kGy (pulse radiolysis allows a high cumulative dose to be given within a relatively short period of time compared to γ-radiolysis), and adjusted to pH 5.6 following irradiation. Under such conditions, about 40% of 2,4,6-trimethylphenol contained in the solution is converted to hydroperoxides and other products [at these high conversions *G*(2,4,6-trimethylphenol consumption) is somewhat lower than its initial value, owing to some diversion of primary radiolytic attack from the reactant to the products]. The hydroperoxides so generated were freed from H_2O_2 (which is also produced, essentially without the participation of the solute) and from most of the starting phenol by extraction with diethyl ether and washing of the ether extract with NaOH (10^{-3} mol dm^{-3}). The ether solution was kept in stock in the refrigerator. These purified hydroperoxides showed the same kinetics of conversion into 2,4,6-trimethylphenol as an untreated irradiated sample, which allowed us to use the *latter* instead of the stock solution for the major part of the kinetic studies. The 2,4,6-trimethylphenol regenerated from the hydroperoxides was quantified by HPLC [12.5 cm Nucleosil-5-C18 (Merck); water containing 60% methanol and 1% acetic acid, detection at 280 nm, baseline separation of 2,4,6-trimethylphenol from other products]; the 2,4,6-trimethylphenol-derived hydroperoxides and H_2O_2 were separated using water containing 40% methanol and 1% acetic acid as eluent (acidification quenches the conversion of the hydroperoxides into the phenol, see below), and quantified by post-column derivatization with molybdate-activated iodide (the concentration of the phthalate buffer required for this assay was doubled to preclude an effect of the acidic eluent on the quantification of the hydroperoxides).

Results and discussion

The free-radical-generating system

In the radiolysis of water the primary species formed are OH radicals, solvated electrons, and H atoms [reaction (3); $G(^{\bullet}OH) \approx$

$$H_2O \xrightarrow[\text{radiation}]{\text{ionizing}} {}^{\bullet}OH, e_{aq}^{-}, H^{\bullet}, H^{+}, H_2O_2, H_2 \qquad (3)$$

$G(e_{aq}^{-}) \approx 2.9 \times 10^{-7}$ mol J^{-1}; $G(H^{\bullet}) \approx 0.6 \times 10^{-7}$ mol J^{-1}].[14] The solvated electrons can be converted into further OH radicals [reaction (4)]. In the presence of O_2 they are converted into

$$e_{aq}^{-} + N_2O \longrightarrow {}^{\bullet}OH + N_2 + OH^{-} \qquad (4)$$

superoxide [reaction (5)]. The H atom is also scavenged by O_2

$$e_{aq}^{-} + O_2 \longrightarrow O_2^{\bullet -} \qquad (5)$$

and contributes to the superoxide yield [reaction (6)]. In the

$$H^{\bullet} + O_2 \longrightarrow HO_2^{\bullet}\ [pK_a(HO_2^{\bullet}) = 4.8] \qquad (6)$$

presence of azide, the OH radical converts N_3 into $N_3^{\bullet}$ [reaction (7)] which is a selective oxidant capable of neatly oxidizing

$$^{\bullet}OH + N_3^{-} \longrightarrow OH^{-} + N_3^{\bullet} \qquad (7)$$

phenols to the corresponding phenoxyl radicals [*e.g.* reaction (8)].[14] The yield of $O_2^{\bullet -}$ relative to $PhO^{\bullet}$ can be enhanced by

$$PhOH + N_3^{\bullet} \longrightarrow PhO^{\bullet} + N_3^{-} + H^{+} \qquad (8)$$

reacting a portion of the OH radicals with formate ions [reaction (9)]. The formate radical $CO_2^{\bullet -}$ then converts O_2 into

Table 2 Compilation of the rate constants determined in the present study, and related values from the literature

