Tetrahedron Vol 49, No 43, pp 9689-9698, 1993
Printed in Great Britain

0040-4020/93 $6 00
Pergamon Press Ltd

Radiation-Induced Effects In Lignin Model Compounds: A Pulse And Steady-State Radiolysis Study

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(*Received in USA* 12 *January* 1993, *accepted* 18 *August* 1993)

Key Words pulse radiolysis, gamma radiolysis, irradiation, lignin, model compounds

Abstract *A lignin model compound **β-(2-methoxyphenoxy)-3,4-dimethoxyacetophenone (I)** was subjected to ^{60}Co gamma irradiation and pulse radiolysis under different conditions (dose, medium, pH) Interaction with hydroxyl radicals resulted in aryl hydroxylation, fragmentation, and cleavage A hydroxylation-cleavage pathway was found at all pH values At alkaline pH, fragmentation reactions were also observed The predominant reaction with solvated electrons was fragmentation of the β-aryl ether bond followed by cleavage reactions*

INTRODUCTION

Lignin, the second most abundant component in wood after cellulose, is produced in large amounts as a waste product in the pulping process[1] In efforts to develop new industrial uses for ionizing radiation, the irradiation on wood, pulp, pulp mill effluent and lignin is being examined in our laboratories In pulping, pretreatment of wood with ionizing radiation resulted in numerous effects including a 20-25% net reduction in energy requirements[2] Diverse effects were observed upon irradiation of both lignin isolated from irradiated wood, and commercial lignin samples[3,4] Depending on the conditions used, polymerization, demethylation and degradative processes could coexist

Because of the complex structure of lignin[5], a lignin model compound **β-(2-methoxyphenoxy)-3,4-dimethoxyacetophenone** (compound I, Fig 2, #13) was chosen for investigation of radiation-induced degradative pathways occurring in lignin Such compounds have been used extensively in mechanistic studies[6-8] as their structures are representative of the arylglycerol-β-aryl ether units that make up 30-50% of the repeating units in lignin[6]

The results of irradiations performed on aqueous solutions of compound I are described here Pulse radiolysis was used to monitor formation of transient species, while HPLC was used to identify stable radiolytic products

1. RESULTS AND DISCUSSION

Reaction pathways are described using the convention initiated by Dimmel[9] "**Fragmentation**" refers to cleavage of the β-aryl ether bond Decomposition, degradation, dehydration and dehydrogenation denote breakage of bonds other than the β-aryl ether bond and are grouped under "**Cleavage**" reactions "**Hydroxylation**" relates to an hydroxyl adduct on an aromatic ring as a σ- or π-complex "**Combination**" relates to polymerization, dimerization and disproportionation reactions "**Demethoxylation**" corresponds to ipso substitution of a methoxyl group by a hydroxyl group

1 1 Radiolysis Under Oxidative Conditions

In N_2O-saturated water, the dominant path to oxidative radiolysis is via hydroxyl radicals[10] Hydroxyl radicals are strong oxidizing species known to abstract hydrogen from aliphatic hydrocarbons[11] With aromatics, the major reaction is addition of hydroxyl radical to the aromatic ring to form hydroxyl adducts[10]

1 1 1 Steady State Radiolysis Thirteen major peaks were found upon HPLC of I (0 1 mM in H_2O) irradiated under oxidizing conditions (Fig 1A) The identified products (Fig 2) could be grouped into 3 sets based on the reactions assumed to be involved in their formation The first set is the hydroxylated and demethoxylated products in peaks **10**, **11**, and **12** The second set is the fragmentation products **8** and **9**, while the third set contains the more polar cleavage products, **1-7**

At pH 3, compound I disintegrated almost linearly between 0 03-1 kGy and exponentially thereafter (Fig 3) The hydroxylated products (peaks **10**,**11**,&**12**) and the fragmentation products guaiacol (**8**), and dimethoxyacetophenone (**9**,DMAP) were formed in low concentrations at 0 03 kGy, increased with increasing dose, then decreased at higher doses as they underwent secondary radiolysis Dimethoxyphenol, DMP, increased with dose to 0 5 kGy, then declined Cleavage products **1-7** appeared as primary products, even at the lowest dose measured

