# THE SYNTHESIS AND REACTIONS OF LIGNIN MODEL COMPOUNDS

by

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I hereby recommend that this dissertation prepared under my direction by David Alexander Ciaramitaro

entitled The Synthesis and Reactions of Lignin Model Compounds

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

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SIGNED: David A Cantwell

To my mother and father

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The author wishes to thank Dr. Cornelius Steelink for his invaluable advice and assistance in the course of this work and Dr. Ward Scott for his helpful comments on the mass spectra.

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## ABSTRACT

A number of 4-hydroxybenzylphenyl ethers and their acetate derivatives were synthesized and used as models for hardwood lignin in acid hydrolysis and enzymatic oxidation reactions. The ethers were of the guaiacyl and syringyl types commonly found in hardwood lignin preparations.

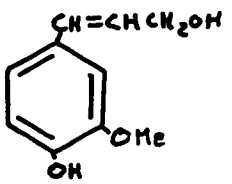
The model compounds were hydrolyzed by dilute acid in dioxane--water solution. It was found that the guaiacyl-substituted benzylphenyl ethers hydrolyzed faster than the syringyl-substituted ones. The ethers were degraded to their component phenols and benzyl alcohols. Evidence for a carbonium ion mechanism was found in these hydrolyses.

Enzymatic oxidations were carried out with horseradish peroxidase and hydrogen peroxide. It was found that the acetate derivatives did not react under these oxidative conditions. The 4-hydroxy ethers, of both types of substitution, oxidized with equal facility, but it was found that the syringyl moieties released in the reaction attained a higher degree of oxidation than did the guaiacyl analogues. It was judged that the guaiacyl radicals formed in the oxidation reaction, lacking in resonance stabilization, tended to polymerize, while the syringyl analogues oxidized to aldehydes and benzoquinones.

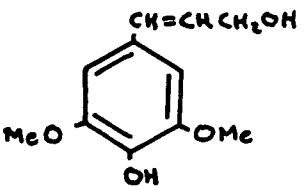
The reaction mechanisms of the syringyl-substituted ethers were examined by electron spin-resonance spectroscopy. It was determined that the first step in the oxidation was the removal of the phenolic hydrogen atom, followed by the loss of either a benzylic hydrogen atom or a phenoxy radical to form the quinone methide, which was hydrolyzed and oxidized to form the aldehyde or quinone.

# INTRODUCTION

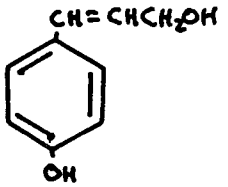
Lignin is a complex natural product which, in combination with cellulose, forms the cell walls of the stems of ferns, club mosses, grasses and deciduous and coniferous trees. According to the definition of Sarkanen, it is a polymer resulting from the enzyme-initiated dehydrogenative polymerization of three primary arylpropyl precursors: trans-coniferyl (I), trans-sinapyl (II), and trans-p-coumaryl (III) alcohols (1, p. 2).



I

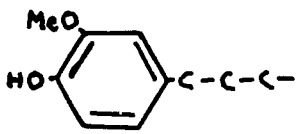


II

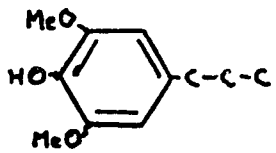


III

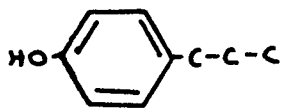
The result of this polymerization is a complex structure of phenylpropane ($C_6C_3$) units linked by carbon-carbon and carbon-oxygen bonds to various positions on neighboring phenylpropane monomers. The guaiacyl group illustrates the ring substitution found in the lignins of conifers while deciduous wood lignins have the syringyl group in addition. Monocotolydenous plants have the aforementioned two monomeric units, and in addition have the p-hydroxyphenyl substituted ring in their lignin.




Guaiacyl

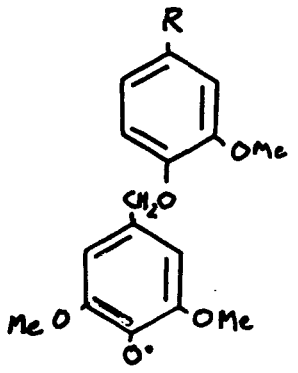



Syringyl




p-Hydroxyphenyl

The subject of this dissertation is hardwood lignin: the degradation reactions of appropriate model compounds and the products of these degradations. Hardwood lignin has been chosen for these studies for a number of reasons. The majority of work on lignin to date has concerned the softwood type (guaiacyl substitution) since this type of lignin is the most abundant, available as a byproduct of the pulp paper industry. Since syringyl as well as guaiacyl substitution is found in hardwoods, a number of reactive sites on the aryl rings in the hardwood lignin polymer are effectively blocked, the result being less chance of polymerization side reactions on the rings during degradation than would be found in the lignins of softwoods and more chance of the recovery of low molecular weight products from the degradative reactions employed. Finally, the added methoxyl group on the syringyl ring stabilizes any paramagnetic species produced by these degradations (i.e., IV) making possible the monitoring of reaction intermediates by electron spin-resonance spectroscopy. Since hardwood lignin itself has stabilized free radicals incorporated into its structure (the content being a function of the method employed in the extraction of the lignin from the wood), the




IV

ESR spectra of any model intermediates can be compared to those of lignin itself to determine the type and extent of these free radical species in the natural product (2).

A constitutional scheme based on studies of a typical hardwood lignin, that of beech, is shown in Figure 1 (3). This diagram is not necessarily the exact structure of the beech lignin polymer, but rather is a statistical average of the phenylpropanoid structural units found in beech lignin based on relative yields of degradation products. The various linkages between the monomers were deduced from the nature of the products obtained by reaction with the various degradative reagents and conditions employed in the study.

The main aspect of this research is concerned with the benzyl-aryl ether bonds which occur to a considerable extent in the lignin polymer. The scheme shown illustrates that almost 31% of the $C_6C_3$ unit bonds in beech lignin are of this type. These linkages are extremely labile--this was deduced by a study of the products of hydrolysis

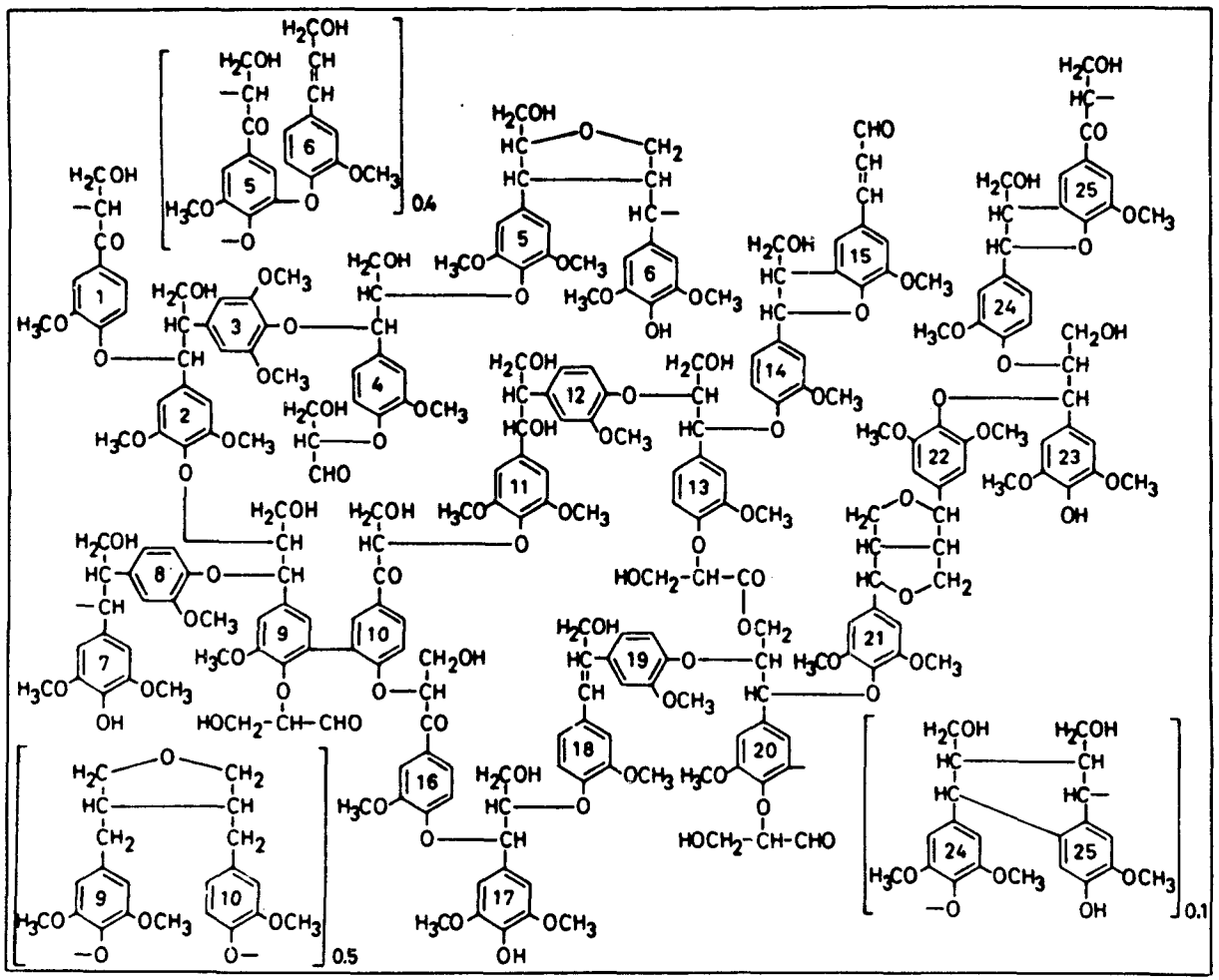


Figure 1. Constitutional Scheme of Beech Lignin. (3)

of the beech lignin with water at pH 7--and since these bonds would tend to break and rearrange under all of the common degradative conditions employed in lignin studies, the behavior of these bonds in model compound studies should afford a clue to the behavior of a large portion of the lignin itself under similar conditions.

The three most common methods of degrading lignin are acid and base hydrolyses and oxidative reactions. The majority of previous work done on lignin has concerned base hydrolysis since alkaline pulping is the most common method of removing the lignin from wood. Very little oxidative work has been done to date, due to the scarcity of good model ethers (4, 5). Investigations in this research are focused on the behavior of the benzyl-aryl ether bond toward acid hydrolysis and enzymatic oxidation conditions.

Lignin in its solid form is an intractible polymer whose composition varies with the method used to extract it from the wood. Until recently, the only uses for this compound have been as well-drilling lubricants, concrete additives, dispersants, emulsifiers and the like (1, p. 846). Most of the lignin produced by the paper industry has had to be disposed of in some way. Since the lignin obtained from industrial sources occurs as a dilute solution of calcium lignosulfonate, called spent sulfite liquor, even the evaporation of the water in order to burn the residue requires enough energy to be economically undesirable.

Lignin has enough heat energy to evaporate its aqueous solution when burned, but the cost of a plant for the continuous evaporation and burning of lignin tends to cut rather deeply into paper profits. Thus, the lignin produced by paper mills is commonly dumped into streams, lakes and oceans, to the ecological detriment of all three (1, p. 79).

Although lignin is a potential source of valuable chemicals, its refinement into generally marketable products has tended to run into the same economic stumbling-blocks as have its modes of disposal. Large amounts of heat must be used to evaporate the sulfite spent liquor and expensive and often non-recoverable chemicals must be used to degrade the resultant solid. Although advances have recently been made in industrial degradative processes, notably the manufacture of vanillin from spruce lignin, these are generally high-pressure and -temperature methods, able in most cases to compete only marginally with the synthetic processes used in the petrochemical industry (6, 7).

