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THE MECHANISMS OF CHROMOPHORE FORMATION OF LIGNIN MODEL COMPOUNDS

by

Sheldon Irvin Clare

A Dissertation Submitted to the Faculty of the

DEPARTMENT OF CHEMISTRY

In Partial Fulfillment of the Requirements
For the Degree of

DOCTOR OF PHILOSOPHY

In the Graduate College

THE UNIVERSITY OF ARIZONA

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THE UNIVERSITY OF ARIZONA

GRADUATE COLLEGE

I hereby recommend that this dissertation prepared under my direction by Sheldon Irvin Clare

entitled The Mechanisms of Chromophore Formation of Lignin Model Compounds

be accepted as fulfilling the dissertation requirement of the degree of Doctor of Philosophy

Cornelius Steelink — Dissertation Director — 8/8/72 — Date

After inspection of the final copy of the dissertation, the following members of the Final Examination Committee concur in its approval and recommend its acceptance:*

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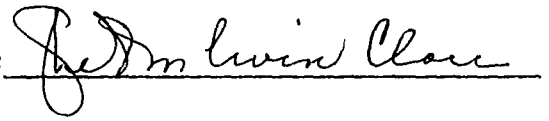
SIGNED: 

To Beth, Andrew, Joseph, and Ellen

ACKNOWLEDGMENT

The author wishes to express his sincere thanks to Dr. Cornelius Steelink for his assistance and advice during the course of this work and to Mrs. Roberta Esker for typing the final copy of this dissertation.

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ABSTRACT

Hardwood lignin model compounds (syringyl phenols) were treated with three different oxidizing agents: 1) NaOH/air, 2) NaOH/H_2O_2, and 3) H_2O_2/horseradish peroxidase enzyme. In the latter two reactions, deeply colored solutions were formed most of the time. In the alkaline-peroxide degradation reaction, the chromophores were characterized as 2,6-dimethoxybenzoquinone (yellow colored) and 2-hydroxy-3,5-dimethoxy-benzoquinone (purple colored). Corresponding guaiacyl (softwood) model compounds seem to be more prone to ring cleavage than these syringyl derivatives. In the enzyme reaction, the red colored compounds were identified as 1-formyl-3-methoxy-4,5-dioxo-1,5-cyclohexadiene and 1-acetyl-3-methoxy-4,5-dioxo-1,5-cylcohexadiene. The formation of these ortho-quinones was predicted by Caldwell and Steelink in 1969. Air oxidation of the model compounds in base produced only slight degradation, in which side chain cleavage and demethylation were competing reactions.

The mechanism of reactions (1) and (2) was examined by electron spin resonance spectrometry. When the syringyl phenols were placed in NaOH and air oxidized, they initially formed phenoxy radicals, which then underwent attack by oxygen radicals at two positions on the ring to give catechols and hydroquinones. In the case of the alkaline peroxide system, the spectrometry showed that an initial Dakin oxidation was followed by a free radical reaction.

INTRODUCTION

The major goal of this research was to investigate the causes of color formation when wood is oxidized. Specifically, attention was focused on the lignin fraction of the wood, because the lignin macromolecule should be easily oxidized due to its phenolic nature (1).

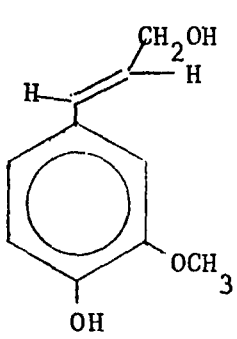
Lignin is an essential component of all terrestrial plants, and is found in the woody stems of arborescent gymnosperms (softwoods) and angiosperms (hardwoods). The other major constituent of wood is cellulose. As a cell wall constituent, lignin performs the following important functions:

1. Decreases the permeation of water through all cell walls of plant tissue and thus regulates internal transport of water, nutrients, and metabolites.

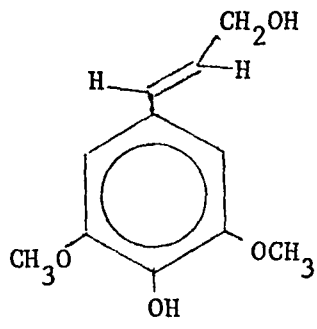
2. Imparts rigidity to cell walls, and in woody parts, acts as a permanent bonding agent between cells which resists impact, compression and bending.

3. Lignified tissue resists macroorganism attack by blocking enzyme penetration into the cell wall (2).

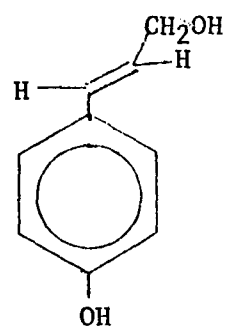
It is generally agreed that there are three primary precursors of lignin in terrertrial plants. These are trans-coniferyl alcohol, trans-sinaply alcohol, and trans-p-coumaryl alcohol. They are polymerized by enzyme-induced dehydrogenation to produce the lignin macromolecule (3).



trans-coniferyl alcohol



trans-sinapyl alcohol



trans-p-coumaryl alcohol

The main mechanism of molecular growth of the lignin macromolecule in nature is dependent on phenoxyl radical coupling processes. This type of polymerization is shared by other naturally occurring substances, e.g., alkaloids, tannins, etc.

Unfortunately, detailed chemical characterization of lignins is very difficult due to its resistance to degradation. All known methods of chemical degradation gives poor yields of small molecular weight products.

Recently, Freudenberg (4,5,6) gathered together all the known information on the analytical data and reactivity of spruce lignin and the dehydrogenative polymerization of coniferyl alcohol and proposed the following composite lignin structure (Figure 1). This represents an <u>average</u> fragment of a larger lignin molecule. A majority of the monomeric units are of the C_6-C_3 type.

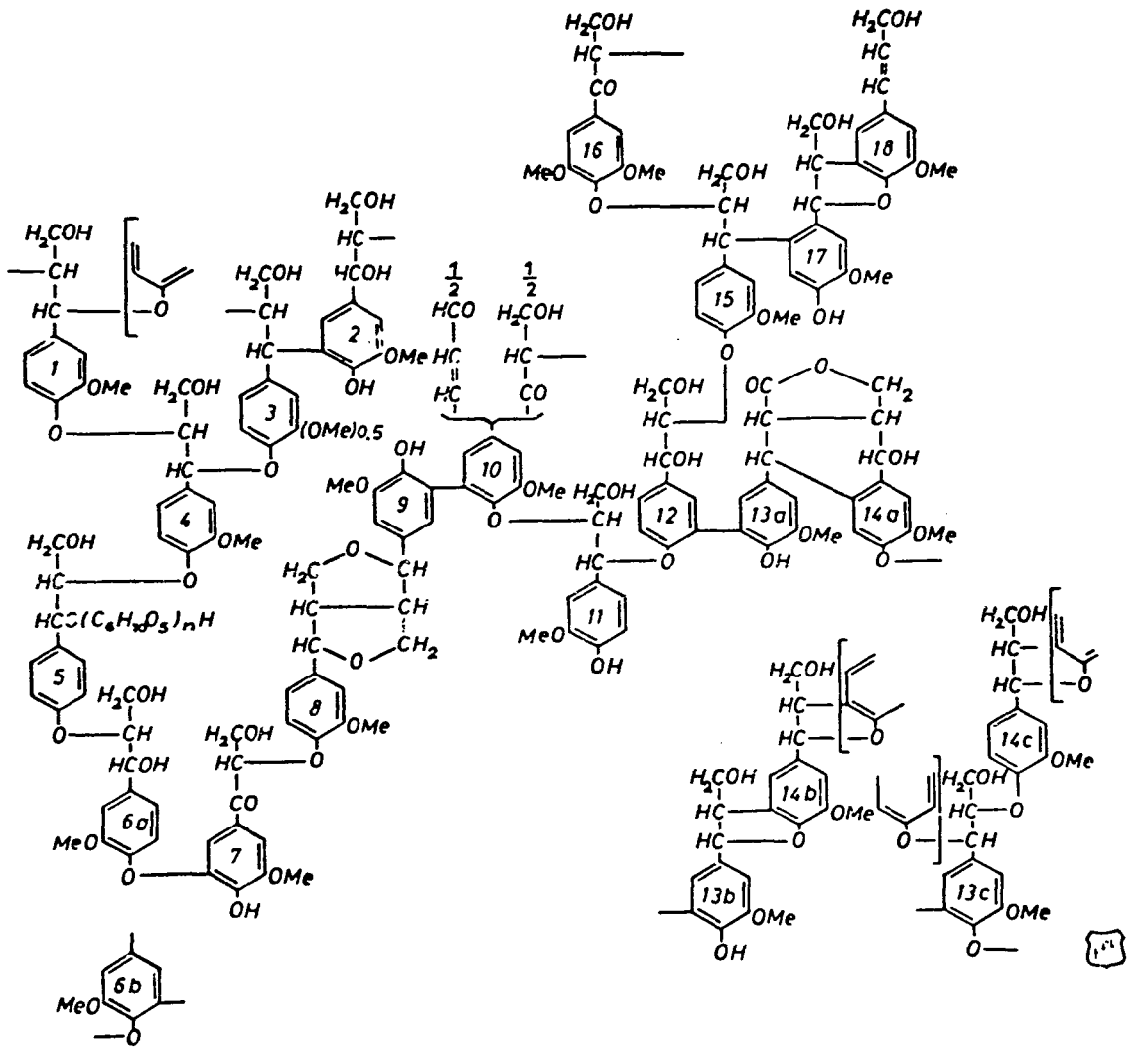
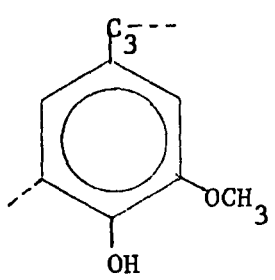
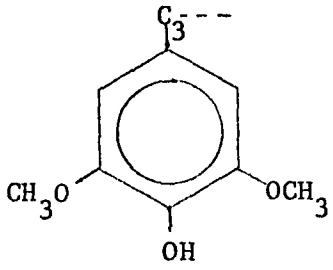


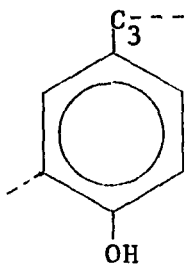
Figure 1. Composite Lignin Formula by Freudenberg.



Guaiacyl or
Vanillyl



Syringyl



p-Hydroxyphenyl

The guaiacyl group is more prevalent in softwood lignins and the syringyl group is found to be more prevalent in the hardwoods. As mentioned before, the resistance of lignins to degradation can be easily understood when one sees that the monomeric units are held together by very stable bonds.

Being phenolic in nature, lignin is prone to oxidation by different pathways depending upon the oxidant and the reaction conditions. Many of these oxidation reactions are of technical as well as academic and biological importance. However, the mechanisms of these reactions have not been carefully studied, and little detailed information is known.

In order for wood to be better utilized as paper and other wood products, it must be first put through the process of pulping. The main objective of the pulping process is the separation of wood into its individual fibers of cellulose. Lignin, which serves to hold the fibers together, must be removed in the pulping process.

Pulping processes are either of the mechanical or chemical type. Mechanical pulp or groundwood is made by grinding logs or blocks of wood against an abrasive stone in the presence of water. This pulp is practically identical to wood in composition and can be used to make newspaper, tissue, towel, and other inexpensive papers. The lignin in this process is not removed and will make the paper yellow (7).

Chemical pulps are classified as either: 1) sulfite, 2) sulfate(kraft), or 3) soda. The sulfite pulping is an acid process utilizing SO_2 which is passed over $CaCO_3$, MgO, or solutions of ammonia, or caustic and produces acid-sulfite pulping liquors. In the soda process, NaOH is used as the major pulping chemical and when sodium sulfide is added to the NaOH, the process is known as sulfate or "kraft" pulping.

Once again, these pulps still contain lignin which must be removed in order to whiten the pulp.

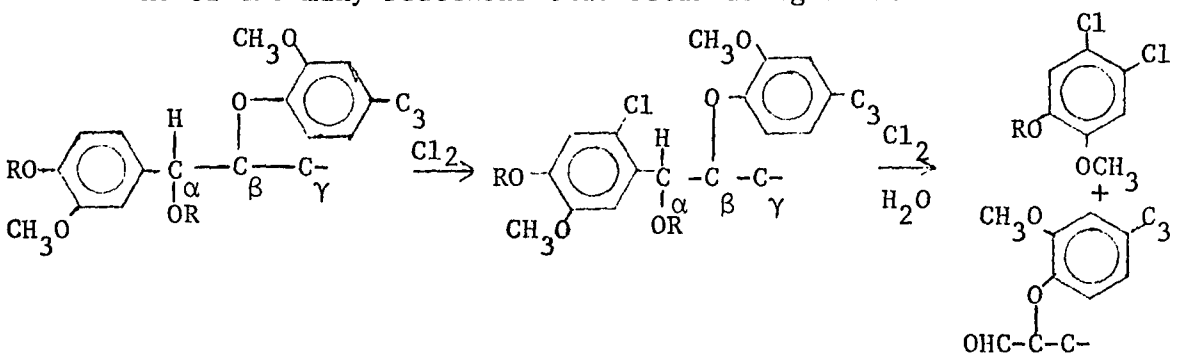
The most effective oxidizing agents for delignification of chemical pulps are:

1) chlorination in acidic medium (pH 2 to 4)

2) hypochlorite bleaching in alkaline solution (pH 9 to 10)

3) alkaline or peroxide bleaching

One of the many reactions that occur using chlorine is:



Until recently, in commerical operations where pulp was produced chemically, lignin was disposed of by burning. Otherwise, the spent liquors were diluted in water and dumped into nearby streams and rivers. As the public outcry toward this type of commercial pollution has increased, the disposal problem of lignin has become more acute. Attempts to create an industry based on lignin liquors have been only slightly successful.

Vanillin has been formed from the oxidation of sulfite lignin by air since 1898 (8). Since then, other studies of vanillin formation from lignin sulfonates have shown that yields can be greatly increased by the correct use of a catalyst along with oxygen in the air under alkaline conditions (2, Chap. 19).

Since the lignin molecule is susceptible to oxidation by many types of oxidants, it is important to investigate a few common lignin oxidants in order to further elucidate the lignin structure and the reactions that occur when pulp is processed commercially from wood.

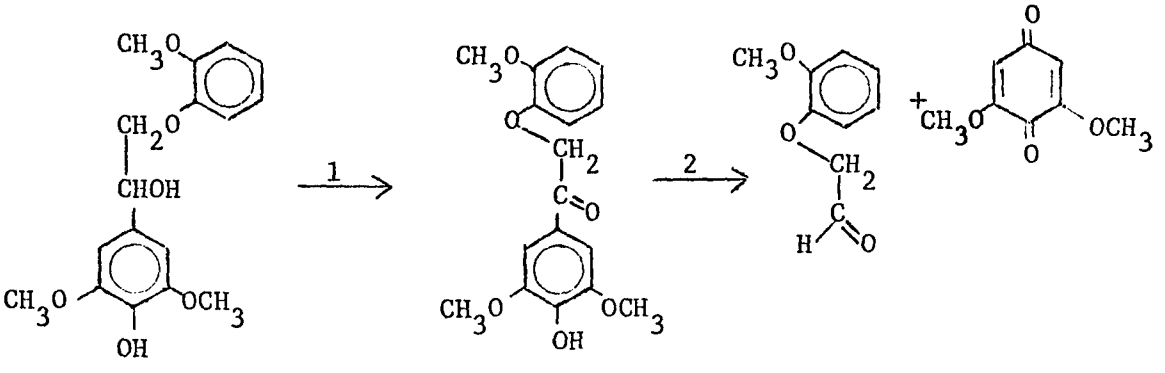
Since 1944, alkaline-peroxide bleaching of pulp has been used as a commercial process (9). Because this oxidant undoubtedly reacts with lignin, it would seem to be important to investigate what happens to the lignin content of the pulp when it is bleached.

Another property of lignins which could be investigated along with degradative oxidation is its paramagnetism (10-15). When hardwood lignins are dissolved in basic solutions, stable radical anions are formed. These anions are responsible for the paramagnetic property of lignin salts. For the last 12 years, electron spin resonance spectrometry has been used to investigate the structure and reactions of

lignin (2. pp. 326-364). Many of the radicals formed from lignin model compounds form chromophores as end products.

It would seem inconsistent that chromophore formation would accompany bleaching reactions. The observation that the formation of chromophores may be a result of free radical processes suggested the use of e.s.r. spectrometry as a technique to monitor formation of these chromophores.

Chromophores are also produced by the microbiological degradation of lignin. Most wood-rotting fungi produce phenol oxidases. These enzymes have been shown to degrade lignin oxidatively (16-19). Using model compounds, investigators have shown that purified laccase as well as fungal cultures were able to cleave aryl-alkyl bonds and form para-benzoquinone derivatives. Free radical intermediates were



postulated for these reactions.

It is also known that decaying hardwood has a much higher spin content than intact hardwood (20). Caldwell and Steelink assumed that the spin content of the wood was due to trapped stable free radicals in the polymeric matrix. After oxidizing hardwood lignin model compounds

with horseradish peroxidase and H_2O_2 under aqueous conditions and physiological pH, e.s.r. spectrometry showed the formation of long lived free radicals. Based upon these results, Caldwell and Steelink concluded that wood-rotting fungi producing phenol oxidases can depolymerize lignin in a series of one-electron steps. They also identified the formation of p-benzoquinones along with unidentifiable bright red compounds. No similar chemical oxidative degradation of lignin produced these chromophoric species.

It would seem important also to note that many lignin model compounds are completely solubilized when degraded enzymatically.

