

ESR Studies of Free Radical Intermediates in the Chemical Oxidation of Phenols and Other Compounds by Cerium (IV)

Brant D. Boucher '17 and Herbert J. Sipe

Department of Chemistry, Hampden-Sydney College, Hampden-Sydney, VA 23943

Introduction

Free radicals are molecules, atoms, or ions with unpaired valence electrons.¹ They are considered a common threat to biological systems because of their highly reactive nature; free radicals in the body have a tendency to interact with and disrupt physiological structure at the molecular level or electronically alter oxygen molecules which in turn may damage proteins.² For some time it has been known that free-radicals are linked to cancer.³ Some have even been noted as possible precursors to Alzheimer's disease.⁴ Because these materials pose a common threat to biological systems, organisms have developed a number of defenses against them. Some enzymes such as Catalase target specific radical precursors such as hydrogen peroxide (H_2O_2) and metabolize them before the molecules can react with iron (Fe^{2+}) to produce highly reactive hydroxyl ions ($OH\cdot$).⁵ Another common defense is chemicals referred to as antioxidants which react with free radicals before the radicals can react with other molecules with more important functions. Because antioxidants are single use and a finite number exist in an organism or a cell at any given time, free radicals can overwhelm the organism's defenses if the radicals' precursors or the radicals themselves exist in high concentrations relative to antioxidants. Many of the chemicals which radicals were generated from in this study are common food additives (**Figure 1**), such as safrole and isosafrole, making them an important interest for scientific study.²

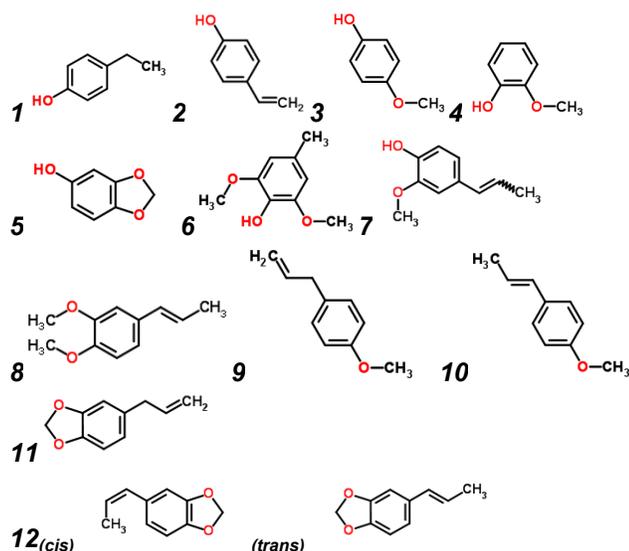


Figure 1. Above are the chemical structures of 4-ethylphenol (1), 4-vinylphenol (2), p-methoxyphenol (3), guaiacol (4), sesamol (5), 2,6 dimethoxy-4-methylphenol (6), isoeugenol (7), methyl-

isoeugenol (8), estragole (9), trans-anethole (10), safrole (11), cis-isosafrole (12_{cis}), and trans-isosafrole (12_{trans}).

Materials and Methods

The chemicals 4-ethylphenol, isoeugenol, methyl-Isoeugenol, trans-anethole, safrole, estragole, 4-vinylphenol, sesamol, safrole, isosafrole (cis/trans), guaiacol, and cerium (IV) sulfate were purchased from Sigma-Aldrich (St. Louis, MO). The chemicals 2,6-dimethoxy-4-methylphenol, and another batch of cerium (IV) sulfate were purchased from Alfa-Aesar (Ward Hill, MA). The chemical p-methoxyphenol was purchased from J.T. Baker chemicals of Avantor Performance Materials (Center Valley, PA). Sulfuric acid and 95% ethanol were from AAPER (Shelbyville, KY). For each experiment, two solutions were prepared. Originally, the sample solutions contained a 2.0mM concentration of experimental compound in 4.0L of DI water. Later experiments included a doubled concentration of experimental compound to 4.0mM and the addition of 180 proof ethanol in 800mL and 1600mL quantities as an accessory solvent. Whenever ethanol was added, DI water volume would decrease to make a complementary 4.0L. These solutions were often heated as well to dissolve the experimental compounds. The oxidizing solution consisted of 1.8mM concentration of $Ce(SO_4)_2$ dissolved in 100mL of H_2SO_4 and diluted to 4.0L with DI water. When the concentration of experimental compound was increased to 4.0mM, the concentration of $Ce(SO_4)_2$ also doubled to 3.6mM. Both of the solutions were stirred with stir bars.