Reaction	k/10^9 dm^3 mol^{-1} s^{-1}
4-CNPhO$^\bullet$ + $O_2^{\bullet -}$→Products	4.5,[a] 0.21[b]
2 4-CNPhO$^\bullet$→Products	2.3[c,a]
Tyrosine-PhO$^\bullet$ + $O_2^{\bullet -}$→Products	1.5,[d] 1.7[e]
2 Tyrosine-PhO$^\bullet$→Products	0.45[c,d]
Tyrosol-PhO$^\bullet$ + $O_2^{\bullet -}$→Products	2.3[a]
2 Tyrosol-PhO$^\bullet$→Products	1.0[c,a]
Tyrosol-PhO$^\bullet$ + $CH_3O_2^\bullet$→Products	1.1[a]
Tyrosol-PhO$^\bullet$ + $HOC(CH_3)_2CH_2O_2^\bullet$→ Products	0.89[a]
Tyramine-PhO$^\bullet$ + $O_2^{\bullet -}$→Products	1.0[a]
2 Tyramine-PhO$^\bullet$→Products	0.47[c,a]
2-MeO-4-Me-PhO$^\bullet$ + $O_2^{\bullet -}$→Products	1.1[a]
2,4,6-Me$_3$-PhO$^\bullet$ + $O_2^{\bullet -}$→Products	1.2[a]
TMPOO$^-$→2,4,6-Me$_3$-PhO$^-$ + O_2	1.4 × 10^{-4} s^{-1} (23 °C)[a]

[a] This work. [b] Ref. 5. [c] 2k. [d] Ref. 6. [e] Ref. 4.

$$^\bullet OH + HCO_2^- \longrightarrow OH^- + CO_2^{\bullet -} \qquad (9)$$

$O_2^{\bullet -}$ [reaction (10)]. Alkylperoxyl radical formation ($^\bullet CH_3$ was

$$CO_2^{\bullet -} + O_2 \longrightarrow CO_2 + O_2^{\bullet -} \qquad (10)$$

produced by the reaction of $^\bullet$OH with dimethyl sulfoxide[15] and $^\bullet CH_2C(CH_3)_2OH$ by H-abstraction from 2-methylpropan-2-ol) is fast as well [reactions (11) and (12)]. For rate constants see refs. 16–19

$$^\bullet CH_3 + O_2 \longrightarrow CH_3O_2^\bullet \qquad (11)$$

$$^\bullet CH_2C(CH_3)_2OH + O_2 \longrightarrow {}^\bullet O_2CH_2C(CH_3)_2OH \qquad (12)$$

Under the present conditions of pulse radiolysis, about 20% of phenoxyl disappears by self-termination and so escapes the reaction with superoxide. In the case of γ-irradiation, $O_2^{\bullet -}$ accumulates relative to phenoxyl since its G value exceeds that of phenoxyl and its self-termination rate constant is very small,[16] so the loss of phenoxyl by self-termination tends to be less than 20%.

Kinetic studies

The rate constants of the reaction of several differently-substituted phenoxyl radicals with superoxide have been determined by pulse-radiolysis, monitoring the disappearance of the phenoxyl radical by optical detection. The results are compiled in Table 2, together with some relevant data from the literature.

In general, the rate of reaction of the superoxide radical with phenoxyl-type radicals is close to diffusion-controlled, *i.e.* ≈ 2 × 10^9 dm^3 mol^{-1} s^{-1} (*cf.* ref. 7 and Table 2), the phenoxyl radical of Trolox (a water-soluble vitamin E analog; reduction potential, +0.19 V) being an exception.[7] Since a definite value for the rate constant of 4-cyanophenoxyl (reduction potential, +1.12 V) is apparently unavailable,[7] it has been redetermined in this study. The spectrum of the 4-cyanophenoxyl radical is shown in Fig. 1. It resembles quite closely those of other phenoxyl radicals. Also, the absorption coefficient at the maximum is similar to those of comparable phenoxyl-type radicals; since the 436 nm band is very narrow, care was taken to choose the minimum possible monochromator slit width setting when running the spectrum. Otherwise the reading for the extinction coefficient would tend to be low.