The steady-state radiolysis pattern of I at pH 7 2 differed from that at pH 3 0 (results not shown) At pH 7 2, I radiolysed easier, with approximately 10% remaining after a dose of 1 kGy The hydroxylated products (**10**,**11**,**12**) reached their maxima at 0 5 kGy, whereas the profile for DMP remained the same as at pH 3 Fragmentation products, guaiacol and DMAP showed increased concentrations at lower doses, indicating increased fragmentation at pH 7 2

At pH 11, almost 75% of I was radiolysed at 0 5 kGy Hydroxylation products found in peaks **10**,**11** & **12** showed similar profiles with sharp declines beyond 0 5 kGy Guaiacol and DMAP formed in significantly higher amounts indicating enhanced fragmentation However, DMP formed in very low concentrations compared to at lower pH

1 1 2 Pulse Radiolysis **The light absorption spectrum of species formed upon pulse radiolysis of I (0 1 mM in H_2O) taken immediately after the 2 6 μs electron pulse gave maxima at 350 and 420 nm (Fig 4) Similar absorption spectra were observed at acidic pH Such "double peak" spectra appearing quickly after the pulse have been related to the formation of hydroxycyclohexadienyl radicals (hydroxyl adducts)[10,12] Thus the absorption spectrum for I is attributed to the instant formation of the hydroxyl adduct The rapid decay of both absorbing species (Table 1) by a second-order process in the microsecond time scale, is also characteristic of bimolecular radical-radical reactions of hydroxyl adducts[13]**

A "long-lived" remaining absorption, which was more prevalent at higher dose per pulse values (i e , 50 Gy/pulse), was also observed at ~350 nm This absorption appeared a few hundred microseconds after the electron pulse (Fig 4)

The optical absorption spectra measured at pH 3 0, 7 2, and 11 0, were similar with the exception that at pH 11, the long-lived absorption at 350 nm was more intense This absorption was stable even in the millisecond time scale In addition, a small absorption was observed in the 370-400 nm range at pH 11 This species was attributed to phenoxyl radical formation in small yield resulting from oxidative displacement of methoxyl groups via ipso attack of hydroxyl radical[14] This demethoxylation pathway has also been found on pulse radiolysis of anisole, (results not shown) where the absorbance of the phenoxyl radical continued to increase long after the hydroxyl adduct had decayed

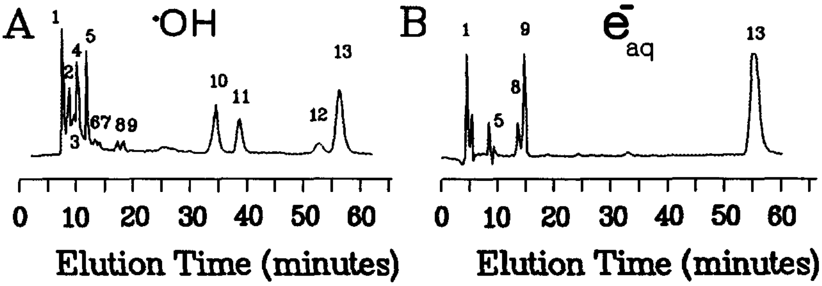

Fig 1 HPLC of I irradiated under oxidizing (A) and reducing (B) conditions at pH 3 and 1 0 kGy