The solutions mentioned above were poured into separate reservoirs positioned in a tilting holder. Both of these solutions were then bubbled with nitrogen gas for 5 minutes to remove paramagnetic dissolved oxygen. A third reservoir holds DI water. Silicone tubing connected each element of the fast-flow apparatus. Two three-way valves regulated flow from DI water to the two prepared solutions as necessary. Tubes ran from each of the down-spouts of the three-way bulbs to a variable speed peristaltic pump. The tubes continued through the pump and connected to the base of the mixing flat-cell, which was firmly secured within the microwave cavity of the ESR. Within the flat cell, the two solutions mixed and then continued to flow out through the top of the cell and through another single tube into a waste container. The overall fast-flow apparatus is shown in **Figure 2**.

Electron spin resonance of the radicals generated via fast-flow technique were measured and

recorded by a JEOL-REX1 spectrometer, an instrument designed to obtain structural information at the molecular level about free radicals through their

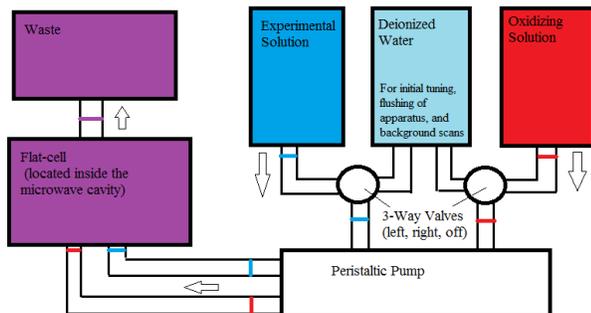


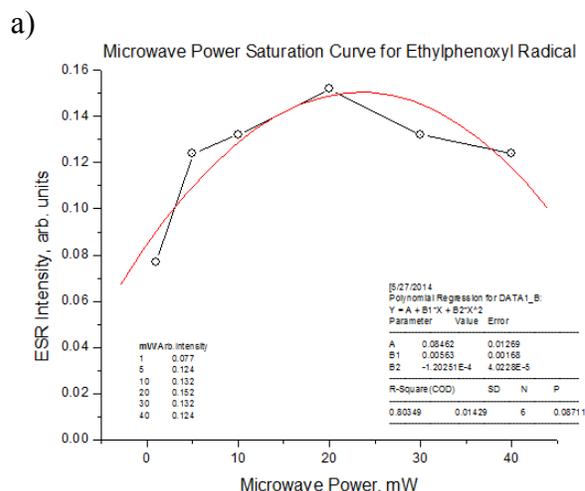
Figure 2. Diagram of the fast-flow apparatus.

specific magnetic properties.⁶ The settings of the instrument varied from compound to compound and from scan to scan, but the overall settings for frequency and center magnetic field were 9.4GHz and 337mT, respectively. The microwave cavity of the instrument was modulated with a magnetic field of 100KHz. A locally written interface designed using LabView 7.1, software purchased from National Instruments (Austin, TX), was used to manipulate and record the ESR digitally. WinSim 2002, software written by researchers from NEIHS (Research Triangle Park, NC), was used to simulate and analyze experimental spectra for Hyperfine Coupling Constants. Because files created and saved in LabView were not directly compatible with WinSim, a locally written file conversion program was used. Molecules were built in GaussView '03 and then Density Functional Theory calculations were run using Gaussian '03 software purchased from Cyberchem (Gainesville, FL).