When wood is subjected to oxidation chemically as well as enzymatically, the lignin content is degraded and the formation of radicals and chromophores are readily observed. However, the mechanisms of their formation have not been studied to any great extent.

EXPERIMENTAL

General

Nuclear magnetic resonance spectra were obtained from solutions in chloroform-d, dimethylsulfoxide-d_6, carbon tetrachloride, and methanol-d_4, with tetramethylsilane(TMS) as an internal standard using a Varian T-60 spectrometer. Chemical shifts measured at the center of each peak are reported in delta (ppm) units.

Infrared spectra were obtained with Perkin-Elmer models 137 and 337 double beam spectrometers using potassium bromide pellets.

Ultraviolet spectra were measured as 95% ethanol solutions on a Cary Recording Spectrometer model 14.

Mass spectra were measured on a Hitachi-Perkin-Elmer Double Focusing RMU-6E Mass Spectrometer.

All electron spin resonance measurements were made on a Varian E-3 spectrometer. Samples were put into a flat quartz aqueous solution cell and were either exposed to air or were degassed with nitrogen. The instrument operated at 9.5 GHz with a frequency modulation of 100 kHz.

Thin layer chromatography was carried out on either pre-coated plates of Silica Gel F-254, layer thickness 0.25 mm, manufactured by E. Merck, Darmstadt, Germany, or on plates which were coated with a slurry of Silica Gel powder 60-200 mesh, J. T. Baker Chemical Co., Phillipsburg, N.J. in 9 to 1 (by volume) solution of water and methanol. For preparative thin layer chromatography, 52 grams of Silica Gel was

stirred with 100 ml. of water, methanol (9:1) mixture, and deposited on a glass plate to a thickness of 2 mm.

Melting points were taken, uncorrected, using a Fisher Mel-Temp melting point apparatus.

The hydrogen ion concentrations of various aqueous solutions was determined by using a Radiometer pH Meter 28.

Enzymatic Oxidation

Enzymatic oxidations were performed using a stock solution of horseradish peroxidase (M.W. 44,100) (21), Sigma Chemical Co., St. Louis, Mo., prepared from 0.0038 grams of the enzyme dissolved in 10 ml. of glass distilled water. This solution was stored at -10° C to prevent decomposition. The approximate molarity of this solution was 8.6×10^{-6} mole/liter.

As needed, stock solution was thawed, diluted (1.4:25), and to 10 ml. of this solution, 0.3 ml. of 3% H_2O_2 was added. A 1×10^{-2} molar solution of a syringyl compound was prepared and mixed (1:1) with the enzyme-peroxide solution. When necessary, appropriate buffers were prepared (Table I) and used as solvents.

At various times, the color of the reaction was noted. At the time when the color of the solution was deep red, the reaction was stopped by extracting the aqueous solution with chloroform or methylene chloride. The organic layer was separated, dried with $MgSO_4$, and concentrated on a rotary evaporator.

Thin layer chromatography was used to identify the products for formed. The developing solvent was usually benzene; absolute

TABLE I. Aqueous Buffer Solutions Used in Model Compound Oxidation

pH	Buffer Solution
2	25.0 ml. of 0.2 M KCl + 6.5 ml. of 0.2 M HCl
3	50.0 ml. of 0.1 M KHphthalate + 22.3 ml. of 0.1 M HCl
4	50.0 ml. of 0.1 M KHphthalate + 0.1 ml. of 0.1 M HCl
5	50.0 ml. of 0.1 M KHphthalate + 22.6 ml. of 0.1 M NaOH
6	50.0 ml. of 0.1 M KH_2PO_4 + 5.6 ml. of 0.1 M NaOH
7	0.01 M N-morpholinopropane sulfonic acid(MOPS) adjusted to pH, 7 by dropwise addition of 1.0 M NaOH
7*	NaH_2PO_4 + KH_2PO_4
8*	NaH_2PO_4 + KH_2PO_4
9*	K_3PO_4 + Na_3BO_3
10*	Na_3BO_3 + Na_2CO_3
11*	Na_3BO_3 + Na_2CO_3
12*	Na_2HPO_4 + Na_3PO_4

*These buffers were prepared by dissolving encapsulated powders in 100 ml. of distilled water to give a 1% solution. They were purchased from Micro Essential Laboratory, Brooklyn, New York and are called pHydrion Buffers.

ethanol (4:1). The plate was developed, dried, and sprayed with a sulfuric acid solution of 2,4-dinitrophenylhydrazine. The spots on the plate were identified as: starting syringyl phenol, 2,6-dimethoxybenzoquinone, and an ortho-quinone (red before spraying, green after spraying). The unknown ortho-quinone was synthesized independently and compared with the unknown. The unknown was identified by comparison with the colors and R_f values of known compounds (Table II).

Alkaline-Peroxide Oxidation

Syringyl model compounds were oxidized by hydrogen peroxide in base by the following procedure (22). A weighed amount of the syringyl compound, usually 1-5 grams, was put into a small round bottom flask containing 75 ml. of 0.01 M NaOH per gram of substrate and a small magnetic stirring bar. The flask was immersed in a water bath and warmed to 45°C. (At this temperature, some of the syringyl compound was dissolved). H_2O_2(30%) was added in an amount equimolar with that of the starting material. The pH of the mixture was determined and adjusted to 10.5 by dropwise addition of 5.0 M NaOH. At this stage, a deep purple developed immediately when syringaldehyde was the substrate. If the substrate were acetosyringone, the purple color would appear within 3-5 minutes and when propiosyringone was the substrate, the color would never darken beyond light pink.

The solution was then returned to the water bath and heated at 45°C with stirring. At the end of four hours, syringaldehyde and acetosyringone had completely dissolved, but propiosyringone had not. The undissolved starting material was removed by filtration, dried, and

TABLE II. Relative R_f Values of Model Compounds and Quinones in Benzene/Absolute Ethanol (4/1)

Compound	R_f
5-hydroxyvanillin	0.45
5-hydroxyacetonvanillone	0.40
1-formyl-5-methoxy-3,4-dioxo-1,5-cyclohexadiene	0.56
1-acety-5-methoxy-3,4-dioxo-1,5-cyclohexadiene	0.59
2,6-dimethoxybenzoquinone	0.59
2,6-dimethoxyhydroquinone	0.49
2-hydroxy-3,5-dimethoxybenzoquinone	0.09
syringaldehyde	0.50
acetosyringone	0.55
propiosyringone	0.59

weighed. All the reaction mixtures were made slightly acidic (pH 5.5-6.5) with dilute hydrochloric acid and extracted three times with chloroform. At this pH, the color of the aqueous layer varied from pink to deep purple. The deep orange chloroform extract was dried over $MgSO_4$ and evaporated to dryness. The residue was weighed and subjected to further analysis. The color of the solid varied from yellow to light orange. The slightly acidic aqueous layer was made more acidic until the purple color had changed to a yellow-orange. The pH of these solutions was between 1.0 and 2.0. Chloroform was used again as the extracting solvent and using the same procedure as before, more solid residue was isolated.

These chloroform extracts were subjected to thin layer chromatography. As the chromatograms developed, two distinct colors became evident on the plates, yellow and purple, the latter color appearing only in the more acidic fraction most of the time. After spraying with either 2,4-DNPH or an aqueous solution of ferric chloride, starting material, 2,6-dimethoxy-p-benzoquinone, 2,6-dimethoxy-p-hydroquinone were immediately identified by comparing the colors after spraying with known sprayed samples. The purple compound was later identified as 2-hydroxy-3,5-dimethoxybenzoquinone. Unidentified substances remained at the origin. A relative percentage composition was obtained by running nmr spectra, integrating the peaks, and calculating the relative abundance of each species.

Isolation and Identification of the Purple Compound

The purple compound was separated from the other oxidation products of the reaction of syringaldehyde with alkaline peroxide by column chromatography. After acidifying the reaction mixture to below pH 2, the aqueous layer was extracted with chloroform and then dried with $MgSO_4$. The dried, concentrated chloroform extracts were chromatographed using a silica gel column, 3 x 60 cm. The purple compound remained on the column, while the other oxidation products were eluted with benzene. The purple product was removed from the column with 10% methanol/benzene. Each fraction was further examined by thin layer chromatography and those fractions consisting of only the purple compound were combined and evaporated to dryness: M.P., decomposes above 290°C; mass spectrum m/e (rel. intensity) 184 (100), 169 (35.3), 151 (39.7); nmr (DMSO-d_6): δ5.64, singlet (1H), 4.17, multiplet (1H), 3.72, singlet (3H), 3.52 singlet (3H) (see Figure 2); λ_{max}: 310 nm, 535 nm; ir (KBr pellet) 1650 (C=O), 1550 (C=O), and 3425 cm^{-1} (O-H).

Two isomers of this compound are known (23,24) 2-hydroxy-3,6-dimethoxybenzoquinone and 5-hydroxy-2,3dimethoxybenzoquinone and are violet-red and blue-violet, respectively. The R_f value for the latter compound in 5 to 1 ethanol-concentrated ammonia is 0.67. The R_f for the purple compound in the same developing solvent is 0.70.

Oxidation of Model Phenols With NaOH in Air

In a typical experiment, 0.5 gm. of a phenol dissolved in 50 ml. of 0.5 M NaOH was allowed to stand overnight (18 hr) in the presence of

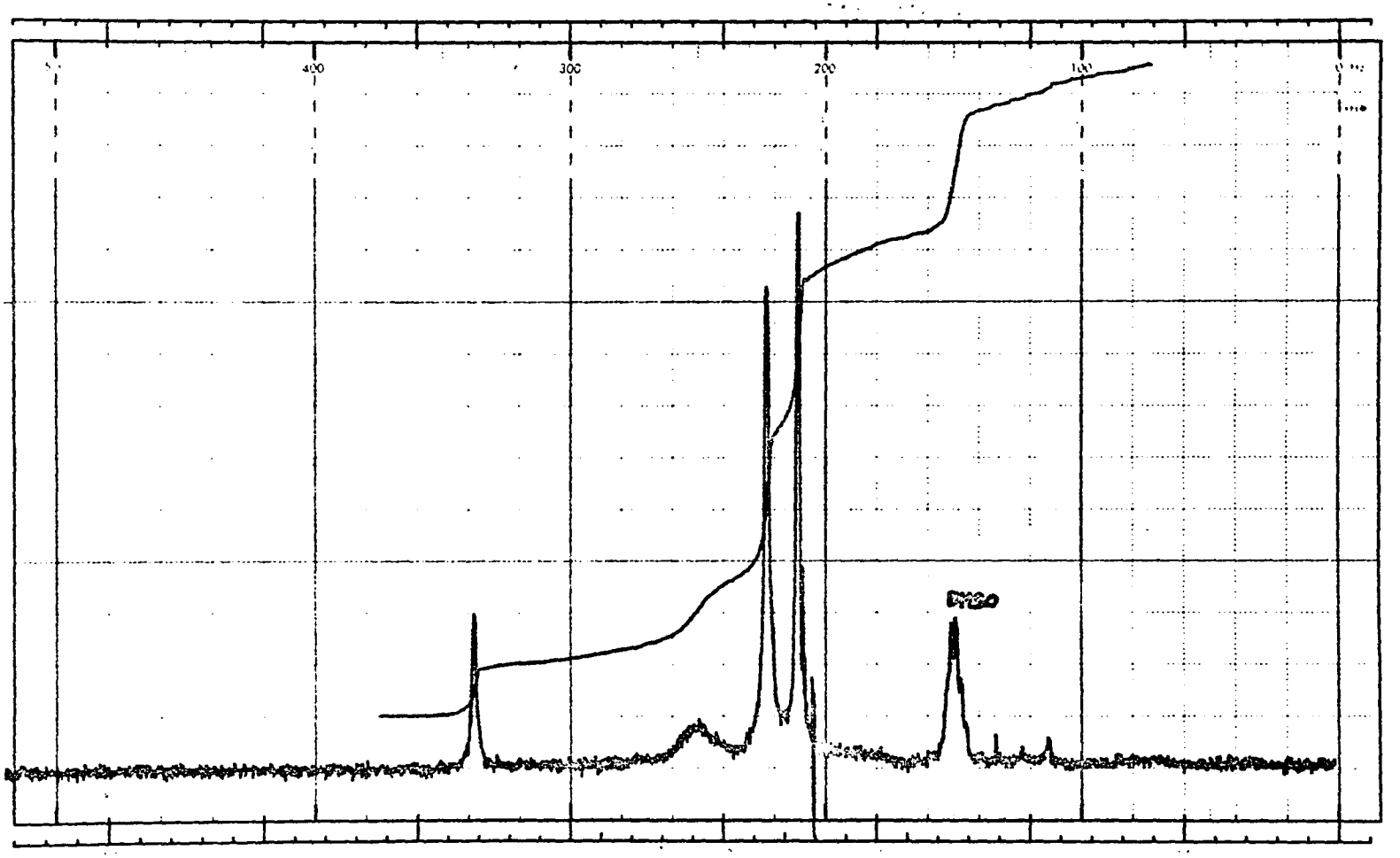


Figure 2. NMR Spectrum of 2-Hydroxy-3,5-Dimethoxybenzoquinone in DMSO-d_6.

air at room temperature. The solution was then acidified, extracted with chloroform, dried with $MgSO_4$, and evaporated to a small volume (5 ml.). The material was then subjected to nmr and thin layer chromatography analysis.

In some of the e.s.r. experiments, the phenol was dissolved in dimethylformamide/water (90/10) and base. This was done in order to stabilize the radical anion species that were formed.

Quantitative Determination of Radical Concentration Using E.S.R.

α-Ethylsyringyl alcohol (VII), (0.100 gm), M.W. 212, was dissolved in 10.0 ml. of 1.0 M NaOH in a volumetric flask. This gave an alcohol concentration of 4.72×10^{-2} mole/liter. Oxygen was bubbled through the solution for five minutes. A sample of the oxygenated solution was put into the quartz solution cell and inserted into the e.s.r. machine. An e.s.r. signal was obtained 20 minutes after insertion. At this time, the color of the solution was light orange. The e.s.r. signal reached maximum intensity after another 30 minutes. The instrumental parameters were; receiver gain 1.25×10^4, scan range $\pm 0.25 \times 10^3$ gauss, modulation amplitude 4 gauss, and scan time 8 minutes. The concentration of the radical was estimated by comparison of the integrated area with that of a standard solution of diphenylpicrylhydrazeyl in benzene (8.4×10^{-4} M). The solution was prepared by dissolving 0.004 gm. of the standard in 10.0 ml. of benzene. A similar experiment was carried out with acetosyringone.

Materials

2,6-Dimethoxybenzoquinone and 2-methoxy-p-benzoquinone were prepared by previously reported methods (15). 2,6-Dimethoxy-p-hydroquinone was prepared by reducing the corresponding quinone with sodium hydrosulfite. Syringaldehyde, acetosyringone, and acetovanillone were purchased from Aldrich Chemical Co., Milwaukee, Wisconsin.

Propiosyringone was prepared as follows: 30.8 grams (0.20 mole) of 2,6-dimethoxyphenol was dissolved in 25 ml. of pyridine with mechanical stirring in a three-necked round bottom flask. After dissolution, 19 grams (0.23 mole) of propanoyl chloride was added, and the mixture was then cooled, poured into a 2% hydrochloric acid solution, and extracted twice with 50 ml. of ether. The ether solution was dried with $MgSO_4$ and evaporated to dryness. Light yellow crystals of the ester were formed which were dried on a Buchner funnel, m.p. 42.5-43°C.

A 13.6 gram (0.06 mole) sample of the ester was refluxed with 18 grams of aluminum chloride in 40 ml. of nitrobenzene for 4 hr. The nitrobenzene mixture was poured into ice water and extracted with ether. The ether fractions were then extracted with aqueous NaOH. The alkaline extract was acidified and the solid Fries product precipitated which was recrystallized from cyclohexane. Yield 8.0 grams. m.p. 104.5-105°C [lit. (25) m.p. 108°C].

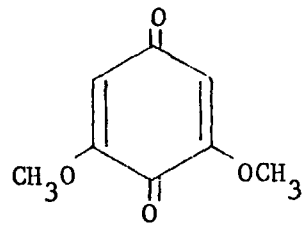
α-Ethylsyringyl alcohol (VII) was prepared from propiosyringone by catalytic hydrogenation over Pd/C, m.p. 93-94°C [lit. (25) m.p. 94-95°C] from benzene. α-Methylsyringyl alcohol (VI) was prepared from acetosyringone in the same manner and was recrystallized from benzene, m.p. 96-97°C.

5-Iodovanillin, 5-hydroxyvanillin, and 5-hydroxyacetovanillone were prepared as reference compounds by the method of Pepper (in 26, 27). The corresponding ortho-quinones were made by oxidizing the catechols with o-chloranil. The mass spectrum of 1-formyl-3-methoxy-4,5-dioxo-1,5-cyclohexadiene gave m/e 166 and a base peak m/e 95. The mass spectrum of 1-acetyl-3-methoxy-4,5-dioxo-1,5-cyclohexadiene gave m/e 180 and a base peak m/e 137. Methyl 3-O-methylgallate and 3-O-methylgallic acid were prepared from gallic acid by the method of Scheline (28). 5-Chlorovanillin and 5-bromovanillin were purchased from the Aldrich Chemical Company, Milwaukee, Wisconsin.