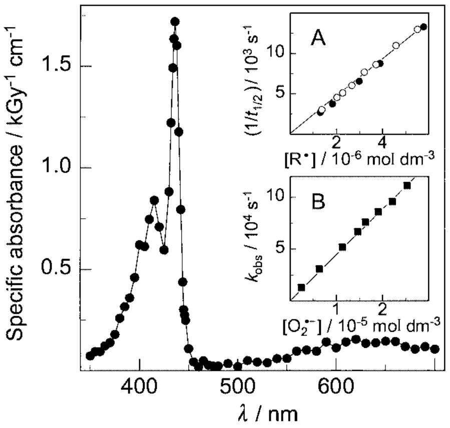


Fig. 1 Pulse radiolysis of aqueous N_2O-saturated solutions containing 4-cyanophenol (10^{-3} mol dm^{-3}) and NaN_3 (2 × 10^{-2} mol dm^{-3}), pH 11, 4.5 Gy per pulse. Inset A: bimolecular decay of the 4-cyanophenoxyl radical in the absence (○) and presence (●) of O_2. The first half-life of the decay at 436 nm is plotted as a function of the total radical concentration (variation of the dose per pulse). Inset B: first-order decay of the 4-cyanophenoxyl radical as a function of the $O_2^{\bullet -}$ concentration (90% of the total radical concentration are $O_2^{\bullet -}$ radicals).

The 4-cyanophenoxyl radicals decay by second order (*cf.* inset A in Fig. 1) with a rate constant of 2k = 2.3 × 10^9 dm^3 mol^{-1} s^{-1}, irrespective of whether or not O_2 is present. This is in agreement with the general observation that phenoxyl-type radicals do not react with O_2 unless substituted with strongly electron-*donating* functions.[20] When the 4-cyanophenoxyl radicals are formed in the presence of a large excess of $O_2^{\bullet -}$ [with formate added at a concentration such as to raise the $O_2^{\bullet -}$ concentration to 90% of the total radical concentration [*cf.* reactions (9) and (10)], they decay by essentially first-order kinetics. Inset B in Fig. 1 shows a plot of the observed rate of decay of the 4-cyanophenoxyl radical as a function of the $O_2^{\bullet -}$ concentration; from these data a rate constant of k(CN-PhO$^\bullet$ + $O_2^{\bullet -}$) = 4.5 × 10^9 dm^3 mol^{-1} s^{-1} is calculated. The rate constants for the reaction of $O_2^{\bullet -}$ with other phenoxyl type radicals investigated in the present study have been obtained in the same way.

Product studies

Studies concerning the products resulting from the reactions of phenoxyl-type radicals with $O_2^{\bullet -}$ are few.[6,21] It turns out that the phenoxyl radical–$O_2^{\bullet -}$ systems are characterized by the lability of the hydroperoxidic intermediates which are the precursors of the final products, but may also lead back to the starting phenol (see below). The study of the tyrosine and related systems in particular clearly shows how the presence of a reactive substituent may alter the pathway to the products. Therefore these transformations have been studied at different levels of structural complexity.

2,4,6-Trimethylphenol is a useful model compound to study, by way of an example, the hydroperoxide-to-phenol restitution reaction since it does not carry a reactive substituent in the above sense. It also carries substituents in the *ortho* and *para* positions, precluding, *e.g.*, a 1,3-hydrogen shift that isomerizes a cyclohexa-2,4-dienone to the corresponding phenol. One might therefore expect that the hydroperoxide(s) formed in this system will efficiently regenerate the parent phenol by dioxygen elimination. This expectation is borne out by the experiment. Fig. 2 shows the decay kinetics of the hydroperoxide(s) (TMPOO$^-$ in Table 2) produced radiolytically as described above, as well as of the regeneration of 2,4,6-trimethylphenol as monitored by HPLC.

In Fig. 3 the rate of regeneration of 2,4,6-trimethylphenol is plotted as a function of pH. These data show that the rate increases with increasing pH showing an inflection point at pH 11.3. It is concluded that the oxygen elimination proceeds at a fast rate from the hydroperoxide anion only and that the inflection point may be attributed to its pK_a. For comparison, the pK_a of hydrogen peroxide is 11.6;[22] apparently, the electron-withdrawing carbonyl group in the 2,4,6-trimethylphenol-derived hydroperoxide lowers the pK_a value of the

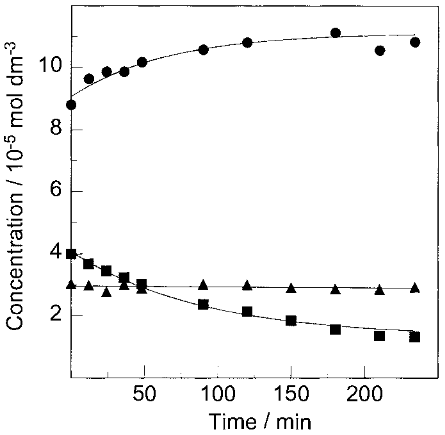


Fig. 2 Reconversion of 2,4,6-trimethylphenol-derived hydroperoxides (■) to 2,4,6-trimethylphenol (●) in a radiolyzed solution (pH 11.7, 23 °C). Note that H_2O_2 (▲) remains unchanged.