Studies of degradation of solutions of model compounds by dilute acids would indicate the products that one could expect by processing the unevaporated sulfite liquors in this manner. In addition, the reaction mechanisms of the resultant carbonium ions could be studied with an eye toward the formation of new polymers in the lignin, as well as degradation products and chromophoric species (8).

A number of bacteria and fungi are known to metabolize and/or degrade lignin, the extent of the degradation depending upon the species of microorganism (9). Polyporus versicolor, the white-rot fungus, possesses the ability to digest 97% of the lignin in some woods (10). Partial degradation of wood by this fungus yields small quantities of monomeric aryl carboxylic acids and aldehydes, the substitution on the ring depending upon the type of wood utilized. Small amounts of other monomeric products are found as well. The residual lignin polymer is found to be similar to the original (11).

Basidomycetes, the brown-rot fungus, metabolizes only the cellulose portion of the wood, but the resultant lignin residue is found to be reduced in its methoxyl content (12). The effectiveness of these fungi varies as to the variety of fungus and the lignin preparation used, but it has been demonstrated that fungi can be "adapted" to a given type of lignin by growing the fungus in a glucose culture medium and slowly replacing more and more of the glucose with lignin until the fungus is subsisting on lignin alone (13).

Bacteria such as Microspora and Candida spp. and Agrobacterium also degrade lignin, although to a lesser degree than do the fungi (14, 15). The majority of these fungi and bacteria contain enzyme systems utilizing polyphenyloxidases, notably peroxidase, tyrosinase and laccase.

Although the exact role that these enzymes play in the parasites' utilization of lignin is not well understood, the large number of free phenolic groups in the lignin polymer would seem to be susceptible to the action of these enzymes. An investigation into the mechanism and resultant products of enzymatic oxidations of some benzyl-aryl ether model compounds could do much toward elucidating the role of polyphenyloxidases in lignin degradation.

Enzymatic experiments with model compounds could have a practical goal as well. These reactions can occur in dilute solution and are most efficient at moderate pH. In addition, the degradability of lignin seems to be enhanced by prior photooxidation with ultraviolet light (16). Since only a small amount of the expensive enzyme need be used, a degradation scheme based on information obtained from model studies could employ sulfite spent liquor as a substrate broken down by sunlight, enzymes and cheap chemicals such as hydrogen peroxide or even atmospheric oxygen. Such a degradative scheme would bypass most of the energy requirements which presently stand in the way of the utilization of lignin in the chemical industry. Since the free-radical process of enzymatic oxidation induces polymerization as well as depolymerization, model studies would show what new polymers might arise from lignin and how they might be degraded in their turn to form new chemicals (17).

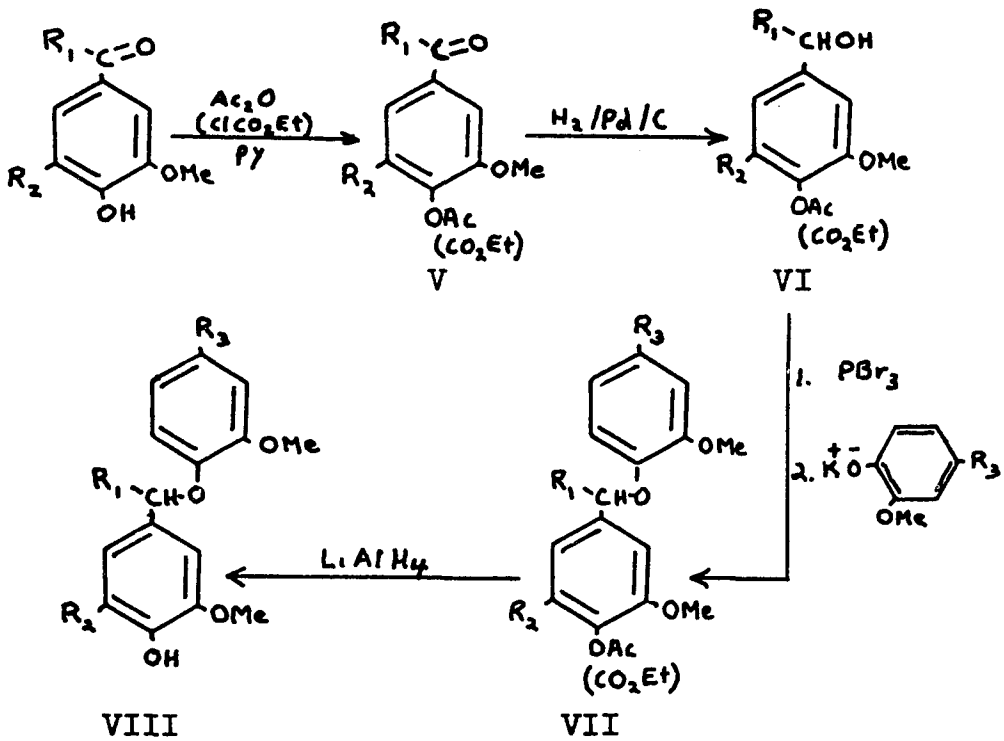
Thus, to evaluate the mechanism of degradation of lignin, it was decided to study the benzyl-aryl ether structure in lignin by synthesizing a series of model compounds and subjecting them to hydrolytic and oxidative conditions.

# DISCUSSION

## Synthetic Aspects

The model ethers prepared in the course of this research were made by a procedure similar to that of Mikawa (18), who acetylated and reduced vanillin to 4-O-acetyl-vanillyl alcohol, chlorinated the product with phosphorus pentachloride and coupled the resulting benzyl chloride with the sodium salt of guaiacol, prepared by the action of metallic sodium on guaiacol in toluene. The procedure to be described, independently arrived at, offers advantages over Mikawa's, notably the freedom to utilize phenols with side chains which would be modified or destroyed by the action of metallic sodium and a higher yield of the coupled product. In addition, a reduction step has been worked out to yield the parent 4-hydroxy ether as a model compound.

The procedure itself is illustrated in Figure 2. Vanillin, syringaldehyde or acetosyringone is reacted with either acetic anhydride or ethyl chloroformate in pyridine to afford a protecting group for the phenol; the resultant acetate or carbethoxylate derivative is hydrogenated to yield the 4-O-acetyl or the 4-carbethoxy benzyl alcohol. Although this step can also be effected with sodium borohydride in isopropanol (19), it was found that side reactions of saponification and the more complex workup




| | Va | Vb | Vc | VIa | VIb | VIc | VIIa | VIIb | VIIc | VIId |
|---|---|---|---|---|---|---|---|---|---|---|
| $R_1$ | H | H | $CH_3$ | H | H | $CH_3$ | H | H | H | H |
| $R_2$ | H | $OCH_3$ | $OCH_3$ | H | $OCH_3$ | $OCH_3$ | H | H | $OCH_3$ | $OCH_3$ |
| $R_3$ | | | | | | | CHO | $CH_2OH$ | H | CHO |

| | VIIe | VIIf | VIIg | VIIIa | VIIIb | VIIIc |
|---|---|---|---|---|---|---|
| $R_1$ | H | $CH_3$ | $CH_3$ | H | H | $CH_3$ |
| $R_2$ | $OCH_3$ | $OCH_3$ | $OCH_3$ | H | $OCH_3$ | $OCH_3$ |
| $R_3$ | $CH=CHCH_3$ | CHO | $CH=CHCH_3$ | $CH_2OH$ | $CH_2OH$ | $CH_2OH$ |

Figure 2. Synthetic Scheme for Lignin Model Ethers.

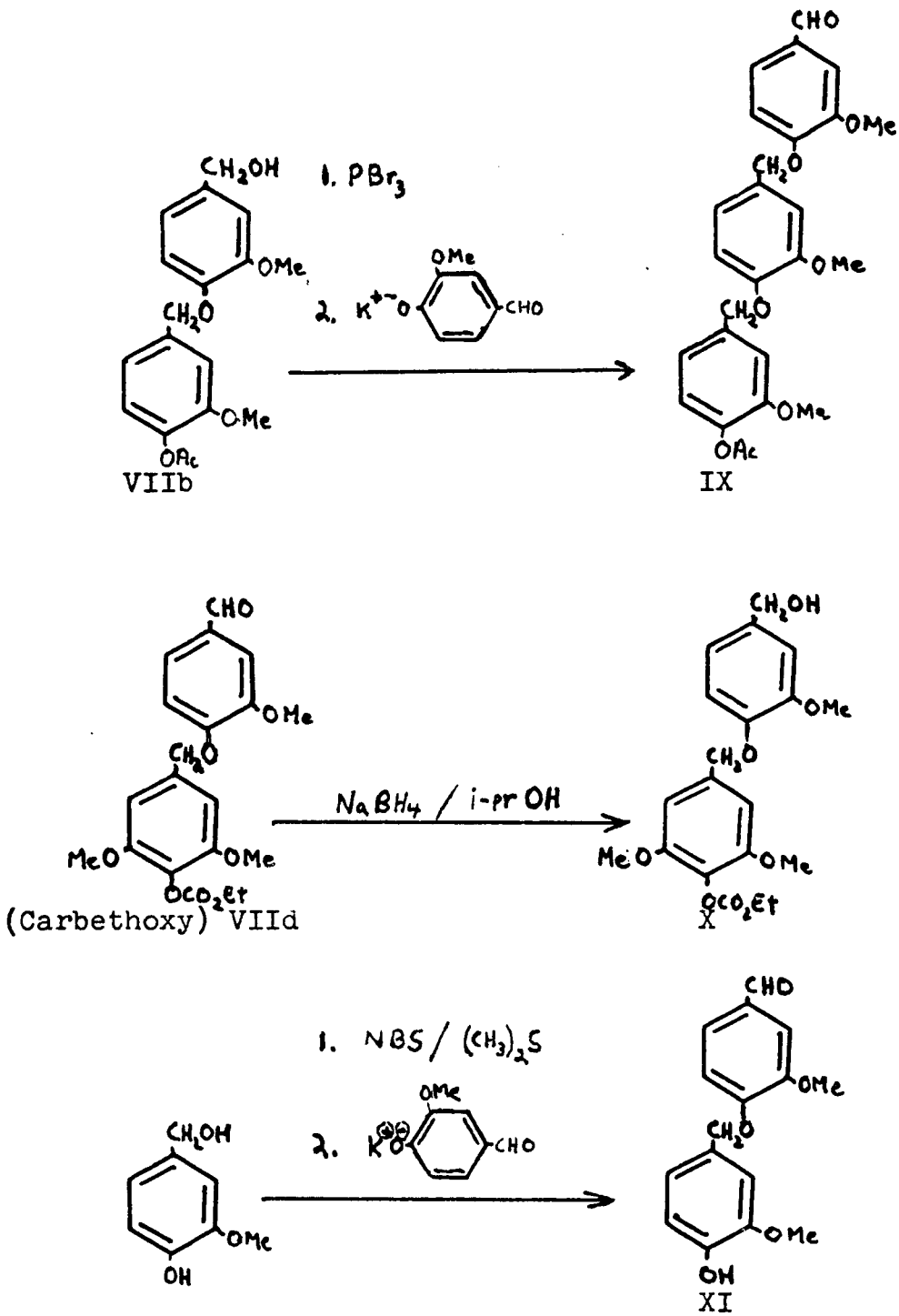



Figure 2. (Continued).

involved tended to reduce the yield for this step. It was noted, however, that recrystallization of the acetoxy or carbethoxy derivative before hydrogenation is essential in order to remove traces of pyridine, which tend to poison the catalyst and inhibit the reaction.