RESULTS AND DISCUSSION

Reaction of Model Compounds With NaOH and Oxidants

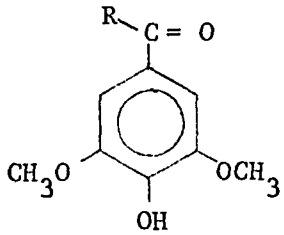
The action of NaOH/oxygen or NaOH/hydrogen peroxide on lignin model compounds has been shown to produce quinones by Dence (in 22) and Kratzl (in 29). Kraft hardwood lignin and Meadol extracts have been shown to contain minor amounts of 2,6-dimethoxybenzoquinone (I) (15). This



I

yellow quinone can form red and purple chromophores in base. It would thus appear that side chain cleavage and quinone formation occur during relatively similar processes which themselves are directly responsible for color formation.

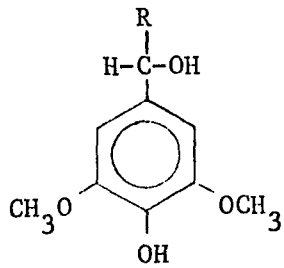
A number of lignin model compounds representative of hardwood and softwood lignins were chosen to study the effects of chemical structure and oxidation medium on these degradation reactions. These compounds are listed below:



II R=H

III R=CH_3

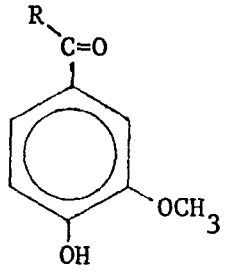
IV CH_2CH_3



V I H

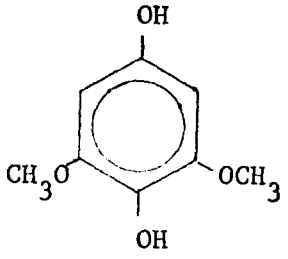
VI R=CH_3

VII R=CH_2CH_3

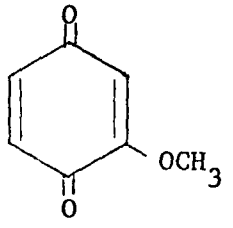


VIII R=CH_3

IX R=CH_2CH_3



X



XI

These compounds were subjected to degradation under the following conditions: 1) NaOH/oxygen, and 2) $NaOH/H_2O_2$.

Reaction of Model Compounds With NaOH and Oxygen

The results of chromatographic analysis of the oxidation products of the reaction of model compounds (room temperature 18 hrs) is shown in Table III. In order to compare these results with those from e.s.r. studies, very mild conditions were employed. At higher temperatures, one would expect more extensive degradation.

It can be readily seen from Table III that the ketones and alcohols undergo only minor side chain cleavage at room temperature. What is more striking however, is that hardwood quinone products of the reaction are significantly changed to chromophores, whereas the softwood quinone products are precipitated as polymers.

These mild conditions should represent the behavior of lignin when it is dissolved in base before it is further treated. Under these conditions, 95% or more of the starting material is recovered after

TABLE III. Reaction Products of Lignin Models with NaOH and O_2

Compound	Conditions	Product	Percent
Ketones	1M NaOH Room Temp.		
III		III	95%
		I	Trace
IV		IV	95%
		I	Trace
Alcohols	0.5M NaOH Room Temp.		
V		V	90%
		I	Trace
		Purple Comp. XII	Trace
VI		VI	95%
		I	Trace
		Purple Comp. XII	Trace
VII		VII	95%
		I, IV	Trace
		Purple Comp. XII	Trace
Quinones			
I		I	2%
		Purple Comps.	Not detn.
XI		XI	50%
		Brown ppt.	Not detn.
Hydroquinones			
X		Purple Comp. XII	25%

18 hr of standing. Analysis by the use of thin layer chromatography reveals minor amounts of quinone (I) and a purple chromophore. Apparently, some side chain cleavage has occurred. Kratzl (in 29) used more drastic conditions (70°C and oxygen) and one would expect an acceleration of this reaction.

Reaction of Model Compounds With NaOH and H_2O_2

The model compounds were heated to 45°C for 4 hr at pH 10.5 in the presence of 3% H_2O_2. Conditions were similar to those used by Bailey and Dence (22). Other sets of conditions were also employed as shown in Table IV.

The results show that extensive Dakin cleavage occurs with all of the ketones (30,31), but that the syringyl alcohols show very little

DAKIN REACTION

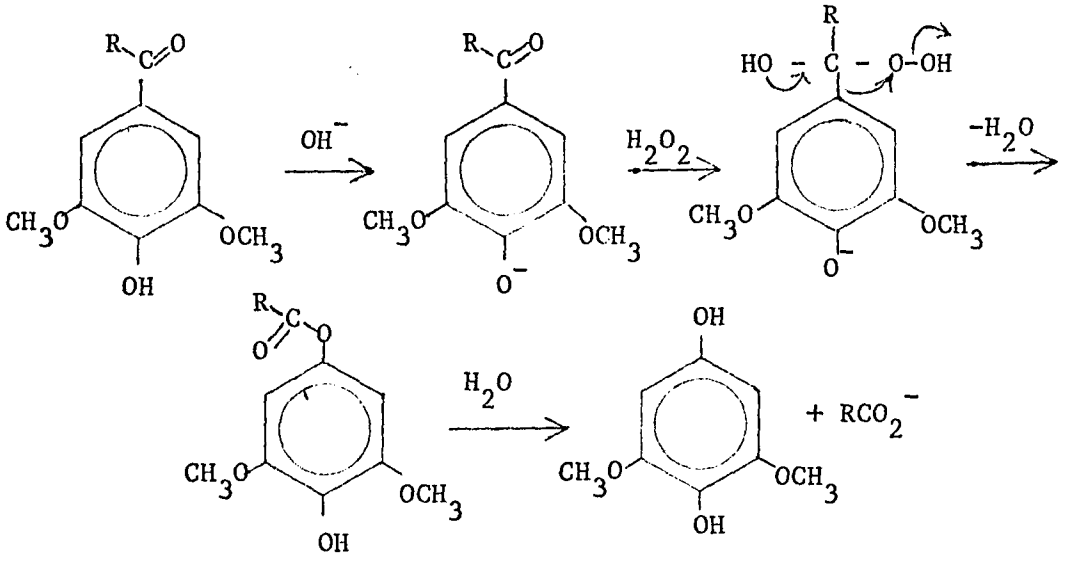


TABLE IV. Reaction Products of Model Phenols with NaOH and H_2O_2

Compound	Conditions	Products	Percent
Carbonyl Compounds			
II	A	II	42%
		I	36%
		X	12%
		Purple Comp. XII	
II	C	II	49%
		I	14%
		X	18%
		Purple Comp. XII	Trace
II	D	II	Above 90%
		I	Trace
		Purple Comp. XII	Trace
III	A	III	60%
		I	25%
		X	Trace
		Purple Comp. XII	
III	B	III	60%
		I	28%
		X	1%
		Purple Comp. XII	Trace
III	C	III	Above 90%
		I	Trace
IV	A	IV	60%
		I	25%
		Purple Comp. XII	
IV	C	IV	95%
		I	Trace
VIII	A	VIII	60%
		Polymer	

TABLE IV. Continued

Compound	Conditions	Products	Percent
Carbonyl Compounds			
VIII	B	VIII No polymer; only water soluble products	60%
IX	A	IX Water soluble products	16%
Benzyl Alcohols			
VI (VII)	A	No change	
	B	VI (VII) I (I)	Above 95% Trace
Quinones			
I	A	Purple Comp. XII	Small Amt.
XI	A	Black ppt.	
Hydroquinones			
X	A	I Purple Comp. XII	50% 30%

A. H_2O_2/Substrate (1:1), pH, 10.5, 45°C, 4hrs.

B. H_2O_2/Substrate (3:1), pH, 10.5, 45°C, 4hrs.

C. H_2O_2/Substrate (1:1), pH, 12.5, 45°C, 4 hrs.

D. H_2O_2/Substrate (1:1), pH, 2 to 9, 25°C, 18 hrs.

change. The presence of ketones in the reaction mixtures of the syringyl alcohols were not found. Thus, under the conditions of the experiments benzyl alcohol oxidation is very insignificant. A fascinating and striking difference between the reactivity shown by the syringyl (hardwood) ketones and those of the guaiacyl (softwood) ketones is that the former produce deep purple chromophores and the latter precipitate brown polymers under these bleaching conditions.

As an example of softwood model compound reaction, one could look at the degradation of propiovanillone (IX). Side chain cleavage is more than 80% complete and the products are almost entirely water soluble. The propanoyl side chain on lignin model compounds react to give anomalous results.

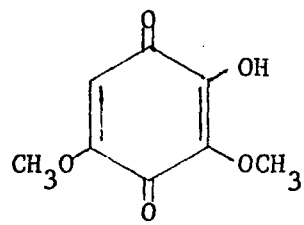
When excess H_2O_2 is used, guaiacyl compounds do not form polymers. However, excess H_2O_2 has little effect on the reactions of syringyl compounds. When the length of the side chain is increased, the amount of side chain cleavage is lessened. However, this may be a solubility effect, since increasing length parallels decreasing solubility of the ketone in the basic solution. As the reaction proceeds, the mixture becomes more acidic and some ketones are precipitated before the reaction is complete.

The yield of product is significantly affected by the acidity of the reaction mixture, but the nature of the products does not seem to be changed. Even as low as pH 2 at room temperature, the quinone (I) and the purple compound are still formed, but in minor amounts. At pH 12.5, the amount of quinone (I) is reduced relative to hydroquinone (X)

compared with the distribution of products obtained at 10.5. This suggests that the reaction conforms more closely to the products of the Dakin reaction. However, syringaldehyde (II) and acetosyringone (III), when oxidized at pH 12.5, showed little change from starting ketone. When syringaldehyde was usbjected to oxidation under classical Dakin conditions (32), the pH then was measured to be 8.4. Therefore, the assumption that under Dakin conditions, the reaction mixture is strongly basic, is incorrect.

Compounds (I), (X), and (XI) were subjected to bleaching conditions. This was done because it was suspected that the Dakin oxidation products could be precursors of the purple compound. Quinone (I) gave a low yield of the purple chromophore, hydroquinone (X) gave a high yield, and as was expected, the guaiacyl derived quinone (XI) gave a black polymer.

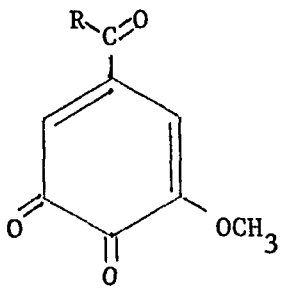
The purple compound was identical to one of the reaction products from the alkaline-peroxide oxidation of syringaldehyde. It was separated from the other products by column chromatography on silica gel and identified as 2-hydroxy-3,5-dimethoxybenzoquinone (XII). This compound has not been previously reported in the literature. The substance is an acid-base indicator with the color changing from yellow to



XII

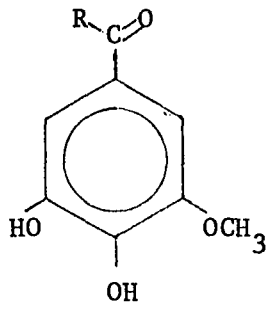
purple in the pH range 2.5-4.0. This compound is identical to the purple compound previously mentioned.

Bailey and Dence (22) have suggested that ortho-quinones are intermediates in the bleaching process. Marton (33) has proposed the presence of reduced ortho-quinone (catechol) moieties in kraft lignin. The oxidation mixtures were examined for the presence of compounds such as (XIII) and (XIV) and their reduced forms, compounds (XIIIa) and (XIVa).



XII R=H

XIV R=CH_3



XIIIa R=H

XIVa R=CH_3

The ortho-quinones are red solids which, when sprayed with 2,4-dinitrophenylhydrazine reagent, give characteristic green colors on thin layer chromatograms. These compounds were not found, although other experiments to be discussed, suggested that some demethylation does occur in the oxidation reaction.

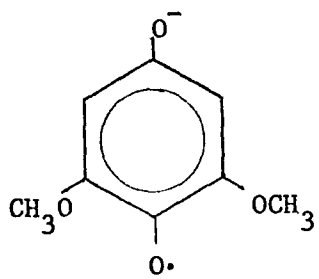
It will be shown that these ortho-quinones are indeed formed when lignin model compounds are oxidized enzymatically. Apparently, the pH of the reaction mixture greatly affects the formation of these chromophores.

For use as chromatographic standards, these ortho-quinones can be easily prepared from syringyl ketones by periodate (34,35) or ceric oxidation (36).

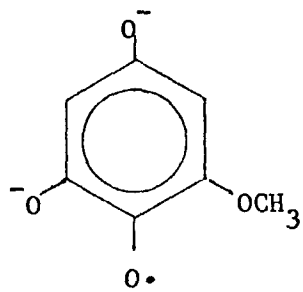
Electron Spin Resonance (E.S.R. Studies)

Reaction of 2,6-dimethoxybenzoquinone (I) in Base

Two radical anions are formed when hardwood lignins are dissolved in base (15). They are 2,6-dimethoxybenzosemiquinone (XV) and 2-methoxy-6-hydroxybenzosemiquinone (XVI).



(XV)

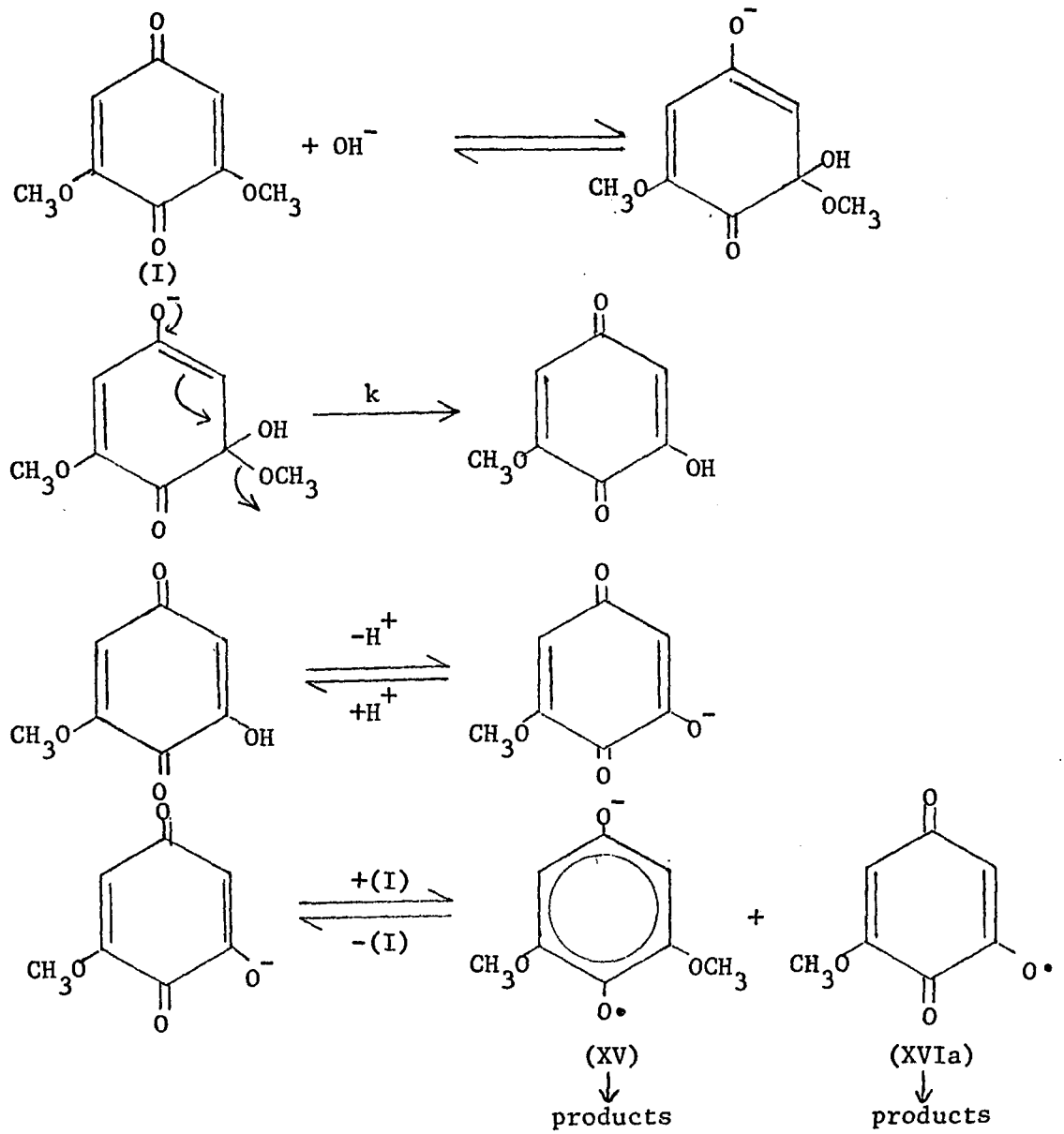


(XVI)

Native hardwood lignins give low yields of these radical anions, but kraft and Meadol lignins give concentrations of (XV) and (XVI) up to 10 mole percent.

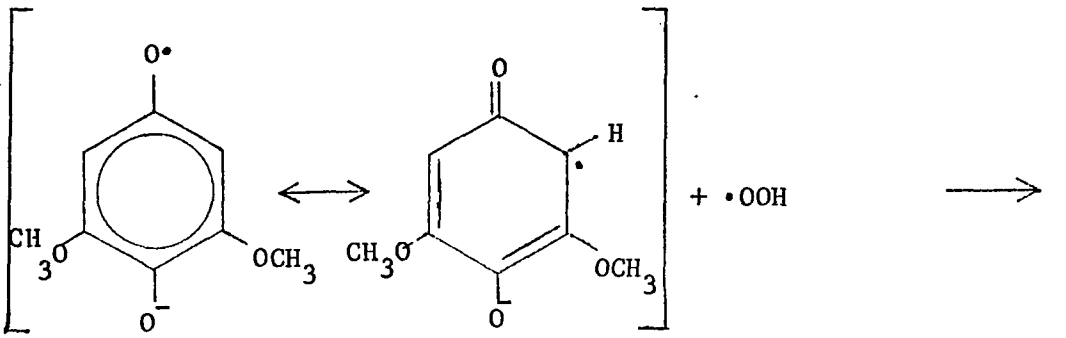
Quinone (I) is probably the source of these radical anions. Chloroform or hot water extracts of kraft and Meadol lignins all show measurable amounts of the quinone. In order to show a relationship between (I) and the radical anions (XV) and (XVI), quinone (I) was dissolved in base under nitrogen. Quinone (I) was slowly converted to radical (XV) in solution at pH 9. At higher base concentration, (XV) formed more quickly and then slowly decomposed to radical (XVI). These reactions were accompanied by the appearance of a maroon color, which remained after the radicals had disappeared.