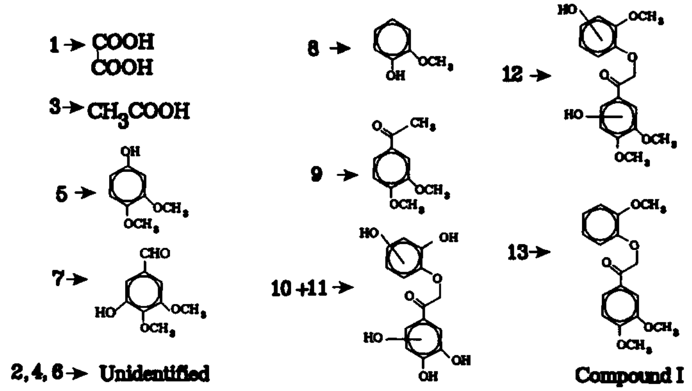

Fig 2 Characterization of major radiolysis products by HPLC and ^1H NMR

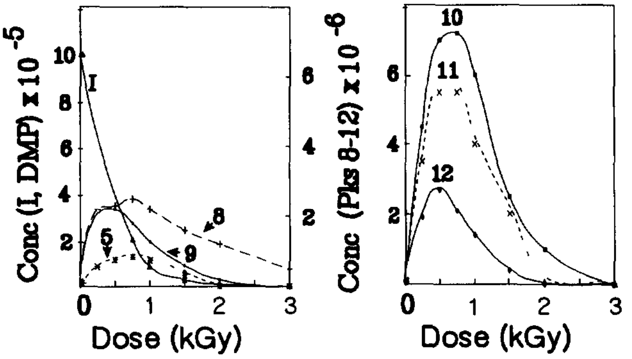

Fig. 3 Area profile of major radiolytic products as a function of dose

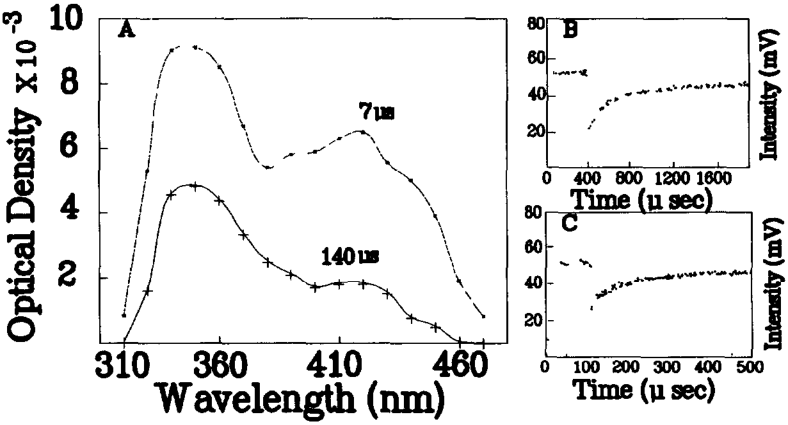

Fig 4 (A) Optical absorption spectra of the hydroxyl adduct of I taken 7 µs and 140 µs after the 2 6 µs electron pulse (pH 7 2), (B) Oscilloscope traces related to the decay of the absorbing species observed at 350 nm, and (C) at 420 nm.

1 1 3 Summary of Radiolysis Under Oxidative Conditions The pulse radiolysis results support the instant formation of hydroxyl adducts of I Phenoxyl radicals are also formed in small amounts presumably via ipso hydroxylation These results agree with steady-state data which show that hydroxyl radical-mediated radiolysis of I occurs rather selectively

Hydroxylated and demethoxylated products (**10**,**11**,**12**) predominated at all pH values Significant fragmentation to **8**, and **9** occured only at higher pH indicating that abstraction of the methylene hydrogen proceeds more easily in alkaline medium Cleavage to form **1**-**7** was observed at all pH values Based on these results, the pathways shown in Fig 5 are postulated

At lower pH the hydroxyl radical preferentially attacks the aromatic rings of I Hydroxylation occurs via formation of a π-adduct on the aromatic ring(s), to form products found in peak **12** These compounds probably result from primary reactions, since they form and disappear at low doses The presence of hydroxylation products (in peaks **10** and **11**) in high concentrations at all pH values supports a pathway involving ipso hydroxylation Because the methoxyl group may direct the attacking hydroxyl radical preferentially into the ortho and para positions on each ring, different hydroxylated products would be expected Ipso hydroxylation can also occur at the aromatic carbons linked to the aliphatic bridge leading to fragmentation The presence of DMP at pH 3 and 7 2 is indicative of ipso hydroxylation at the bridging carbon of the DMAP ring

