

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

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Introduction

Free radicals are highly reactive molecules that have unpaired electrons and may have negative physiological effects. Much of the harm caused by radicals results from their interaction with biological membranes. Cellular protective agents within the body, such as glutathione, superoxide dismutase, and ascorbate help cells defend against oxidative damage from reactive oxygen species (ROS) by scavenging the free radicals. However, these enzymes and antioxidants are depleted when they interact with large quantities of radicals. This is due to the radical's ability to pull off electrons from molecules and consequently damage these cellular protective agents. Therefore, if a compound is consumed that increases the quantity of free radicals in the body, oxidative damage may also increase. This may cause the body to go into oxidative stress, and may eventually lead to cell death by apoptosis from radical induced cellular mutation.

This study focuses on one type of free radical: phenoxy radicals ($\text{PhO}\cdot$) which easily form within the body. $\text{PhO}\cdot$ have a harmful effect in the body when they interact with certain molecules. About 5% of all oxygen inhaled is oxidized to superoxide radicals that react quickly with $\text{PhO}\cdot$ and lead to the development of mitochondrial diseases.¹ Mitochondria make energy for the cell, using oxygen as a reactant. Therefore, there is a greater concentration of superoxide radicals with which $\text{PhO}\cdot$ can interact. Furthermore, since there is more interaction between reactive oxygen species (ROS) and mitochondrial DNA, as opposed to interaction with nuclear DNA, the likelihood of oxidative damage increases. This oxidative damage augments the DNA mutation rate by halting DNA replication and cell division. By doing so, it becomes more probable for a lesion to slip past the DNA error-correcting polymerases. The oxidative damage from free radicals can ultimately lead to the formation of cancerous cells by increasing the probability of genetic error in DNA. It is believed that the $\text{PhO}\cdot$ of ochratoxin A specifically attacks guanine molecules in DNA strands.²

To determine if phenoxy free radical intermediates were present, ESR spectroscopic measurements were performed. The radical intermediates were detected by fast flow ESR experimentation. Unless noted, each fast flow ESR experiment of the studied compounds (**Figure 1**) was conducted with the same procedure. The results of

each experiment were also analyzed using the GaussView and WinSim software.

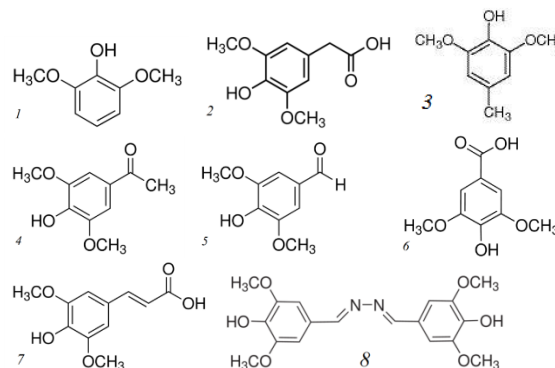


Figure 1. Chemical structures of 2,6-dimethoxyphenol (1), 3,5-dimethoxy-4-hydroxyphenylacetic acid (2), 2,6-dimethoxy-4-methylphenol (3), acetosyringanone (4), syringaldehyde (5), syringic acid (6), sinapic acid (7), and syringaldazine (8).²⁰

Materials and Methods

2,6-dimethoxyphenol; syringaldehyde; syringic acid; acetosyringanone (3',5'-dimethoxy-4'-hydroxyacetophenone); sinapic acid (3,5-dimethoxy-4-hydroxycinnamic acid); syringaldazine; cerium (IV) sulfate were purchased from Sigma-Aldrich (St. Louis, MO). 3,5-dimethoxyphenol-4-hydroxyphenylacetic acid; 2,6-dimethoxy-4-methylphenol were purchased from Alfa Aesar (Ward Hill, MA). Sulfuric acid; 95% ethanol; and pure ethanol were purchased from AAPER (Shelbyville, KY). Ethylene glycol was purchased as antifreeze from Walmart (Bentonville, AR). Two solutions were prepared for each experiment. One solution contained 2mM of the studied compound, 400 mL 95% ethanol, and 1600 mL deionized water. The other solution was the oxidizing agent which contained 1.8mM or 1.1961 g cerium (IV) sulfate, 50 mL sulfuric acid, and 1950 mL deionized water. Each solution was prepared in a 2L beaker and mixed using a magnetic stirring bar and a magnetic stirring plate until the compound was dissolved in solution. Sometimes it was necessary to heat the solution in order to dissolve the compound being studied. The only exception to the above procedure was in the fast flow ESR of syringaldazine experiment. There were significant problems in dissolving the 2mM of syringaldazine into solution. Therefore, the solution was diluted to 1.64mM syringaldazine by the addition of ethanol.

