Free Radical Induced Oxidation of Phloroglucinol. A Pulse Radiolysis and EPR Study

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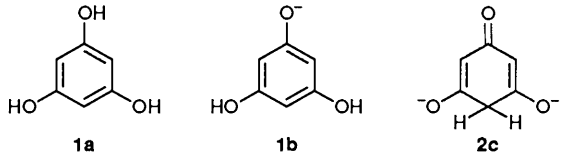
Phloroglucinol-derived radicals have been studied using pulse radiolysis and EPR spectroscopy. Phloroglucinol **1a** (pK_a = 8.0) and its anion **1b** (pK_a = 9.2) have phenolic structures while the 3,5-dihydroxycyclohexa-2,5-dienone structure predominates in the dianion **2c**. The neutral OH-adduct radicals (λ_{max} = 345 nm) rapidly eliminate water ($k = 2 \times 10^5$ s^{-1}) yielding the 3,5-dihydroxyphenoxyl radical **4a** (λ_{max} = 495 nm, pK_a = 6.5). This radical as well as its monoanion **4b** (λ_{max} = 550 nm, pK_a = 8.6), its isomer **5b**, derived from **2c** (λ_{max} > 800 nm), and the dianion **4c** (λ_{max} = 640 nm) can be generated directly with the N_3 radical ($k = 1.4 \times 10^9$ dm^3 mol^{-1} s^{-1} at pH 6). All four radicals have been characterized by EPR spectroscopy. Radical **5b** reacts with the phloroglucinol monoanion **1b** with a rate constant of 2×10^7 dm^3 mol^{-1} s^{-1}. Formation of an adduct is excluded by EPR. Therefore, electron transfer from the phloroglucinol monoanion **1b** to radical **5b** is favoured as an explanation for this reaction.

While **4a** does not react with O_2 ($k < 4 \times 10^5$ dm^3 mol^{-1} s^{-1}), the anions **4b** and **5b** do so quite rapidly ($k = 2.1 \times 10^8$ and 1.9×10^8 dm^3 mol^{-1} s^{-1}, respectively) and at pH 7, O_2 is consumed with $G = 15 \times 10^{-7}$ mol J^{-1}.

Although $Br_2^{\bullet -}$ mainly produces radicals **4a** and **b**, bromination occurs with an efficiency of at least 10%.

Phenolic compounds play an important role as antioxidants with technical applications and in Nature. Tocopherols, *tert*-butyl derived monophenols, and gallic acid derived polyphenols are used in food preservation and for inhibition of lipid oxidation in biological material. A variety of antioxidants in food is provided by the large number of polyphenolic compounds in plants, *i.e.*, flavonoids and tannins. Pulse radiolysis experiments have been carried out to study the reactivity of flavonoids with oxidizing radicals.[1,2] Because of the complexity of the systems, model compounds were required for the interpretation of the results. In order to gain insight into the complexity of the protonation–deprotonation and tautomerization reactions expected for polyphenol-derived radicals we chose, as a model compound for pulse radiolysis and EPR studies, phloroglucinol (1,3,5-trihydroxybenzene), which is an integral structural component of flavonoids and which in its free form is found in some eucalyptus and acacia species. Recent ^{13}C NMR studies showed that it has pK_a values at 8.0, 9.2 and ~ 14.[3] The neutral molecule **1a** and the monoanion **1b** exist predominantly in the phenolic forms whereas the dianion has largely the 3,5-dihydroxycyclohexa-2,5-dienone structure **2c**. Relaxation kinetics showed that besides **1a**, **1b** and **2c** seven further forms of the molecule contribute in minor amounts to the dissociation and keto–enol equilibria.[4]

We now present our results on the reaction of OH, N_3 and Br_2^- radicals with phloroglucinol in acidic and alkaline solutions. The radicals generated in this way from the polyphenol were characterized by UV–VIS and by EPR spectroscopy.



1a **1b** **2c**

Experimental

Phloroglucinol (*pa*, Fluka), sodium azide (for synthesis, Schuchardt), *N*,*O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA, Macherey-Nagel) and potassium bromide (*pa*, Merck) were used as received. Solutions were made up in Millipore Milli-Q-filtered water. Nitrous oxide (Messer-Griesheim) was freed from remaining traces of oxygen by an Oxisorb column (Messer-Griesheim). A mixture of N_2O–O_2 and pure O_2 was obtained from the same supplier. Gas mixtures were prepared using a Brooks gas mixer.

The pulse radiolysis technique and set-up used in these experiments was as described recently.[5]

γ-Radiolysis experiments were carried out in a ^{60}Co panorama source (Nuclear Engineering Ltd.) at a dose rate of 0.45 Gy s^{-1}. Bromophloroglucinol (**8a**) was identified by GC–MS (Hewlett–Packard Model HP5890 II; 15 m PS-343.5, MSD 5971A) after trimethylsilylation with BSTFA. Its mass spectrum is characterized by *m*/*z* 420 (70%), 405 (24), 377 (6), 327 (10), 325 (7) and 73 (100). The same procedure was applied to products originating from reaction of O_2 with the phloroglucinol OH-adduct **3a**. The compound eluting directly after the TMS-ether (TMS = trimethylsilyl) of phloroglucinol showed a mass spectrum with *m*/*z* 430 (100%), 415 (10), 342 (10), 327 (13), 299 (5), 147 (5) and 73 (60). This product was tentatively assigned as the TMS-ether of 1,2,3,5-tetrahydroxybenzene (**11**).

EPR-spectra were measured with X-band spectrometers equipped with flow systems. Free radicals were generated by *in-situ* photolysis or by *in-situ* radiolysis techniques.

In the photolysis experiments Ar-saturated solutions containing phloroglucinol, NaN_3 and H_2O_2 were pumped through the EPR quartz cell by a continuous flow arrangement with a rate of 0.1 cm^3 s^{-1} for a 0.3×8 mm^2 cross section. *In-situ* photolysis was achieved by irradiation with an argon plasma light source (GAT-PB 1500 from GAT *Gamma Analysentechnik*, Bremerhaven). Spectra were recorded with a modified Varian V-4500 EPR spectrometer with 100 kHz modulation. The *g* factors were determined with a side-band technique.[6]

In radiolysis experiments the radicals were generated in the cavity of a Varian E-9 EPR spectrometer at 4 °C by *in-situ* irradiation of N_2O-saturated solutions with a beam (diameter 1 mm) of 2.4 MeV electrons. The solutions containing phloro-

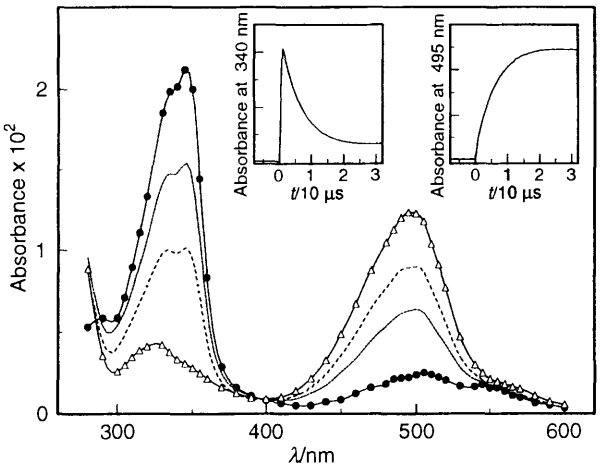


Fig. 1 Pulse radiolysis of N_2O-saturated phloroglucinol (2×10^{-3} mol dm^{-3}) at pH 5.8. Transformation of the OH-adduct radical **3a** (λ_{max} = 345 nm) into the phenoxyl radical **4a** (λ_{max} = 495 nm). The spectra were taken 1 μs (●), 4 μs (· · ·), 7 μs (- - -) and 24 μs (△) after the pulse. Insets: decay at 345 nm and buildup at 495 nm.

Table 1 Compilation of rate constants (dm^3 mol^{-1} s^{-1} unless stated otherwise)[a]

Reaction	
OH + Ph-ol	$\geqslant 1 \times 10^{10}$
Ph-ol $^{\bullet}$OH-adduct→Ph-O$^{\bullet}$	2×10^5 s^{-1}
$N_3^{\bullet}$ + Ph-ol	1.2×10^9
$N_3^{\bullet}$ + Ph-ol anion	3.9×10^9
$N_3^{\bullet}$ + DCH-ol dianion	3.9×10^9
DCH-O$^{\bullet}$ anion + Ph-ol anion	2×10^7
Ph-ol $^{\bullet}$OH-adduct + O_2	1.7×10^9
Ph-O$^{\bullet}$ + O_2	nil ($< 4 \times 10^5$)
Ph-O$^{\bullet}$ anion + O_2	2.1×10^8
DCH-O$^{\bullet}$ anion + O_2	1.4×10^8
Ph-O$^{\bullet}$ + Ph-O$^{\bullet}$	1.7×10^9
DCH-O$^{\bullet}$ anion + Ph-ol anion	2×10^7

[a] Ph-ol = phloroglucinol; DCH-ol = 3,5-dihydroxycyclohexa-2,5-dienone; Ph-O$^{\bullet}$ = phenoxyl radical derived from Ph-ol; DCH-O$^{\bullet}$ = radical derived from DCH-ol.

glucinol and NaN_3 were saturated with N_2O which was freed from O_2 by passage through columns packed with Oxisorb. The flow rate was 0.5 cm^3 s^{-1}.