Table 1 Spectral Characteristics and Decay Rate Constants of Hydroxyl Adducts Formed in Compound I

pH	λ_{max},nm	Gε (350 nm)	Gε (420 nm)	2k/ε,cm s^{-1} (350 nm)	2k/ε,cm s^{-1} (420 nm)
3 01	345	7 6 x 10^3	5 9 x 10^3	1 2 x 10^6	1 3 x 10^6
7 20	350	6 5 x 10^3	4 4 x 10^3	1 6 x 10^6	1 5 x 10^6
11 0	350	8 4 x 10^3	5 0 x 10^3	-	4 0 x 10^6

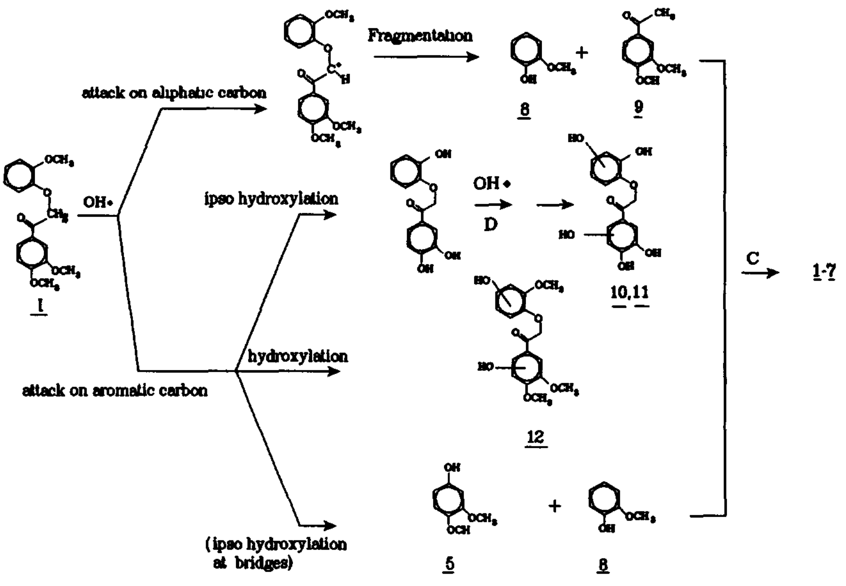

Fig 5 Possible mechanistic pathways for radiolysis of I under N_2O

Hydroxyl radical may also attack the aliphatic methylene carbon at high pH forming 8 and 9, although other possibilities exist, ie O^- formed via reaction 1, may abstract the methylene proton from I causing fragmentation, as the pKa for this

$$OH^- + OH \rightarrow H_2O + O^- \qquad (1)$$

reaction is 11 9[15] Increased amounts of 8 and 9 at pH 11 0 imply the pH effect contributes to this mode of fragmentation

1 2 Radiolysis Under Reducing Conditions

Electrons, one of the major transient products in radiolysis of water, are quickly solvated forming the strongly reducing species, e^-_{aq} This species forms either anions or free radicals by bond scission reactions with substrates A very selective reaction occurs with aromatic ketones, the adherance of electrons to the carbonyl group at diffusion controlled rates[16] As shown below, I undergoes very specific reactions under reducing conditions

1 2 1 Steady State Radiolysis. Fewer radiolytic products were formed under reducing conditions (Fig 1B) compared to oxidizing conditions The major products were the fragmentation products guaiacol (8) and dimethoxyacetophenone (9,DMAP) I decomposed almost linearly between 0-1 kGy (Fig 6) Guaiacol (8) and DMAP (9) formed in higher concentration than under oxidizing conditions, increased with dose to 2 kGy then declined DMP (5) decreased beyond 1 kGy No hydroxylated derivatives of I were found

Compound I fragmented much faster at higher pH At pH 7 2 fragmentation reactions yielded guaiacol and DMAP The DMP concentration remained stable up to 1 5 kGy At pH 11, guaiacol and DMAP increased in concentration, while DMP was absent, possibly due to faster degradation through a combination of pH and radiation effects