Acetoxy and carbethoxy ethers were produced by bromination of the protected benzyl alcohols with phosphorus tribromide and addition of the resulting bromides to an acetone solution of the potassium salt of the chosen phenol. The syringyl alcohol derivative brominated much more readily than the vanillyl derivative, which in turn reacted faster than the α-methyl syringyl analogue. Steric effects probably contribute to the difference in reaction rates of the first and last compound, since an intermediate alkyl phosphite, $ROPBr_2$, forms when an alcohol ROH is brominated in this way (20). The syringyl compound reacts much more vigorously than the vanillyl since the additional electron-donating methoxyl group further activates the benzyl position toward nucleophilic substitution. Unfortunately, this enhanced reactivity tends to lessen the yield of ether product; the starting material is degraded to tar by the exothermic vigor of the reaction. Dilution of the reactants and careful control of heat can lessen this tendency somewhat. The respective benzyl bromides were never isolated as such; their chloroform solutions were washed with water to remove phosphorus compounds and hydrogen

bromide, dried with a strong drying agent such as magnesium sulfate, and used directly.

The phenoxide ion used to couple the ether was generated in acetone by refluxing the phenol under nitrogen over anhydrous potassium carbonate. The use of more acidic phenols, i.e., vanillin, invariably resulted in enhanced yields of ether derivative, while less acidic phenols such as guaiacol and isoeugenol remained to a large degree unreacted. At times a small amount of potassium hydroxide was used in conjunction with potassium carbonate in an effort to raise the concentration of phenoxide ion, but this practice also led to a greater degree of condensation of solvent molecules to form diacetone alcohol (21), making crystallization of the product difficult and necessitating chromatography to purify the resultant ether derivative. Methanol was tried as a solvent in order to avoid this condensation problem, but no reaction resulted.

The removal of the phenol blocking group presented many problems and remains the lowest-yield step in the reaction sequence. The carbethoxy-protected ethers were originally made in an attempt to simplify the last step by effecting hydrolysis of this group in an alkaline solution of sufficient dilution that the resultant free-phenol ether would not be degraded by side reactions. Extremely dilute hydroxide ion did indeed cause hydrolysis of the carbethoxy group, but in every instance no ether was recovered.

Monitoring of this reaction by NMR spectroscopy (in $D_2O$-acetone-$d_6$ with a small amount of NaOD) demonstrated that the ether broke up into its component alcohol and phenol as rapidly as the blocking-group hydrolysis took place.

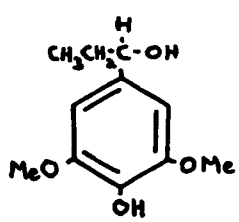
A method for neutral-pH removal of acetates by the action of imidazole (22) was tried on the 4-O-acetyl ether derivatives. Compound VIIa acetate was refluxed with imidazole, but apart from a slight amount of decomposition, the starting material was unchanged. Extended refluxing degraded the sample entirely.

The blocked ether derivatives were all extremely resistant to acid hydrolysis and when this was effected, the entire compound degraded. Dilute hydrochloric acid and concentrated acetic and phosphoric acids had no effect upon the compounds, either at room temperature overnight or after 2 hr of heating at 90°. Methyllithium solutions likewise had no effect on either the ether or the blocking group.

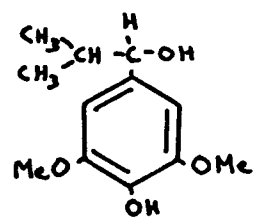
Some success in overcoming the problem of the production of the free-phenol ether was achieved with the use of lithium aluminum hydride. This reagent does not provide an entirely satisfactory solution, however, since any reducible group (such as the 4'-formyl) would not survive the final step unchanged. An alkyl side chain on either of the benzyl carbon atoms seems to encourage degradative side reactions and lower yields; extremely low yields of VIIIc from VIIf were obtained, and attempts to

deblock VIIe and VIIg failed entirely. However, the overall yield of VIIIb was 38%, and the yield from the deblocking step was 68%, indicating that this method has some potential for the production of some model compounds.

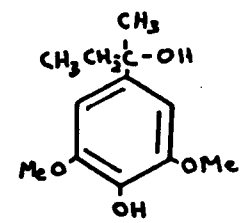
Two attempts were made to entirely bypass the problem of blocking and deblocking model ethers. Johansson and Miksche (23) generated quinone methides from substituted 4-hydroxybenzyl alcohols, such as vanillyl alcohol, and reacted these directly with excesses of phenols to produce small amounts of phenolic benzyl-aryl ethers directly. Accordingly, compounds XII, XIII and XIV were prepared in an attempt to make the corresponding α-aryl benzyl ethers:




XII



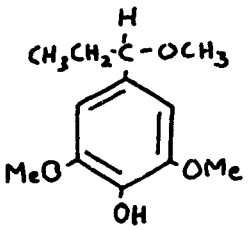

XIII



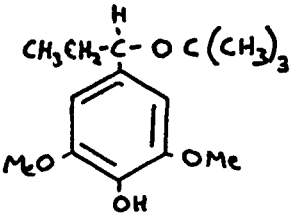

XIV

When the quinone methide of XII was reacted in chloroform with vanillin, no discernible product was obtained. A ten-fold excess of phenol was used in the literature reference, and a 21% yield of product with the recovery of some starting material was reported. The alkyl side chain of XII served to stabilize the resultant quinone methide so that in chloroform solution it would persist for days; the reactivity of this material with a solution of phenol was thus of a very low order. Better results were obtained by

using this procedure to obtain benzyl-alkyl ethers, using methanol and t-butanol as solvent and reactant. Both XV and XVI were produced, albeit in low yields.




XV




XVI

However, since the usefulness of this type of ether as a lignin model is debatable, this general approach was abandoned.

A synthetic attempt in some respects similar to the one above was performed utilizing the bromination procedure of Corey, Kim and Takeda (24) for benzylic alcohols. Both vanillyl and syringyl alcohols were reacted with a dimethyl sulfide-N-bromosuccinimide brominating complex at low temperature in dilute solution to produce the corresponding bromides, which were coupled with potassium vanillate in acetone as previously described. The method worked fairly well for vanillyl alcohol, producing a moderate yield of XI (see Figure 2), but the syringyl analogue reacted to the extent of only a few percent. Refinements in this procedure, however, may provide the best route to these model ethers in the future.

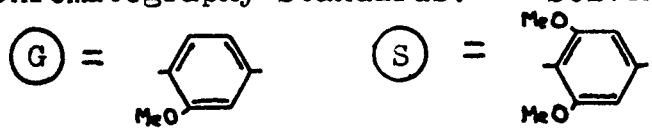
A route to ether trimers as possible lignin model oligomers was also investigated. The reduction of

carbethoxy ether VIId with sodium borohydride afforded X, which might then be treated as a blocked benzyl alcohol with phosphorus tribromide and a potassium phenolate to yield the trimer. An alternative method to produce a dimer with a benzyl alcohol function was the coupling of VIa acetate with vanillyl alcohol to produce VIIb. The reaction of this dimer with phosphorus tribromide and potassium vanillate afforded IX, which obviously could be reduced to the benzyl alcohol, brominated and reacted again. This sequence could be continued until the resultant oligomer was no longer soluble in the bromination solvent.

## Acid Hydrolysis Reactions

Both the 4-O-acetyl and the 4-hydroxy ethers were hydrolyzed to some extent by 0.2 M hydrochloric acid solutions heated at 50° for 8 or 24 hr. In order to keep the reaction homogeneous, the ethers were dissolved in 9:1 dioxane--water mixtures which were 0.2 M in HCl and allowed to stand in a hot oil bath for the requisite times. The products of the hydrolysis reactions were relatively easy to identify by thin-layer chromatography. Table 1 shows the approximate Rf values of starting materials and standards used for identification, as well as the colors obtained when the compounds were developed with ferric nitrate solution and dinitrophenylhydrazine reagent. The Rf values varied slightly from one thin-layer plate to another, and the

Table 1. Rf Values and Development Colors for Thin-Layer Chromatography Standards. -- Solvent: Benzene--Methanol 9:1




| Compound | Rf | Sprayed w/$Fe(NO_3)_3$ | Sprayed w/DNPH |
|---|---|---|---|
| HO(G)CHO | 0.57 | violet | orange |
| HO(S)CHO | 0.49 | blue | dark orange |
| HO(S)$COCH_3$ | 0.45 | violet | red-orange |
| HO(G)$CH_2OH$ | 0.27 | blue | yellow-gray |
| HO(S)$CH_2OH$ | 0.25 | red-brown | yellow |
| HO(S)$CH(CH_3)OH$ | 0.20 | red-brown | yellow |
| O=(G)=O | 0.76 | ---- | yellow-violet |
| O=(S)=O | 0.63 | ---- | yellow-brown |
| HO(S)COOH | 0.25 | dark brown | ---- |
| HO(S)$C(CH_3)$=$CHCH_3$ | 0.75 | red-brown | brown |
| Dehydrodivanillin | 0.35 | ---- | yellow |
| Dehydrodivanillyl alcohol | 0.14 | brown | brown |
| 4-Hydroxymethyl-6-methoxy-orthoquinone | 0.11 | purple | brown |
| Va acetate | 0.40 | ---- | brown |
| Vb acetate | 0.44 | ---- | brown |
| VIIa acetate | 0.81 | ---- | orange |
| VIIb acetate | 0.37 | ---- | brown |
| VIId acetate | 0.72 | ---- | orange |
| VIIf acetate | 0.92 | ---- | orange |
| VIIIa | 0.25 | gray | brown |

Table 1. (Continued).

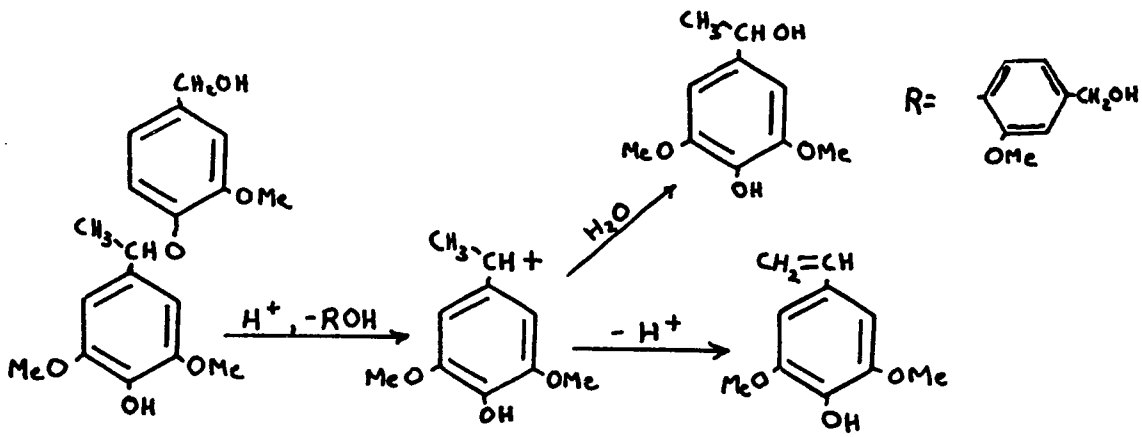
| Compound | Rf | Sprayed w/$Fe(NO_3)_3$ | Sprayed w/DNPH |
|---|---|---|---|
| VIIIb | 0.24 | red-brown | faint yellow |
| VIIIc | 0.25 | red-brown | yellow |
| IX | 0.72 | ---- | orange |
| XI | 0.62 | violet | orange |

colors of the spots varied likewise with the relative concentrations of material, so typically a group of standards would be chromatographed alongside the product-mixture being investigated in order to eliminate uncertainty. When a given spot on the plate was ambiguous, it was separated from the other reaction products by column chromatography and a mass spectrum was taken.