Results and Conclusion

Fast-flow experimentation on 4-ethylphenol produced a signal in ESR, indicating the formation of radicals. The following solutions were involved in the fast-flow apparatus. The sample solution contained 0.2mM 4-ethylphenol dissolved in 4.0L of deionized (DI) water while the oxidizing solutions contained 0.18mM $\text{Ce}(\text{SO}_4)_2$ and approximately 100mL of sulfuric acid (H_2SO_4). Once mixed in the flat-cell within the microwave cavity of the ESR, the final concentrations of the combined solution were 1.0mM 4-ethylphenol and 0.9mM $\text{Ce}(\text{SO}_4)_2$. Firstly, a microwave saturation study (**Figure 3a**) was conducted on the sample to determine optimal power. After running scans on power levels from 1mW to 40mW, the optimal power level was determined to be approximately 20mW as demonstrated by the parabolic curve plotted in Origin. Afterwards, a flow-rate study (**Figure 3b**) was conducted on the sample from 30mL/min to 100mL/min. After plotting the data in Origin, a linear curve was determined, indicating that the faster the flow-rate, the larger the signal.

Because of seal concerns, the flow-rate was capped at 100mL/min. The best resolved spectrum is shown in (**Figure 4a**). This spectrum was captured with the following instrument settings: 9.40146GHz microwave frequency, 20mW power, 337.400mT +/- 2.5mT center field, 0.05mT field modulation width, 1000 receiver gain, 1 second time constant, 16 minute sweep time for one sweep, and 100mL/min flow rate. The experiment also produced a polymer which built up in the thin mixing cavity. This polymer had to be removed with ethanol to prevent pressure buildup and a leak in the fast-flow apparatus. The simulated spectra made in WinWin is shown in **Figure 4b** and has a correlation of $R^2=0.966453$ with the experimental spectrum. This correlation indicates a simulation that is fairly representative of the experimental spectrum. **Figure 4c** displays the residual spectrum. Little structure is seen in the residual spectra, though it is visually evident that the simulated spectrum is not a perfect match to the experimental simulation. The HFCC's from the simulated spectrum were $a_{\text{CH}_3}^{\text{H}} = 0.321\text{G}$ (3H), $a_{\text{H}}^{\text{H}} = 1.386\text{G}$ (2H), $a_{\text{H}}^{\text{H}} = 6.117\text{G}$ (2H), $a_{\text{CH}_2}^{\text{H}} = 10.421\text{G}$ (2H). DFT calculations in Gaussian yielded the following values for HFCC's: $a_{\text{CH}_3}^{\text{H}} = 0.016\text{G}$ (3H), $a_{\text{H}}^{\text{H}} = 1.697\text{G}$ (2H), $a_{\text{H}}^{\text{H}} = 4.871\text{G}$ (2H), and $a_{\text{CH}_2}^{\text{H}} = 6.331\text{G}$ (2H). These results are also summarized in Table 1.



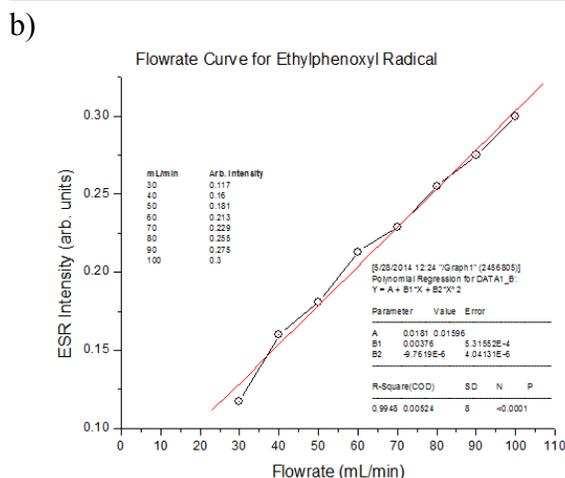


Figure 3: Results from microwave power saturation and flowrate studies, respectively.

