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Radical Spectra and Product Distribution following Electrophilic Attack by the OH$^\bullet$ Radical on 4-Hydroxybenzoic Acid and Subsequent Oxidation

Robert F. Anderson,* Kantilal B. Patel and Michael R. L. Stratford
Gray Laboratory of the Cancer Research Campaign, Mount Vernon Hospital, Northwood, Middlesex HA6 2RN

The distribution of electrophilic OH$^\bullet$ radical addition to 4-hydroxybenzoic acid (HBA) has been determined by oxidizing the radical intermediates (substituted hydroxycyclohexadienyl radicals) with quinones and viologens, to yield products. It is deduced from product analysis using high-performance liquid chromatography that the fractions of OH$^\bullet$ attack at the 1:2:3:4 positions of HBA are 0.16:0.04:0.65:0.15 respectively. Pulse radiolysis studies show that the rate of electron transfer from the radical intermediate formed by OH$^\bullet$ addition to position 3 of HBA is dependent on the one-electron reduction potential of the oxidant. This electron-transfer process is in competition with the elimination of water by general acid–base catalysis to yield the phenoxyl radical. Catalysis by OH$^-$ proceeds through the formation of the deprotonated species, $pK_a = 8.4 \pm 0.2$, followed by the elimination of water, $k_e = 3.0 \pm 0.3 \times 10^5\ s^{-1}$. The addition of an OH$^\bullet$ radical *ipso* to the hydroxy group of HBA also gives rise to the phenoxyl radical through rapid water elimination, $k = 2 \times 10^7\ s^{-1}$. The addition of an OH$^\bullet$ radical to position 3 of HBA gives rise to an absorption band centred at 365 nm.

Hydroxylation of substituted benzenes can be achieved in a two-step process in which the electrophilic OH$^\bullet$ radical adds to form a substituted hydroxycyclohexadienyl radical, followed by electron transfer to an oxidant to yield products. The most commonly employed oxidants in this process have been metal ions[1-5] which often yield a varying range of product distribution, dependent on the nature and concentration of the metal ions. Accurate determinations of the pattern of OH$^\bullet$ radical addition to anisole[6] and phenol[7] have been made using quinones as oxidants, by observing both the kinetics of oxidation of the isomeric radicals and product analysis. A strong preference for addition to occur *ortho* and *para* to both the hydroxy and methoxy groups was observed.

It was noted in the course of a study on the reaction of OH$^\bullet$ radicals with benzoic acid[4] in the presence of ferricyanide that hydroxybenzoic acids also react with OH$^\bullet$ radicals, but the resulting products were not identified. In this study we have used quinones and viologens as oxidants to study the product distribution in a disubstituted benzene following OH$^\bullet$ radical attack on 4-hydroxybenzoic acid (HBA). This compound is a substrate for the enzyme *p*-hydroxybenzoate hydroxylase, which is a flavin containing mono-oxygenase. Although some intermediates involved in the functioning of this class of enzyme have been identified by comparing u.v. spectra[8] with model compounds, the intermediate considered to be closely related to that responsible for oxygen transfer has not been successfully modelled. In this study we also report the spectrum of the OH$^\bullet$ radical adduct to HBA.

Experimental

4-Hydroxybenzoic acid (HBA)† was obtained from Aldrich (gold label product) and found to be pure using h.p.l.c. 2,4-Dihydroxybenzoic acid (2,4-DHBA) and 3,4-dihydroxybenzoic acid (3,4-DHBA) were obtained from BDH and hydroquinone (HQ) was obtained from Sigma and repurified by sublimation. Other reagents were of the highest purity commercially available.

The pulse radiolysis studies were carried out using a 3.5 MeV Van de Graff accelerator to irradiate [3–20 Gy (J kg^{-1}) in 30 ns] aqueous solutions (21 °C) contained in a 2-cm path-length cell. The associated charge monitoring device and optical detection system have been described.[9] The absorbed radiation dose was determined by measuring the optical density at 472 nm in aerated KSCN[10] (10 mmol dm^{-3}) assuming an extinction coefficient, ε of 758 m^2 mol^{-1} and a radiation chemical yield $G\varepsilon$‡ of 0.29 μmol J^{-1}. Transients were recorded on a Tektronic 7612D digitizer interfaced to a PDP 11/34 computer for data analysis. [Absorptions are presented as the product of the radiation chemical yield, G (mol J^{-1}) and the extinction coefficient, ε(m^2 mol^{-1}).]