1 2 2 Pulse Radiolysis The transient optical absorption spectrum of I varied little with pH At pH 4, the spectrum taken immediately after the 2 6 μs electron pulse had maxima at 350, 365, 385, and 420 nm The maxima at 350 nm and 420 nm are characteristic of ketyl radicals formed by reaction of e_{aq}^- with aromatic carbonyl compounds These species formed rapidly after the pulse and decayed by second-order kinetics (Table 2) in agreement with data by Hayon et al [16]

The similarity in absorption spectra at pH 4 and 13 implies that the ketyl radical anion does not form at pH 13 For example we found different spectra for anisoin at pH 3 and 11 (results not shown) The different spectra were assumed to be due to the ketyl radical and the ketyl radical anion of anisoin Similar results were found by Hayon et al [16]

The maxima at 365 and 385 nm are similar to that found upon pulse radiolysis of guaiacol in N_2O-saturated solution (results not shown) With guaiacol, this absorption was probably due to the phenoxyl radical, since a very similar absorption was found when NaN_3 (used to oxidize aromatic compounds to phenoxyl radicals) was included

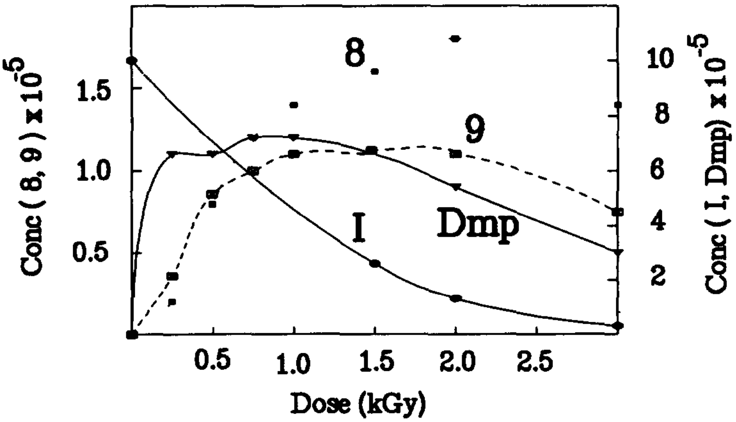

Fig 6 Area profile of major components from I irradiated under reducing conditions

Table 2 Spectral Characteristics and Decay Rate Constants of Species Formed in the Pulse Radiolysis of I

	λ_{max},nm	Gε	2k/ε,cm s^{-1}
ketyl radical			
pH = 3 0	350	3 8 x 10^3	4 0 x 10^6
pH = 7 1	350	3 8 x 10^3	-
pH =13 0	350	5 3 x 10^3	5 2 x 10^6
phenoxyl radical			
pH = 3 0	380	-	2 8 x 10^6
pH = 7 1	380	2 4 x 10^3	-
pH =13 0	380	3 2 x 10^3	1 7 x 10^6

Further evidence for phenoxyl radical formation was provided by the absorption found in the 365-390 nm region upon pulse radiolysis of I at pH 10 and 13 This absorption was much more pronounced and decayed much more slowly (Table 2)

1 2 3 Summary of Radiolysis Under Reducing Conditions Fragmentation reactions forming guaiacol and DMAP predominate under reducing conditions Radiolysis proceeds by initial formation of ketyl and phenoxyl radicals The ketyl radical presumably forms by reaction of hydrated electron with the carbonyl group followed by protonation[17]

$$R_2C{=}O \ + \ e_{aq}^- \xrightarrow{H^+} R_2C\text{-}O^- \longrightarrow R_2C\text{-}OH \qquad (2)$$

The ketyl radical does not undergo ionization at high pH Phenoxyl radicals are formed by fragmentation of the β-aryl bond These radicals decay by second-order kinetics resulting in guaiacol, a major radiolysis product Based on these observations, the following reaction scheme may be drawn (Fig 7)