Generally speaking, the free-phenol ethers hydrolyzed more readily than the 4-O-acetyl derivatives and of these, the vanillyl dimers VIIa and VIIb would typically be totally hydrolyzed within 8 hr, while the syringyl analogue VIId would persist in the reaction medium for more than 8 hr but less than 24.

Hydrolyses of the 4-hydroxy ethers VIIIa-VIIIc and XI probably progress through the benzyl carbonium ion and result in the phenol and the 4-hydroxybenzyl alcohol. An indication that the carbonium ion is the intermediate was given in the hydrolysis of VIIIc. The product-mixture included a spot of Rf 0.64, which sprayed red-brown with ferric nitrate and turned brown in iodine vapor. It was deduced that this product was the styrene derivative of the hydrolyzed α-methyl syringyl fragment of ether VIIIc. A model compound of much the same type, <u>cis</u>- and <u>trans</u>-2-(3,5-dimethoxy-4-hydroxyphenyl)-2-butene, was synthesized and was found to react in an identical manner with the spray reagents as the unknown, although its Rf value was slightly

higher (0.75), most likely because of its added alkyl substitution. Although a sample for mass spectral analysis could not be obtained in this case, the later instance of enzymatic oxidation of VIIIc produced the same moiety in the product-mixture, due to hydrolytic side-reactions; a mass spectrum of this moiety had a parent peak of 180 and the characteristic breakdown pattern of a 4-hydroxy styrene. The reaction yielding this product is shown below.

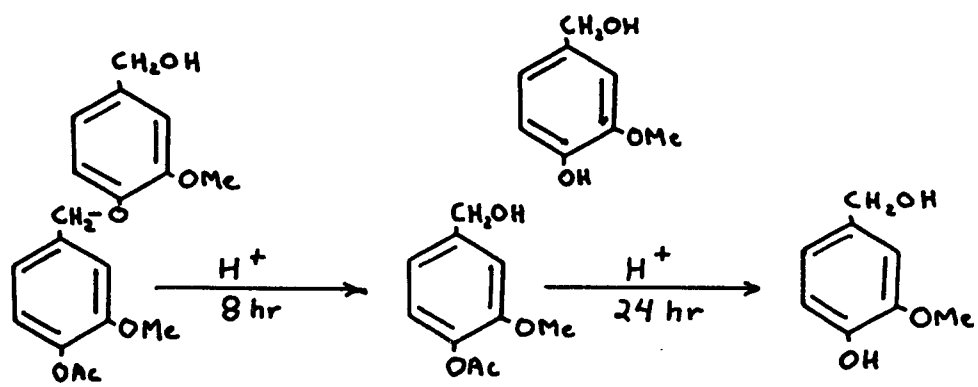



VIIIc

The acidities of the phenol hydrogens on these dimers and their ability to ionize to phenolate ions probably account for the retarded reaction rates of the 4-hydroxysyringyl and 4-O-acetyl ethers relative to the guaiacyl derivatives. A negative charge on the ring caused by a loss of phenol hydrogen would help to further stabilize the benzyl carbonium ion formed upon hydrolysis. Since the steric and resonance effects of an added methoxyl group would make the phenyl proton on the syringyl derivatives less acidic and interfere with its solvation, the resulting

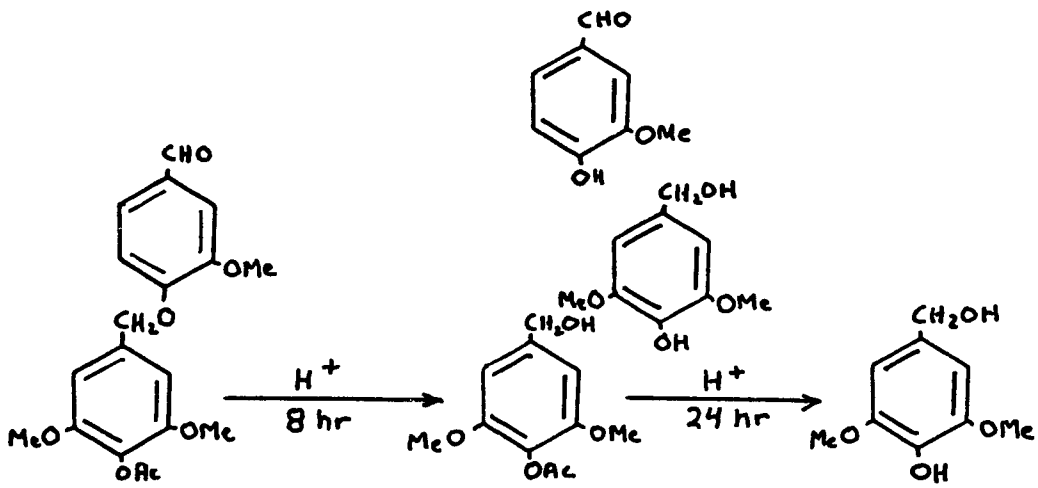
carbonium ion is somewhat less stable and the reaction is slower. An acetate-blocked phenol group has no chance to induce a negative charge at all and the benzyl carbonium ion must form with still less added stabilization. An α-methyl group would generate a secondary carbonium ion, which is apparently stabilized enough to enhance the reaction rate without the necessity of the phenolate negative charge. Thus, the dimer VIIIc hydrolyzed as rapidly as the guaiacyl analogues VIIIa and XI.

The acetate-protected ether dimers underwent hydrolytic cleavage at different sites depending upon which compound was undergoing the reaction. The guaiacyl dimer VIIb hydrolyzed first to the phenol and the 4-O-acetylbenzyl alcohol; the acetate was hydrolyzed somewhat later:




VIIb VIa

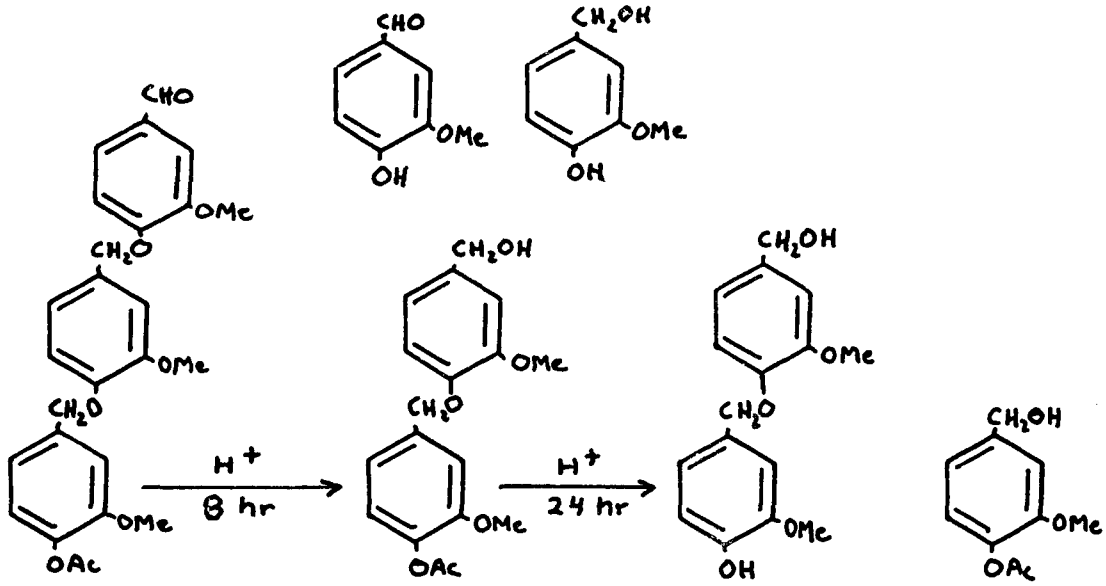
The acetate VIId was much more resistant to hydrolysis. At the end of 24 hr, starting material was still very much in evidence. That portion of the sample which did hydrolyze, however, showed a different mode of cleavage than did the case of VIIb above:




VIId VIb

This difference in products found would indicate that the ether bond in VIId has the same or greater resistance to the hydrolysis than the acetate blocking group. After 8 hr, the small amount of ether that was hydrolyzed was broken up into its component phenol and the acetylated and phenolic benzyl alcohols. After 24 hr, with much starting material still undecomposed, the intermediate VIb acetate has assumed so low a concentration as to be unnoticeable on the thin-layer plate, although large concentrations of vanillin and syringyl alcohol are in evidence.

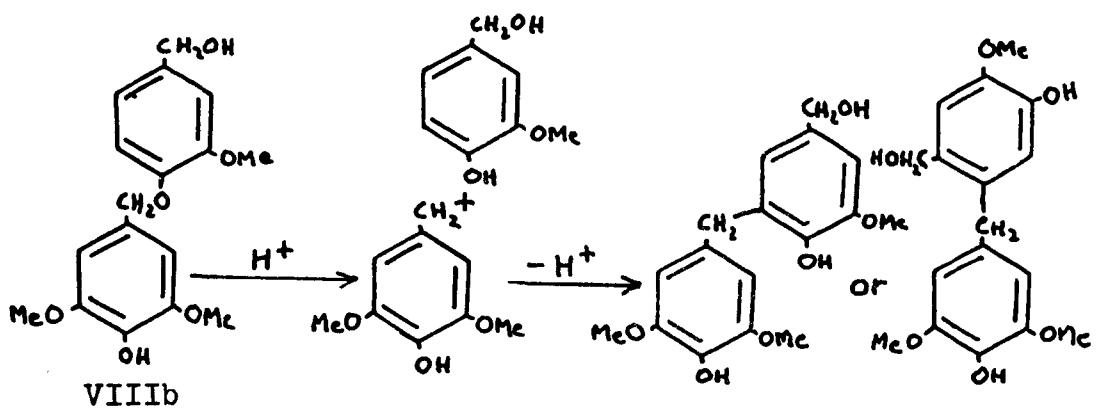
The trimer IX showed a strong resistance to ether bond hydrolysis, reacting at a faster rate than the syringyl derivative VIId, but more slowly than the guaiacyl dimers VIIa and VIIb. Apparently the trimer allows hydrolysis of the 4-O-acetyl group and the terminal vanillin moiety at approximately equal rates, but after the terminal vanillin is gone, the remainder of the molecule, now the dimer VIIb




IX VIIb VIIIa VIa

acetate, begins to hydrolyze preferentially at the ether linkage. Much starting material remained, even after 24 hr.

The benzyl carbonium ion generated in these hydrolysis reactions has other options besides alcohol and styrene formation. It could attack the departing phenol, forming a diphenylmethane derivative, as illustrated using VIIIb as an example:




VIIIb

Formation of compounds of this type have occurred upon acid hydrolysis of model compounds (23) and lignin itself (25); however, compounds of this type were not isolated from the product mixtures obtained from the reactions of the models employed here. A small amount of material of low Rf (less than 0.2) was always present in these mixtures, and it is possible that compounds of this type are responsible. The similar small Rf values of the components of this fraction made separation in quantities large enough to be properly analyzed impossible. The incidence of this type of reaction could well be a feature of reactions employing large concentrations of ether substrate, where the hydrolyzed phenol could properly compete with water for the benzyl ion.