Results and Discussion

Generation of Free Radicals.—Ionizing radiation was used to generate the free radicals. When dilute aqueous solutions of a given substrate such as phloroglucinol are subjected to ionizing radiation practically all the energy of the ionizing radiation is absorbed by the solvent water leading to solvated electrons, OH-radicals and H-atoms as free-radical intermediates [reaction (1)].[7] The solvated electrons can be converted into further OH-radicals by saturating the water with N_2O [reaction (2)].

$$H_2O \xrightarrow{\text{ionizing radiation}} e_{aq}^-,\ {}^{\bullet}OH,\ H^{\bullet},\ H^+,\ H_2O_2,\ H_2 \qquad (1)$$

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

Hydroxyl-radicals and H-atoms are electrophilic radicals[8,9] and react with phenols by adding preferentially to the *ortho*- and *para*-positions of the OH groups [*cf.* reaction (8)].[10] Phenols can also be oxidized by radicals which are less reactive than the OH-radical, *e.g.*, the azide radical $N_3^{\bullet}$, or the $Br_2^{\bullet -}$ radical. These radicals can be conveniently formed by reacting azide and bromide ions with OH-radicals [reactions (3)–(5)].[11–13] In the case of the azide ion the H-atom may also be converted into an N_3-radical [reactions (6)–(7)].[14,15]

$$N_3^- + {}^{\bullet}OH \longrightarrow N_3^{\bullet} + OH^- \qquad (3)$$

$$Br^- + {}^{\bullet}OH \longrightarrow Br^{\bullet} + OH^- \qquad (4)$$

$$Br^{\bullet} + Br^- \rightleftharpoons Br_2^{\bullet -} \qquad (5/-5)$$

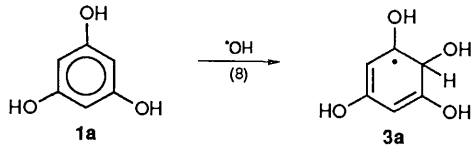
$$N_3^- + H^{\bullet} + H^+ \longrightarrow {}^{\bullet}NH_2 + N_2 \qquad (6)$$

$$^{\bullet}NH_2 + N_3^- + H^+ \longrightarrow NH_3 + N_3^{\bullet} \qquad (7)$$

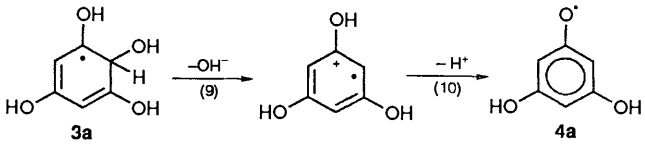
The rate constants of these various reactions are well documented, and the rate constants of the OH-radical with phenols are generally very fast ($k \approx 1 \times 10^{10}$ dm^3 mol^{-1} s^{-1}).[16] Choice of appropriate concentration conditions can enable the OH-radicals to react practically exclusively with the inorganic ions rather than with the phenols themselves.

Formation of Phenoxyl-type Radicals from Phloroglucinol.—Free-radical attack on phenols leads to the formation of phenoxyl radicals. Often an adduct radical can be detected as the precursor, *e.g.*, in the case of the OH-radical.

In Fig. 1 is given the UV–VIS absorption spectrum of the products formed 1, 4, 7 and 28 μs after a 7 Gy pulse of 0.4 μs duration in N_2O-saturated aqueous phloroglucinol at pH 5.8. The intermediate formed is characterized by an absorption maximum at 345 nm. This spectrum is compatible with the formation of the hydroxycyclohexadienyl radical **3a** [reaction (8)]. For benzene, OH-radical addition leads to an intermediate



with λ_{max} = 310 nm.[17] A spectral shift to longer wavelength for the radical **3a** due to the presence of three OH groups is to be expected, as the OH-adduct radical of phenol absorbs at λ = 330 nm.[18] From the build-up of this absorption at low phloroglucinol concentration (10^{-5} mol dm^3) the rate constant for reaction of phloroglucinol with the OH radical has been estimated at $k_8 \geqslant 1 \times 10^{10}$ dm^3 mol^{-1} s^{-1} (for a compilation of rate constants determined in this study see Table 1). Within a few microseconds the spectrum changes. The absorption at 345 nm decays while a build-up at 495 nm is observed (inset in Fig. 1). The final spectrum is attributed to the phenoxyl radical with some contribution from the H-adduct radical. The conversion of the OH-adduct radical into a phenoxyl radical is a well-known process in the free-radical chemistry of phenols.[18,19] It may occur as a spontaneous reaction [reaction (9)] followed by reaction (10).[18] The radical cations of phenols are very strong acids (*e.g.*, pK_a = −8.1 for the phenol radical cation and −5.5 for the hydroquinone radical cation)[20] so that reaction (10) becomes very fast. Thus the rate-limiting step is



reaction (9), which in the phloroglucinol system occurs with a rate constant of (2 ± 0.5) × 10^5 s^{-1} (measured at pH 5–6). The conversion of the OH-adduct radical into the phenoxyl radical is acid/base catalysed, and the reaction becomes faster in both acidic and basic media (*e.g.*, k_{obs} > 10^6 s^{-1} at pH 3). The spontaneous water elimination reaction is so fast there is very little room to measure the acid/base catalysis in some detail with our set-up which is limited to measure rate constants ⩽ 10^6 s^{-1}.

With phenols, there is yet a more direct way to the phenoxyl radical. The azide radical, $N_3^{\bullet}$, can react with phenols/phenolates by producing the phenoxyl radical directly [reactions (11) and (12), Scheme 1].[11] No intermediate adduct radical has yet been detected. The rate constant of the reaction of the azide radical with phloroglucinol has been measured as a function of pH (Fig. 2). At low pH the rate constant of the reaction of the N_3-radical with phloroglucinol is (1.2 ± 0.1) × 10^9 dm^3 mol^{-1} s^{-1}. It rises to a value of (3.9 ± 0.1) × 10^9 dm^3 mol^{-1} s^{-1} when the phenolate predominates (pK_{1a} = 8.0), but no further change in the rate constant is observed when the azide radical has to react with the 3,5-dihydroxycyclohexa-2,5-dienone dianion **2c**.

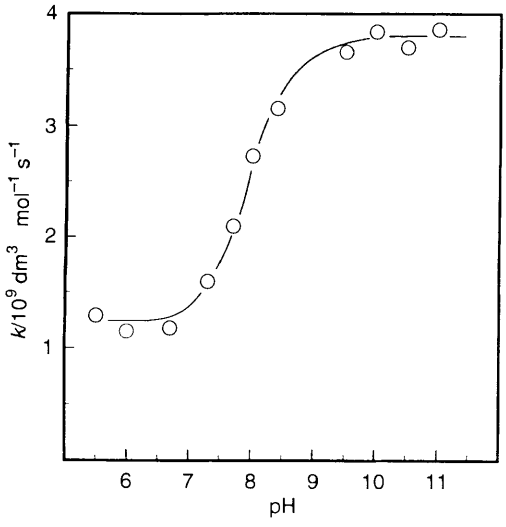
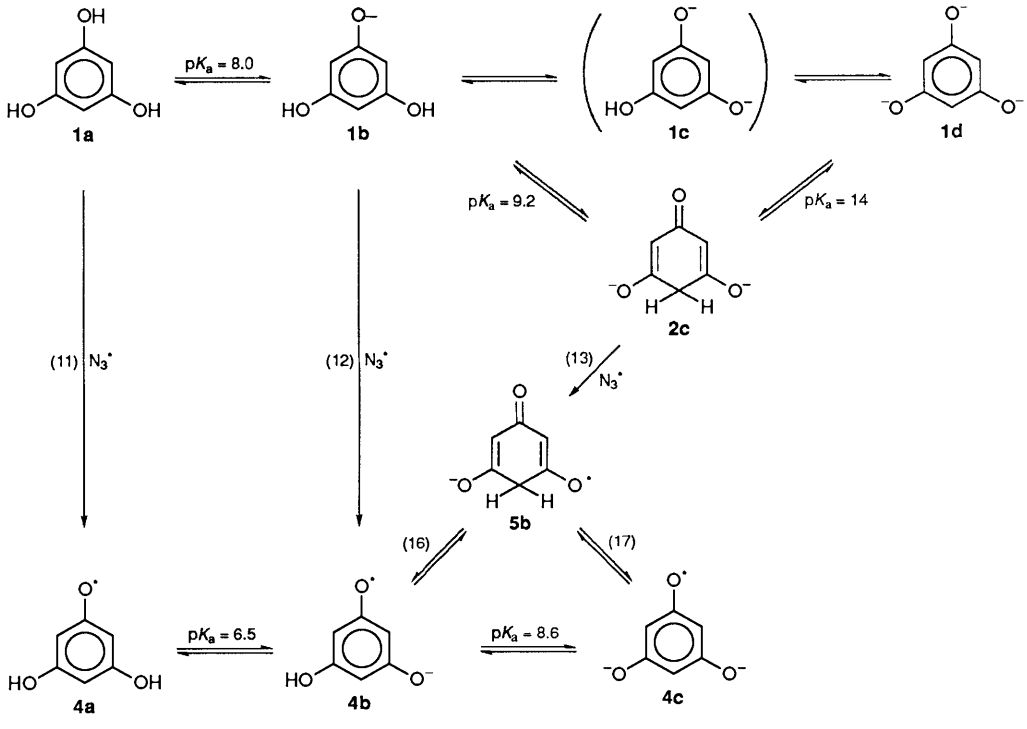