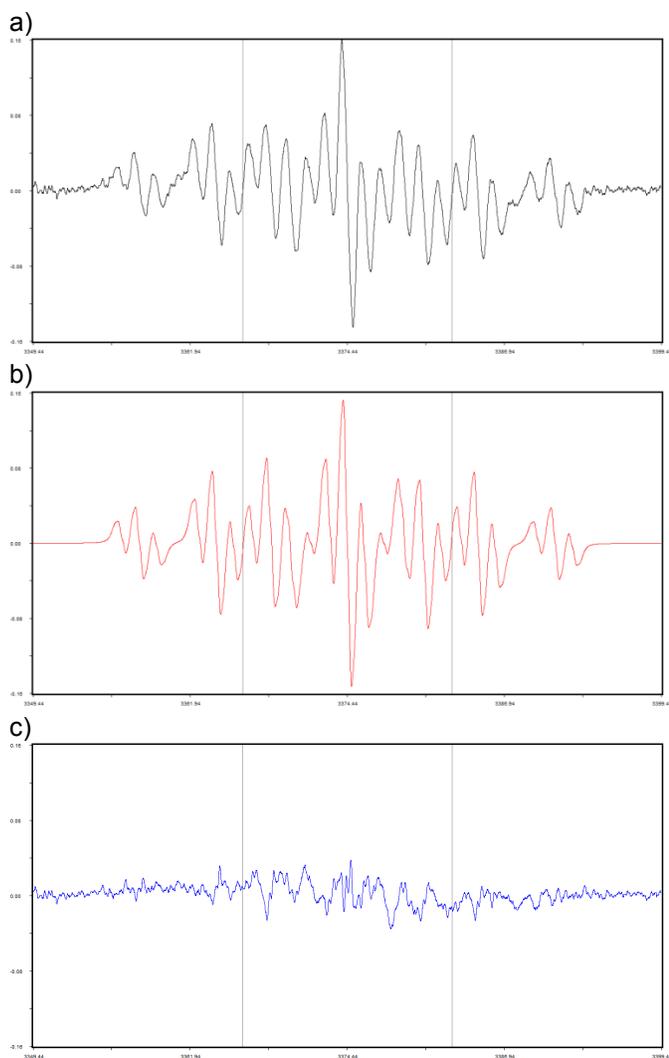


Figure 4: Associated Fast Flow ESR spectra of radicals formed from 4-ethylphenol. The final concentrations of 4-ethylphenol and $\text{Ce}(\text{SO}_4)_2$ were 1.0mM and 0.9mM, respectively. A) is the experimental spectrum with the parameters: 9.40146GHz microwave frequency, 20mW power, 337.400mT +/- 2.5mT center field, 0.05mT field modulation width, 1000 receiver gain, 1 second time constant, 16 minute sweep time for one sweep, and

100mL/min flow-rate. B) is the simulated spectrum with $R^2=0.966453$. C) is the residual spectrum.

As indicated by the spectra and other data gathered in these experiments, 4-thylphenol produces a radical that stable that retains structure long enough to be observed via fast-flow ESR technique. The HFCC's gathered from the simulation show similar grouping, but slight variation in value to the HFCC's gathered from the DFT calculations. This is partially attributable to the fact that the simulated spectrum did not have near exact correlation such as $R^2 > 0.98$

This study was conducted in order to observe the free radical structure of radical organic compounds using chemical oxidation. The compounds in order of appearance in the paper are