The prepared solutions of the studied compound and chemical oxidation system were poured into separate solution reservoirs. These solutions were then bubbled with nitrogen gas for 5 minutes. Afterwards, the solution reservoirs were mounted in the reservoir stabilizing device, and attached to a 3 way bulb valve purchased from Cole-Parmer (Vernon Hills, IL) with Tygon 1/8 x 1/4 3350 silicone tubing purchased from Saint-Gobain (Millville, NJ). One length of tubing went from the bulb valve to a beaker of DI H₂O, and the other went to a Cole-Parmer Masterflex L/S peristaltic pump purchased from Cole-Parmer (Vernon Hills, IL). Tubing from the pump led to the TE-011 cavity which was fitted with a Wilmad WG-814H mixing flat cell, purchased from Wilmad-Lab Glass (Buena, NJ). The peristaltic pump was set to flow rates between 10mL/min and 60mL/min depending on the studied compound.

Measurements were made using a JEOL-REX1 spectrometer that operated at a frequency near 9.4 GHz and a magnetic field near 3370G. The microwave cavity was modulated with a magnetic field of 100 kHz. The spectral analysis and simulation were performed with LabView 7.1 interface written locally and WinSim 2002 software from NIEHS (Research Triangle Park, NC). LabView itself was purchased from National Instruments (Austin, TX).

Files were converted from LabView to a WinSim usable format through the use of a conversion program written locally. The density functional theory (DFT) calculations were completed using GaussView and Gaussian '03 software purchased from Cyberchem (Gainesville, FL).

Results and Conclusion

A chemical oxidation fast flow ESR system containing the studied compound and Ce(SO₄)₂ produced an observable signal. The system consisted of two 2L solutions. The first solution consisted of 2mM studied compound in an 80/20 (v/v) ratio solution of deionized (DI) H₂O and 95% ethanol. The second solution consisted of 1.8mM Ce(SO₄)₂, in 0.225M H₂SO₄. The final concentrations of components in the flat cell were: 1.0mM studied compound and 0.9mM Ce(SO₄)₂. An ESR signal was observed at a variety of flow rates ranging from 10mL/min to 60mL/min. The observed spectra, were produced with different parameters based on the studied compound's spectrum. The simulated spectrum with an R²>0.922574 (correlating the simulation to the original spectrum) and consisted of three to five coupling constants. This value, R², is calculated by measuring how similar the experimental spectrum is to the simulated spectrum. The

Compound	R-substituent	HFCC				Calc. Time	Sim. Corr.
		Position	Group	Experimental	DFT		
2,6-Dimethoxyphenol	-H	ortho	a ^H _{CH₃}	1.464 (6H)	2.141	23 hrs, 7 mins	(Spectrum 2) 0.995304
		meta	a ^H _H	1.376 (2H)	2.217		
		para	a ^H _H	8.168 (1H)	7.24		
Acetosyriganone	-COCH ₃	ortho	a ^H _{CH₃}	1.464 (6H)	2.459	51 hrs, 14 mins	(Spectrum 4) 0.997357
		meta	a ^H _H	1.748 (2H)	2.403		
		para	a ^H _{COCH₃}	0.332 (3H)	0.112		
Syringaldehyde	-CHO	ortho	a ^H _{CH₃}	1.468 (6H)	2.383	46 hrs, 51 mins	(Spectrum 5) 0.994464
		meta	a ^H _H	1.753 (2H)	2.365		
		para	a ^H _{CHO}	0.510 (1H)	0.283		
Syringic Acid	-COOH	ortho	a ^H _{CH₃}	1.483 (6H)	2.535	64 hrs, 21 mins	(Spectrum 4) 0.996748
		meta	a ^H _H	1.754 (2H)	2.406		
		para	a ^H _{COOH}	0.084 (1H)	0.391		
Sinapic Acid	-COHCHCOOH	ortho	a ^H _{CH₃}	1.282 (6H)	2.057	148 hrs, 29 mins	(Spectrum 3) 0.97338
		meta	a ^H _H	1.342 (2H)	2.336		
		para	a ^H _{CH}	1.619 (1H)	0.258		
		para	a ^H _{CH}	3.415 (1H)	2.301		
		para	a ^H _{COOH}	4.005 (1H)	4.983		
			0 (Gaussian Curve)	-			
Syringaldazine	-CHN	ortho	a ^H _{CH₃}	1.557 (6H)			(Spectrum 5) 0.922574
		meta	a ^H _H	0.361 (2H)			
		para	a ^H _{CH}	1.667 (1H)			
		para	a ^H _{CN}	0.267 (1N)			
					0 (Gaussian Curve)		
2,6-Dimethoxy-4-methylphenol	-CH ₃	ortho	a ^H _{CH₃}	1.257 (6H)	1.839	121 hrs, 20 mins	(Spectrum 5) 0.995916
		meta	a ^H _H	1.368 (2H)	1.957		
		para	a ^H _{CH₃}	9.576 (3H)	7.631		
3,5-Dimethoxy-4-hydroxy-phenylacetic Acid	-CH ₂ COOH	ortho	a ^H _{CH₃}	1.395 (6H)	2.102	86 hrs, 21 mins	(Spectrum 11) 0.970632
		meta	a ^H _H	7.162 (2H)	2.126		
		para	a ^H _{CH₂}	1.395 (2H)	4.643		
		para	a ^H _{COOH}	0.093 (1H)	0.178		

simulation correlation has great validity between the 0.98 to 0.99 range, which was achieved for five of the studied compounds. The simulation parameters were different depending on the studied compound. Conditions were optimized using WinSim software. The residual of the simulation was obtained by subtracting the simulation from the original spectrum. **Figure 2** shows an example.