Fig. 2 Rate constant of the azide radical with phloroglucinol as a function of pH

When the phenoxyl radical is generated *via* the azide radical {[N_3^-] = 10^{-1} mol dm^{-3}; $pK_a(HN_3)$ = 4.74}[14] or *via* the OH-adduct radical in the presence of 10^{-2} mol dm^{-3} phosphate, the azide or phosphate buffers catalyse the acid/base equilibrium of the phenoxyl radical in neutral and pH < 9 solutions. At pH ⩾ 9, H_3BO_3 (10^{-2} mol dm^{-3}) was used as buffer, and the pH was adjusted with KOH.

At pH 4 the protonated phenoxyl radical has a UV–VIS spectrum with λ_{max} = 495 nm (Fig. 3). When the absorbance at 470 nm is plotted *vs.* the pH an inflection point is observed at pH 6.5 which is attributed to the pK_a of the fully protonated phenoxyl radical (inset in Fig. 3). At pH 7 the absorption maximum is at 550 nm, with a shoulder at about 500 nm (Fig. 4). We believe that the spectrum is mainly due to the phenoxyl radical monoanion with some contribution of the fully protonated phenoxyl radical (λ_{max} = 495 nm). When the absorbance at 570 nm is plotted against the pH an inflection point is observed at pH 6.5 (inset in Fig. 4), which again reflects the acid/base equilibrium between the protonated phenoxyl radical and its monoanion. From this pK_a value and the spectra shown in Figs. 3 and 4 the spectrum of the phenoxyl radical monoanion can be calculated. It is also shown in Fig. 4.

At pH 8.3 and 9.0 spectra are obtained with an absorption maximum at 640 nm (Fig. 5). The 640 nm absorption is more pronounced at the higher pH value. A plot of the absorbance at 570 nm shows an inflection point at pH > 8.5. Concomitantly the absorption at 620 nm rises (data not shown). The new species with λ_{max} = 640 nm is most likely the phenoxyl radical dianion **4c**. The determination of the pK_a value of the phenoxyl radical monoanion **4b** must be around 8.6, but its determination is fraught with a considerable error (⩾ ± 0.2), because in this pH range the N_3-radical starts to react with the 3,5-dihydroxycyclohexa-2,5-dienone **2c**, thereby forming **5b**. The spectrum of **5b** can be taken at pH ⩾ 10.2 (Fig. 6). Taking pK_a (**1b**) = 9.1 and $k_{12} \approx k_{13}$ = 3.9 × 10^9 dm^3 mol^{-1} s^{-1} (see Fig. 2) and assuming a pK_a value of 8.6 for **4b**, the spectrum of **4c** has been



Scheme 1

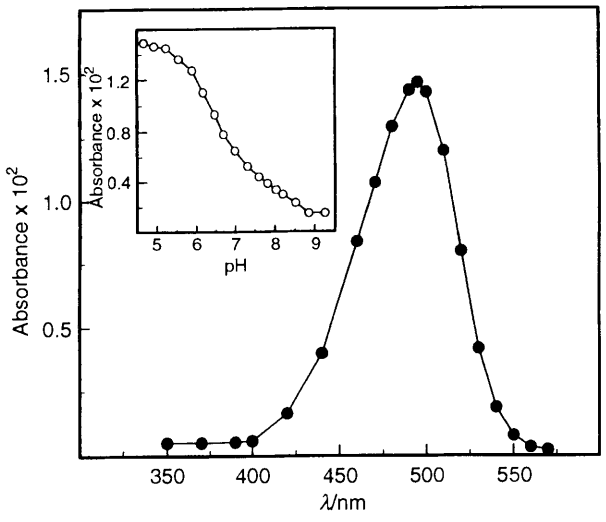


Fig. 3 Pulse radiolysis of N_2O-saturated phloroglucinol (1×10^{-3} mol dm^{-3}) in the presence of NaN_3 (0.1 mol dm^{-3}) at pH 4.0. The spectrum of the phenoxyl radical **4a** was taken 5 μs after the pulse. Inset: absorbance at 470 nm as the function of pH.

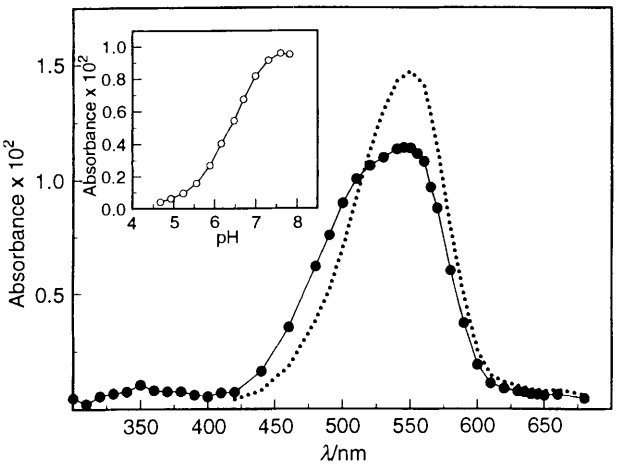


Fig. 4 Pulse radiolysis of N_2O-saturated phloroglucinol (1×10^{-3} mol dm^{-3}) in the presence of NaN_3 (0.1 mol dm^{-3}) and KH_2PO_4 (1×10^{-2} mol dm^{-3}) at pH 7.0. (●), Experimental spectrum of the mixture of the protonated phenoxyl radical **4a** and phenoxyl radical monoanion **4b** at 5 μs after the pulse; (· · ·), calculated spectrum for phenoxyl radical monoanion. Inset: absorbance at 570 nm as the function of pH.

calculated on the basis of the spectra given in Fig. 5 (dotted line).

At pH ⩾ 10, the 3,5-dihydroxycyclohexa-2,5-dienone **2c** is the predominating species. Its oxidation by either OH- or N_3-radicals yields **5b** [reaction (13)]. This species barely absorbs between 350 (cut-off by the strong absorption of **2c**) and 600 nm, but there is a steady increase in absorption towards > 800 nm, our detection limit (Fig. 6). The absorption at 750 nm immediately after the pulse is plotted as a function of pH in the inset of Fig. 6. This plot shows an inflection point at pH 9 ± 0.1. Recalling that in the plot of k_{obs} *vs.* pH of the reaction of the azide radical with phloroglucinol (Fig. 2) no noticeable change in the rate constant between **1b** and **2c** was observed, we conclude that the second pK_a value of the phloroglucinol[3] (9.2 ± 0.15) must be close to 9.1 rather than to 9.2 to accommodate the two sets of data.

The intermediate **5b** decays by first-order kinetics into another species absorbing at 640 nm (Fig. 7). At a given pH (*e.g.*, pH 9.2) the observed rate constant is proportional to the total phloroglucinol concentration (Fig. 8). When at a given total phloroglucinol concentration the pH is raised to between

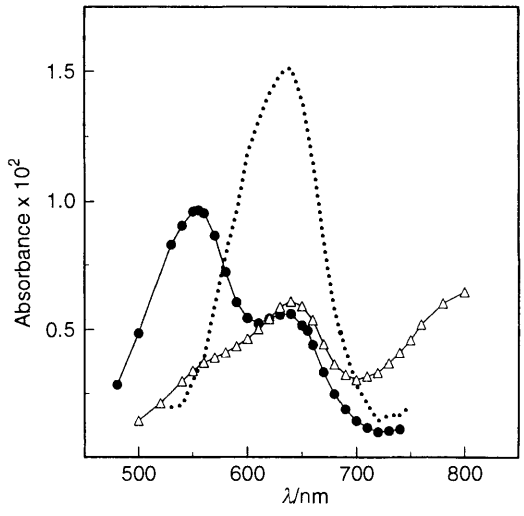


Fig. 5 Pulse radiolysis of N_2O-saturated phloroglucinol (1×10^{-3} mol dm^{-3}) in the presence of NaN_3 (0.1 mol dm^{-3}). (●), KH_2PO_4 (1×10^{-2} mol dm^{-3}), pH 8.3, 5.7 μs after the pulse; (△), H_3BO_3 (1×10^{-2} mol dm^{-3}), pH 9.0, 8.3 μs after the pulse; (· · ·), calculated spectrum for the phenoxyl radical dianion **4c** (based on a pK_a value for the phenoxyl radical monoanion of 8.6; the pK_a value for the phloroglucinol monoanion is 9.1).