Table 1

Compound	R-Substituent	Position	Group	HFCC		Sim. Corr.
				Experimental	DFT	
4-ethylphenol	CH_2CH_3	ortho	$\text{a}^{\text{H}}_{\text{H}}$	1.386 (2H)	1.697	0.966453
		meta	$\text{a}^{\text{H}}_{\text{H}}$	6.117 (2H)	4.871	
		para	$\text{a}^{\text{H}}_{\text{CH}_2}$	10.421 (2H)	6.331	
4-vinylphenol	CHCH_2	ortho	$\text{a}^{\text{H}}_{\text{CH}_3}$	0.321 (3H)	0.016	0.859158
		ortho	$\text{a}^{\text{H}}_{\text{H}}$	5.243 (2H)	2.738	
		meta	$\text{a}^{\text{H}}_{\text{H}}$	6.779 (2H)	4.875	
p-methoxyphenol	OCH_3	para	$\text{a}^{\text{H}}_{\text{CH}}$	1.336 (1H)	1.359	0.995597
		ortho	$\text{a}^{\text{H}}_{\text{CH}_2}$	1.457 (2H)	2.418	
		ortho	$\text{a}^{\text{H}}_{\text{H}}$	0.206 (2H)	1.750	
guaiacol	H	meta	$\text{a}^{\text{H}}_{\text{H}}$	5.026 (2H)	4.918	0.950290
		ortho	$\text{a}^{\text{H}}_{\text{OCH}_3}$	2.057 (3H)	0.016	
		ortho	$\text{a}^{\text{H}}_{\text{H}}$	1.666 (1H)	4.789	
sesamol	OCH	ortho	$\text{a}^{\text{H}}_{\text{OCH}_3}$	3.914 (3H)	0.058	0.964461
		meta	$\text{a}^{\text{H}}_{\text{H}}$	2.146 (2H)	1.787	
		para	$\text{a}^{\text{H}}_{\text{H}}$	2.852 (1H)	5.345	
2,6-dimethoxy-4-methylphenol	CH_3	ortho	$\text{a}^{\text{H}}_{\text{H}}$	7.852 (1H)	5.404	0.994148
		ortho	$\text{a}^{\text{H}}_{\text{H}}$	1.898 (1H)	3.953	
		meta	$\text{a}^{\text{H}}_{\text{H}}$	1.061 (1H)	1.708	
isoeugenol	CHCHCH_3	meta	$\text{a}^{\text{H}}_{\text{OCH}}$	6.784 (1H)	1.838	0.963074
		para	$\text{a}^{\text{H}}_{\text{OCH}}$	6.784 (1H)	1.838	
		ortho	$\text{a}^{\text{H}}_{\text{OCH}_3}$	1.305 (6H)	0.294	
		meta	$\text{a}^{\text{H}}_{\text{H}}$	1.230 (2H)	1.424	
		ortho	$\text{a}^{\text{H}}_{\text{CH}_3}$	9.570 (3H)	5.068	
isoeugenol	CHCHCH_3	ortho	$\text{a}^{\text{H}}_{\text{H}}$	6.800 (1H)	4.285	0.963074
		ortho	$\text{a}^{\text{H}}_{\text{OCH}_3}$	3.092 (3H)	0.495	
		meta	$\text{a}^{\text{H}}_{\text{H}}$	1.603 (2H)	2.566	
		para	$\text{a}^{\text{H}}_{\text{CH}}$	0.194 (2H)	0.240	
para	$\text{a}^{\text{H}}_{\text{CH}_3}$	3.857 (3H)	9.789			

4-ethylphenol, 4-vinylphenol, p-methoxyphenol, guaiacol, sesamol, 2,6 dimethoxy-4-methylphenol, isoeugenol, methyl-isoeugenol, estragole, trans-anethole, safrole, cis-isosafrole, and trans-isosafrole. Cis-isosafrole and trans-isosafrole were studied at the same time as they were both in the same sample of isosafrole. Using fast-flow ESR technique, the experimental compounds were oxidized and observed within a brief window of time. All of the experimental compounds produced radicals when oxidized; however, not all of them provided spectra that could be analyzed. For those compounds which provided well-resolved spectra, simulations were

made to determine Hyperfine Coupling Constants. These HFCC's were then compared to other sets of HFCC's obtained from computer Density Functional Theory calculations. While the values of the sets of HFCC's did not always match, their pairings did, suggesting that the HFCC data were valid. Some of the compounds, such as safrole, are found naturally or as additives in certain foods. It should be noted that these chemicals may potentially be oxidized into hazardous carcinogens inside the body by weaker oxidizers than cerium (IV).

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