Steady-state irradiations were carried out using a ^{60}Co γ-ray source. Solutions were prepared and irradiated in either air-tight syringes (25 cm^3) from which samples were sequentially removed for analysis or (in the case of DMBQ) in separate sealed h.p.l.c. vials for each dose point. The dose rate (*ca.* 30 Gy min^{-1}) was determined using the Fricke dosimeter.[11]

Product analysis was carried out by high-performance liquid chromatography (h.p.l.c.) using a Waters 840 chromatograph equipped with a Waters 490 multi-wavelength detector. Detection of the dihydroxybenzoic acids and hydroquinone was at 295 nm and the benzoquinones at 254 nm. The column used was a Waters Novapak C18 cartridge (100 × 8 mm) in a Waters Z-module. Separation was achieved with a binary gradient, the eluants being: A, acetonitrile (95%) and water (5%) and B; ortho-phosphoric acid (20 mmol dm^{-3}) and sodium dihydrogen orthophosphate (20 mmol dm^{-3}). All the analyses used the same initial linear gradient of 10–20% A in 8 min. For 1,4-benzoquinone and methyl-1,4-benzoquinone the gradient then returned to 10% A in 1 min. For the less polar quinones, an additional gradient from 20–40% A in 6 min was employed, again returning to 10% A in 1 min.

Results and Discussion

Reactions initiated by the OH˙ radical are conveniently studied in irradiated aqueous solution saturated with N_2O gas to convert e^-_{aq} to OH˙.

$$H_2O \rightsquigarrow OH^{\bullet}, e^-_{aq}, H^{\bullet}, H_2O_2, H_2, H_3O^+, OH^- \qquad (1)$$

$$N_2O + e^-_{aq} \longrightarrow OH^{\bullet} + OH^- + N_2 \qquad (2)$$

yielding [OH˙] = 0.62 μmol J^{-1} and [H˙] = 0.06 μmol J^{-1}.[12] The rate of reaction of OH˙ with HBA, reaction (3), was determined from the appearance of an absorption band centred at 370 nm following pulse radiolysis (10 Gy in 30 ns). The rate constant was determined using three concentrations of HBA (0.5–2.0 mmol dm^{-3}), yielding $k_3 = 6.0 \times 10^9$ dm^3 mol^{-1} s^{-1}. To study the subsequent reaction, (4),

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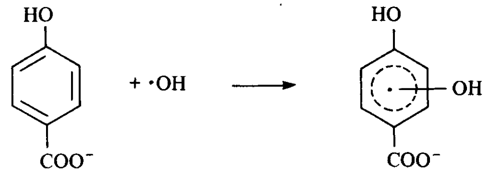
† Abbreviations used: HBA (4-hydroxybenzoic acid) 2,4-DHBA (2,4-dihydroxybenzoic acid), 3,4-DHBA (3,4-dihydroxybenzoic acid); Q (quinone), $Q^{\bullet-}$ (semiquinone), QH_2 (fully reduced quinone); pBQ (1,4-benzoquinone), HQ (hydroquinone), MBQ (methyl-1,4-benzoquinone), DMBQ (2,5-dimethyl-1,4-benzoquinone), AQS (anthraquinone-2-sulphonic acid), TQ (7,8-dihydro-6*H*-dipyrido[1,2-*a*:2′,1′-*c*][1,4]diazepinedi-ium dibromide), V41 (6,7,8,9-tetrahydro-2,13-dimethyldipyrido[1,2-*a*:2′,1′-*c*][1,4]diazocinedi-ium dibromide), OX (oxidant), OXH_2 (two-electron reduced oxidant), $OX^{\bullet-}$ (one-electron reduced oxidant), TMPD (*NNN′N′*-tetramethyl-*p*-phenylenediamine).

‡ A G-value of 1 molecule (100 eV)$^{-1}$ corresponds to a radiation chemical yield, G = 0.1036 μmol J^{-1}.