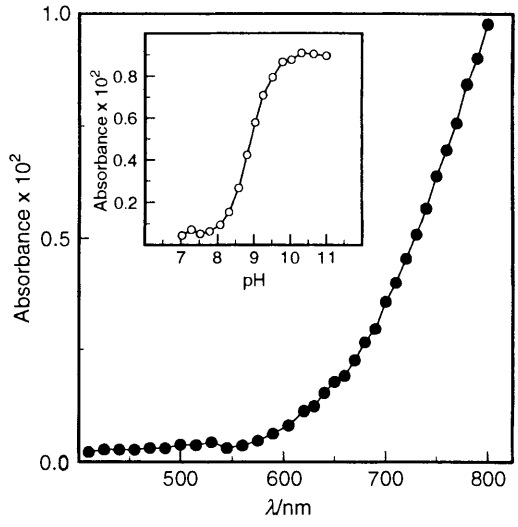
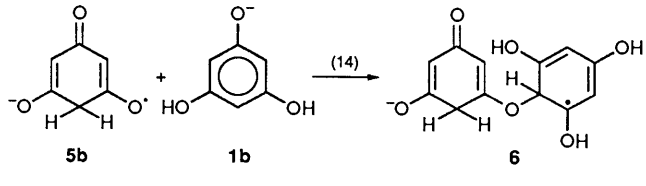


Fig. 6 Pulse radiolysis of N_2O-saturated phloroglucinol (1×10^{-3} mol dm^{-3}) in the presence of NaN_3 (0.1 mol dm^{-3}) and H_3BO_3 (1×10^{-2} mol dm^{-3}) at pH 10.9. The spectrum was taken 5 μs after the pulse. Inset: absorbance at 790 nm as the function of pH.

9.3 and 10.2 the observed rate constant falls (inset in Fig. 8). These results indicate that the observed reaction is that of **5b** with phloroglucinol, and more specifically with its monoanion **1b**. In fact when the data in Fig. 8 were used to calculate k(**5b** + **1b**) a value of 1.7×10^7 dm^3 mol^{-1} s^{-1} was obtained. From the data given in the inset of Fig. 8 when transformed into k_{obs} *vs.* [**1b**] a straight line was obtained with a slope of 2×10^7 dm^3 mol^{-1} s^{-1} (plot not shown). These two values agree to within experimental error.

Two types of reaction might be envisaged. In the first one [reaction (14)] a stable adduct radical (**6**) should be formed by



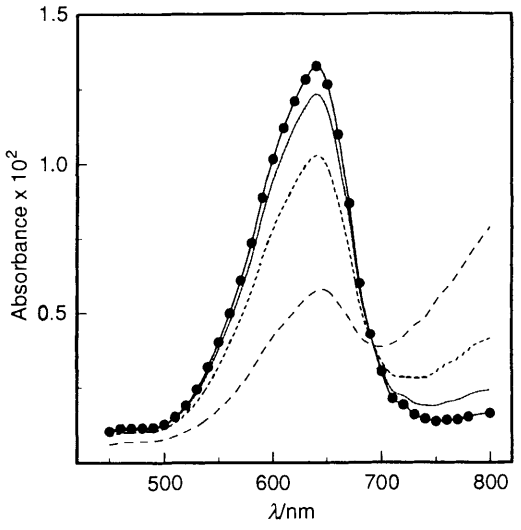


Fig. 7 Decay of the spectrum in Fig. 6 and the build-up of a new spectrum with λ_{max} = 640 nm. Spectra were taken at 40 μs (- - -), 130 μs (- - -), 220 μs (——) and 400 μs (●) after the pulse.

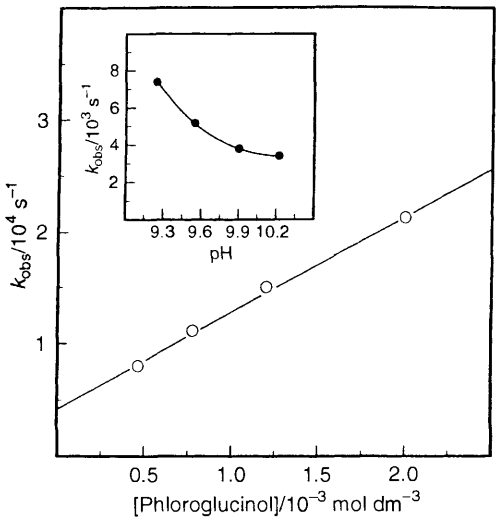
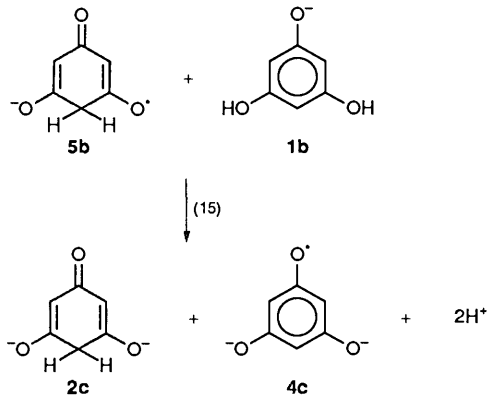


Fig. 8 Pulse radiolysis of N_2O-saturated phloroglucinol in the presence of NaN_3 (0.2 mol dm^{-3}) at pH 9.23. First-order decay rate constant of the absorption at 800 nm as the function of total phloroglucinol concentration. Inset: first order decay rate constant at 750 nm as the function of pH at a given total phloroglucinol concentration (5 × 10^{-4} mol dm^{-3}).

addition of radical **5b** to the monoanion of the parent compound (**1b**). In a similar system for dihydroxyindole, rapid addition of the indole-derived phenoxyl radical to the phenolate has been suggested recently.[21] On the other hand, electron transfer reactions of the phenoxyl radical with substituted phenols have been shown to be potentially very fast ($k \approx 2 \times 10^9$ dm^3 mol^{-1} s^{-1}).[22] Thus one could envisage, by analogy, an electron transfer from **1b** to **5b** and the formation of the corresponding phenoxyl radical **4b** or **4c** in reaction (15), (*i.e.*, **5b** would be a metastable species in this system). The spectral similarities of the estimated spectrum of **4c** (dotted line in Fig. 5) and of the transient absorption due to reaction of **5b** with **1b** (see Fig. 7) as well as the failure to detect an adduct radical in the EPR experiments (see below) are in line with such a proposal.

Reaction (13), *i.e.*, the OH^--induced oxidation of **2c** by the N_3-radical is not the only route to **5b**. It was found that the radical anion **4b** formed at pH 8–9 in reaction (12) transforms into radical **5b** in the presence of 0.4 mol dm^{-3} phosphate (to



speed up protonation–deprotonation reactions) with a rate of 6 × 10^2 s^{-1} [reaction (16)].

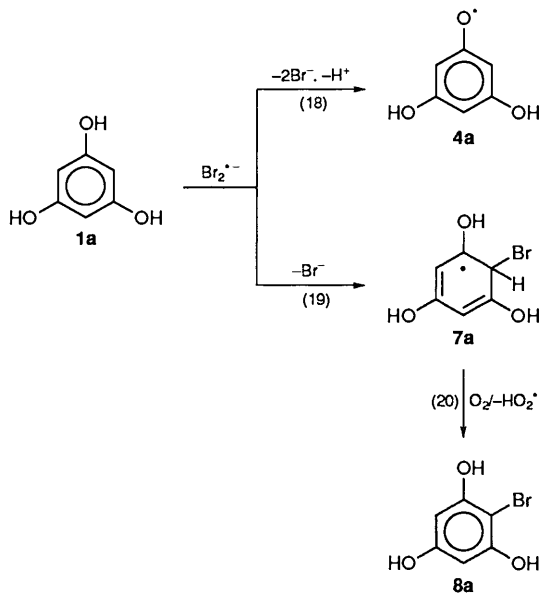
The rather complex situation in the alkaline region is illustrated in Scheme 1.