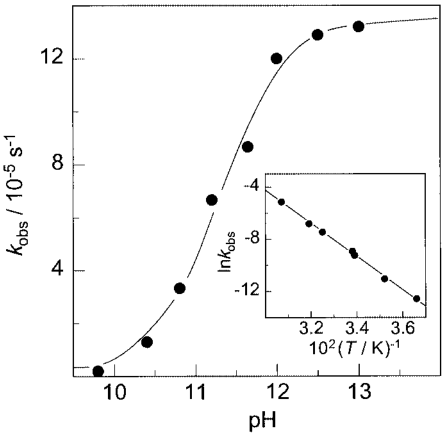
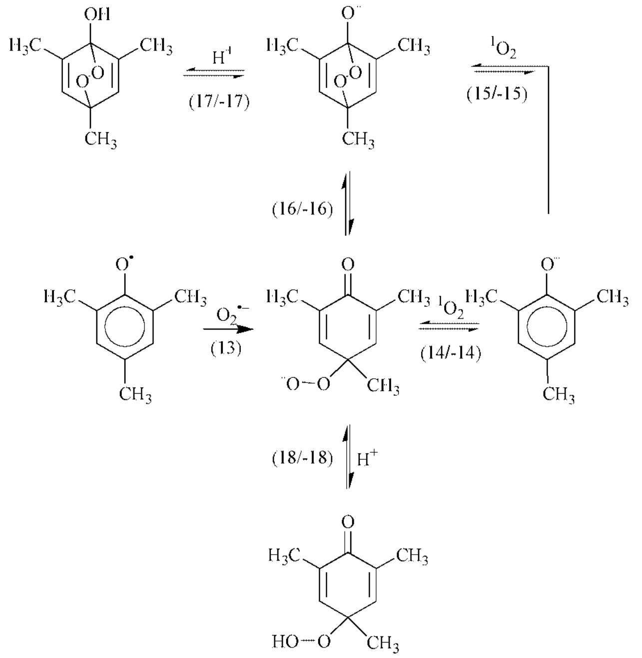


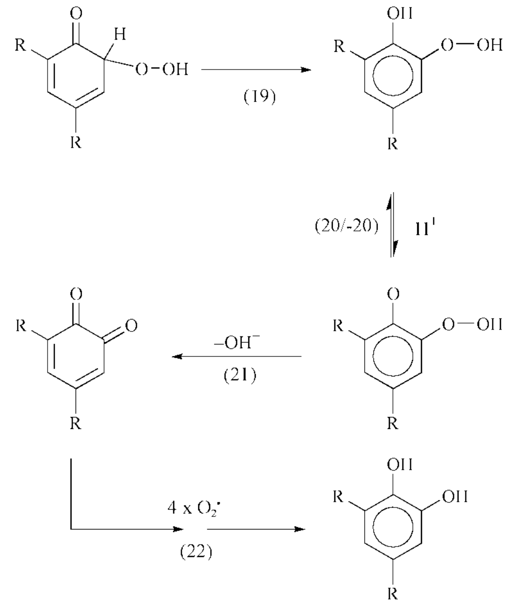
Fig. 3 Rate of decomposition of the 2,4,6-trimethylphenol-derived hydroperoxides as a function of pH at 23 °C. The fitted curve corresponds to a pK_a of 11.3, with $k_{obs}^{max} = 1.4 \times 10^{-4}$ s^{-1}. Inset: Arrhenius plot at pH 13.

hydroperoxide function slightly, as would be expected. From the Arrhenius plot of the rate of 2,4,6-trimethylphenol regeneration at pH 13 (inset in Fig. 3) an activation energy of 105 kJ mol^{-1} (frequency factor, 3.5×10^{14} s^{-1}) is calculated.