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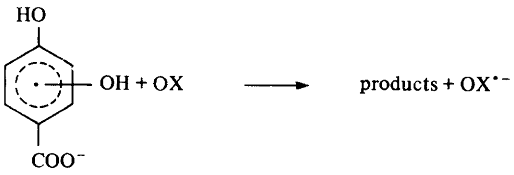
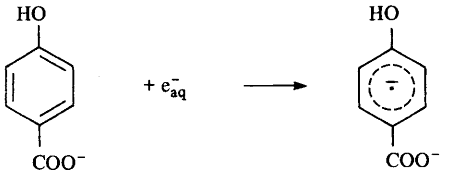


(4)

it was necessary to employ a high concentration of HBA (10–40 mmol dm^{-3}) relative to the oxidants, OX (50–400 μmol dm^{-3}), to ensure complete scavenging of the $OH^{\bullet}$ radicals by HBA, which in turn resulted in some direct scavenging of e^-_{aq} by HBA, reaction (5):



(5)

The rate constant for reaction (5) was measured by observing the decay of e^-_{aq} absorption at 715 nm in the presence of HBA (100–400 μmol dm^{-3}) in N_2-saturated solutions containing 2-methylpropan-2-ol (0.2 mol dm^{-3}, pH 6). The second-order rate constant, $k_5 = 2 \times 10^9$ dm^3 mol^{-1} s^{-1}, is similar to that reported for benzoic acid.[13] This side reaction ($\leqslant$ 25% of $[e^-_{aq}]$) reduced the maximum yield of products arising from $OH^{\bullet}$ radical adducts to 0.545 and 0.595 μmol J^{-1} when using 40 or 10 mmol dm^{-3} HBA, respectively.

Product Yields

Addition of $OH^{\bullet}$ to HBA forms isomeric dihydroxycyclohexadienyl radicals which can undergo a variety of unimolecular and bimolecular reactions. From pulse radiolysis studies, see below, it was found that $\leqslant$ 400 μmol dm^{-3} of the quinones of relatively high reduction potential (pBQ, MBQ, DMBQ) resulted in a constant level of electron transfer to form semiquinone as observed on the microsecond time-scale. Yield–dose plots using 400 μmol dm^{-3} of the oxidants were linear, giving the results presented in table 1. The total radiation dose delivered, 300 Gy (given in 6 serial doses), resulted in $<$ 1% conversion of substrate (HBA) and $<$ 25% conversion of the oxidants.

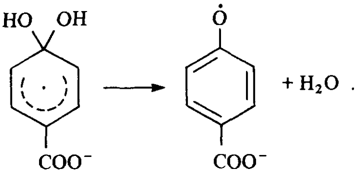
With the three most powerful oxidants (highest reduction potentials, table 1), the major product, 3,4-DHBA, accounted for 65 ± 2% of the total yield of $OH^{\bullet}$ while 2,4-DHBA (4 ± 1%) and HQ (16 ± 2%) were the only other measured products. The results are consistent with the addition of $OH^{\bullet}$ to positions 3,2 and 1 of HBA, respectively, followed by electron transfer to the oxidant to yield products [reaction(4)]. The remaining 15 ± 2% of the $OH^{\bullet}$ presumably adds to position 4 of HBA and by analogy

Table 1. Yields[a] of products and change in oxidants[b] following the reaction of hydroxyl radicals with 4-hydroxybenzoic acid

oxidant [E(1) at pH 7[c]]	[HBA]/ mmol dm^{-3}	pH	[products]/μmol J^{-1}			[oxidants]/μmol J^{-1}	
			3,4-DHBA	2,4-DHBA	HQ	OX	OXH$_2$
pBQ (+99 mV)	40	5	0.358±0.011 (66±2)	—	0.378±0.016	0.308±0.014 (45±2)	*d*
	10	6	0.332±0.004 (56±1)	0.023±0.002 (4±1)	0.358±0.010	0.331±0.010 (49±1)	*d*
MBQ (+23 mV)	40	5	0.359±0.008 (66±2)	—	0.087±0.006 (16±2)	0.352±0.013 (52±2)	0.321±0.015 (47±2)
	40	6	0.353±0.005 (65±1)	0.020±0.001 (4±1)	0.083±0.002 (15±1)	0.351±0.014 (52±2)	0.301±0.015 (44±2)
	10	6	0.384±0.010 (65±2)	0.015±0.001 (3±1)	0.094±0.003 (16±1)	0.340±0.016 (50±2)	0.373±0.015 (55±2)
DMBQ (−66 mV)	40	6	0.351±0.004 (64±1)	—	0.086±0.003 (16±1)	*e*	0.275±0.018 (40±3)
	10	6	0.413±0.006 (69±2)	0.014±0.001 (2±1)	0.098±0.003 (16±1)	*e*	0.250±0.007 (37±1)
AQS (−380 mV)	40	6	0.279±0.007 (51±2)	< 0.01	0.114±0.026 (21±5)		
TQ (−548 mV)	40	6	0.184±0.007 (34±2)	< 0.01	0.015±0.009 (3±2)		
V41 (−740 mV)	40	6	0.162±0.003 (30±1)	< 0.01	0.010±0.005 (2±1)		
none	1	6	0.168±0.009 (27±2)	< 0.01	0.020±0.010 (4±2)		
none	1	9	< 0.01	< 0.01	< 0.01		
none (N$_2$ gas)	1	6	< 0.01	< 0.01	< 0.01		