The two phenolic radical ions **4b** and **4c** and the isomer **5b** are related by equilibria [tautomerization reactions (16) and (17) and protonation–deprotonation at pH 8.6]. Upon oxidation at pH values < 8.6, *i.e.*, below the pK_a value of the monoanion **1b**, radical **4b** is the predominating species in the pulse radiolysis experiments because at low buffer concentrations tautomerization [reaction (16)] is slow on the microsecond time-scale. For the same reasons at pH values 8.6–9.2 the dianion radical **4c** is favoured whereas at pH values > 9.2, **5b**, formed *via* reaction (13), is detected as a metastable species which transforms into the radical dianion **4c** in reaction (17) on the microsecond time-scale. Extrapolation of the plot in Fig. 8 to low phloroglucinol concentrations results in an intercept of $k_{obs} \approx 4 \times 10^3$ s^{-1}. This value may be due to contributions from the self-termination reaction of **5b**, from the back reaction of **4c** to **5b** according to equilibrium (17) and from the first-order transformation of **5b** into **4c** not involving **1b**. It was not possible to distinguish between these processes.

The Reaction of $Br_2^{\bullet-}$ *with Phloroglucinol.*—The $Br_2^{\bullet-}$ radical has been used quite often to produce the phenoxyl radical, especially in neutral and acid solutions when the reaction of the azide radical is slow with a given phenol. Also in the present case $Br_2^{\bullet-}$ proved to be a convenient route to phenoxyl radicals. However, when we investigated the products of this reaction in the presence of O_2 at about pH 6.5, we observed that, with a G value of 0.6 × 10^{-7} mol J^{-1}, 2-bromophloroglucinol **8a** was formed. This observation indicates that besides an electron transfer (or H-abstraction) [reaction (18)] $Br_2^{\bullet-}$ can transfer a bromine atom to phloroglucinol [reaction (19)]. It is suggested that the intermediate Br-adduct radical **7a** reacts with oxygen, after $HO_2^{\bullet}$-elimination and isomerization giving **8a** [see reaction (20)].

The interesting formation of **8a** raises the question whether also with other systems Br-atom transfer competes significantly with electron transfer and/or H-abstraction. Here bromination occurs to an extent of at least 10%.

In a recent study on the reaction of $Cl_2^{\bullet-}$ with *tert*-butyl alcohol it has been shown that the reactivity observed at Cl^- concentrations ≤ 0.02 mol dm^{-3} is mainly due to the Cl-atom in equilibrium with $Cl_2^{\bullet-}$ (Mertens and von Sonntag, unpublished results). Hence bromination of phloroglucinol by $Br_2^{\bullet-}$ could be due to such a side reaction of the Br-atom in equilibrium. The above experiments have been carried out at a Br^- concentration of 0.1 mol dm^{-3}. The equilibrium constant of equilibrium $[Br_2^{\bullet-}]/[Br^{\bullet}][Br^-]$ is reported at $K < 7.8 \times 10^6$ (ref. 12) and $K > 2.3 \times 10^5$ dm^3 mol^{-1} (ref. 23), and it is rapidly



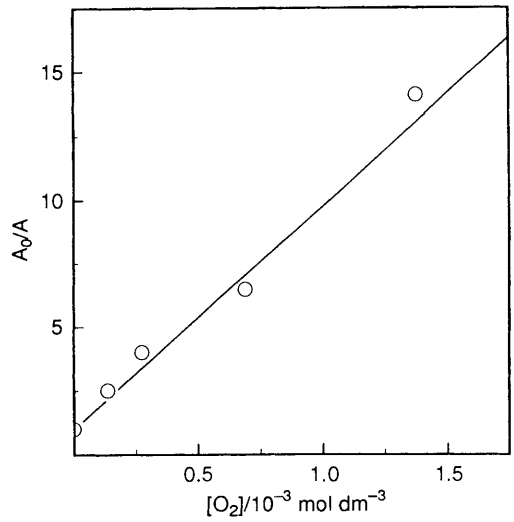


Fig. 9 Pulse radiolysis of phloroglucinol (1×10^{-3} mol dm^{-3}) saturated with N_2O or N_2O–O_2 at pH 5.8. Absorbance A_0 (without O_2) at 495 nm divided by the absorbances A (with various O_2 concentrations) at the same wavelength as a function of O_2 concentration.

established. At the above Br^- concentration free Br-atoms are thus present at a fraction of 0.005% of $Br_2^{\bullet -}$ at the upper limit. The observed rate constant of $Br_2^{\bullet -}$ with phloroglucinol at pH 4 is 6.6×10^8 dm^3 mol^{-1} s^{-1}. In order to yield 10% bromination *via* the free Br-atom its rate constant with phloroglucinol would have to be at least $k > 1.5 \times 10^{12}$ dm^3 mol^{-1} s^{-1}, an unreasonable value. We therefore conclude that bromination of phloroglucinol is indeed a side reaction of $Br_2^{\bullet -}$ and not due to the reaction of the Br-atom in equilibrium.

Reaction of the Phloroglucinol OH-*Adduct Radical with Oxygen.*—The rate of the reaction of O_2 with carbon-centred radicals often approaches the diffusion-controlled limit.[24,25] For the unsubstituted hydroxycyclohexadienyl radical, and OH-adduct radicals derived from phenylalanine, oxygen addition is reversible.[17,26] This seems not to be the case with the OH-adduct to phenol, where oxygen addition is followed by a rapid $HO_2^{\bullet}$ elimination from the ensuing peroxyl radical.[27] In the present system the rapid conversion of the OH-adduct radical into the phenoxyl radical makes a detailed study of this kind more difficult. However one can pulse-irradiate phloroglucinol at pH close to 7 in a solution saturated with N_2O–O_2 (1:1) or with O_2 alone. At these high oxygen concentrations the OH-adduct radicals will be scavenged very rapidly by O_2, and only a fraction will be converted into phenoxyl radicals. At pH 5.8 the fully protonated phenoxyl radical **4a** predominates and this radical does not react with oxygen on the pulse radiolysis time scale (see below). Thus the absorbance at 495 nm at about 5–10 μs after the pulse is a strong indication of how many of the OH-adduct radicals have been converted into phenoxyl radicals and how many scavenged by oxygen. At four different oxygen concentrations we have measured the absorbance at 495 nm at about 6 μs after a 0.4 μs pulse. The absorbance at 495 nm without oxygen divided by the absorbances at the same wavelength in the presence of various oxygen concentrations has been plotted against the oxygen concentrations (Fig. 9) From this competition reaction we get $k(\mathbf{3a} + O_2) = 1.7 \times 10^9$ dm^3 mol^{-1} s^{-1}.

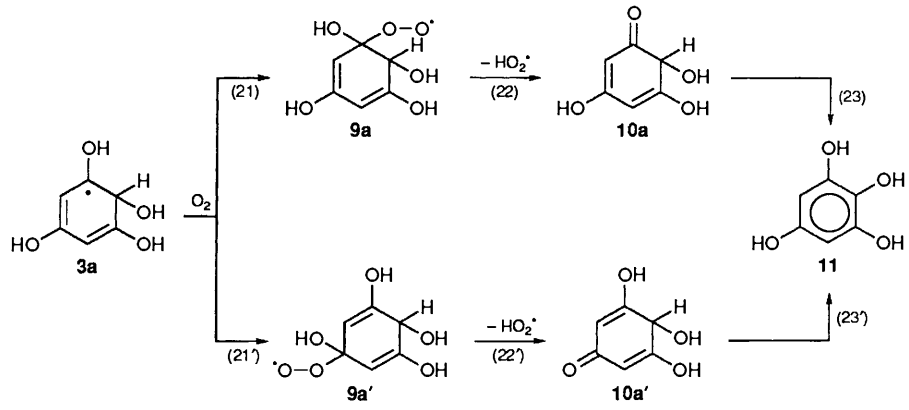
From Fig. 10 it can be seen that while oxygen reacts with the OH-adduct radicals an absorption is formed with an absorption maximum of 340 nm, close to the one of the OH-adduct radical itself. This species is stable for quite some time and only decays with a half-life of 0.26 s (inset in Fig. 10) to yield the spectrum shown in Fig. 11. The build-up kinetics at 280 nm (inset in Fig. 11) agrees with the decay kinetics at 345 nm (inset in Fig. 10).

These results are interpreted as being due to a rapid addition of oxygen to **3a** [reaction (21), (21'), $k = 1.7 \times 10^9$ dm^3 mol^{-1} s^{-1}] followed by a rapid elimination of $HO_2^{\bullet}$ [reaction (22), (22')]. The resulting products would be the trihydroxycyclohexadienones **10a** and **10a'**. The absorption at 345 nm has been attributed to these intermediates.

When cyclohexadienones have H atoms in the 2- or 4-position there is a rapid isomerization into the corresponding phenol.[27] The decay rate constant of 2.7 s^{-1} of the 345 nm transient has been attributed to this reaction [reaction (23), (23')]. Tetrahydroxybenzene **11**, to which the UV–VIS absorption spectrum formed at 1 s after the pulse (Fig. 11) is attributed, has been identified as one of the products.