The behavior of the model ethers synthesized here toward acid hydrolysis is strikingly similar to the behavior of lignin under the same conditions. Styrene derivatives such as compounds I, II and III are lignin hydrolysis products (1, p. 3). Lundquist and Miksche (26) found that oligolignols, upon acid hydrolysis, lost a proton or a formaldehyde molecule and were converted into stilbene derivatives. Stilbenes also arise upon hydrolysis of lignin phenylcoumaran derivatives (27). α-hydroxyguaiacylpropane structures, upon hydrolysis, yield styrene derivatives resembling isoeugenol (28). Finally, some low molecular

weight phenols, including vanillin, vanillyl alcohol derivatives and styrene derivatives all have been isolated from hydrolyzed spruce lignin (25).

Table 2 summarizes the products and estimated yields in the acid hydrolyses of the model ethers. The models are listed in order of their relative rates of hydrolysis, from most rapid to least.

## Enzymatic Oxidations

Enzymatic oxidations of model compounds were carried out in 10-35% methanol--water solutions with hydrogen peroxide and horseradish peroxidase at pH 6-7. The products were analyzed by thin-layer chromatography and, when more rigorous characterization was required, column-chromatographed fractions were analyzed by their mass spectra.

The phenol-protected ethers were difficult to dissolve in solutions of a low enough concentration of methanol to render the enzyme viable and it was found that a solution of these blocked ethers could stand indefinitely at room temperature under reaction conditions without any reaction taking place. Dehydrogenation of the phenol is thus shown to be an important step in the oxidation reaction. This observation corroborates findings made by electron spin-resonance studies done on radicals produced by the oxidation of model phenol monomers (29).

Table 2. Products and Estimated (to Within 5%) Yields from Acid Hydrolyses of Model Compounds. -- Listed in Order of Decreasing Reactivity with 0.2 M Hydrochloric Acid in 9:1 Dioxane--Water.

| Compound | Time | Product | Percent Yield |
|---|---|---|---|
| VIIIa | 8 hr | vanillyl alcohol | 95 |
| | | low Rf products | trace |
| XI | 8 hr | vanillyl alcohol | 45 |
| | | vanillin | 45 |
| | | low Rf products | trace |
| VIIIc | 8 hr | 1-(syringyl)-ethylene | 15 |
| | | α-methyl syringyl alcohol | 20 |
| | | vanillyl alcohol | 20 |
| | | low Rf products | 25 |
| VIIIb | 8 hr | starting material | 20 |
| | | syringyl alcohol | 40 |
| | | vanillyl alcohol | 40 |
| | 24 hr | syringyl alcohol | 45 |
| | | vanillyl alcohol | 45 |
| | | low Rf products | trace |
| VIIb | 8 hr | vanillyl alcohol | 50 |
| | | 4-O-acetylvanillyl alcohol | 30 |
| | | low Rf products | 20 |
| | 24 hr | vanillyl alcohol | 70 |
| | | low Rf products | 30 |
| VIId | 8 hr | starting material | 60 |
| | | vanillin | 20 |
| | | 4-O-acetylsyringyl alcohol | 10 |
| | | syringyl alcohol | 10 |
| | 24 hr | starting material | 50 |
| | | vanillin | 25 |
| | | syringyl alcohol | 20 |

Table 2. (Continued).

| Compound | Time | Product | Percent Yield |
|---|---|---|---|
| IX | 8 hr | starting material | 60 |
| | | vanillin | 25 |
| | | vanillyl alcohol | 10 |
| | | 4-O-acetylvanillyl alcohol | 5 |
| | 24 hr | starting material | 50 |
| | | vanillin | 25 |
| | | compound XI | 10 |
| | | vanillyl alcohol | 10 |
| | | low Rf products | 5 |

In contrast to the acid hydrolysis reactions, all of the free-phenol ethers reacted with equal facility with enzyme and oxidant; except in cases where a supersaturated solution of starting material precipitated out, no starting material was found in the product mixtures.

The syringyl ether VIIIb reacted with peroxide and enzyme to yield at least six different products, visible on a thin-layer plate. Table 3 shows the products and estimated yields for the oxidations of VIIIb and other model compounds. The major identifiable products of the enzymatic reaction of VIIIb with 2 equivalents of hydrogen peroxide were syringaldehyde, 2,6-dimethoxy-p-benzoquinone, syringyl alcohol, vanillyl alcohol and occasionally a small amount of vanillin.

In a similar fashion, the α-methyl syringyl compound VIIIc, oxidized with enzyme and 2 equivalents of peroxide, yielded acetosyringone, a larger proportion of vanillin than in the previous example, vanillyl alcohol and a small amount of α-methyl syringyl alcohol. No p-quinone was obtained from this reaction.

When compound VIIIb was reacted with an excess of peroxide, the identifiable products formed were syringaldehyde, 2,6-dimethoxy-p-benzoquinone, a larger quantity of vanillin than in the previous reaction of this model, and small amounts of 2-methoxy-p-benzoquinone, vanillyl alcohol and syringyl alcohol.

Table 3. Products and Estimated (to Within 5%) Yields from Enzymatic Oxidations of Model Compounds.

| Compound | Method* | Product | Percent |
|---|---|---|---|
| VIIIa | A | vanillin | 10 |
| | | vanillyl alcohol | 10 |
| | | compound Rf 0.37 | 15 |
| | | compound Rf 0.30 | 15 |
| | | compound Rf 0.16 | 15 |
| | | compound Rf 0.09 | 15 |
| VIIIb | A | syringaldehyde | 42 |
| | | 2,6-dimethoxy-*p*-benzoquinone | 9 |
| | | syringyl alcohol | 12 |
| | | vanillyl alcohol | 22 |
| | | vanillin | 5 (variable) |
| | | compound Rf 0.27 | 3 |
| | | chromophore Rf 0.15 | 3 |
| | | compound Rf 0.10 | 3 |
| VIIIb | B | syringaldehyde | 30 |
| | | 2,6-dimethoxy-*p*-benzoquinone | 20 |
| | | 2-methoxy-*p*-benzoquinone | 5 |
| | | vanillin | 10 |
| | | syringyl alcohol | 8 |
| | | vanillyl alcohol | 10 |
| | | chromophore Rf 0.12 | trace |
| | | low Rf products | 10 |
| VIIIb | C | 3,5-dimethoxy-4-hydroxybenzyl methyl ether | 45 |
| | | starting material | 10 |
| | | syringyl alcohol | 20 |
| | | vanillyl alcohol | 25 |
| VIIIb | D | 3,5-dimethoxy-4-hydroxybenzyl methyl ether | 45 |
| | | starting material | 10 |
| | | syringaldehyde | 5 |
| | | 2,6-dimethoxy-*p*-benzoquinone | trace |
| | | vanillyl alcohol | 25 |
| | | syringyl alcohol | 15 |

Table 3. (Continued).

| Compound | Method* | Product | Percent |
|---|---|---|---|
| VIIIc | A | acetosyringone | 35 |
| | | vanillin | 20 |
| | | 1-(syringyl) ethylene | 5 |
| | | vanillyl alcohol | 10 |
| | | α-methyl syringyl alcohol | 5 |
| | | chromophore Rf 0.17 | 6 |
| | | low Rf products | 10 |
| XI | A | vanillin | 25 |
| | | 2-methoxy-p-benzoquinone | 20 |
| | | vanillyl alcohol | 10 |
| | | compound Rf 0.39 | 10 |
| | | compound Rf 0.34 | 15 |
| | | compound Rf 0.24 | 15 |
| Equimolar mixture of vanillyl and syringyl alcohols | A | syringaldehyde | 45 |
| | | vanillin | 5 |
| | | 2,6-dimethoxy-p-benzoquinone | 15 |
| | | vanillyl alcohol | 10 |
| | | syringyl alcohol | 10 |
| | | low Rf compounds | 10 |

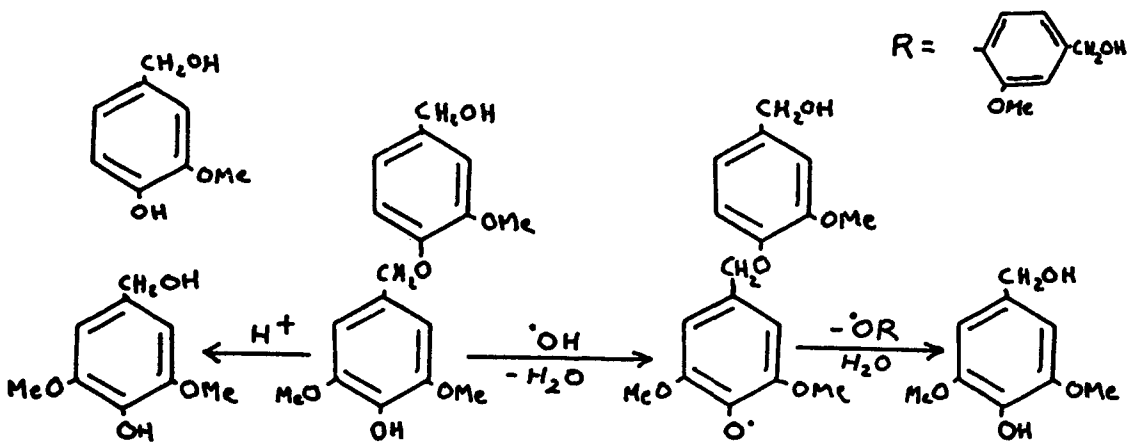
*A. 2 equivalents peroxide, 0.5 mg peroxidase, 24 hr.

B. excess peroxide, 1.0 mg peroxidase, 24 hr.

C. 2 equivalents peroxide, no peroxidase, 1 week.

D. 2 equivalents peroxide, no peroxidase, 4 weeks.

Several mechanisms seem to be at work in these oxidations. The formation of syringyl alcohol and vanillyl alcohol could proceed by simple hydrolysis to form the carbonium ion and the phenol, or by a radical process:

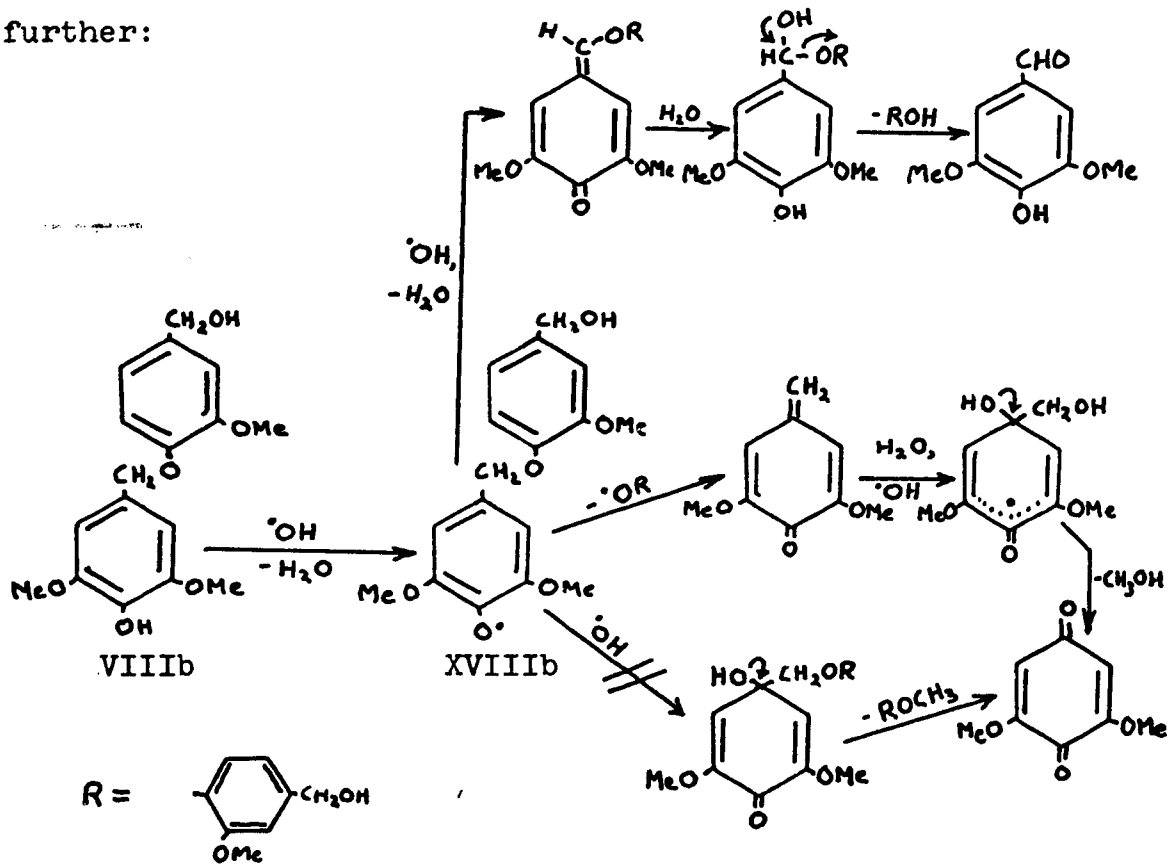



VIIIb XVIIIb

As a control experiment, a 20% methanol--water solution of VIIIb was allowed to stand with 2 equivalents of hydrogen peroxide and no enzyme for time periods of 1 week and 4 weeks. The shorter time period yielded some precipitated starting material, small amounts of syringyl and vanillyl alcohols and a relatively large amount of 4-hydroxy-3,5-dimethoxybenzyl methyl ether. The 4-week reaction gave the above products plus small amounts of syringaldehyde and 2,6-dimethoxy-p-benzoquinone. It is fairly certain, then, that the syringyl and vanillyl alcohols formed in the enzymatic oxidations are largely the products of a hydrolysis reaction. In the presence of enzyme, the amount of hydrolysis occurring is minute in

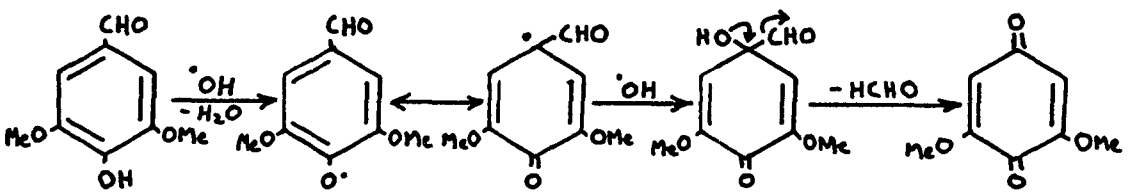
relation to the amount of oxidation; removal of the enzyme reverses the relationship of the rates of oxidation and hydrolysis.

The following mechanisms are postulated for the formation of the aldehyde and the p-quinone. The failure of the acetate-blocked ether to oxidize and reports of enzyme reactions with syringyl alcohols (29) indicate that the phenolic hydrogen is homolytically cleaved in the first step of the reaction, yielding the radical XVIII, which can react further:




The reaction sequence leading to the 4-methoxy ether and the p-quinone does not occur under the reaction conditions employed here. No veratryl alcohol or veratraldehyde was

ever found in the product mixtures. The quinone methide intermediates shown are common postulated intermediates in studies of oxidation mechanisms of phenolic models (30). The 2,6-dimethoxy-p-benzoquinone can be formed from the aldehyde as well as from the intermediate alcohol, with the liberation of formaldehyde as a byproduct:

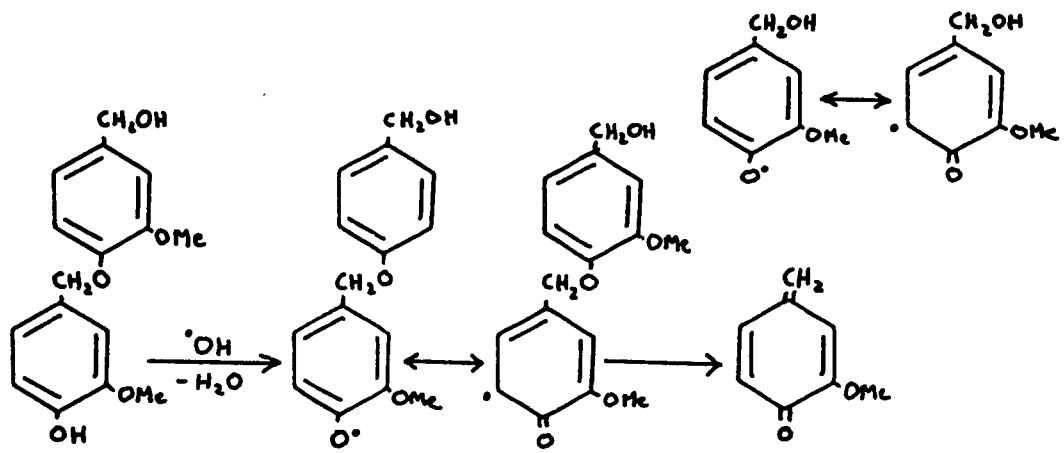



Evidence for this reaction was obtained by carrying out the oxidation in methanol--water as a standard, and in methanol and Nash's solution (31), and observing the relative rates of visible light absorption at 412 nm. Formaldehyde reacts with ammonium ion and acetylacetone at neutral pH to produce diacetyldihydrolutidine, an intense yellow chromophore, and the increase in absorption of the reaction in Nash's reagent was much more rapid and complete than in the water solution. The possibility of a side reaction between methanol and peroxide to produce formaldehyde was ruled out by running a blank. No yellow color was observed.

The oxidation of the syringyl alcohol intermediate to the quinone could not be followed by the techniques (32) given in the literature, since the small amount of methanol formed would not be detectable in methanol--water solution.

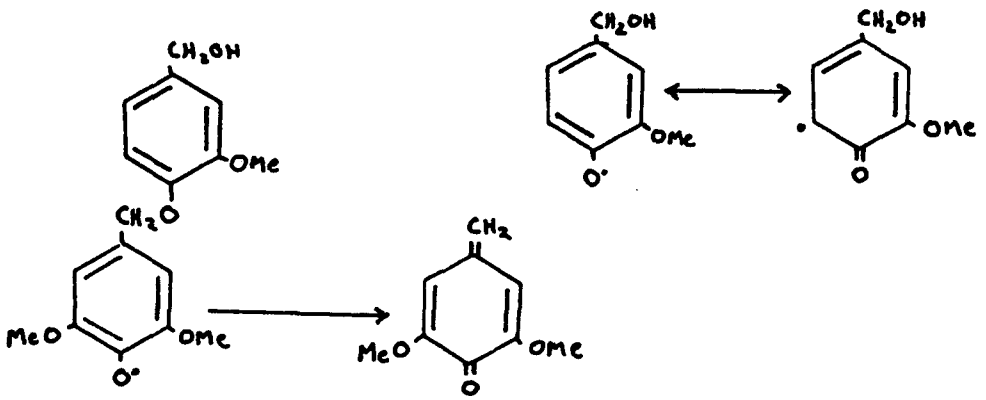
It has been shown that when the concentration of p-benzoquinone reaches a certain point, it begins to impede the functioning of the enzyme and the oxidation ceases. This accounts for the observation that, unless large concentrations of enzyme are used, the majority of the aldehyde formed will not react further and the p-benzoquinone will remain a minor product.

The guaiacyl dimers VIIIa and XI reacted enzymatically with 2 equivalents of peroxide to yield products analogous to those of the reactions of the syringyl compounds. Because of the lack of substitution on position 5 of the guaiacyl moieties, the radical formed upon oxidation can resonate:



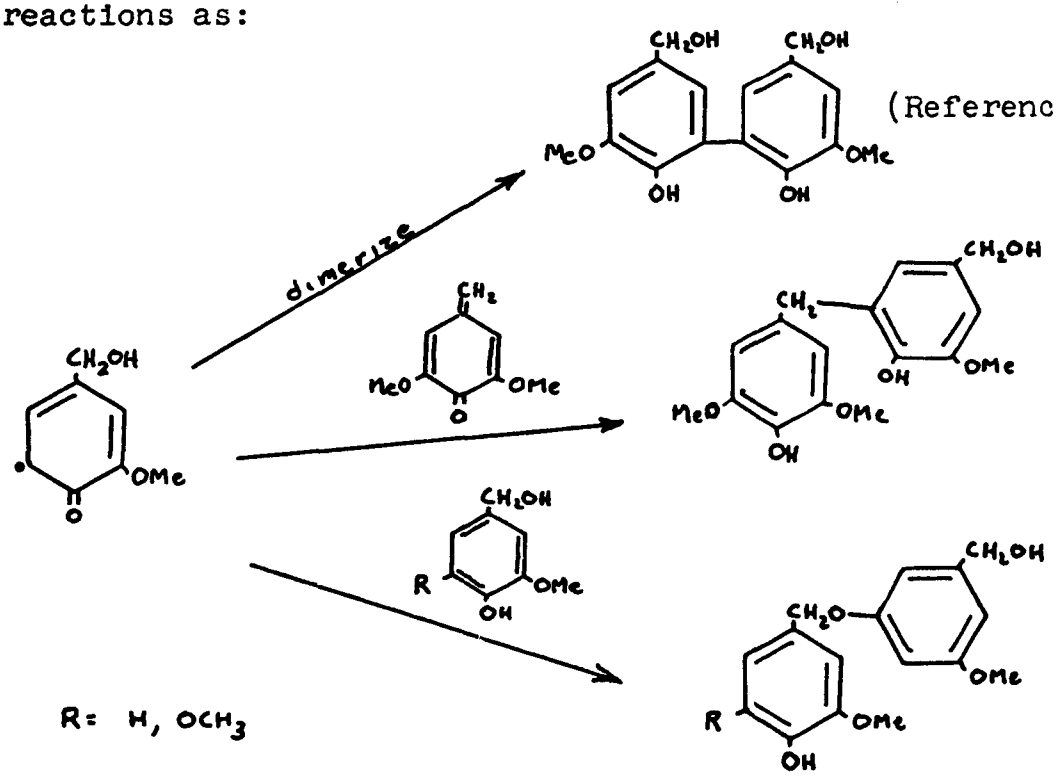

VIIIa XVIIIa

This resonance form can also form from the aforementioned syringyl compounds, although it forms at lower concentrations because of the blocking effect of the added methoxyl group on the syringyl ring.



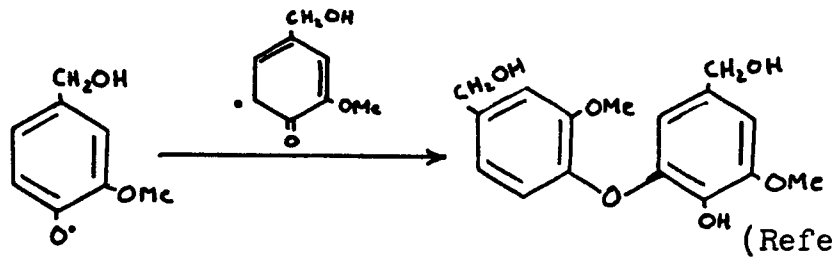
XVIIIb

This resonance form may then undergo addition reactions on position 5. The unidentified spots on thin-layer plates of the product mixtures of these enzymatic oxidations are presently thought to be due to compounds produced by such reactions as:




(Reference 33)

The phenoxy radical itself can react to form the diphenyl ether structure shown:



(Reference 34)

Two compounds, dehydrodivanillin (35) and dehydrodivanillyl alcohol (36) were used as TLC standards in an attempt to match Rf values and color reactions with the unknown products of the reactions. Dehydrodivanillin was shown to be definitely absent, even in the products of the oxidation of XI, and though dehydrodivanillyl alcohol had an Rf value in the requisite range (0.15), its bright blue fluorescence under ultraviolet light had no counterpart among the products of the oxidation reactions.