The dioxygen eliminated upon regeneration of the parent phenol from the hydroperoxides will be $O_2(^1\Delta_g)$ if the rule of spin conservation is obeyed along the reaction pathway. Therefore, the observation that the reaction of $O_2(^1\Delta_g)$ with phenols and indoles can give the same products as are formed[6] when the phenoxyl (indolyl) radical is reacted with $O_2^{\bullet -}$ could throw some additional light on the mechanism because it suggests[23,24] that there is a common intermediate to both the forward ($O_2^{\bullet -}$ + phenoxyl) and the reverse ($O_2(^1\Delta_g)$ + phenolate) reaction. In the reaction of the 2,4,6-trimethylphenolate ion with $O_2(^1\Delta_g)$, just as in the reaction of the 2,4,6-trimethylphenoxyl radical with $O_2^{\bullet -}$ [reaction (13)], a product is formed which upon standing at high pH (but not at low pH) readily regenerates the parent phenolate.[25] Until now this phenomenon had eluded interpretation. We now suggest that the 2,4,6-trimethylphenolate and $O_2(^1\Delta_g)$ undergo reactions (14) and/or (15); for the sake of simplicity *ortho*-additions are not shown but must occur as well. These intermediates are in equilibrium with their corresponding protonated forms [equilibria (17) and (18)]. In addition, one must consider equilibrium (16). The elimination of $O_2(^1\Delta_g)$ and the regeneration of the parent phenolate can only occur from the anionic forms [reactions (−14) and (−15)]. Hence only in basic media will the regeneration of the parent phenol be relatively fast.



The next level of reactional diversity is represented by phenols with a substituent-free *ortho* position. When in such a case $O_2^{\bullet -}$ adds to this position, a cyclohexadienone is formed which can rearrange into the corresponding phenol [reaction (19)].[26,27] When deprotonated, the aromatic hydroperoxide is expected to lose OH^- and give rise to an *o*-quinone [reactions (20) and (21)]. This type of hydroperoxide decomposition is also observed in other systems.[28,29] Especially at the low dose rates of γ-radiolysis, where $O_2^{\bullet -}$ builds up to relatively high steady-state concentrations the *o*-quinones will not be the final products. Quinones react readily with the superoxide radical.[16] This reaction is reversible and has been used to determine the reduction potential of the superoxide radical. The resulting semiquinone radicals are known to disproportionate. In a sequence of such reactions the observed catechol may be formed as indicated by the overall reaction (22).



Cases in point are the 2-methoxy-4-methylphenol system, where 4-methyl-6-methoxycatechol is formed in considerable amounts; 4-methylphenoxyl (from *p*-cresol) and 2,6-dimethylphenoxyl (from 2,6-dimethylphenol) yield (among other products) 2-methylcatechol and 2,6-dimethyl-1,4-dihydroxybenzene, respectively. We explain the formation of these diphenolics according to the above scheme, considering that at

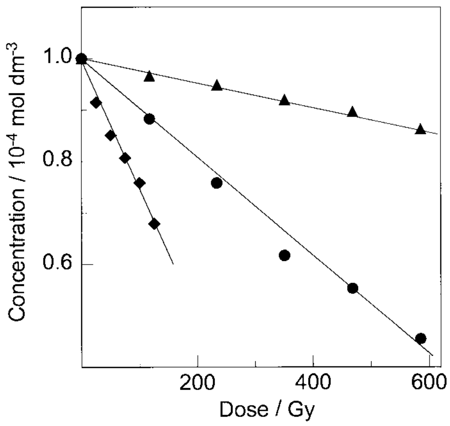
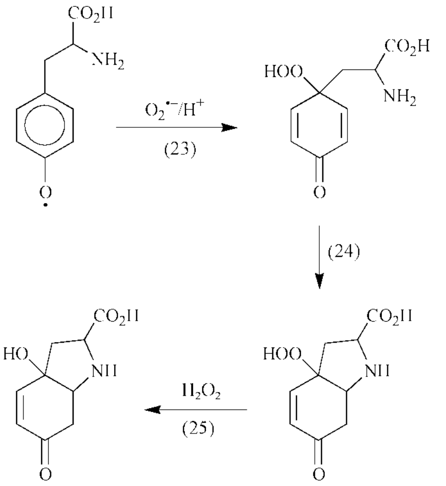


Fig. 4 γ-Radiolysis of O_2-saturated aqueous solutions (pH 10.5) of tyrosine (◆), tyramine (●) and tyrosol (▲) in the presence of an excess of sodium azide. Phenol consumption as a function of dose.