The Reaction of the Phenoxyl Radicals **4a** *and* **4b** *and the Isomer* **5b** *with Oxygen.*—Some phenoxyl radicals do not react with oxygen.[28–30] One such is the fully protonated phenoxyl radical **4a** at pH 2.5 where practically no **4b** exists in equilibrium. In Fig. 12 the inverse of the first half-life of the absorption at 495 nm has been plotted against the dose per pulse. The solutions were saturated with a 4:1 mixture of N_2O–O_2, thus the oxygen concentration was 2.7×10^{-4} mol dm^{-3}. As can be seen from this Fig. the straight line obtained goes through the origin. Any reaction with oxygen would appear as an intercept, provided the rate constant $k(\mathbf{4a} + O_2)$ is faster than 4×10^5 dm^3 mol^{-1} s^{-1}. From the slope of this graph the bimolecular rate constant is calculated to be $2k(\mathbf{4a} + \mathbf{4a}) = 1.7 \times 10^9$ dm^3 mol^{-1} s^{-1} [taking $G(R^{\bullet}) = 4.9 \times 10^{-7}$ mol J^{-1}].

However, at pH 7 the phenoxyl radical monoanion **4b** decayed much more rapidly when oxygen was present. As can be seen from Fig. 13 the observed first-order decay rate constant increases with increasing oxygen concentration. In order to correct for a contribution of the second-order self-termination of the phenoxyl radicals, experiments were done at different doses/pulse. The inverse of the first half-life of the 550 nm absorption was plotted as a function of dose/pulse and the values obtained by extrapolation to zero dose/pulse were used in Fig. 13. This procedure is required to obtain reliable data at low oxygen concentrations. From the slope in Fig. 13 the rate constant for reaction with O_2 of the equilibrium mixture of **4a** and **4b** at pH 6.9 ($k_{obs} = 1.5 \times 10^8$ dm^3 mol^{-1} s^{-1}) can be



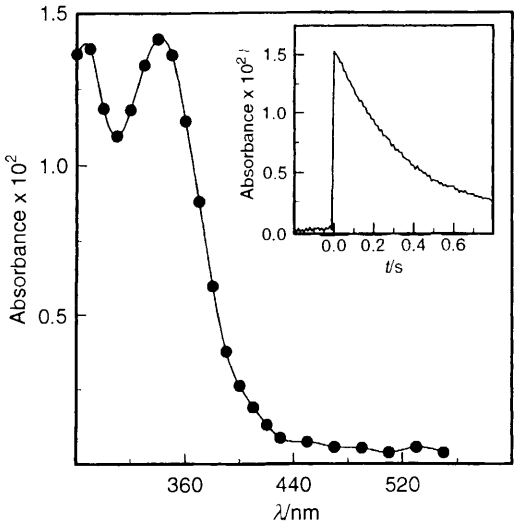


Fig. 10 Pulse radiolysis of N_2O–O_2(4:1)-saturated phloroglucinol (2×10^{-3} mol dm^{-3}) at neutral pH. The spectrum was taken 1 ms after the pulse. Inset: Decay of the absorbance at 345 nm.

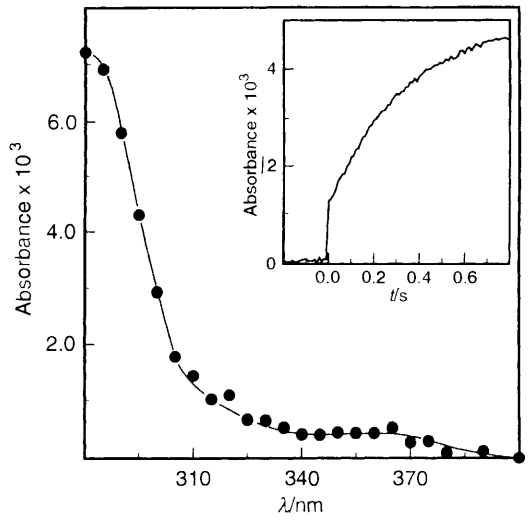


Fig. 11 The UV–VIS spectrum of the stable product formed from the isomerization of the trihydroxycyclohexadienones. Inset: build-up of the absorbance at 280 nm.

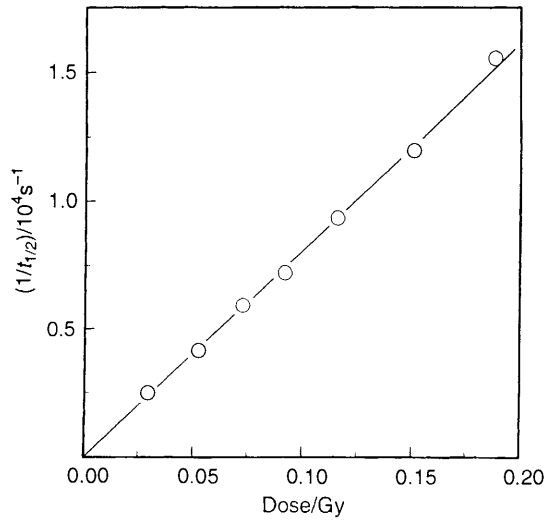


Fig. 12 Pulse radiolysis of N_2O–O_2(4:1)-saturated phloroglucinol (3×10^{-3} mol dm^{-3}) at pH 2.5. The inverse of the first half-life of the decay at 495 nm as the function of the dose.

calculated. From the pK_a value of **4a** which is estimated at 6.5 (see above) and the fact that **4a** does not react with oxygen the rate constant of **4b** with oxygen is calculated at 2.1×10^8 dm^3 mol^{-1} s^{-1}.

In steady-state experiments (0.3 Gy s^{-1}) oxygen was measured at pH 5.5 in the presence of N_3^- (0.2 mol dm^{-3}; phloroglucinol concentration 2×10^{-3} mol dm^{-3}). At this pH autoxidation is still sufficiently low to measure radiation-induced oxygen uptake with some accuracy and the equilibrium concentration of **4b** is high enough to allow quantitative reaction (negligible bimolecular decay of **4a**/**4b**). Under these conditions G(oxygen uptake) = 15×10^{-7} mol J^{-1} has been measured. This indicates that for every one molecule of **4b** that reacts with O_2, 2.5 molecules of oxygen are consumed. Such a high G value of oxygen uptake may be due to either excessive fragmentation and/or a chain reaction. Reduction of the substrate by a factor of two had very little effect [G(O_2-consumption) = 13.5×10^{-7} mol^{-1}]. This small drop is within experimental error, and more work would be needed to prove or disprove the contribution of a chain reaction. As it stands, the high azide concentration prevented an attenpted product study.

At pH 10.5 radical **5b** predominates. To measure its reaction with oxygen, an alkaline solution containing N_2O and phloroglucinol (2×10^{-4} mol dm^{-3}) was mixed with an alkaline oxygen-containing solution immediately before entering the pulse radiolysis cell. Thus the rapid autoxidation of phloroglucinol which would otherwise occur is drastically reduced. The rate of oxygen addition to the anion **5b** can be measured at 800 nm. Although the reaction of **5b** with **1b** must be taken into account in addition to the bimolecular decay, the concentration of **1b** at this pH is only 1×10^{-5} mol dm^{-3}, so the contribution of **5b** + **1b** ($k = 2 \times 10^7$ dm^3 mol^{-1} s^{-1}) is less than 2% of the observed pseudo-first-order rate constant (1.9×10^4 s^{-1});

because the experiment was performed also at low dose rate, we can estimate that the rate constant $k(\mathbf{5b} + O_2)$ is about 1.4×10^8 dm^3 mol^{-1} s^{-1}, *i.e.*, one order of magnitude below diffusion controlled.

In Fig. 13 the line through the experimental data extrapolates through the origin. This indicates that the reaction of the anion **4b** with oxygen is not significantly reversible at room temperature, as has been found for the hydroxycyclohexadienyl radical.[17] Thus the low reactivity of **4a**, the tyrosine phenoxyl radical[29,30] and also of the phenoxyl radical anions derived from 1,2-dihydroxy- and 1,2,3-trihydroxy-benzenes[28] must be due to reasons other than rapid reversibility of (rapid) oxygen addition. Thus the parameters that determine the rate of peroxyl radical formation (and the reverse reaction) are not yet fully understood. In this context it may be worth mentioning that hydroxycyclohexadienyl radicals that carry a CN- or NO_2-group also do not react with oxygen within a few hundred microseconds (X.-M. Pan and C. von Sonntag, unpublished results).

Table 2 EPR Parameters of the radicals **4** and **5b**[a]

		Hyperfine splittings ($G = 10^{-4}$ T)				
Radical	pH	*a*(H2)	*a*(H4)	*a*(H6)	*a*(OH)	*g*
4a	4–8	5.9	11.8	5.9	0.4 (2 H)	2.0042
4b	7–9	1.6	10.8	10.8	0.45 (1 H)	2.0039
4c[a]	8–10	7.5	7.5	7.5	—	2.0042
5b	8–12	2.6	11.1 (2 H)	2.6	—	2.0042

[a] Linewidth of **4c** = 1 G, for the other species 0.3 G.