[a] Values in parentheses are the percentage yields of (i), products, from a maximum yield of OH$^{\cdot}$ of 0.525 and 0.575 μmol J^{-1} for 40 and 10 mmol dm^{-3} [HBA] and (ii), the change in oxidants, from a total radical yield ([OH$^{\cdot}$]+[H$^{\cdot}$]) of 0.68 μmol J^{-1}. [b] The concentration of all oxidants was 0.4 mmol dm^{-3}. [c] One-electron reduction potentials from ref. (19) and (20). [d] Included in the yield of HQ. [e] Unable to be determined using bubbled vials.

with similar studies with anisole[6] and phenol[7] undergoes rapid water elimination to form a phenoxyl radical



(6)

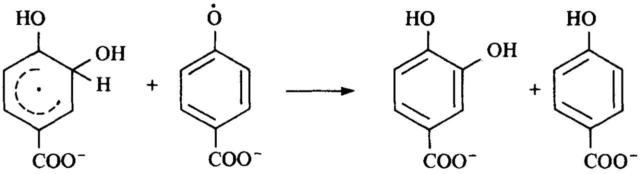
The dependence of product yields on the concentration of the oxidants (<400 µmol dm^{-3}) is understood in that OH$^{\bullet}$ radical adducts in other than the 4 position on HBA can also undergo water elimination in competition to electron transfer [reaction(4)], albeit on a longer time-scale.

Electron transfer from the OH$^{\bullet}$ radical adducts of HBA to the quinones form semiquinones which disproportionate [reaction (7)].

$$2Q^{\bullet -}+2H^{+} \longrightarrow Q+QH_2 \tag{7}$$

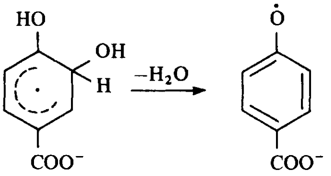
The loss of pBQ and MBQ and the production of their hydroquinones account for the full radical yield. This indicates that the products arising from phenoxyl radicals are oxidized by pBQ and MBQ (but possibly not by DMBQ).

A considerable amount of 3,4-DHBA was formed in the absence of oxidant at pH 6 but not at pH 9, table 1, where only some unidentified less polar products were observed. The 3,4-DHBA product might arise from a radical–radical reaction between the substituted cyclohexadienyl radical and the phenoxyl radical [reaction (8)]



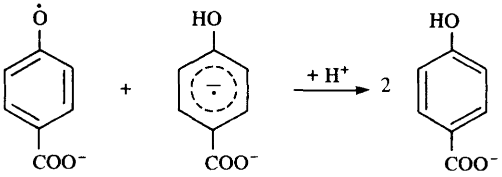
(8)

in competition with an acid–base catalysed elimination of water similar to that known for phenol[14] [reaction (9), see below] to yield the phenoxyl radical, which in turn decays bimolecularly to give unidentified products.