Fitzpatrick and Steelink (15) have proposed the following mechanism:

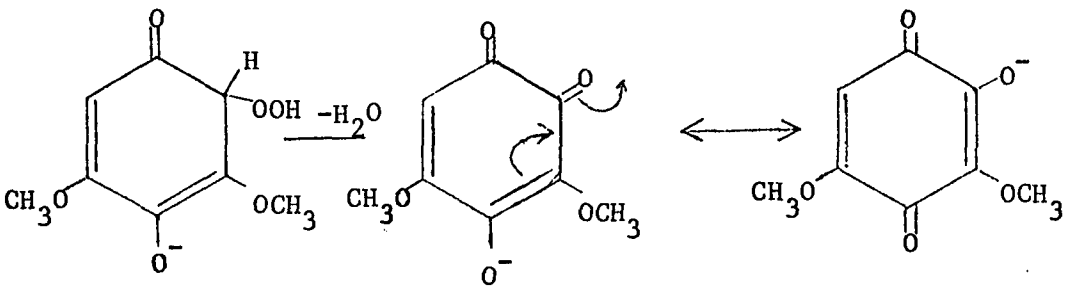


Therefore, an autoreduction reaction produces radical anions when quinone dissolves in aqueous base. Upon exposure to air, radical anion (XV) is quickly scavenged and a maroon color appears.

A possible means for the formation of quinone (XII) from radical (XV) is by direct oxidation with oxygen (or its equivalent) or H_2O_2 by means of a peroxide radical.



(XV)



(XII) Anion

The slow disappearance of radical (XV) can be correlated with the appearance of the maroon chromophore, and appears to support this mechanism. Another deeply colored, but very unstable, highly oxygenated quinone could be formed from the oxidation of radical (XVIa).

Therefore, even when lignin solutions are bleached, deeply colored chromophores could form if quinone (I) is present.

Mechanism of Side Chain Cleavage

The results of model compound oxidation showed that oxygenated alkaline solutions as well as aqueous solutions of H_2O_2 are both capable of producing quinones and hydroquinones from para-substituted phenols, as well as demethylating these quinones. Chromophores can be formed by the quinones and hydroquinones reacting further via a free radical process.

The nature of the side chain has a profound effect on the course of the peroxide reaction. Side chain cleavage is extensive with syringaldehyde (II), but decreases significantly in the cases of acetosyringone (III) and propiosyringone (IV). All the carbonyl compounds give quinones (I) and (XII), and very little water-soluble material. On the other hand, the softwood model, propioguaiacone (IX) undergoes almost total cleavage to yield brown, water-soluble substances. Therefore, one should expect bleaching conditions to give bright chromophores with hardwood species, and brown materials from softwood species.

However, extreme caution must be used in deducing any conclusions about the chemistry of lignin from the reactions of very simple monomeric compounds. A minor variation such as length of side chain can cause widely differing reactivities. Misleading conclusions could be easily drawn about the behavior of the lignin macromolecule from the investigation of only a single model compound.

Benzyl alcohols are structurally more respresentative in lignin than α-carbonyl compounds. The reactions of benzyl alcohols (V), (VI), and (VII) are more consistent than the carbonyl compounds. None of these compounds underwent more than a 5% decomposition under the

conditions of the peroxide reaction. This seeming lack of reactivity appears to bear out the observations of Dence (in 22), who treated apocynol (α-methylvanillyl alchol) with H_2O_2 and base under identical conditions and found only minor degradation of the starting material.

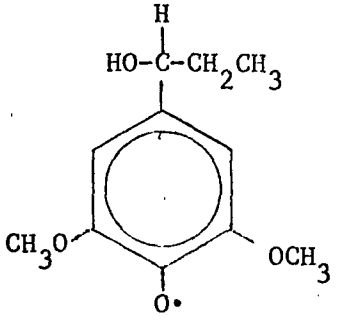
pH has a profound effect on the peroxide reaction of the carbonyl compounds. Degradation to the quinones I and XII is observed at all pH values between 2 and 12 in the hardwood species, although not appreciable in the acid range. The most efficient Dakin degradation conditions appear to be in the range 8.5-9.0. Above or below this range, the reaction is suppressed. Excess peroxide does render the softwood quinoid species soluble, but causes little change in the hardwood compounds.

A question arises as to whether or not a free radical process also operates in the converstion of lignin model phenols into quinones. In order to answer this question, e.s.r. spectrometry was used to investigate the process of side chain cleavage. Room temperature conditions were employed, with the hope that the rates of reaction would be slow enough to measure in a static system.

E.S.R. Studies of Model Phenols in Base and Air

When carbonyl compounds (II), (III), and (IV) were dissolved in aqueous base, differing radical species were slowly generated. Depending upon the strength of the alkaline solution, syringaldehyde (II) gave radicals (XV) and (XVI). This reaction clearly shows a relatively easy removal of the one-carbon side chain. It appears that initially, the side chain remains intact, but that ring demethylation has occurred.

The benzyl alcohol derivatives (VI) and (VII) also rapidly give radicals (XV) and (XVI) under the same conditions, showing side chain cleavage and demethylation. As the concentration of base is lowered, radical (XV) predominates and the fleeting existence of the phenoxyl radical (XVII) was observed in a single case, where α-ethylsyringyl alcohol was oxidized.



(XVII)

The results of these oxidation experiments are summarized in Table (V). Sample e.s.r. spectra are shown in Figures (3-17).

Kratzl (29) and Kleinert (37) have hypothesized that delignification is a free radical process. Our results confirm their hypotheses. Under very mild conditions, the model phenols undergo carbon-carbon and carbon-oxygen bond cleavage reactions. The demethylation reactions occur rapidly at high pH and are base dependent. Side chain removal [a Dakin type process (29)] occurs readily with the benzyl alcohol derivatives. The radical anions are more predominant and long-lived at higher pH. A 4.72×10^{-2} mole/liter solution of α-ethylsyringyl alcohol (VII) in 1.0 M NaOH gave a radical concentration of 1.24

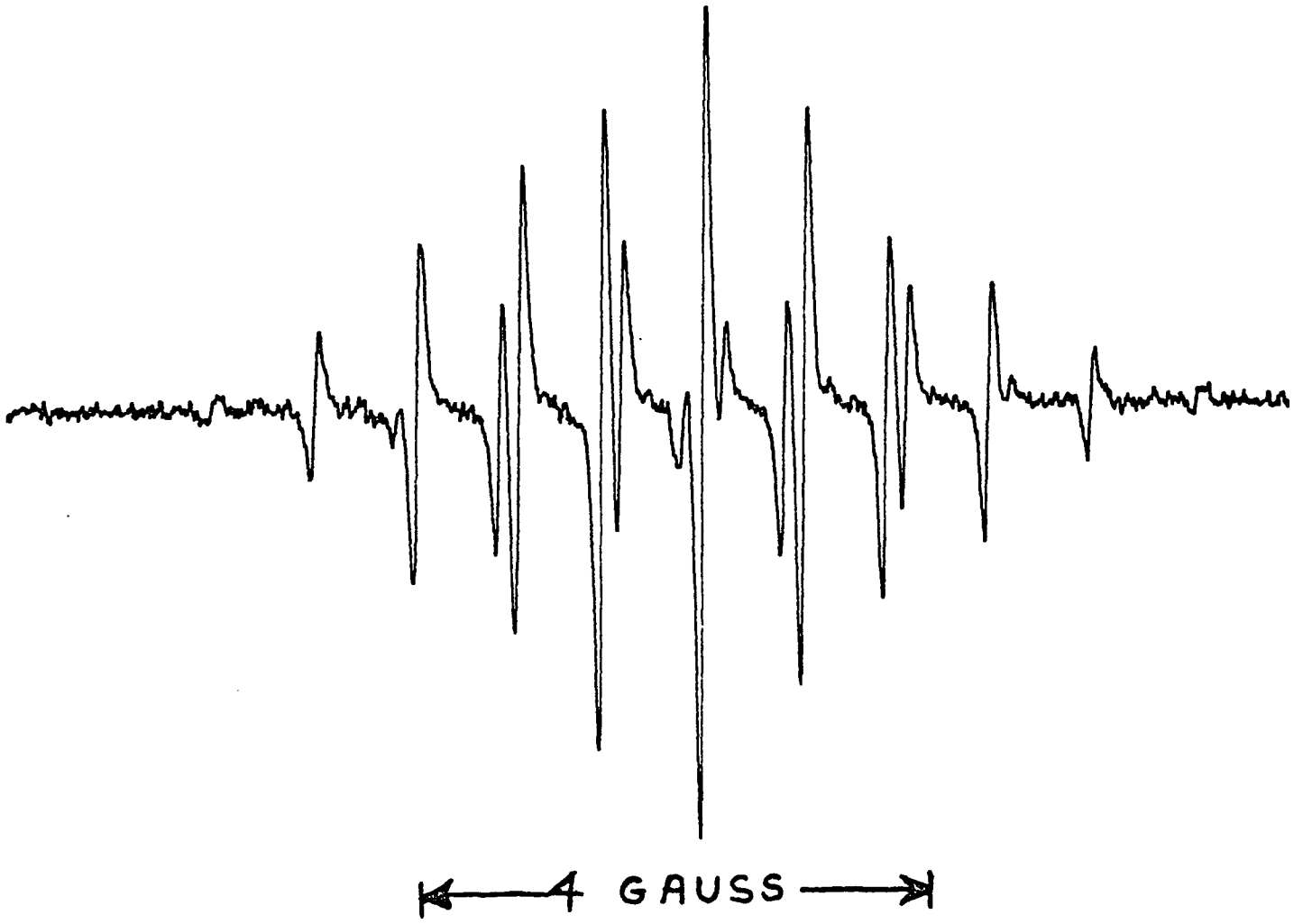


Figure 3. E.S.R. Spectrum of 2,6-Dimethoxy-p-benzosemiquinone.

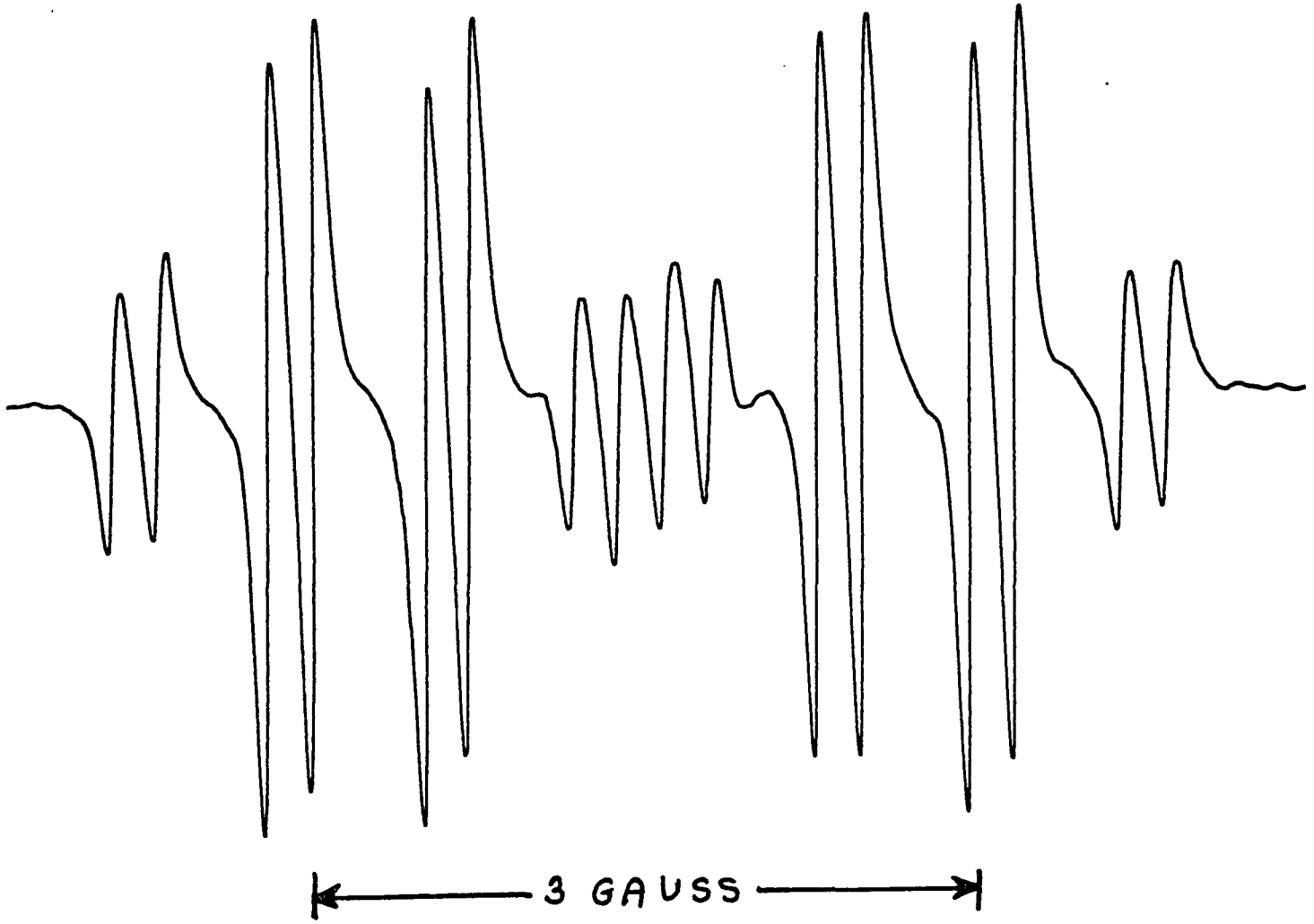


Figure 4. E.S.R. Spectrum of 2-Hydroxy-6-methoxy-p-benzosemiquinone.

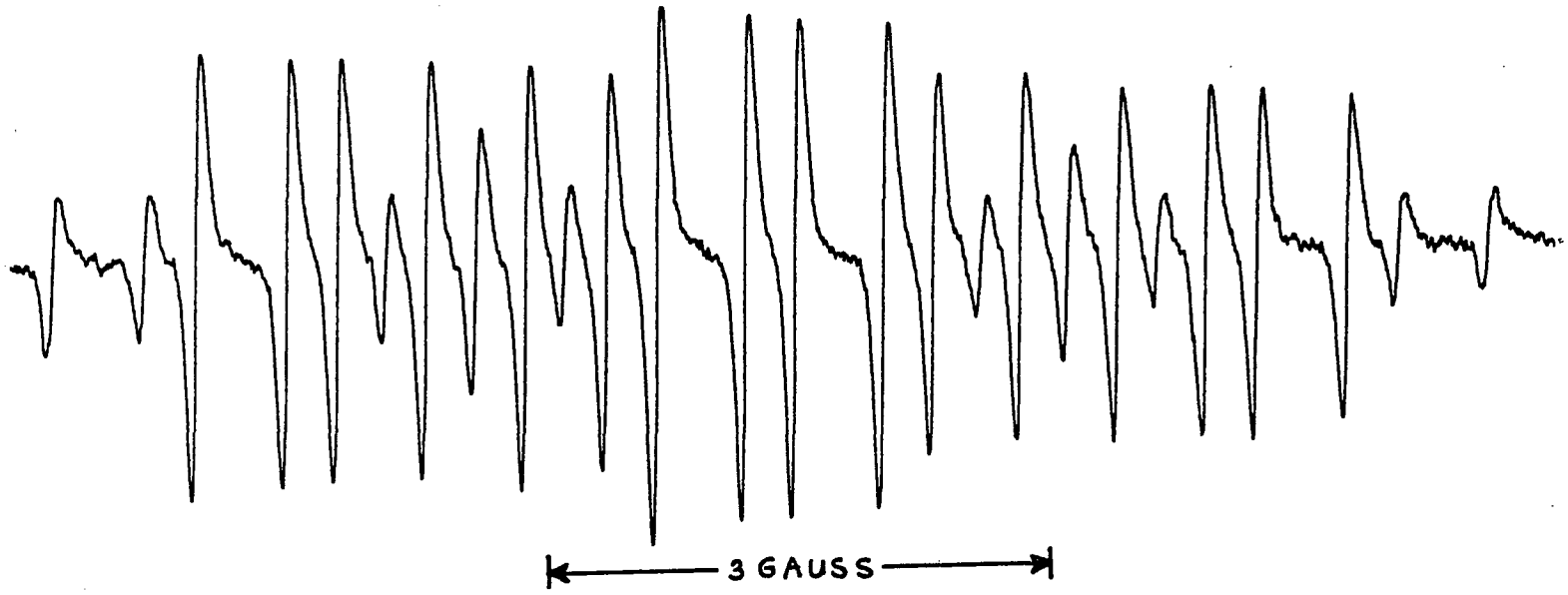


Figure 5. E.S.R. Spectrum of 2-Methoxy-p-benzosemiquinone.