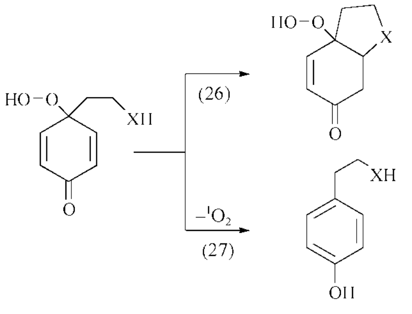
the low dose rate of γ-radiolysis a sufficiently high steady-state concentration of the *o*-quinone can build up. This has also been demonstrated for other phenolic antioxidants.[30]

Tyrosine is an example of a phenol where the restitution reaction is of little importance while the tyramine and tyrosol systems show a considerable degree of restitution of the phenoxyl radical to the parent phenol (Fig. 4 and Table 1). The hydroperoxides from these phenols have a third option available, *i.e.* ring closure.

In these tyrosine-related phenoxyl radicals the restitution of the phenol [reaction (27)] may be preempted to a greater or lesser extent by intramolecular addition[6] [reactions (24) and (26)], and hydroperoxide hydrolysis such as reaction (21). Similar expressions may be written for the addition of $O_2^{\bullet -}$ at the *ortho* position; for details see ref. 6.



The relative rates of O_2-elimination to these reactions would then determine the ratio of restitution to product formation. Reaction (26), a Michael-type addition, will proceed at a fast rate when the amino group (tyrosine and tyramine) or the OH group (tyrosol) is unprotonated. Rate constants in the order of 0.1 to 10 s^{-1} have been reported for such cyclizations in similar systems.[31] In the former two compounds, the pK_a values of the relevant functions are reported at 9.11[32] and 9.2[33] [although a higher value seems possible, considering the disposition of the pK_a values of tyrosine, phenylalanine (9.24)[32] and 2-aminoethylbenzene (9.83)[34]], while the OH group as such is much less reactive and its pK_a for deprotonation is much higher (assumed at > 15; *cf.* benzyl alcohol, 15.4 and *n*-propanol, 16.1).[35] One would therefore expect that at pH 10.5 tyrosine and also tyramine undergo reaction (26) faster than tyrosol. This expectation is borne out by the experimental results (Fig. 4 and Table 1) which show that in the case of tyrosine, little restitution of the educt is observed, while with tyramine some, and with tyrosol a high degree, of restitution occurs.



In the case of tyrosine, the decay of the *cyclized* [*cf.* reaction (25)] organic hydroperoxide is accompanied by a corresponding increase in the yield of H_2O_2 [*cf.* reaction (25)],[6] while there is no H_2O_2 release in the 2,4,6-trimethylphenolate system (Fig. 2).

The kinetics of the restitution process *via* dioxygen elimination from a hydroperoxide has not been followed in the systems other than 2,4,6-trimethylphenol. It may be assumed that at least in those cases where cyclization is an option, *e.g.* with tyrosine and tyramine, restitution must be quite fast (even though relatively unimportant in yield terms) since the kinetics of restitution is the reverse of the kinetics of the disappearance of the superoxide adduct, which is governed by the relatively large rate constant[31] of the cyclization reaction.

Conclusions

From the data presented above we conclude that in the reactions of $O_2^{\bullet -}$ with phenoxyl radicals, addition to the aromatic ring is the preferred process. In comparison, an electron transfer leading to the restitution of the parent phenol seems generally to be of minor importance. Restitution of the phenol may occur by the elimination of oxygen from labile hydroperoxides. In competition, other processes that may involve ring addition of a reactive substituent or the hydrolysis of the hydroperoxidic function, may lead to a permanent phenol loss. A minor contribution to restitution *via prompt* electron transfer [reaction (1)] is not ruled out.

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