(9)

No loss of HBA was observed in irradiated N$_2$-saturated solution which implies that the phenoxyl radical formed from the OH$^{\bullet}$ radical adduct oxidises the e$^-_{aq}$ adduct to reform substrate [reaction (10)]



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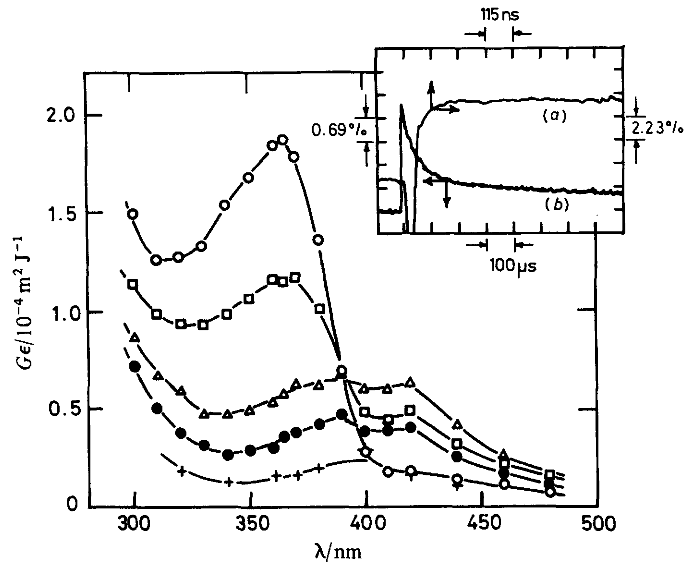


Fig. 1. Spectral changes observed following the pulse radiolysis of a N_2O-saturated solution of HBA (5 mmol dm^{-3}) adjusted to pH 7 [NaOH, phosphate (1 mmol dm^{-3})]. Spectra measured: ○, 0.5 μs; □, 50 μs; △, 200 μs; ●, 800 μs after the pulse (4.7 Gy in 30 ns). Insert: oscilloscope trace of percentage absorption at 360 nm (ordinate) against time (abscissa) for (*b*), conditions as above and (*a*), for HBA (10 mmol dm^{-3}) following a radiation dose of 12.7 Gy. Spectrum + was found by subtraction of the spectrum measured at 70 ns (40 ns after a 30 ns pulse) from that measured at 0.5 μs.

Pulse Radiolysis

The transient spectrum observed following pulse radiolysis (5 Gy in 30 ns) of a N_2O-saturated solution [pH 7, phosphate (5 mmol dm^{-3})] containing HBA (5 mmol dm^{-3}) is presented in fig. 1. The transient species exhibiting an absorption band centred at 365 nm (measured 0.5 μs after the pulse) decayed with first-order kinetics to a new species (isosbestic point at 390 nm) that exhibits a broad absorption band near 400 nm (measured 200 μs after the pulse). A two-stage process was seen in the formation of the 365 nm species where a small increase in absorption (by 0.5 μs) was seen superimposed on the initial OH$^{\bullet}$ adduct measured at 70 ns (40 ns after a 30 ns pulse) (insert, fig. 1). The rate of build-up of this small increase in absorption is independent of the concentration of HBA (10–40 mmol dm^{-3}) with $k \approx 2 \times 10^7$ s^{-1}. The spectrum corresponding to this increase is found by subtracting the spectrum at 70 ns from that at 0.5 μs, fig. 1. This spectrum has similar features to the spectrum measured at 200 μs and accounts for the observed absorption at $\geqslant$ 400 nm in the spectrum measured at 0.5 μs. The spectrum is similar to known phenoxyl radical spectra.[15] Furthermore a two-step oxidation of added *NNN′N′*-tetramethyl-*p*-phenylenediamine (TMPD) to its radical cation was observed consistent with the known reaction between TMPD and phenoxyl radicals.[16] By comparing the formation of the TMPD radical cation following pulse radiolysis (4 Gy in 30 ns) with that formed by the OH$^{\bullet}$ radical directly it was found that *ca.* 15% was formed during the 0.5 μs time period (independent of pH), rising to 100% at pH $\geqslant$ 7.5 when measured within the lifetime of the 365 nm species, fig. 2. This secondary increase in the production of the TMPD radical cation is interpreted as arising from the base-catalysed formation of the phenoxyl radical (see below) in competition with bimolecular reactions of the 365 nm species.