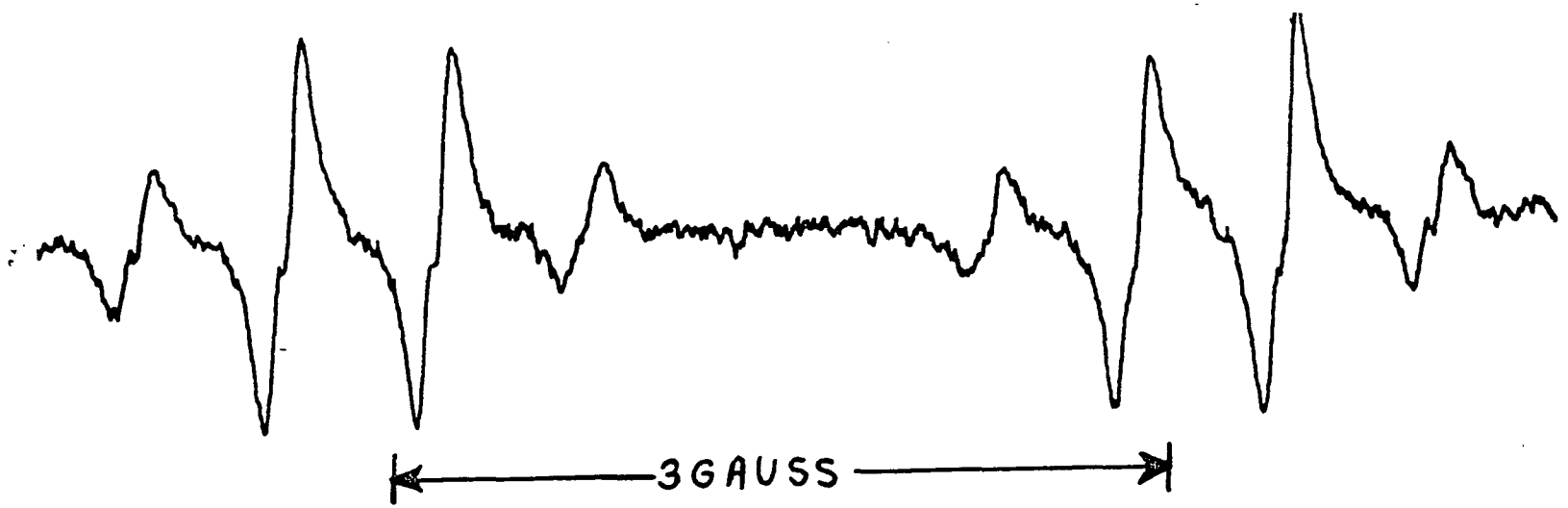


Figure 6. E.S.R. Spectrum of 5-Formyl-4-hydroxy-3-methoxy-o-benzosemiquinone.

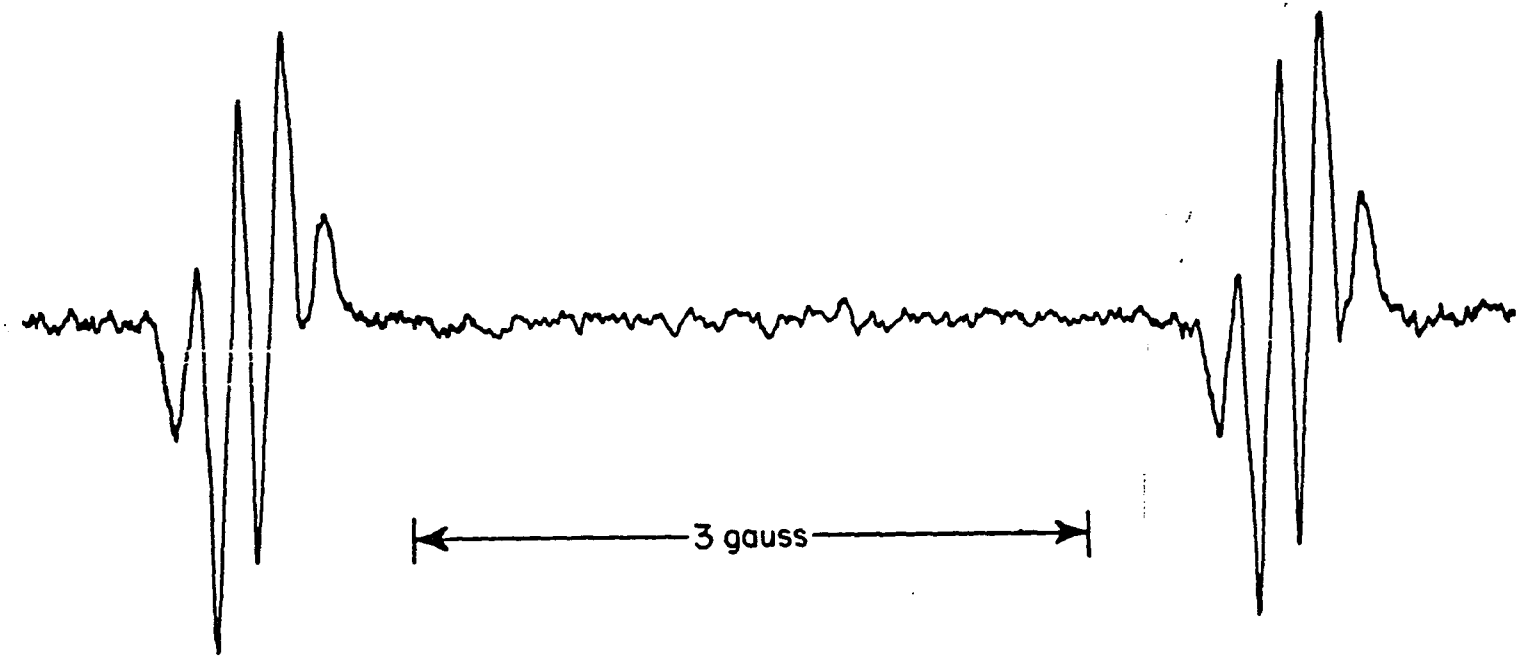


Figure 7. E.S.R. Spectrum of 5-Acetyl-4-hydroxy-3-methoxy-o-benzosemiquinone.

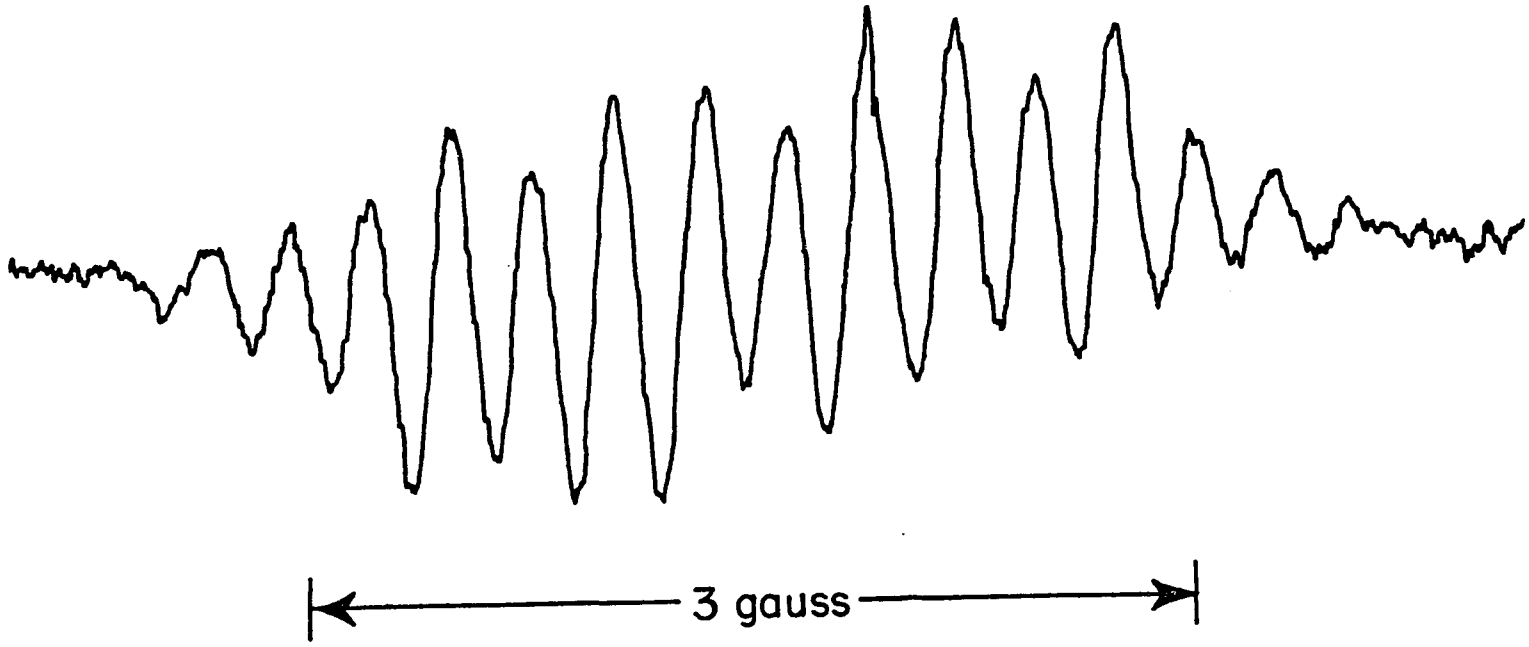


Figure 8. E.S.R. Spectrum of 5-Propanoyl-3-methoxy-o-benzosemiquinone.

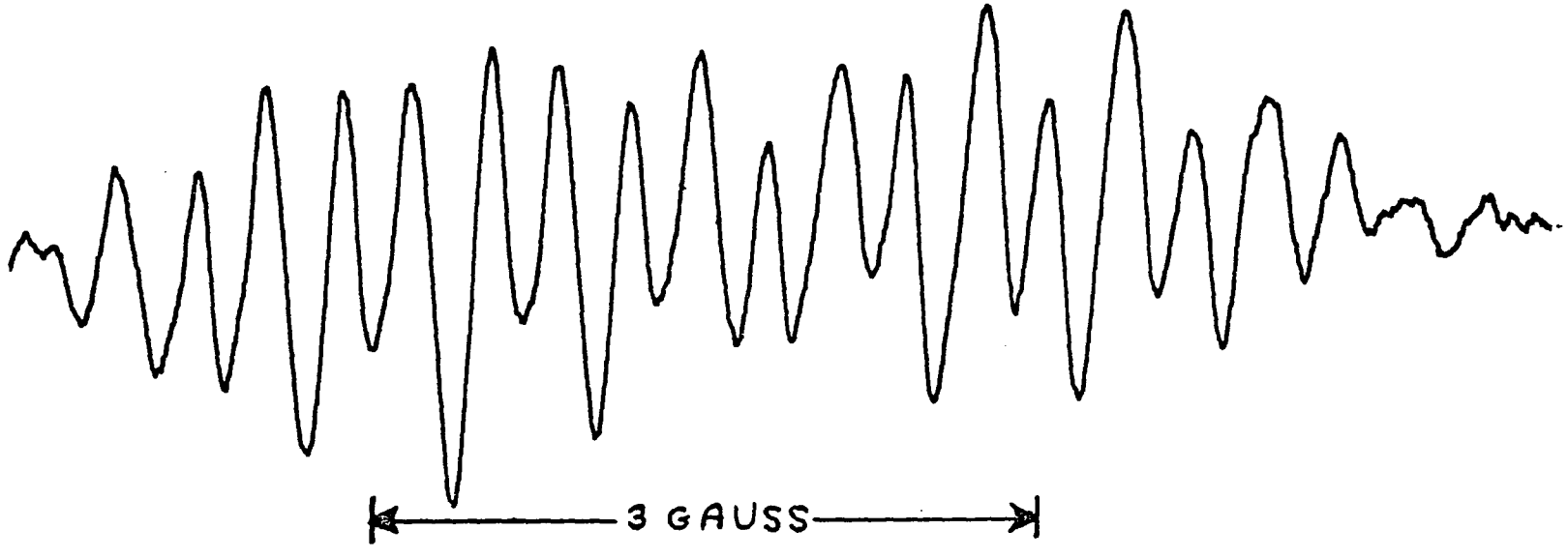


Figure 9. E.S.R. Spectrum of 2,6-Dimethoxy-4-(1-hydroxypropyl)-phenoxyl.

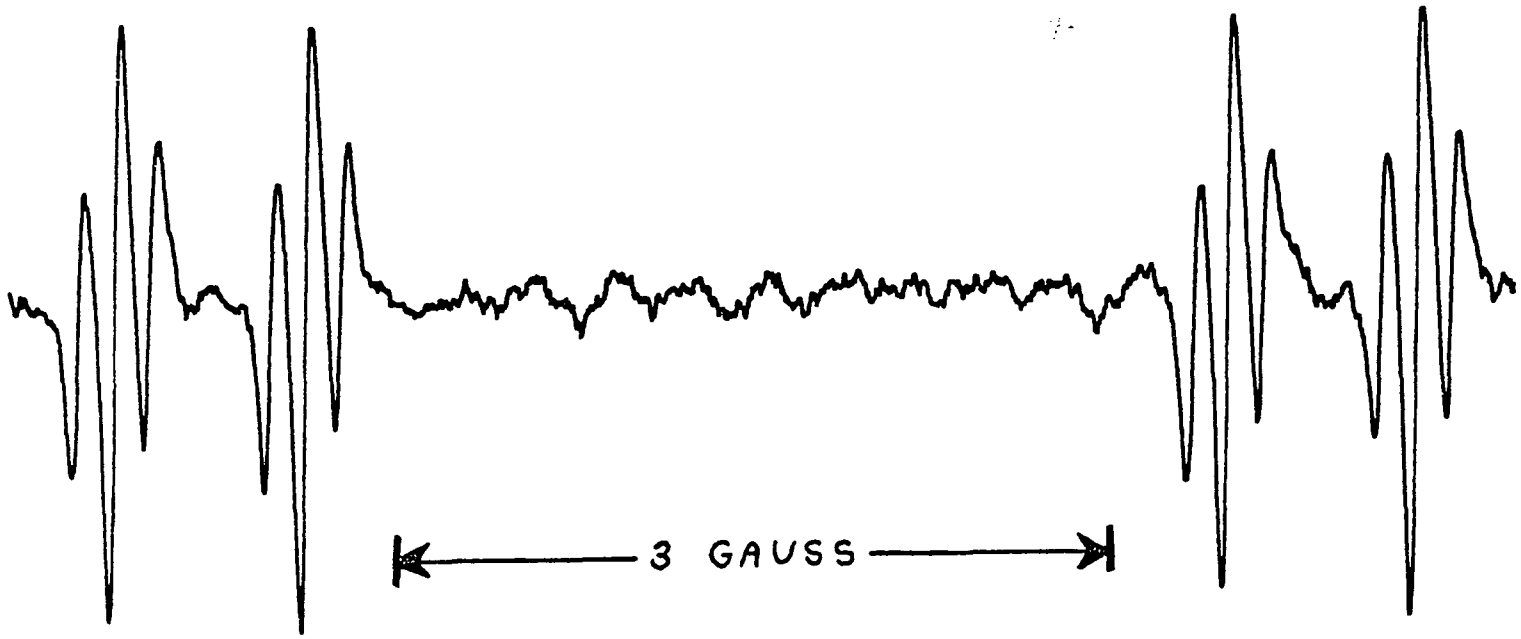


Figure 10. E.S.R. Spectrum of 3-Methoxy-4-hydroxy-5-(1-hydroxypropyl)-o-benzosemiquinone.

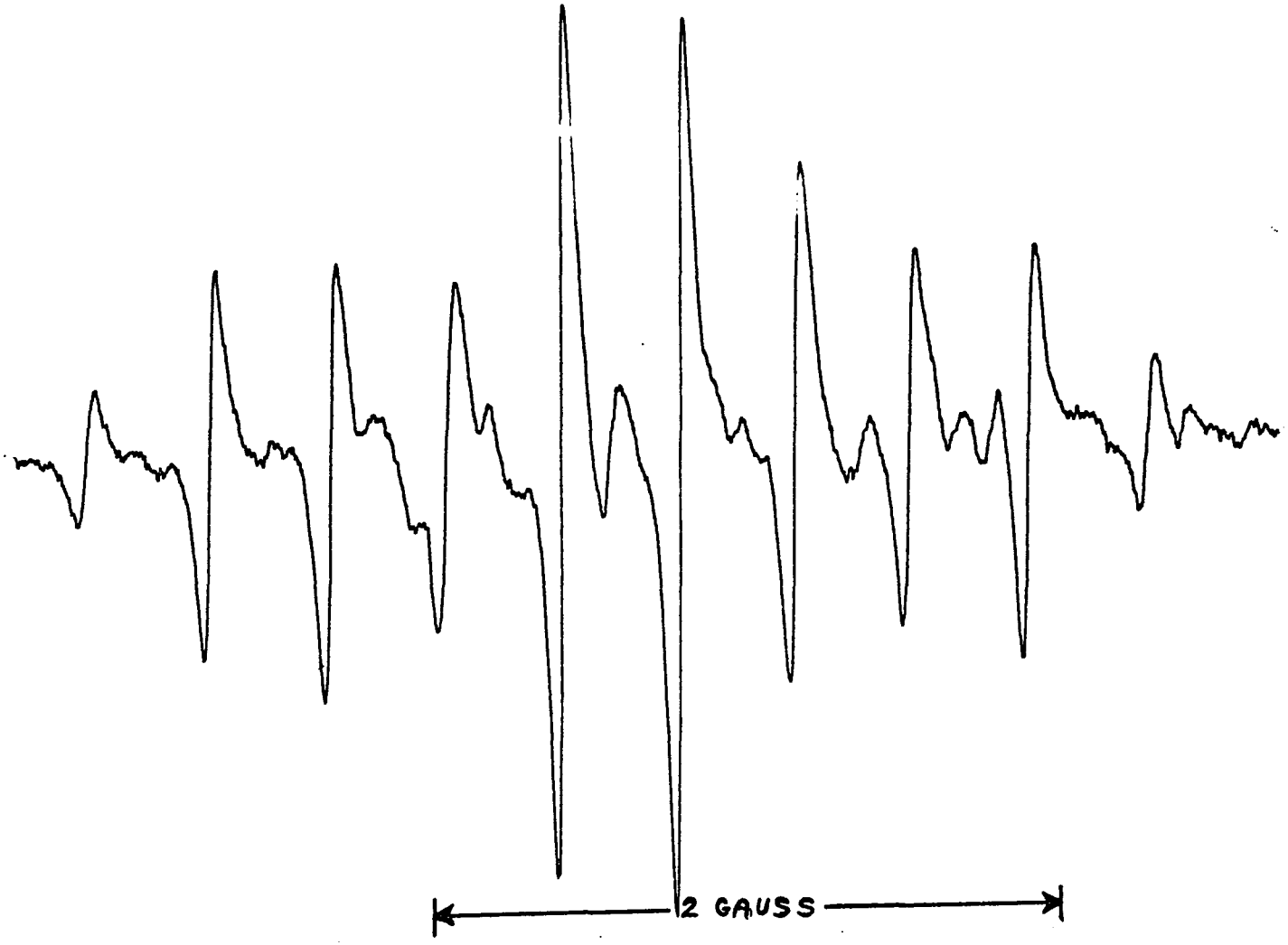


Figure 11. E.S.R. Spectrum of Methyl Gallate Anion Radical.