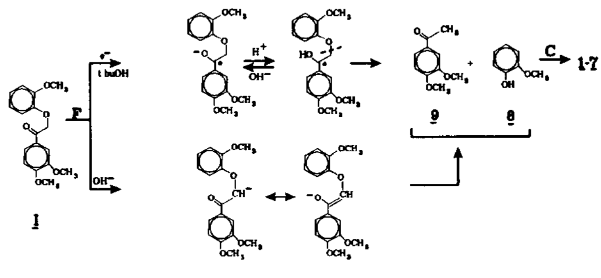

Fig 7 Possible reaction pathways for the radiolysis of I under reducing conditions at all pH ranges

1 3 Fragmentation Pattern of Compound I

It may be worthwhile to compare the facile radiolytic fragmentation of I with chemical fragmentation of similar compounds Lundquist and Lundgren[17] studied several lignin models including arylglycerol derivatives of I Derivatives containing a hydroxyl group at the para position of the arylglycerol moiety were found to be much more susceptible to acid hydrolysis than analogs containing a methoxyl group.

Ciaramitaro and Steelink[18] studied the acid hydrolysis of 4-hydroxybenzylphenyl ethers and their acetates (to mimic ester structural linkages in lignin). The acetates required 24 h for fragmentation whereas the free phenols were completely hydrolysed in 8 h. Sarkanen and Hoo[19] determined a relative rate of 4:1 for fragmentation of the β-aryl ether bond in a phenolic model compared to its methoxyl analog. Several cases in the literature support this fact[19,20].

Thus non-phenolic lignin model compounds appear to be much less reactive to chemical fragmentation. In this context, it is interesting that **I** fragments very easily under ionizing radiation, especially in the presence of solvated electrons. As non-phenolic units containing α- and β-aryl ether linkages are the dominant components in both softwood and hardwood lignins[1], the cleavage of these bonds contributes significantly to lignin degradation. Hence the positive effects of ionizing radiation in pulping[2] may be related to the ease of fragmentation of lignin. A better understanding of these fragmentation processes may also provide possibilities for obtaining lower molecular weight products from lignin.

2. CONCLUSIONS

i) Pulse radiolysis, HPLC, NMR and UV data are consistent with the mechanistic schemes shown in Figs. 6 and 11.

ii) Under oxidative conditions a hydroxylation-cleavage mechanism dominates at acidic and neutral pH while fragmentation also becomes significant at high pH.

iii) A fragmentation pathway, yielding dimethoxyacetophenone (DMAP) and guaiacol predominates under reducing conditions. These products are fairly stable to further radiolysis.

iv) In alkaline medium, under both oxidizing and reducing conditions, radiolytic decomposition of **I** is significant despite the absence of a phenolic group.

3. EXPERIMENTAL

All chemicals were of high research-grade purity, obtained from commercial sources and were used without further purification. ^{1}H NMR was done with a Bruker Aspect 3000 and Bruker AMX 500 instruments operating at 300 and 500 MHz respectively, referenced to $CHCl_3$ (at the Chemistry Department, University of Manitoba, Manitoba, Canada). GC-MS experiments were conducted on a Hewlett Packard Instrument, GC model 5890 and MS model 5970 with an HP-5 column, 12 m, and a linear velocity of 50 cm/s. UV spectra were recorded using a Hewlett Packard 8451A spectrophotometer.

Compound **I** was synthesized in 64% yield according to published procedures (m.p. 90 to 91°C, lit. 85 to 86°C[21]). ^{1}H NMR and GC-MS data confirmed the structure.

3.1 Pulse Radiolysis Experiments

Pulse radiolysis was conducted at the Institute of Isotopes of the Hungarian Academy of Sciences, Budapest, Hungary, using a 4 MeV linear electron accelerator with 80 ns or 2.6 μs pulse width. Dosimetry was carried out using 10 mM KSCN. The dose per pulse value was varied between 5 and 20 Gy for the 80 ns pulses, and between 8 and 100 Gy for the 2.6 μs pulses. Absorbances (A) were converted to $G\varepsilon_\lambda$ values by the following formula