Disyringylmethane was rejected as a standard for comparison, since the compounds possibly produced which are of this structure are sufficiently different in substitutions as to make a judgment about their presence in the reaction mixtures impossible. The low yields and similar small Rf values of the unidentified products of all of the ether model reactions make them very difficult to separate in quantities large enough to analyze; work upon them will continue.

Although the syringyl (VIIIb, VIIIc) and guaiacyl (VIIIa, XI) ethers react with hydrogen peroxide and enzyme

with equal facility (colored products forming in both cases within minutes of the addition of enzyme and oxidant) it is apparent that there is a large difference in the extent of oxidation of the products of these model ethers. Comparison of the product mixtures of the oxidations of compounds VIIIa, VIIIb, VIIIc and XI shows that vanillyl alcohol, or the vanillyl alcohol moiety (one equivalent produced from the cleavage of VIIIb, VIIIc and XI; two equivalents produced from the cleavage of VIIIa) reacts relatively slowly to produce the corresponding aldehyde and more slowly yet to produce the p-benzoquinone. When vanillin is releases in the reaction (as in the case of XI) some 2-methoxy-p-benzoquinone is produced, but the greater part of the residues remain less oxidized, i.e. as vanillin and vanillyl alcohol.

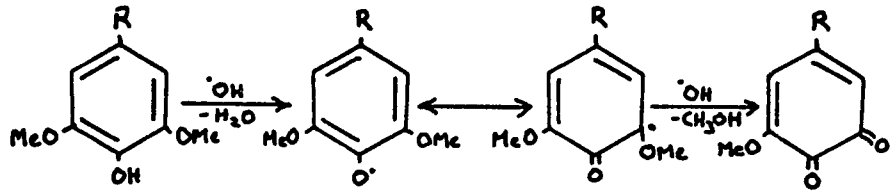
The syringyl compound VIIIb is oxidized with much greater facility; only a small amount of syringyl alcohol is produced, the major product being syringaldehyde. A small amount of 2,6-dimethoxy-p-benzoquinone is always produced as well. When an excess of peroxide is reacted with VIIIb, even less syringyl alcohol remains in the product mixture, the major products being syringaldehyde and the p-benzoquinone. In addition, some vanillin is produced, and a small amount of 2-methoxy-p-benzoquinone is observed in the product mixture.

Oxidation of VIIIc with 2 equivalents of peroxide yields acetosyringone as the major product, followed by vanillin and vanillyl alcohol. No p-benzoquinone is produced in this reaction. Since a secondary benzyl carbonium ion is formed upon hydrolysis of VIIIc, hydrolysis products are slightly more prevalent in the product mixture of this reaction. Both α-methyl syringyl alcohol and 1-(syringyl)-ethylene are observed.

In order to investigate more fully the relative ease of oxidation of syringyl and vanillyl residues, a competing reaction was carried out with equimolar amounts of syringyl and vanillyl alcohols with 2 molar equivalents of peroxide. The product mixture, analyzed by thin-layer chromatography, showed syringaldehyde as the major product with smaller amounts of 2,6-dimethoxy-p-benzoquinone and vanillin appearing and small and approximately equal amounts of starting materials left in the product mixture. From the results of this reaction and those of the model ethers, it is evident that the syringyl moiety is the easiest to oxidize, forming syringaldehyde, which in turn oxidizes easily to the corresponding p-quinone until the concentration of quinone becomes great enough to interfere with the function of the enzyme catalyst. The α-methyl syringyl analogue oxidizes to acetosyringone with equal facility, but apparently the elimination of acetaldehyde, analogous to the syringyl case of the removal of formaldehyde, to form the

corresponding p-quinone is a higher-energy reaction than oxidation of the vanillyl alcohol residue to form vanillin. Vanillyl alcohol moieties react most sluggishly to form vanillin, and only in those cases where vanillin is already present (as with XI) or where there is a large excess of oxidant does the 2-methoxy-p-quinone form. The lack of resonance stability and resultant diversion of the radical to the 5-position on the vanillyl moieties is the probable cause of the apparently higher oxidation potential of these moieties. The presence of unidentified low-Rf spots on chromatograms of the product mixture of the vanillyl and syringyl alcohol oxidation is probably due to reactions of the 5-position radical of vanillyl alcohol.

A small amount of low-Rf red chromophoric product was a feature of the oxidations of VIIIb, VIIIc and the syringyl alcohol-vanillyl alcohol mixture. This chromophore was conspicuously absent in all the oxidations of the guaiacyl model ethers. Since the chromophore appeared only to the extent of a few percent and since its Rf value was too low to allow for easy separation and identification, it remains an unknown quantity. However, since o-quinones have a red color, it is speculated that a side reaction such as the following could be involved in the production of the red chromophore.




For a comparison, 5-hydroxyvanillin was oxidized to the corresponding o-quinone and chromatographed. This compound had the requisite red color but its Rf value was much too high (0.85) to consider it as a possibility. However, it was noted that solutions of the syringyl ether product mixtures, when allowed to stand in air for a few days, produced relatively high-Rf red chromophores, while the low-Rf analogues disappeared. On the suspicion that the red chromophore was a benzyl alcohol derivative which might air-oxidize to the aldehyde after a few days, the 5-hydroxy-vanillin was reduced to the benzyl alcohol and oxidized as before to the o-quinone. The result was a mixture of products with the red compound of the requisite low Rf. A mass spectrum of the chromatographed product mixture was run and a parent peak of 168, the molecular weight of 5-hydroxy-vanillyl alcohol o-quinone, was obtained. Confirmation of this structure awaits the separation of the actual compound from the product mixtures of the oxidations of the model ethers, but the evidence obtained thus far seems to point to a structure of this type.

As in the acid hydrolysis experiments, the model ethers seem to compare well with lignin itself under conditions of enzymatic oxidation. Vanillin and syringaldehyde (11) and 2,6-dimethoxy-p-benzoquinone (17) all have been isolated from the products of hardwoods attacked by fungal oxidizing enzymes. Various syringyl and vanillyl ketone and alcohol derivatives have also come from the same source. The synthesis of a model ether with an α-ethyl group on the benzyl carbon would, in all probability, result in closer analogues to some of the more complex structures isolated from hardwood lignins after oxidation and hydrolysis.

## Electron Spin-resonance Studies

In order to gain insight into the nature of the radical species produced during the enzymatic oxidations of the ether model compounds, a number of their enzymatic oxidations were monitored by electron spin-resonance spectroscopy. Solutions of substrate, enzyme and oxidant were mixed rapidly, poured into a flat quartz cell and the buildup and decay of radical species were monitored by successive scans on the spectrometer.

It was found that the radical species produced by the guaiacyl ethers VIIIa and IX were too rapidly quenched to become concentrated enough to be visible. However, the syringyl analogues VIIIb and VIIIc formed stable radicals,

and by reducing the concentration of enzyme in the reaction, the oxidation was slowed enough to give the radical species time to form in observable concentrations without quenching. For best results, it remained necessary to use a minimum of 2 equivalents of peroxide in these reactions.

Figure 3 shows the progress of the oxidation of compound VIIIb. The first radical species formed gives what appears to be a symmetrical septuplet surrounded by two quintets. Except for a slightly larger set of hyperfine coupling constants, this pattern strongly resembles that of the radical formed upon oxidation of syringyl alcohol (29). It is therefore postulated that this first spectrum is that of radical XVIIIb, with the change in coupling constants due to the ether substitution instead of the alcohol on the benzyl carbon. A true spectrum of this radical would be a triplet of nonuplets, but the aqueous solutions and high dilution necessary for the reaction of the model ethers causes a loss in resolution and the outer peaks are thus obscured.

After a period of time, this radical disappears, probably by conversion to the nonparamagnetic quinone methide intermediate. Then, another pattern begins to form, this time apparently a doublet of quintets. This pattern is reminiscent of that of 2,6-disubstituted benzosemiquinones (8, p. 47) so it is speculated that this species may be one of the two radicals shown below:

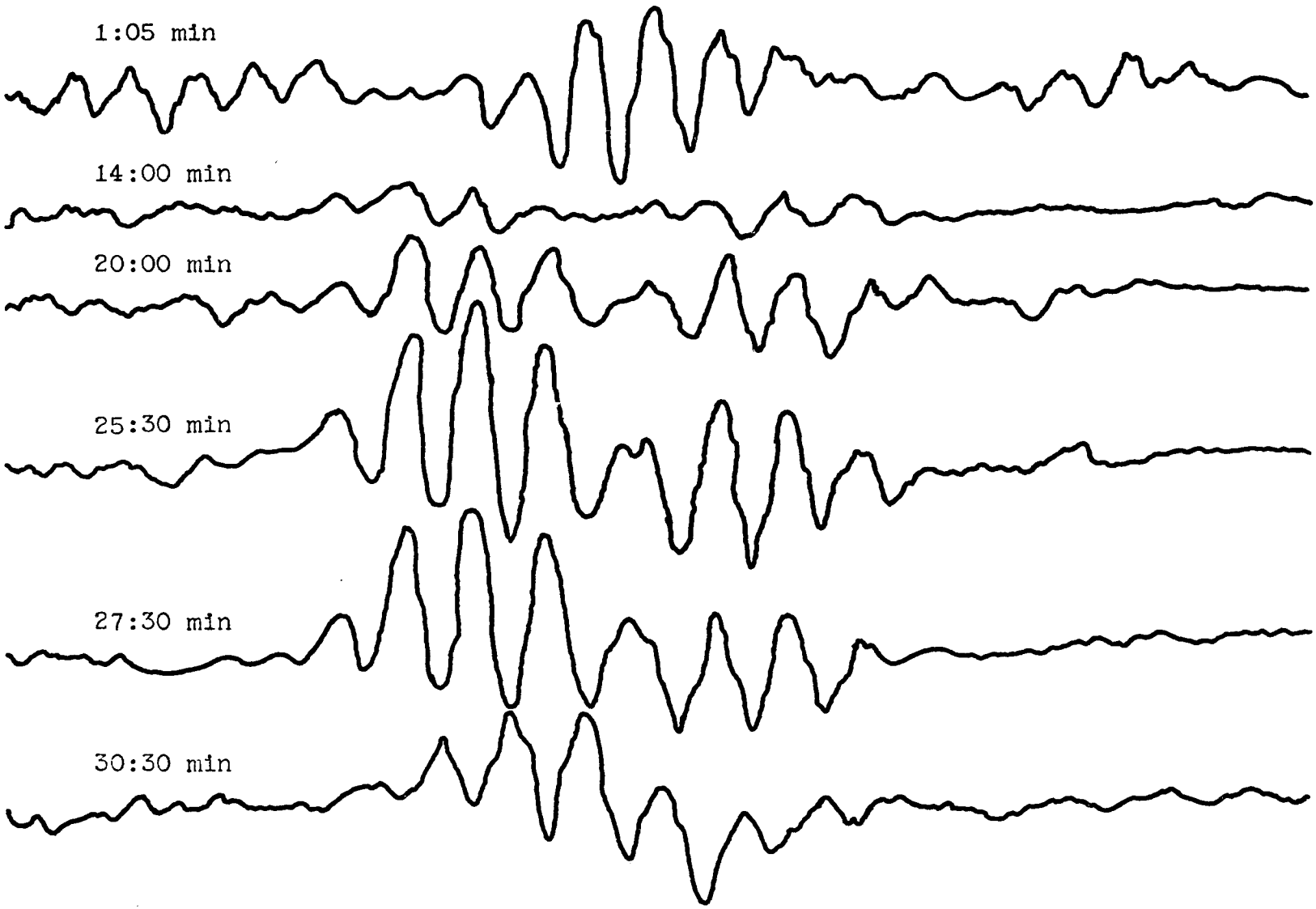
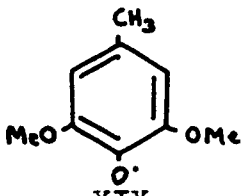
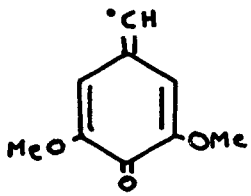



Figure 3. ESR Spectrum of Enzymatic Oxidation of 3,5-Dimethoxy-4-hydroxybenzyl-2'-methoxy-4'-hydroxymethylphenyl Ether. -- Scan Length is 27.5 Gauss.