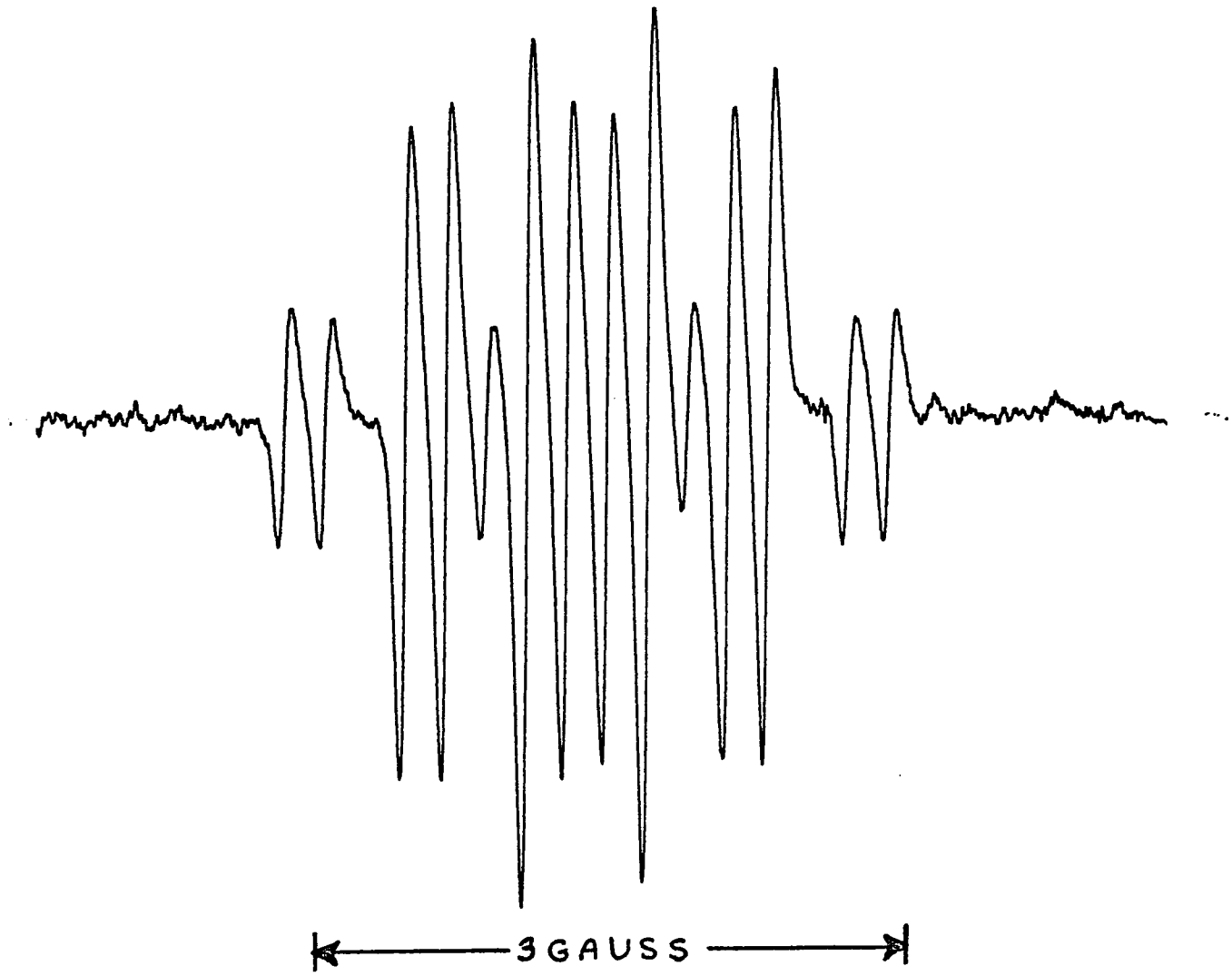


Figure 12. E.S.R. Spectrum of 3-O-Methylagallate Anion Radical.

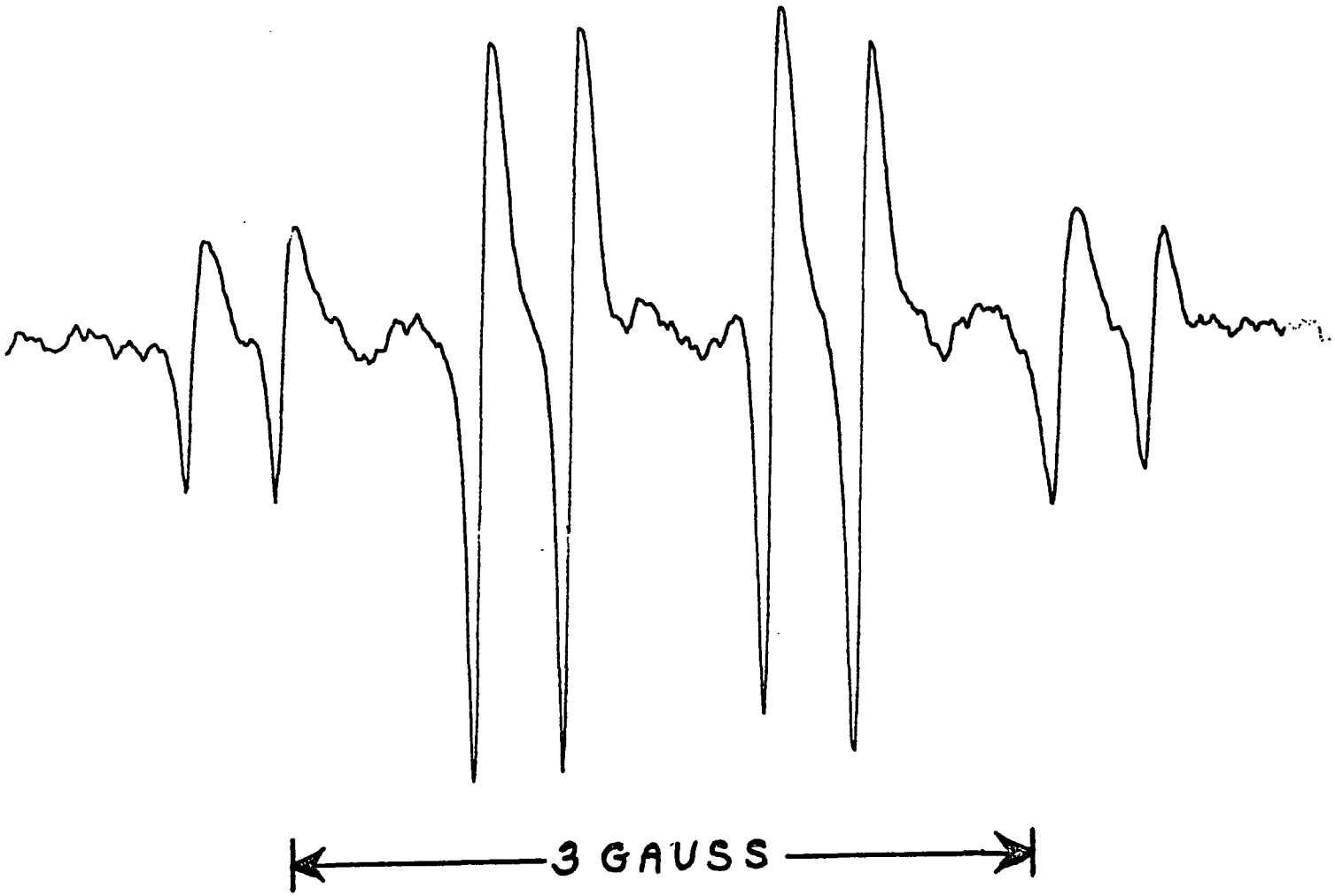


Figure 13. E.S.R. Spectrum of 2-Methoxy-3,6-dihydroxy-p-benzosemiquinone.

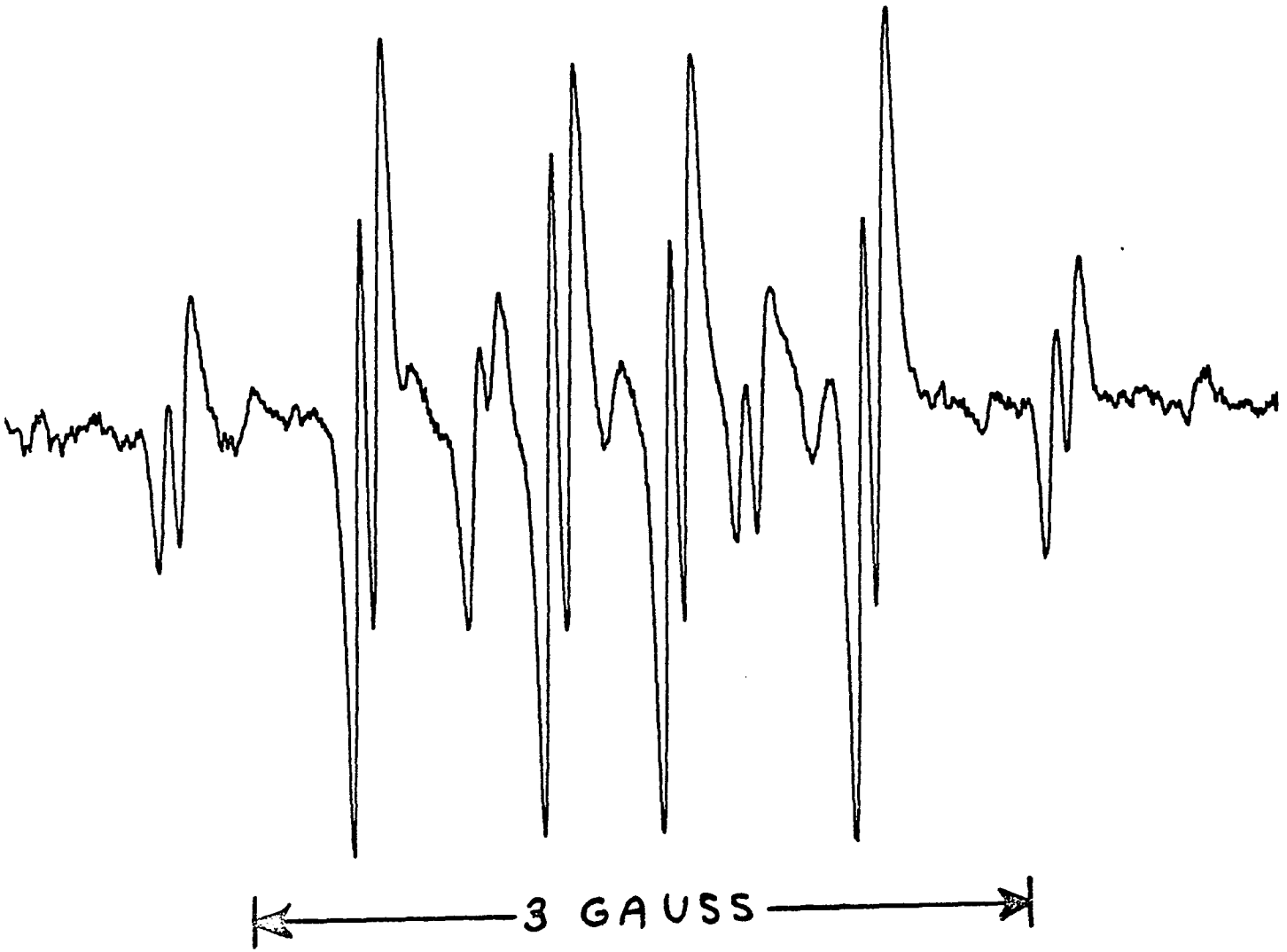


Figure 14. E.S.R. Spectrum of 2,5,5-Trihydroxy-1-methoxycyclohexadiene-6-one-3-oxyl.

Figure 15. E.S.R. Spectrum of 2-Chloro-6-methoxy-p-benzosemiquinone.

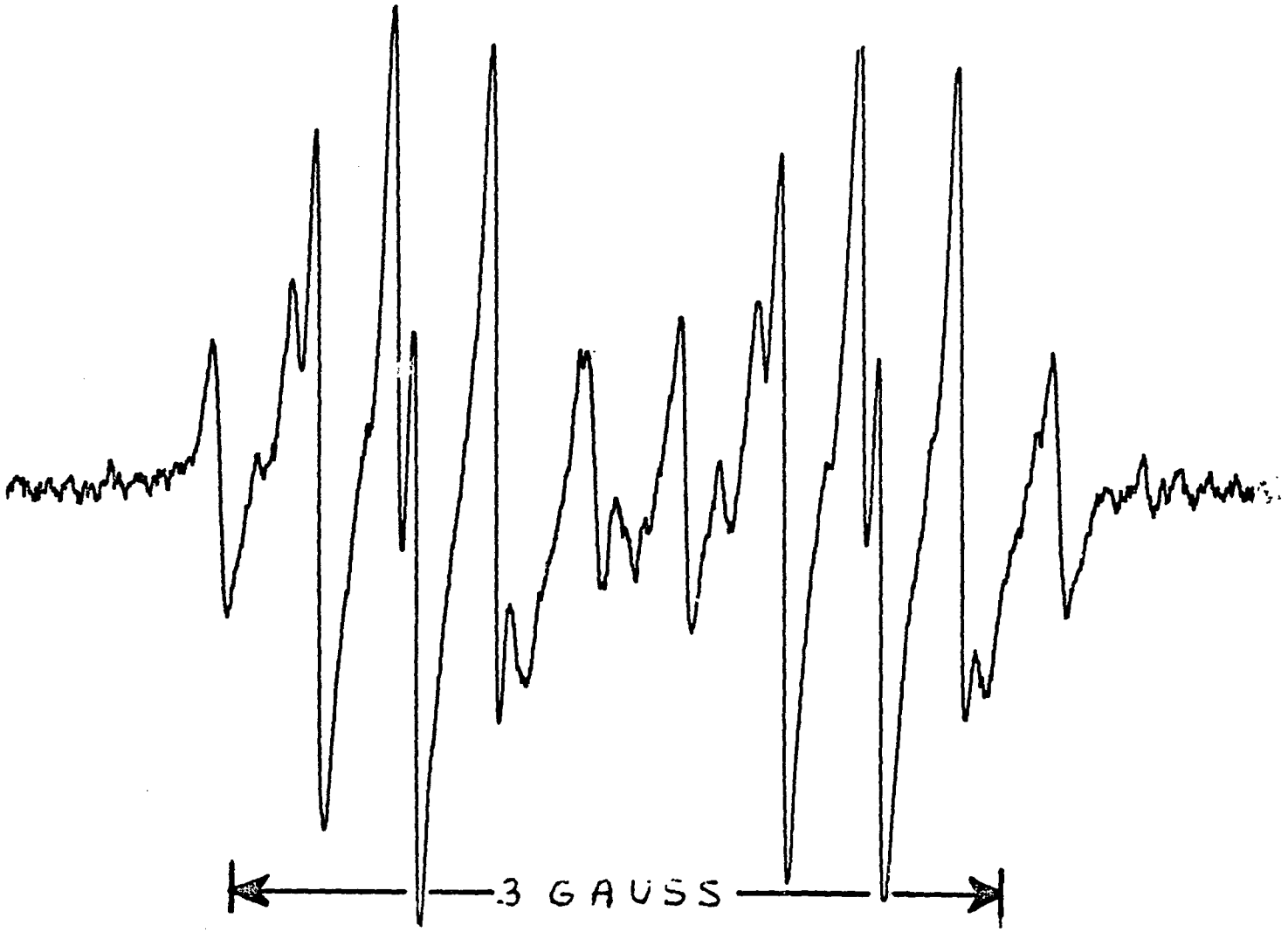


Figure 16. E.S.R. Spectrum of 2-Bromo-6-methoxy-p-benzosemiquinone.