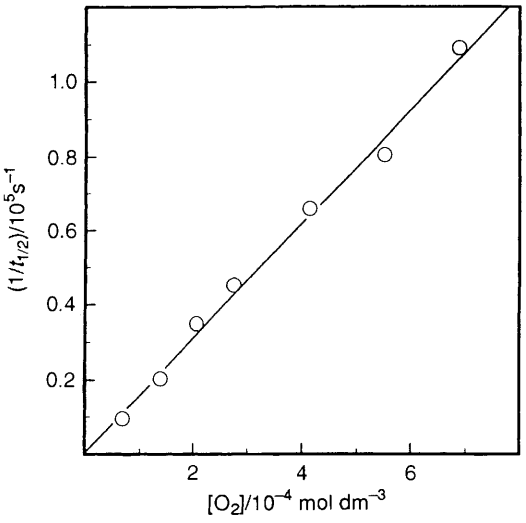


Fig. 13 Pulse radiolysis of phloroglucinol (3×10^{-3} mol dm^{-3}) in the presence of NaN_3 (0.3 mol dm^{-3}) and KH_2PO_4 (1×10^{-2} mol dm^{-3}) saturated with N_2O–O_2 at pH 6.9. The inverse of the first half-life of the decay at 550 nm extrapolated to zero dose/pulse as the function of oxygen concentrations.

EPR Spectra

Short-lived aryloxyl radicals of the polyhydroxybenzenes resorcinol (1,3-dihydroxybenzene),[31] catechol (1,2-dihydroxybenzene)[32] and pyrogallol (1,2,3-trihydroxybenzene)[33] in aqueous solution have been successfully studied by EPR. The radicals were generated by oxidation of the substrates with potassium ferricyanide, ceric sulfate or Ti^{3+}–H_2O_2 in a flow system. From phloroglucinol in alkaline solution a 1:3:3:1 quartet was obtained under such conditions which was assigned to the dianion radical **4c** with three equivalent protons at the 2-, 4-, and 6-positions whereas 'a strong but unanalysable spectrum was obtained on acidic oxidation'.[31] In contrast with those results, the spectra generated in our experiments by reaction of N_3 radicals with phloroglucinol could be easily analysed and compared with the pulse radiolysis data over the whole pH range 4–11 (see Table 2).

The spectrum obtained at pH by *in situ* photolysis of a solution containing phloroglucinol, H_2O_2 and NaN_3 [Fig. 14(*a*)] is described by a doublet splitting (a = 11.8 G), two triplet splittings (a = 5.9 and 0.4 G) and a g factor of 2.0042. These parameters are characteristic for 3,5-distributed phenoxyl radicals with high spin density at C4 and lower spin density at C2 and C6.[34] Therefore the spectrum is assigned to the neutral phenoxyl-type radical **4a**. The small triplet of 0.4 G is due to hyperfine splitting of the two equivalent hydroxyl protons at positions 3 and 5 (*cf.* ref. 33).

Upon increasing the pH the signals of **4a** decrease in intensity and new signals appear. At pH 8 the spectrum shows a large triplet splitting (a = 10.8 G), two doublet splittings (a =

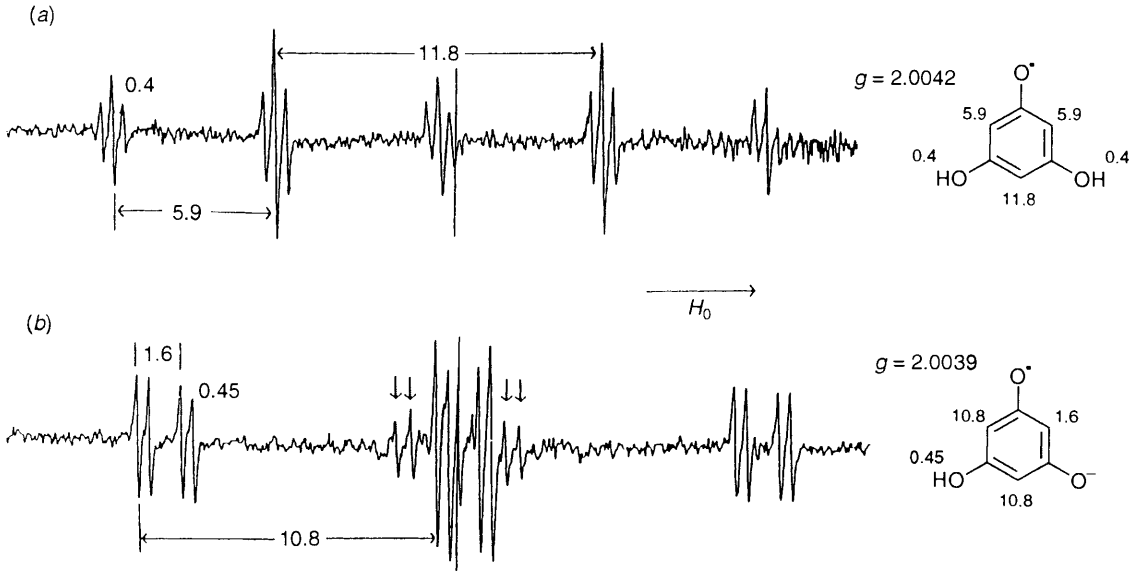


Fig. 14 EPR spectra obtained by *in situ* photolysis of solutions of phloroglucinol (1×10^{-3} mol dm^{-3}), H_2O_2 (3×10^{-3} mol dm^{-3}) and NaN_3 (6×10^{-3} mol dm^{-3}); (*a*) pH 6.5; (*b*) pH 8.0 (signals marked by arrows are due to radical **14**); microwave power, 1.2 mW; modulation amplitude, 0.09 G; receiver gain, 2×10^3.

1.6 and 0.45 G) and a g factor of 2.0039 [Fig. 14(*b*)]. The spectrum is assigned to the radical anion **4b** which can be described as a 5-substituted resorcinol radical anion with a nodal plane through C2 and C5, possessing high spin densities at C4 and C6 and low spin densities in the nodal plane.[31] The hydroxyl proton at position 5 gives rise to the small doublet splitting of 0.45 G.

The spectra obtained in radiolysis experiments in the pH range 4–9 (not shown) were characterized by the same parameters as the spectra in Fig. 14 but their intensities were lower than those obtained by photolysis.

The fact that we observe hydroxyl proton structure from **4a** and **4b** was rather unexpected in view of results reported for a number of radicals derived from polyhydroxybenzenes.[33,35] In general, hyperfine splittings of the hydroxyl protons were not observed because of rapid proton exchange with the solvent. An exception was encountered for the semiquinones of pyrogallol[33] and of napthazarin,[35] where proton exchange was slowed down. It was suggested that this was due to intramolecular hydrogen bonding between neighbouring OH groups.[33] However, this explanation does not hold for the phloroglucinol radicals **4a** and **4b** and we must conclude that factors other than intramolecular hydrogen bonding may be responsible for slowing down proton exchange rates in this type of radicals.

In alkaline solutions at pH > 9.5 in radiolysis experiments a triplet of triplets (a = 2.6 G and 11.1 G) centred at g = 2.0042 was detected [Fig. 15(*a*)]. It is known that at pH > 9.2 phloroglucinol exists in the keto-form **2c**. Therefore we assign this spectrum to radical **5b**, the oxidation product of **2c**, with two pairs of equivalent protons.

The fourth optical transient besides **4a**, **4b** and **5b** detected in pulse radiolysis experiments was assigned to the radical dianion **4c**. The quartet expected for **4c** in the EPR experiments showed much broader lines than the other radicals derived from phloroglucinol and was rather difficult to detect (see below).

Reaction of the Radical Anion **5b** *with Phloroglucinol.*—Pulse radiolysis experiments have shown that the decay rate of the transient absorbing at λ > 800 nm increases with increasing phloroglucinol concentration (Fig. 8). This result has been interpreted in terms of reaction of the cyclohexadienone radical **5b** with the phloroglucinol monoanion **1b** either by adduct formation [reaction (14)] or electron transfer [reaction (15)] resulting in either the adduct radical **6** or the radical dianion **4c**. In principle, it should be possible to decide between these two reaction pathways by EPR spectroscopy, because the two products should give rise to rather different spectral parameters. Under the assumption of asymmetrical distribution of spin density and charge, the radical dianion **4c** should give rise to a 1:3:3:1 quartet as mentioned above[31] with a splitting of 7.5 G whereas the spectrum of the adduct radical should be characterized by a large hyperfine splitting of the β-proton, like, *e.g.*, in the spectrum of the phenolate radical anion (a = 42 G).[36]

In order to decide between the two possibilities EPR spectra were recorded from samples with differing phloroglucinol concentrations. The results of radiolysis experiments with phloroglucinol concentrations of 1×10^{-3} and 5×10^{-3} mol dm^{-3} are compared in Fig. 15(*a*) and (*b*). In addition to the signals of **5b** at higher phloroglucinol concentrations a narrow spectrum was obtained at pH 8–10. The additional signals were observed with better intensity in photolysis [Fig. 15(*c*)]. By computer simulation [Fig. 15(*d*)] they were assigned to a species giving rise to a triplet of triplets (a_1 = 0.55 G, a_2 = 1.7 G, g = 2.0042).