$$G\varepsilon_\lambda = \frac{A}{D\ d}\,9\ 6\ \ 10^6\,(100\ \mathrm{eV})^{-1}$$

where D is the dose (Gy), d the density of the solution (g cm^{-3}), and G the number of species formed, decayed or transformed per 100 eV absorbed The time-dependent concentration of the optically absorbing species, i e , the transient absorption, was measured by an Applied Photophysics K-347 kinetic spectrophotometer On the oscilloscope screen the time-dependence of the light intensity (Δ I (t), mV) passing through the cell appears in mV units On the basis of the light intensity before the electron pulse (I_o, mV) the time dependent light intensity is calculated by the data acquisition system

$$OD_\lambda = \log \frac{I_o}{I_o - \Delta I(t)} = \varepsilon_\lambda\ c\ 1$$

where ε_λ is the extinction coefficient at λ wavelength, c the concentration of absorbing species, and 1 the cell light path Three storage oscilloscopes (100, & 60 MHz Phillips, 100 MHz Iwatsu Ts 6123), coupled with an IBM compatible computer were used for data acquisition[22]

The decay of transient species usually followed second order kinetics and fittings were made using the standard formula

$$\frac{1}{G\ \varepsilon_\lambda} = \frac{1}{(G\ \varepsilon_\lambda)_o} + \frac{2k}{\varepsilon_\lambda}\,t$$

where 2k is the second order rate parameter of the self-termination reaction of the intermediates

Experiments were performed on solutions of **I** (0 1 mM) in double-distilled water deaerated using N_2 or saturated with N_2O Solubilization of I was enhanced by ultrasonication The pH was adjusted with 7% $HClO_4$ or 0 1 M NaOH Hydrated electrons (e_{aq}) were generated in N_2-saturated solutions containing 1 0 M t-butanol which scavenges hydroxyl radicals and also increases the solubility of **I** Azide radicals produced by irradiation of solutions containing 100 mM NaN_3 were used to oxidize phenolic type aromatic substrates

3 2 *Steady State Radiolysis*

Solutions were prepared as mentioned above Irradiations were performed in an AECL Gammacell 220 HPLC was performed using a Beckman model 344 pump upgraded with a data acquisition system run by a Nelson Analytical Model 2600 Chromatographic Software system loaded on an IBM PC Samples were injected onto a Waters Nova-Pak C_{18} cartridge (8 x 100 mm) eluted isocratically with MeOH Water (1 1) at 0 5 mL/min The eluent was monitored at 274 nm All HPLC discussed in this paper was performed using these conditions

HPLC peaks **1**, **3**, **5**, **8**, and **9** were identified by comparison of retention times with authentic compounds and also by spiking irradiated solutions of **I** with authentic compounds prior to HPLC The radiolytic product mixtures seen as peaks **10**, and **11**, were isolated by repeated HPLC The collected samples were pooled, concentrated, and analysed by ^1H NMR Both **10** and **11** showed complex spectra suggesting a mixture of demethoxylated products A signal at 3 8 ppm, characteristic of OCH_3, was absent indicating complete demethoxylation of all OCH_3 groups The exact position of hydroxyl groups attached to the aromatic rings could not be determined due to overlap and signal broadening stemming from the mixture of products

For indirect characterization of **10**, and **11**, Compound **I** (3 mg) was refluxed in 5 mL 57% HI for 10 min to produce demethoxylated products Peaks **10'** and **11'**, corresponding in retention time to peaks **10**, and **11** from irradiated samples of

I, were separated by HPLC, collected, and concentrated The ^{1}H NMR of **10'** and **11'** showed complex spectra of aromatic and aliphatic peaks similar to those found for peaks **10** and **11** from irradiated I A peak at 5 2 ppm, characteristic of CH_2, indicated that the β-aryl ether bond was not fragmented Integration of signals at 3 8 to 4 0 ppm, (characteristic of $O\text{-}CH_3$) afforded a reduced ratio of OCH_3 to CH_2, indicating a mixture of partially demethoxylated compounds

To identify the radiolysis products in peak **12**, compound I was hydroxylated by standard synthetic techniques The reaction mixture was analyzed by HPLC Material corresponding in retention time to peak **12** from irradiated samples was collected by repeated injections and concentrated ^{1}H NMR showed multiple hydroxylation on both rings, with the methylene group and methoxy groups left intact

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