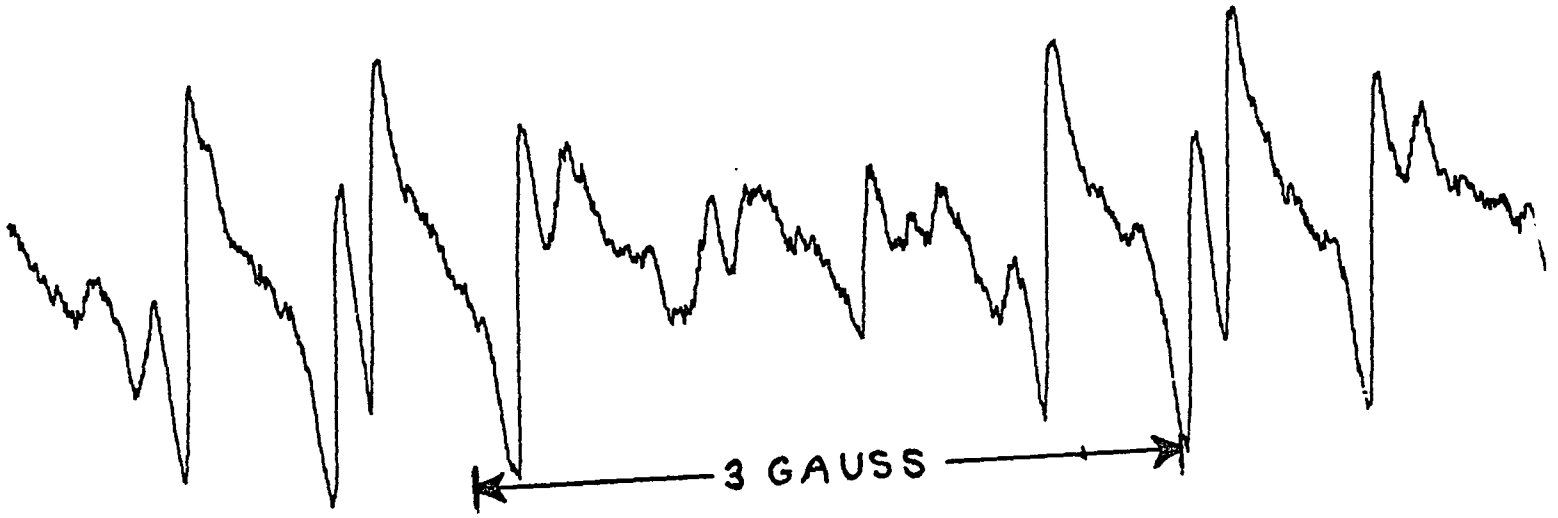
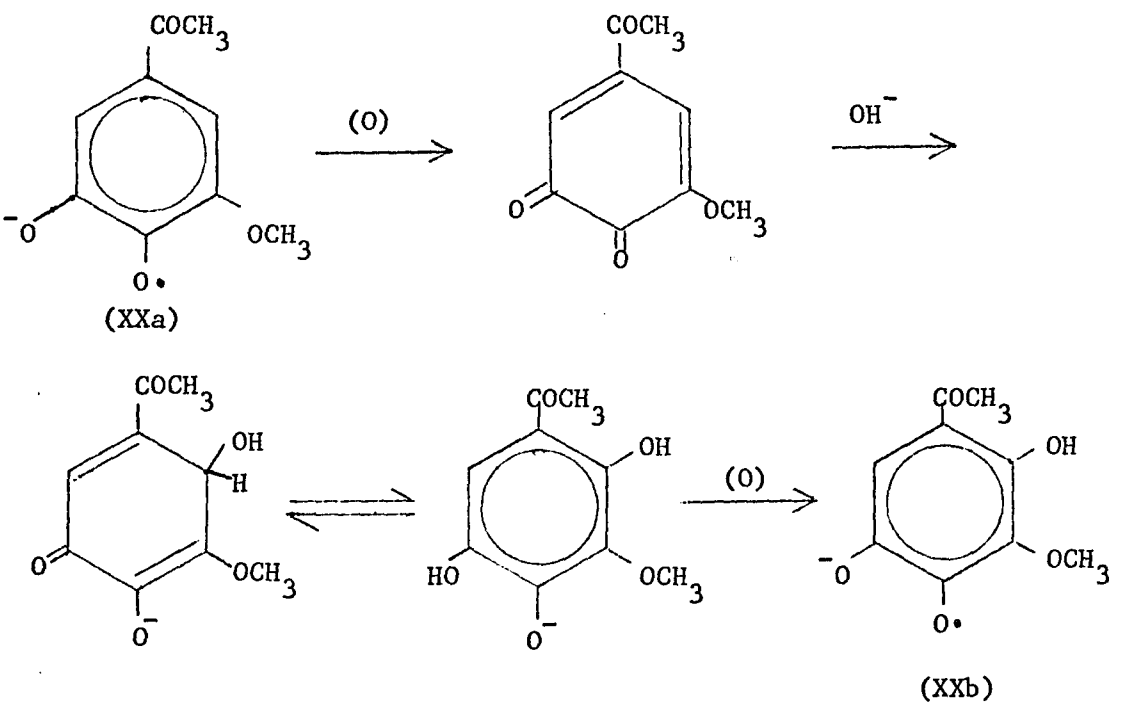


Figure 17. E.S.R. Spectrum of 2-Iodo-6-methoxy-p-benzosemiquinone.

mole-percent as compared to a 5.1×10^{-2} mole/liter solution of acetosyringone (III) in a pH, 10 buffer which gave a mole-percent value of 4.82×10^{-2}.

In the one case where the transitory phenoxy radical (XVII) was detected, it seemed to decompose by two routes: (a) carbon-carbon bond cleavage leading to a hydroquinone anion and radical (XVI) and (b) carbon-oxygen bond cleavage leading to a catechol anion radical. With (b), the side chain remains intact, and further hydroxylation can occur. This possibility has been proposed by Kratzl (in 29) to account for his ortho-benzoquinone and para-benzoquinones products.

The catechol anion radical (XXa) can be converted to anion radical (XXb) by attack of hydroxide ion. This is seen in the e.s.r.



spectra by the collapse of the complex spectrum to a simple doublet of quartets. This means that one ring proton has disappeared. Stone and Waters (38) have observed similar behavior with related systems.

When Kleinert (37) heated wood samples and lignin in alkali, he observed a steady increase in free radicals. He deduced from this observations that homolytic carbon-carbon bond breaking had occurred and that a primary depolymerization process had taken place. It would, therefore, appear on the basis of our results that Kleinert detected stable semiquinone radicals such as (XV) and (XVI) and not transitory radicals such as (XVII). This would be the first step in carbon-carbon bond breaking of lignin phenols. As was previously shown, semiquinone formation is the result of a secondary process.

From these experiments, it can be readily seen that demethylation plays an important role in the initial reaction. Furthermore, a radical mechanism is responsible for the initial bond breaking of the side chain. The presence of a radical such as (XVII) indicates that the first step is the removal of one electron to form a phenoxy radical. Attack at the ortho- or para- position to the phenol group can occur by either the oxygen diradical or a hydroperoxyl radical. This then leads to catechol or hydroquinone species, which are further oxidized to radicals (XV), (XVI), (XX), (XXI), and (XXII).

The demethylation reaction predominates in 1.0 M NaOH and the sensitivity of this reaction to a change in pH is demonstrated by the reactions of the gallic acid model compounds (XXIII), (XXIV), and (XXV), (Table V).

TABLE V. Radical Anion Products of 4-substituted Phenols in Base

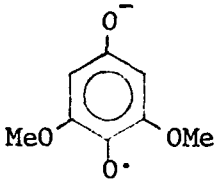
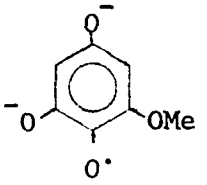
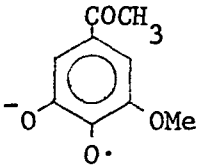
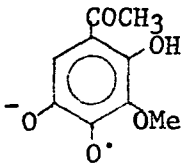
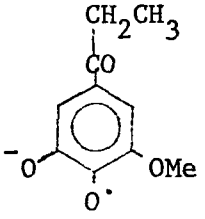
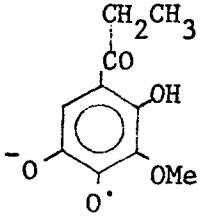
Compound	Radical Products			
	0.1 M NaOH		1.0 M Base	
Carbonyl Compounds		Comments		Comments
II	 XV	Stable	 XVI	Stable
III	No signal		 XXa  XXb	Appears very slowly. Weak signal.
IV			 XXI  XXII	Appears slowly. Moderate intensity.

TABLE V. Continued

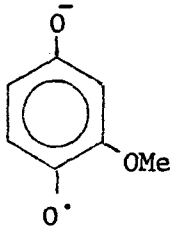
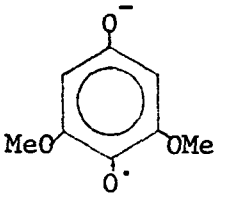
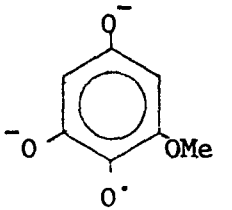
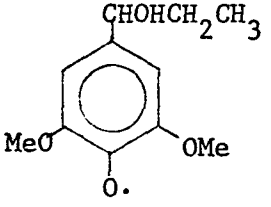
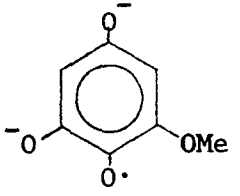
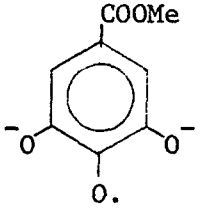
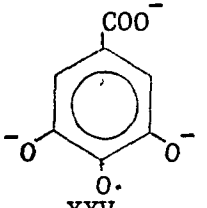
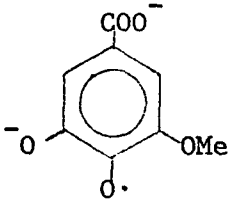
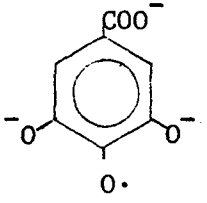
Compound	Radical Products			
	0.1 M NaOH		1.0 M Base	
Carbonyl Compounds		Comments		Comments
IX		Weak unresolved signal	Short lived	
Benzyl Alcohols				
VI	 XV	Weak signal. Fades rapidly	 XVI	Stable

TABLE V. Continued

Compound	Radical Products			
	0.1 M NaOH		1.0 M Base	
		Comments		Comments
VII	 XVII		 XVI	Stable
Acids				
XVIII[a]	 XXIII	Strong signals	 XXV	Strong signals
XIX[b]	 XXIV	Strong signals	 XXV	Strong signals

a. 3-O-methylgallic acid methyl ester
b. 3-O-methylgallic acid

It is also interesting to note that the benzyl alcohol derivatives (VI) and (VII) appear to be more susceptible to side chain cleavage than the corresponding carbonyl compounds (III) and (IV). The former both give high concentrations of radical (XVI).

E.S.R. Studies of Model Phenols With Base and H_2O_2

The reaction of carbonyl compounds with NaOH and H_2O_2 led exclusively to the anion radical (XV), and a large amount of the purple quinone (XII) (See Table VI). The lifetime of the radical, as well as its concentration, was very much shorter than in aqueous base alone. This was due to the conversion of radical (XV) into compound (XII) by peroxide. In an experiment where the reaction was carried out at pH, 13, no radical signal appeared for 6 hr. This is consistent with the data in Table IV, where the negligible production of quinone (I) with H_2O_2 at high pH values is shown.

This suggests that initial attack of H_2O_2 on the α-carbon of the carbonyl compounds (II), (III), and (IV) is a rapid, non-radical conversion to quinone (I) by the accepted Dakin mechanism (30,31,32). No demethylation occurs at pH values below 11. The quinone (I) can then form radical anions and can be further oxidized to the purple quinone (XII) by the reaction with peroxide.

Syringyl alcohol derivatives seem to suffer the same fate as the carbonyl compounds, except that the radical anion concentration is much less. Hyperfine coupling constants of the radicals are found in Table VII.

TABLE VI. Radical Anion Products of 4-Substituted Phenols in Base and H_2O_2*

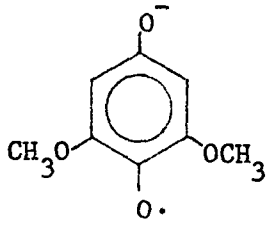
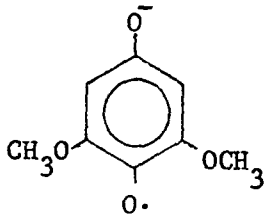
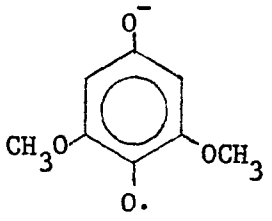
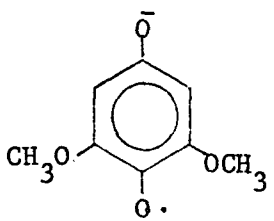
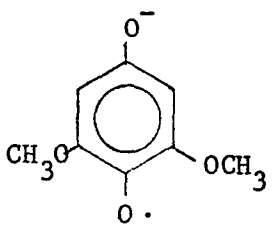
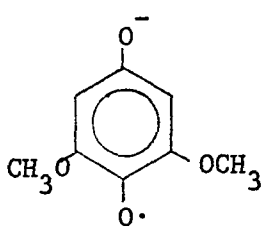
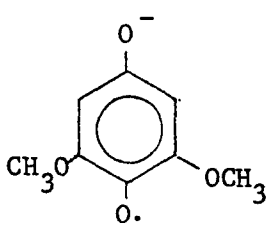
Compound	Radical Products			
	pH 9		pH 10.5	
Carbonyls		Comments		Comments
II		Disappears rapidly. Purple compound appears.		
III				Signal disappears rapidly. Purple compound appears.
IV				Signal disappears rapidly. IV precipitates and purple compound appears.

TABLE VI. Continued

Compound	Radical Products			
	pH 9		pH 10.5	
		Comments		Comments
Alcohols				
VI		Immediate signal, disappears very rapidly. Purple cpd. appears.		Small signal disappears very rapidly. No purple cpd.
VII		Immediate signal, disappears very rapidly. Purple cpd. appears.		Small signal disappears very rapidly. No purple cpd.

*Substrate: H_2O_2 (1:1)

TABLE VII. Hyperfine Coupling Constants (in gauss) of Radicals in NaOH

Radical	Solvent	Coupling Constants[a]		
		$A^H_{OCH_3}$	A^H_{ring}	$A^H_{side\ chain}$
2,6-dimethoxy-p-benzosemiquinone (XV)	water	0.80(6)	1.49(2)	
2-hydroxy-6-methoxy-p-benzosemiquinone (XVI)	water	0.70(3)	2.50(1); 0.20(1)	
2-methoxy-p-benzosemiquinone	water	0.85(3)	3.65(1); 1.95(1); 0.54(1)	
5-formyl-4-hydroxy-3-methoxy-o-benzosemiquinone (from 5-hydroxyvanillin)	DMF/water[b]	0.60(3)	3.5(1)	
5-acetyl-4-hydroxy-3-methoxy-o-benzosemiquinone (XXb)	water		4.65(1)	0.20(3)
5-propanoyl-3-methoxy-o-benzosemiquinone (XXI)	DMF/water[b]	0.55(3)	1.75(1)	0.28(2)
5-propanoyl-4-hydroxy-3-methoxy-o-benzosemiquinone (XXII)	water		4.5(1)	0.15(?)
2,6-dimethoxy-4-(1-hydroxypropyl)-phenoxyl (XVII)	water	0.30(6)	0.30(2)	3.35(1)
3-methoxy-4-hydroxy-5-(1-hydroxypropyl)-o-benzosemiquinone (XVIIa)	water		0.8(1)	4.65(1); 0.15(2)
Methyl gallate anion radical (XXIII)	water		1.11(2)	0.38(3)

TABLE VII. Continued

Radical	Solvent	Coupling Constants[a]		
		$A^H_{OCH_3}$	A^H_{ring}	$A^H_{side\ chain}$
3-O-methylgallate anion radical (XXIV)	water	0.60(3)	1.0(1); 0.20(1)	
Gallic acid anion radical (XXV)	water		1.30(2)	

a. Numbers in parentheses indicate number of protons.

b. DMF/water: 90% DMF/10% water. Coupling constants may be 20-30% changed from those in pure water.

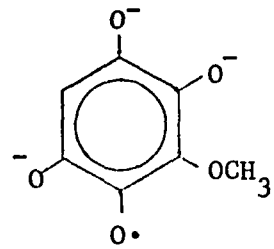
With the aid of e.s.r. spectrometry, one can gain an insight into the reaction mechanism. The initial reaction appears to be an immediate Dakin cleavage to the hydroquinone. No intermediate radical species are detected and one must assume that this is a two-electron step. The second step is the one-electron oxidation of the hydroquinone to the semiquinone (XV), which has a steady state concentration of 0.1% of the original carbonyl compound. In the third step (XV) is converted to the purple quinone (XII) as well as quinone (I). Comparing the fate of radical (XV) with its fate under conditions without peroxide, one can see two striking differences. In the presence of peroxide, the radical is very short-lived and in very low concentration compared to its stable high concentration in NaOH without peroxide. This suggests a rapid scavenging of the radical by peroxide.

Another important feature is that using the rather mild bleaching conditions of the e.s.r. experiments (room temperature, pH, 9-11, substrate: peroxide ratio 1:1), no demethylation was observed during the initial phases of the reaction.