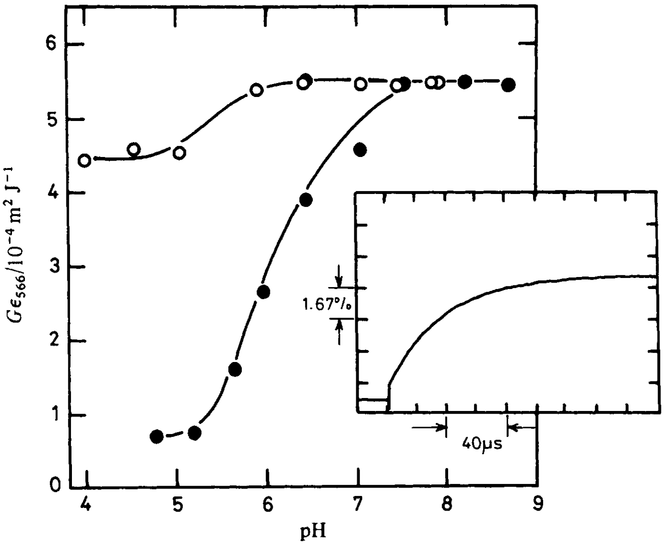


Fig. 2. The maximum spectral change observed at 565 nm *versus* pH following the pulse radiolysis (4 Gy in 30 ns) of N_2O-saturated solutions containing TMPD (400 μmol dm^{-3}) (○) and added HBA (20 mmol dm^{-3}) (●). Insert: oscilloscope trace of percentage absorption (ordinate) against time (abscissa) for pH 6.5.

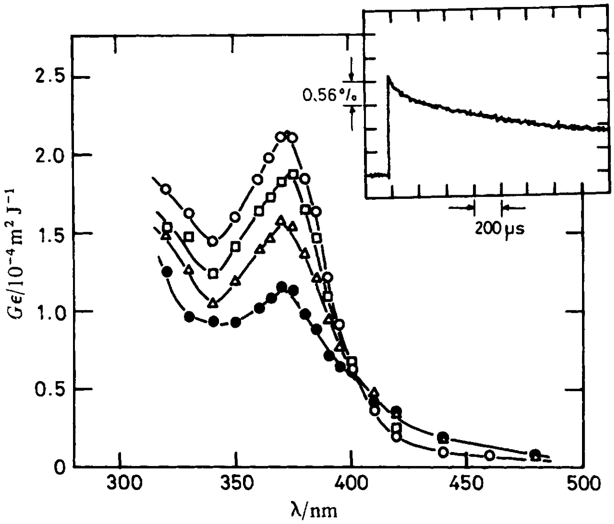
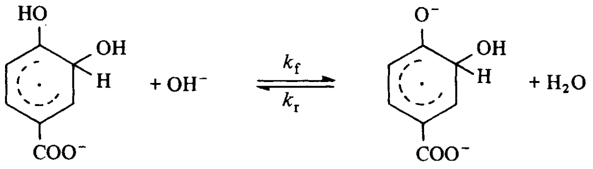


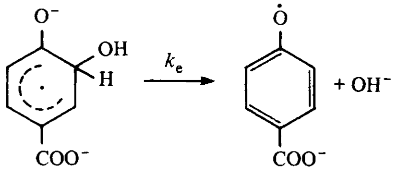
Fig. 3. Spectral changes observed following the pulse radiolysis of a N_2O-saturated solution of 3,5-dimethyl-4-hydroxybenzoic acid (5 mmol dm^{-3}) adjusted to pH 7 [NaOH, phosphate (1 mmol dm^{-3})]. Spectra measured: ○, 2 μs; □, 50 μs; △, 200 μs; ●, 800 μs after the pulse (2.5 Gy in 30 ns). Insert: oscilloscope trace of percentage absorption at 370 nm (ordinate) against time (abscissa).

These results support the suggested scheme in which a small proportion of the OH$^\bullet$ radicals add *ipso* to the hydroxyl group of HBA followed by rapid elimination of water to form the phenoxyl radical, reaction (6). The majority of the OH$^\bullet$ radicals add to position 3 of HBA followed also by water elimination, reaction (9). That the observed first-order decrease in the 365 nm peak arises from this pathway was confirmed on using 3,5-dimethyl-4-hydroxybenzoic acid as the substrate, fig. 3. In this case OH$^\bullet$ addition to position 3 (and 5) is unfavourable and the observed radical intermediate decayed only with second-order kinetics, $2k/\varepsilon = 6.2 \pm 0.9 \times 10^4$ cm s^{-1} measured at 370 nm. The phenoxyl radical of HBA decayed with second-order kinetics, $2k/\varepsilon = 1.8 \pm 0.3 \times 10^5$ cm s^{-1} measured at 370 nm and using $\varepsilon_{370} = 110$ m^2 mol^{-1} gave $2k = 2 \times 10^8$ dm^3 mol^{-1} s^{-1}. This rate constant is similar to that reported for the decay of the anionic form of the phenoxyl radical formed from hydroquinone.[17]