In view of the failure to detect a large hyperfine splitting characteristic for an adduct radical, our tentative interpretation of the effect of increasing phloroglucinol concentration on the

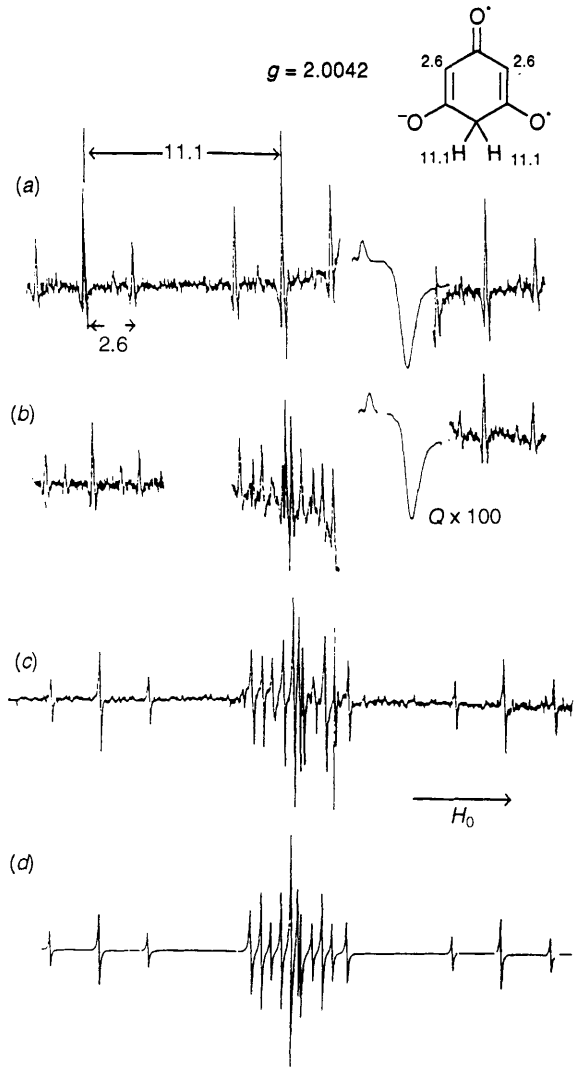


Fig. 15 EPR spectra obtained by *in situ* radiolysis of N_2O-saturated solutions: (*a*) phloroglucinol (1×10^{-3} mol dm^{-3}), NaN_3 (5×10^{-3} mol dm^{-3}; (*b*) phloroglucinol (5×10^{-3} mol dm^{-3}), NaN_3 (2.5×10^{-2} mol dm^{-3}) (Q denotes the signal from the quartz cell); (*c*) *in-situ* photolysis of a solution containing phloroglucinol (1×10^{-3} mol dm^{-3}), NaN_3 (3×10^{-3} mol dm^{-3}); (*d*) Superposition of simulated spectra of the radical **5b** and of the bisphenol-derived radical **14** [a(2 H) = 0.55 G, a(2 H) = 1.70 G, see the text]. The pH value was 9.5 (Hyperfine couplings are given in G = 0.1 mT).

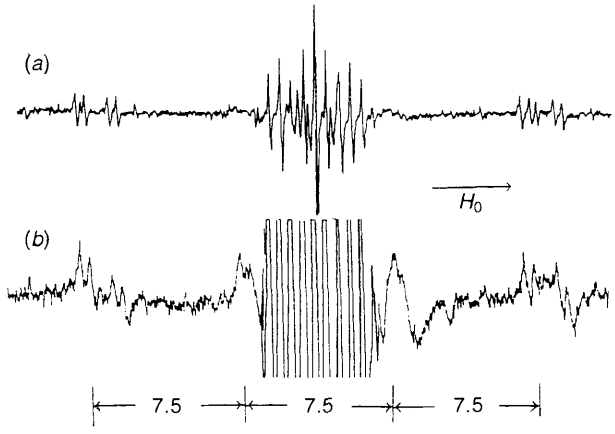
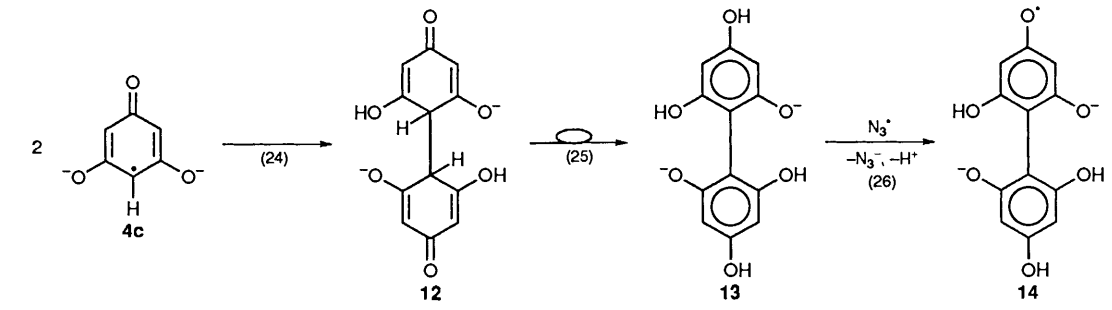
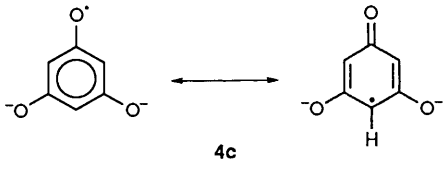


Fig. 16 EPR spectra obtained by *in situ* photolysis of a solution containing phloroglucinol (1×10^{-3} mol dm^{-3}), H_2O_2 (3×10^{-3} mol dm^{-3}), NaN_3 (6×10^{-3} mol dm^{-3}), pH 8; (*a*) modulation amplitude, 0.09 G; (*b*) modulation amplitude, 0.22 G





EPR spectra is as follows: radical **5b** reacts with phloroglucinol *via* electron transfer [reaction (15)]. The resulting radical dianion **4c** is difficult to detect because of line broadening effects. For example, *in situ* photolysis at pH 7.5 with a modulation amplitude of 0.09 G results in the spectrum shown in Fig. 16(*a*). The two broad bumps in Fig. 16(*a*) are more prominent at higher modulation [Fig. 16(*b*)] and could well be part of a 1:3:3:1 quartet with a splitting of 7.5 G. The outer lines would be hidden under the narrow peaks of radical **4b**. It is not known whether this line broadening is due to exchange of the ring protons of **4c** with the water protons, or to slow electron transfer between asymmetrically solvated oxygen atoms in positions 1, 3 and 5, in analogy to the effects described for radical anions of *m*-dinitrobenzene and methyl-substituted *m*-dinitrobenzenes[37] and the *Z*,*E*-isomer of benzene-1,3-dicarbaldehyde.[38] To explain the narrow spectrum near the centre we assume that radical **4c** dimerizes [reaction (24)]. The dimerization product **12** rearranges [reaction (25)] to yield the substituted biphenyl **13** which is oxidized by N_3-radicals [reaction (26)] under formation of the corresponding aroxyl radical **14**. This radical could well give rise to two small triplet splittings as is shown by comparison with the spectrum obtained from the aroxyl radical generated with N_3 radicals from 4,4'-dihydroxybiphenyl [a_1(4 H) = 1.75 G, a_2 (4 H) = 0.97 G].

According to reaction (15) the production rate of **4c** increases with increasing phloroglucinol content of the solutions (see Fig. 8). Therefore the rate of dimerization of **4c** [reaction (24)] and finally the intensity of the signals of **14**, generated in reactions (24)–(26) from **4c**, will also depend on phloroglucinol concentration [compare Fig. 15(*a*) and 15(*b*)]. Considering the high rate of transformation of **5b** into **4c** ($k_{obs} \approx 5 \times 10^4$ s^{-1}, corresponding to a lifetime of ~20 μs for **5b** at 5×10^{-3} mol dm^{-3}, see Fig. 8) it seems surprising, at first glance, that the EPR signals of **5b** are observed. Obviously, by the back reaction [equilibrium (17)] the steady-state concentration of **5b** reaches a value high enough for EPR detection.

Contribution of a radical–radical recombination [reaction (24)] in formation of the bisphenol-derived radical **14** is supported by comparison of the spectra in Fig. 15(*b*) and 15(*c*). In photolysis experiments with larger rate of OH-radical production the spectrum of **14** was generated at much lower phloroglucinol concentrations than in the radiolysis experiments with a lower radical production rate. The rate of the rearrangement [reaction (25)] is probably increased by OH and therefore the EPR signals of **14** are detected in alkaline solutions only.

Acknowledgements

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