E.S.R. Studies of 2-Hydroxy-3,5-dimethoxybenzoquinone in Base

In order to further characterize the properties of the purple chromophore, quinone (XII) was dissolved in base to observe possible radical formation. This indeed was found to be the case. When dissolved in 0.1 M NaOH, the color of the solution turned cranberry red and a radical anion was detected. The spectrum (Figure 13) which was very narrow, showed a quartet split into doublets. Apparently, the quinone

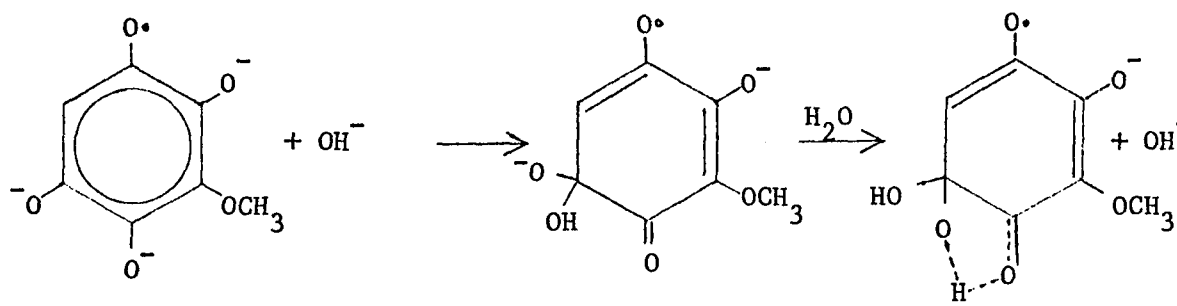
has been demethylated to give:



(XXVI)

On standing, the e.s.r. spectrum changed slowly to give an e.s.r. spectrum of another radical anion. The new spectrum (Figure 14) consisted of two overlapping quartets which were then split into doublets. It is apparent that a second proton has been picked up by the first radical anion. This secondary radical anion appears immediately in stronger base (0.1 M NaOH). Both these radical anions appear when the quinone is dissolved in 0.1 M KOH or in saturated $Ca(OH)_2$ in air or under nitrogen. In the weaker base, calcium hydroxide, the primary radical has a much longer lifetime.

It seems plausible that in strong base, the primary radical has been attacked by hydroxide ion.

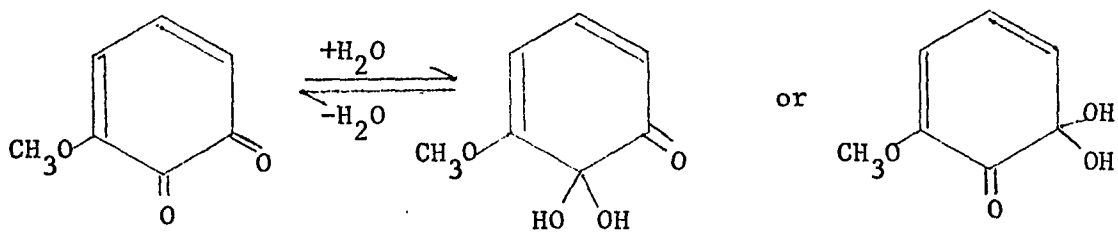


(XXVII)

One of the alcohol protons could then be stabilized by hydrogen-bonding with the carbonyl oxygen. This proton could then account for the small splitting as seen in the e.s.r. spectrum of the secondary radical.

In a similar case, Adler, Magnusson, Berggren, and Thomelius (39) theorized that a change in an ultraviolet spectrum they were observing for 3-methoxy-o-benzoquinone (XXVIII) was due to a reversible addition of a water molecule to one of the carbonyl groups.

Hyperfine coupling constants of radicals (XXVI) and (XXVII) are shown in Table VIII.



(XXVIII)

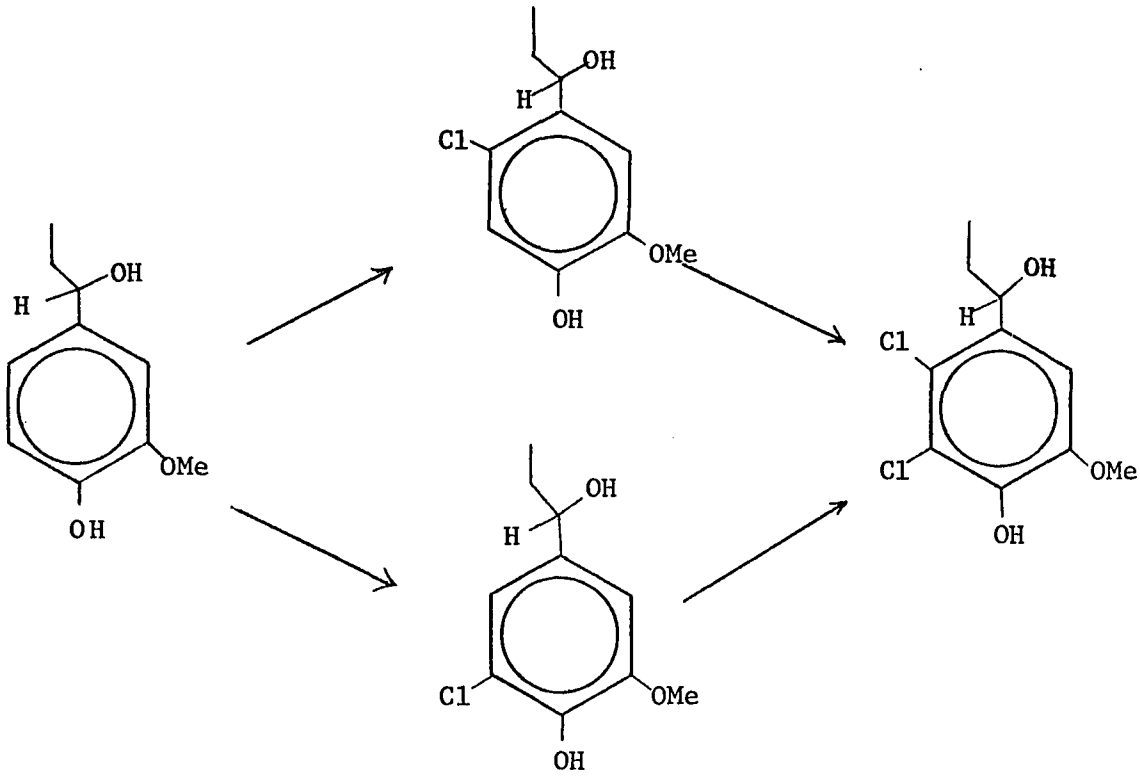
E.S.R. Studies of 5-Halovanillins With Base and H_2O_2

One of the most important bleaching operations makes use of chlorine as the oxidant (40). It seems quite conceivable that chlorine could attack an aromatic ring and substitute a chlorine atom for a hydrogen or some other group in lignin. Van Buren and Dence (41) have shown that guaiacyl units are substituted in both the 5- and 6- positions during chlorination.

TABLE VIII. Hyperfine Coupling Constants (in gauss) of Radicals From 2-hydroxy-3,5-dimethoxybenzoquinone (XII) in NaOH

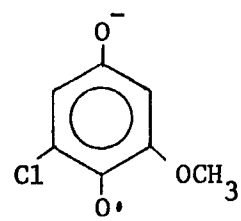
Radical	Coupling Constants[a]		
	$A^H_{OCH_3}$	A^H_{ring}	A^H
2-methoxy-3,6-dihydroxy-p-benzosemiquinone (XXVI)	1.18(3)	0.38(1)	
2,5,5-trihydroxy-1-methoxy-cyclohexadiene-6-one-3-oxyl (XXVII)	0.76(3)	1.21(1)	0.09 (1)

a. Numbers in parentheses indicate number of protons.



In a multistage pulp bleaching process, a hot alkaline treatment generally follows the chlorination step. The halogen content undergoes a marked reduction (42). Braddon and Dence (43) found that during alkaline hydrolysis of chlorine substituted model compounds, chlorine substituents of side chain moieties were rapidly removed, but aromatic chlorine substituents were resistant to alkali.

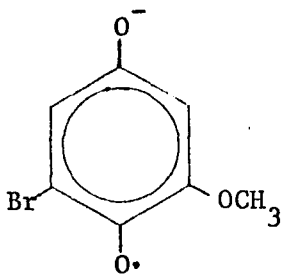
The reaction of 5-chlorovanillin with NaOH and H_2O_2 led exclusively to anion radical (XXIX). As the radical decayed, the color of the



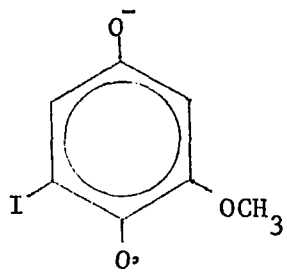
(XXIX)

solution changed from yellow to purple. This would suggest that the anion radical is being further oxidized by O_2 or a peroxide radical as was the case of radical XV forming the purple chromophore XII. This e.s.r. study also confirms the resistance of the aromatic chlorine substitutent to removal by alkali. If this were not the case, radical anion XVI would be one of the species formed. This was not found.

Allowing 5-bromovanillin and 5-iodovanillin to react with H_2O_2 in base also led to the formation of halogenated anion radicals (XXX) and (XXXI) similar to that obtained from 5-chlorovanillin, and upon decay of the signals, purple chromophores were formed. Hyperfine coupling constants of the halo-radicals are shown in Table IX.



(XXX)



(XXXI)

Enzymatic Oxidation of Model Phenols

The action of H_2O_2 on lignin model compounds in the presence of horseradish peroxidase enzyme has been shown to produce radicals and quinones by Caldwell and Steelink (20). These experiments also showed the formation of deeply colored chromophores. The colors of the reaction varied from yellow to orange to red. The color hues and intensities depended upon the substrate reacted, the length of reaction time, and the ratio of substrate to peroxide. One of the chromophoric species

TABLE IX. Hyperfine Coupling Constants (in gauss) of Halosemiquinone Radicals in NaOH and H_2O_2[a]

Radical	Coupling Constants[b]	
	$A^H_{OCH_3}$	A^H_{ring}
2-chloro-6-methoxy-p-benzosemiquinone (XXIX)	0.75(3)	3.4(1); 0.75(1)
2-bromo-6-methoxy-p-benzosemiquinone (XXX)	0.78(3)	3.65(1); 0.78(1); 0.18(?)
2-iodo-6-methoxy-p-benzosemiquinone (XXXI)	0.70(3)	3.6(1); 0.70(1); 0.2(?)

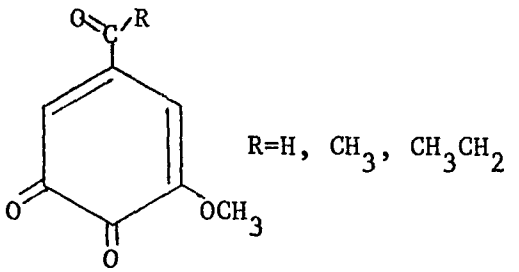
a. 0.1 M NaOH + 2 drops 3% H_2O_2

b. Numbers in parentheses indicate number of protons.

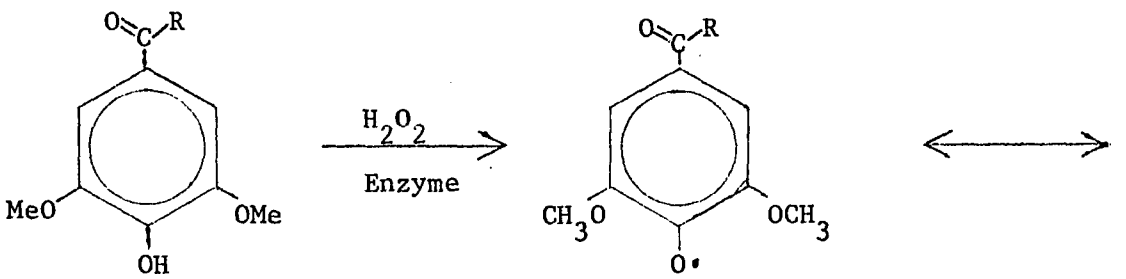
was identified as quinone I. Quinone I was identified by ultraviolet spectroscopy, thin layer chromatography, and comparison of these properties with those of an authentic sample of the quinone.

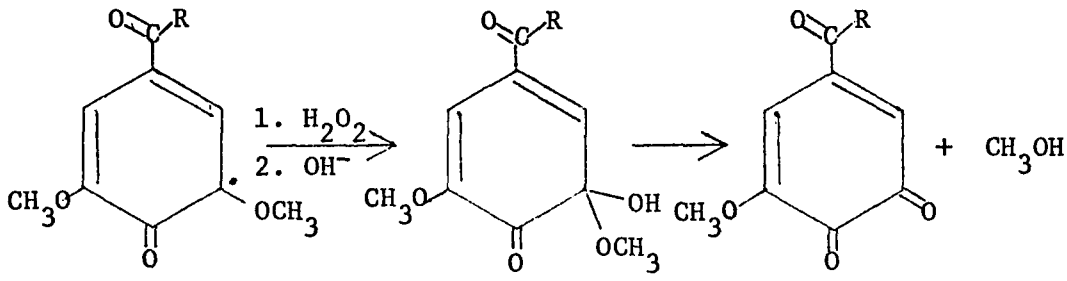
Another red-colored chromophoric substance formed during the enzymatic oxidation. Using an appropriate developing solvent, the red product could be easily separated from the yellow chromophore using thin layer chromatography. Depending upon what substrate is oxidized, the red product has different R_f values. One can conclude that, e.g., the different carbonyl side chains were still present in the otherwise similar red compounds.

It was suspected that the compounds formed during the oxidations were orthoquinones of the type

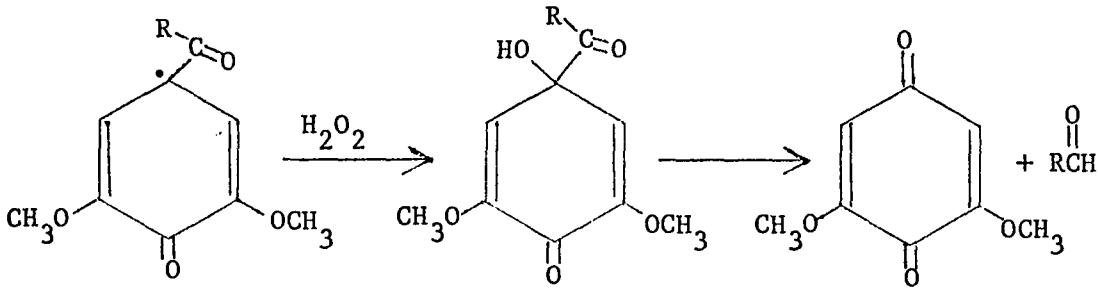


and were formed from the demethylation of the original syringyl compound.





Reaction of the initial phenoxy radical at the ring carbon attached to the carbonyl group could lead to the formation of the para-benzoquinone:



Therefore, each syringyl substrate would lead to the formation of the same paraquinone.

The suspected ortho-quinones were synthesized from the corresponding catechols, 5-hydroxyvanillin and 5-hydroxyacetovanillone. 5-Hydroxypropiovanillone could not be prepared but periodate oxidation of propiosyringone gave the same red compound as did the enzymatic oxidation of propiosyringone. These ortho-quinones were compared with the red compounds from the enzymatic oxidations and were found to be the same. Thin layer chromatography and ultraviolet spectroscopy were used.

The enzyme reaction carried out in glass distilled water with the model compound and at the end of four hours, the pH of the solution was measured to be 3.8. When the reaction was carried out in either a pH, 7 phosphate or non-phosphate buffer (see Table II), at the end of four hours, the pH of the solution was 5.1. What is the most striking difference however, between the two reactions is that no red compound was found in either of the buffered solutions.

In other buffered solutions, where the pH varied from 4 to 6, once again, no ortho-quinones were detected.

Brockhaus (44) oxidized pyrocatechol with $Ce(SO_4)_2$ in acid media and formed o-benzoquinone which he found to be stable in acid up to pH, 4. Above the pH, the ortho-quinone decomposed spontaneously.

Compounds (XIII) and (XIV) each have electron withdrawing carbonyl groups on the ring and this property would tend to make these quinones more stable. Also, the methoxy group blocks any coupling in the 3-position. Thus these quinones are fairly stable as a solid (in a moisture-free atmosphere) up to three months. They are extremely stable in non-aqueous solvents such as benzene and chloroform.

SUMMARY

Oxidation of hardwood lignin model compounds by either chemical or enzymatic means, has clearly shown the formation of chromophoric ortho- and para-quinones. Mechanisms of their formation have been proposed and a previously unreported para-quinone was isolated and characterized.

E.s.r. spectrometry was used to follow the formation of radicals during the chemical oxidation reactions and was an invaluable tool in gaining an insight into the mechanism of the reactions.

Characterization of the further reactions of the ortho-quinones is incomplete due to their relative instabilities. E.s.r. signals were obtained from the reactions of the ortho-quinones in base, but these spectra were not interpreted.

Other cromophores were obtained from the oxidation reactions and were detected using thin layer chromatography. However, there identitites are not known.

The difference in reactivity between the softwood and hardwood model compounds was clearly demonstrated by the former showing more tendency for polymerization. However, this polymer formation was not investigated to any great extent.

Both ortho- and para-quinones were formed by enzymatic oxidation, but the factors which favor demethylation over side chain cleavage were not investigated. Two-electron chemical oxidants such as periodate and ceric ions favor demethylation. It would be of interest to use a

two-electron enzymatic oxidant such as tyrosinase in order to see if ortho-quinones are also formed exclusively.

Although model compound oxidation gives an insight into the reactions of lignin, it would be of greater importance to extend these studies to lignins and wood.

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