The log plot of the observed first-order rate constant, k, for the conversion of the 365 nm species (the substituted hydroxycyclohexadienyl radical) to the 400 nm species (the phenoxyl radical) against pH exhibits regions that are H$^+$ or OH$^-$ catalysed, fig. 4. Catalysis by the phosphate buffer also occurs with a second-order rate constant of *ca.* 5×10^5 dm^3 mol^{-1} s^{-1} (insert, fig. 4), leading to a region where k is independent of pH, k_0. Whereas there are insufficient data at low pH to derive mechanistic conclusions on H$^+$ catalysis a full description of OH$^-$ catalysis is possible. The absorbance at 365 nm decreases at high pH (fig. 4), which is consistent with the fast establishment of an equilibrium between the substituted hydroxycyclohexadienyl radical and its deprotonated form, reaction (11), before an elimination reaction (k_e) which yields the phenoxyl radical, reaction (12).



(11)



(12)

The kinetic description for when $k_f \approx k_r$ and $k_e \gg k_r$ is given by[18] $k = k_f k_e (k_f + k_r)^{-1}$, and for HBA between pH 4 and 9 by

$$k = k_0 + k_e k_{11}[\mathrm{OH}^-](K_W + K_{11}[\mathrm{OH}^-])^{-1}. \qquad (13)$$

Fixing $k_0 = 3 \times 10^3$ s^{-1} and performing regression analysis on equation (13) gave $k_e = 3.0 \pm 0.3 \times 10^5$ s^{-1} and $K_{11} = 2.9 \pm 0.3 \times 10^{-9}$. The value for K_{11} is in good agreement with that found independently from fig. 4, where a least-squares fit to the optical absorption data gave $\mathrm{p}K_a = 8.4 \pm 0.2$ and hence $K_{11} = 4.0 \pm 2.5 \times 10^{-9}$.

The rate constants for electron transfer from the OH$^\bullet$ radical adduct of HBA to oxidants (OX) were determined by following the formation of the absorbance bands[20–23] of the one-electron reduced oxidants (OX$^{\bullet -}$). The second-order rate constants, determined using at least three concentrations of OX, are presented in table 2. The fractions of the total OH$^\bullet$ concentration that are associated with the observed electron

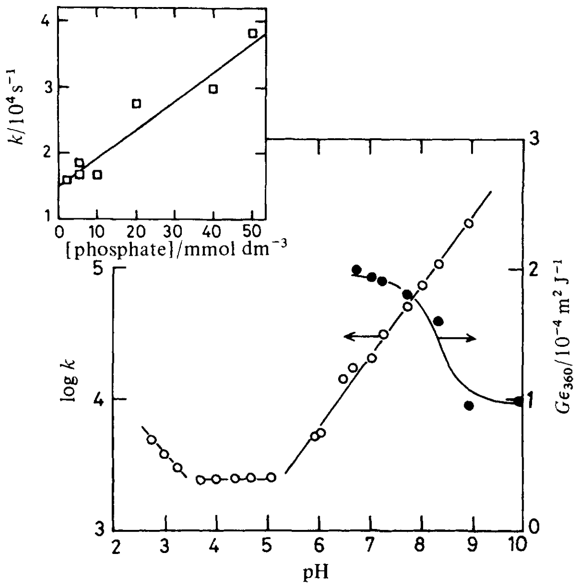


Fig. 4. The pH dependence of the initial absorbance at 360 nm (●) formed upon the reaction of the $OH^{\bullet}$ radical with HBA, and the first-order rate constant of its decay (○). Insert: the dependence of the first-order rate constant at pH 6.5 on phosphate concentration.