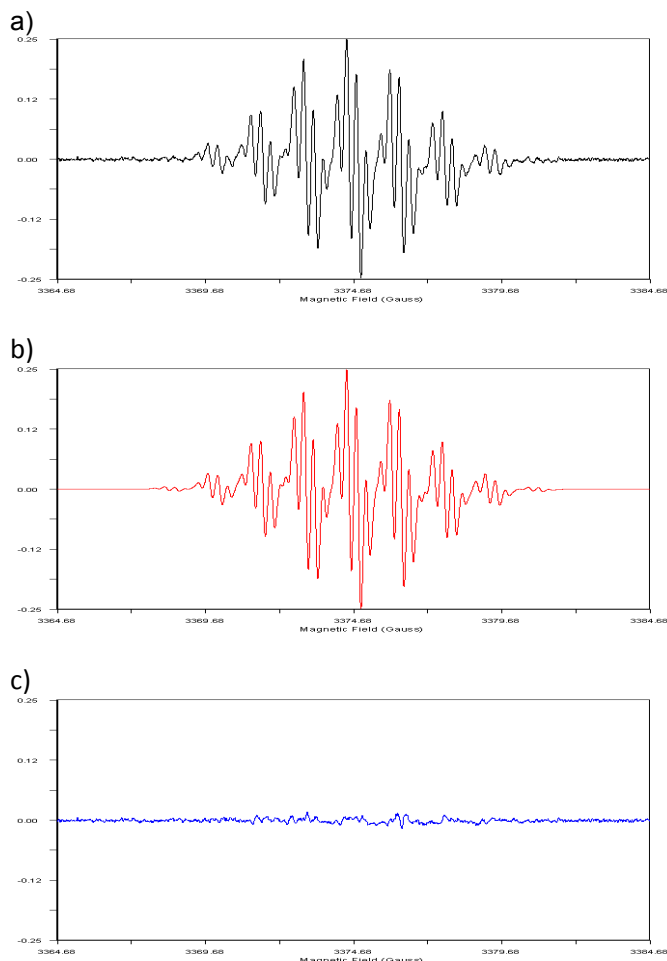


Figure 2. Fast Flow ESR spectrum of acetosyringane-derived radicals: a) experimental spectrum, b) simulated spectrum with $R^2=0.997357$, and c) the residual from the simulated spectrum.

DFT calculations were performed using a variation of the 3-21G basis set called “epr-ii” basis set. The results yielded coupling constants that resembled those found via WinSim analysis. These results are summarized in the table.

These results showed that the studied compounds will produce a stable radical that lives long enough to be observed under the specified experimental conditions. The experimental results were similar to those produced by the DFT calculations. Therefore, the experimental hyperfine coupling constants are likely to be valid. In addition,

molecular orbital depictions were generated for some of the studied compounds using Gaussian software.

This study was conducted in order to observe free radicals produced from syringaldazine and related compounds using a chemical oxidation system. The related compounds studied were: 2,6-dimethoxyphenol, 3,5-dimethoxy-4-hydroxyphenylacetic acid, 2,6-dimethoxy-4-methylphenol, acetosyringane, syringaldehyde, syringic acid, and sinapic acid. An ESR spectrum was produced for each compound using fast-flow ESR experimentation. However, the spectrum observed for sinapic acid was difficult to analyze and did not produce a simulation correlation great enough to be considered valid. Coupling constants were assigned for all spectra and compared to the DFT calculation results. The experimentally found coupling constants closely resembled those predicted by DFT calculations. The similarity between the experimental coupling constants and the DFT calculations suggests that these values are likely to be valid. It was determined from the results that syringaldazine’s free radical was localized and did not travel to the opposite side of the molecule. In addition, molecular orbital visualizations were produced for several of the compounds. The molecular orbital visualizations are displayed with green and red colored orbital lobes that represent MO symmetry.

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References

1. Atkins, P.; de Paula, J. *Atkins’Physical Chemistry*. 8th ed. W.H. Freeman and Company: Oxford, 2006.
2. Halliwell, B.; Gutteridge, J. M. C. *Free Radicals of Biology and Medicine*. 4th ed. Oxford Biosciences: Oxford, 2007.