Table 2. Observed rate constants and radical yields for the formation of oxidant radicals following the addition of $OH^{\bullet}$ radicals to 4-hydroxybenzoic acid[a]

oxidant [E(1) at pH 7]	pH	$k(4)$/ $dm^3\ mol^{-1}\ s^{-1}$	$[OX^{\bullet-}]$/ $[{}^{\bullet}OH]_0$
pBQ (+99 mV)	5	2.7×10^9	0.72[b]
MBQ (+23 mV)	6	1.8×10^9	0.70[b]
DMBQ (−66 mV)	6	1.5×10^9	0.68[b]
O_2 (−155 mV)	6	5.5×10^8[c]	
AQS (−380 mV)	7	3.0×10^8[d]	0.38[e]
TQ (−548 mV)	6	4.0×10^7[d]	0.43[e]
V41 (−740 mV)	6	$\leqslant 2 \times 10^7$	

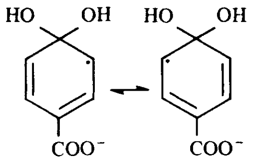
[a] [HBA] = 20 mmol dm^{-3}. [b] Plateau value in $[OX^{\bullet-}]$ observed for [OX] $\leqslant$ 0.4 mmol dm^{-3}. [c] Rate constant measured for decay of the 360 nm absorption band. [d] Values derived from reciprocal plots. [e] Ratio observed using [OX] 0.4 mmol dm^{-3}.

transfers were determined against the yield of $OX^{\bullet-}$ formed in solutions containing sodium formate (0.1 mol dm^{-3}) and the OX, table 2. The observed yields {$[OX^{\bullet-}]$ in the presence of pBQ, MBQ and DMBQ (0.70 ± 0.02)} are similar to the yield of 3,4-DHBA, which implies that the observed rate constants are for the oxidation of the intermediate formed upon the addition of $OH^{\bullet}$ to position 3 of HBA. The rate constants decrease with the one-electron reduction potential of OX, which accounts for the observed decrease in the yields of $OX^{\bullet-}$, as an increasing proportion of the $OH^{\bullet}$ radical adduct will form the phenoxyl radical.

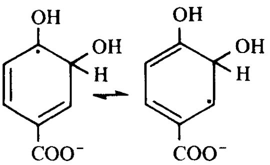
Conclusions

These results indicate that the OH$^{\bullet}$ radical undergoes electrophilic addition to the ring positions which are activated by the electron donating hydroxy group. This pattern of reactivity is expected from data obtained in similar systems.[3,5,7,24-26] However, in the case of HBA clearly steric effects are also important in the pattern of OH$^{\bullet}$ reactivity, where addition to the position *ortho* to the hydroxy group predominates over the substituted *para* position. Also a greater percentage of the OH$^{\bullet}$ radicals react *ipso* to the hydroxy group than in the case of phenol.[7]

The spectrum measured 0.5 μs after the pulse is the sum of the absorption bands due to OH$^{\bullet}$ radical addition to all 4 positions on HBA. The fast formation of the fraction of the phenoxyl radical absorption associated with OH$^{\bullet}$ addition to position 4 is observed as an increase in absorbance at all measured wavelengths. This observation implies that the initial OH$^{\bullet}$ radical adduct to position 4 absorbs at $\lambda \leqslant 320$ nm, in the region where the cyclohexadienyl radical absorbs.[27] The difference in the absorption spectra between addition (*a*) *ipso* to the hydroxy group and (*b*) to position 3 might be related to the different natures of the two substituted cyclohexadienyl radicals produced. Whereas in case (*b*) a resonance form of the cyclohexadienyl radical can be drawn with a hydroxy-substituted carbon radical, this is not so for case (*a*). A similar consideration could apply for addition *ipso* to the carboxylate group.



(*a*)



(*b*)

Since OH$^{\bullet}$ addition to position 2 of HBA is *ca.* 4% of the radical yield it is clear that the band at 365 nm arises from OH$^{\bullet}$ addition at position 3. During the oxidation of certain substrates for *p*-hydroxybenzoate hydroxylase it is observed that the absorption spectrum associated with its flavin hydroperoxide, species I ($\lambda_{\text{max}} = 350$–$400$ nm, $\varepsilon \approx 900$ m^2 mol^{-1}), increases to give species II ($\varepsilon \approx 1400$ m^2 mol^{-1}).[8] We are currently investigating these other substrates of *p*-hydroxybenzoate hydroxylase to see if part of the absorption spectrum of species II can be accounted for by the radical mechanism described above.

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