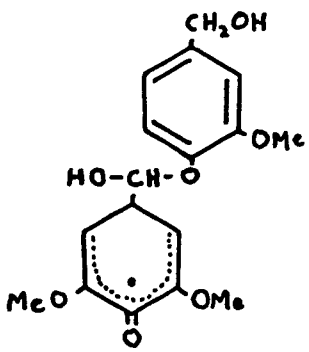



XIX




XX

These species can be formed either by enzymatic dehydrogenation of the quinone methide intermediate of XVIIIb, or they may come about via the disproportionation of two molecules of quinone methide. Since in time this pattern merges into the spectrum of the radical of syringaldehyde (4), a third possibility exists for the structure of this radical intermediate, structure XXI, below:




XXI

This structure may come about via the addition of a hydroxy radical to the quinone methide intermediate. Upon elimination of the phenoxy group, this structure can smoothly tautomerize to syringaldehyde. For comparison, the two routes to the aldehyde from these postulated intermediates are shown below:

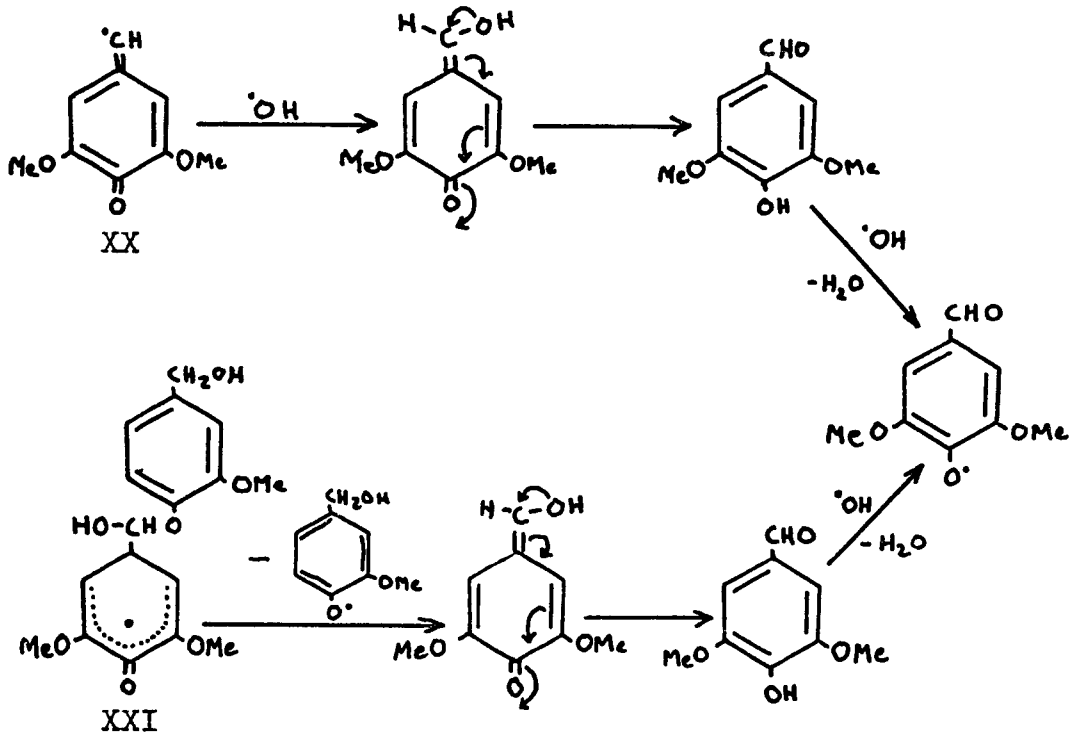



Figure 4 shows the oxidation of VIIIc. This compound, because of problems of product solubility, was run in a cellosolve--water solution (37), instead of methanol--water. A complex spectrum of 15 lines, reminiscent of that of α-methyl syringyl alcohol under the same conditions (29), forms, fades and is replaced by the spectrum of the radical of acetosyringone (4). In this case, no intermediate radical of the above type forms, possibly because of a lack of benzyl hydrogen atoms to lose. Rather than a mechanism such as that which forms XXI occurring, it appears that the quinone methide simply hydrates, eliminates the phenol and tautomerizes to acetosyringone:

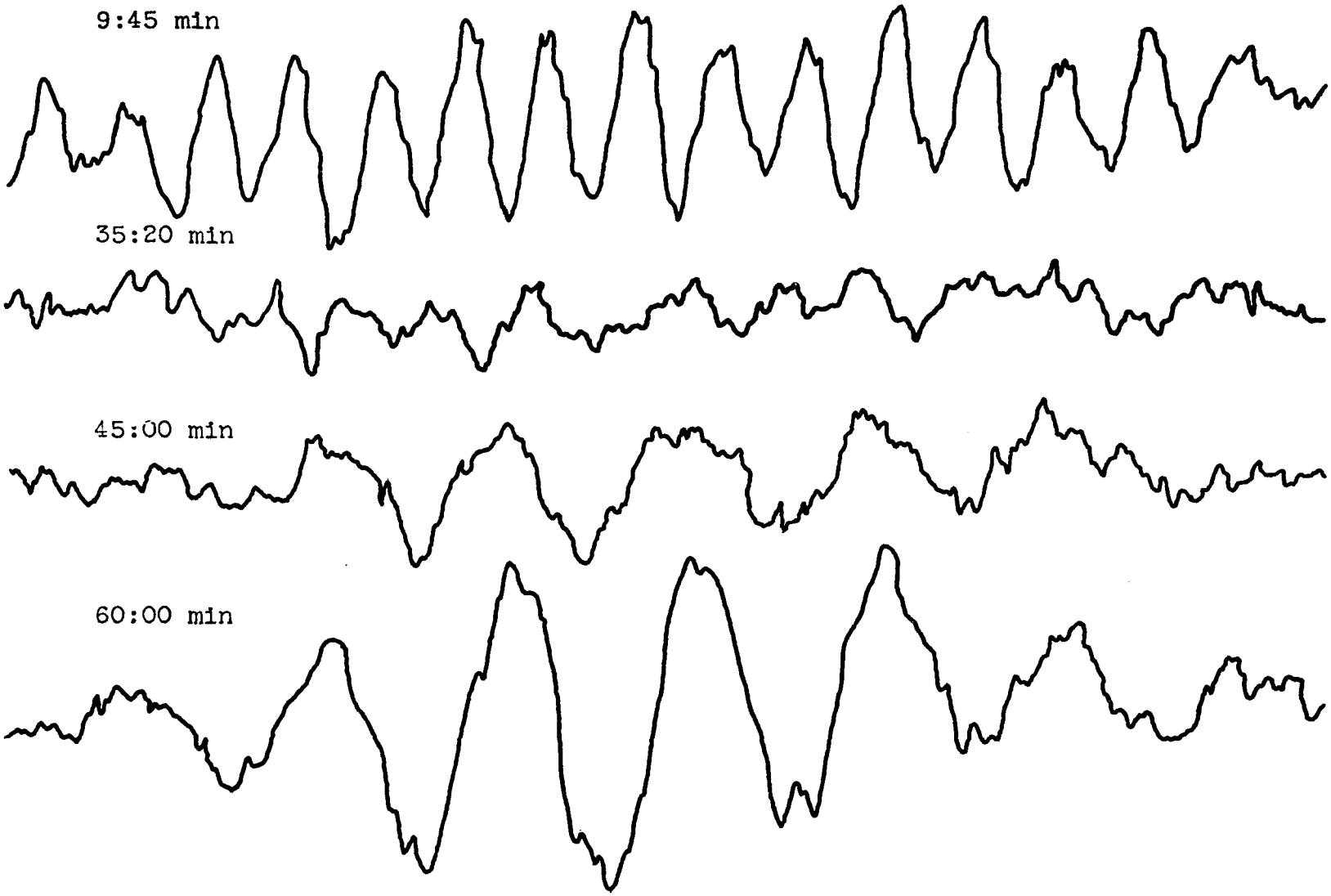
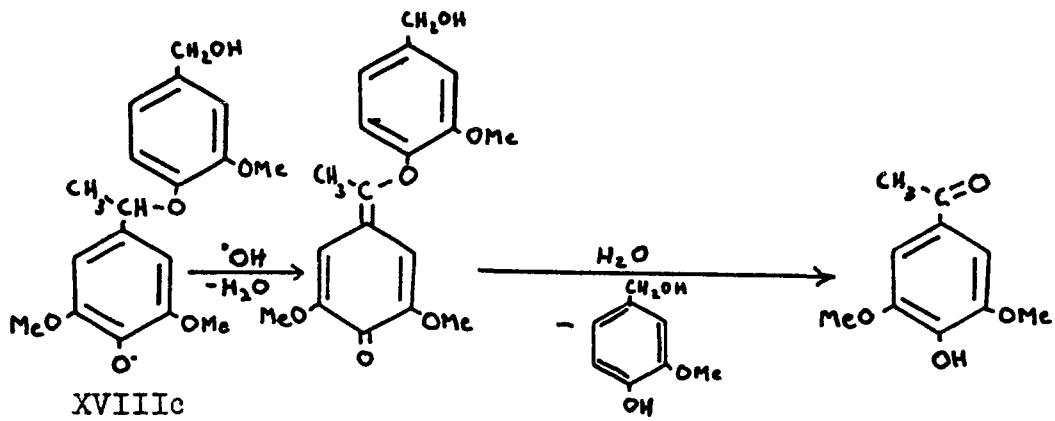



Figure 4. ESR Spectrum of Enzymatic Oxidation of 1-(3,5-Dimethoxy-4-hydroxyphenyl)-ethyl-2'-methoxy-4'-hydroxymethylphenyl Ether. -- Scan Length is 11.0 Gauss.



XVIIIc

Hyperfine coupling constants for the radical species produced from compounds VIIIb and VIIIc are given in Table 4. For comparison, the constants for the radicals produced from the analogous alcohols are also given. The ether compounds differed from the alcohols not only in their coupling constants, but also in the times required for the oxidations to the carbonyl derivatives. Compound VIIIb required 37 min for complete oxidation, while syringyl alcohol was oxidized in 20 min. Compound VIIIc oxidized in 61 min, but the analogous alcohol required 161 min to oxidize to acetosyringone. The difference in the reaction times probably reflects the greater stability of the phenoxy-substituted quinone methide over the hydroxy-substituted analogue, and also the steric effects which would make the phenoxy a better leaving group than the hydroxy.

Table 4. Hyperfine Coupling Constants (in Gauss) of Radical Intermediates and Products of Ether and Alcohol Oxidations.

| Radical | Solvent* | Coupling Constants | |
|---|---|---|---|
| | | $A^{H}_{ring,\ OCH_3}$ | $A^{H}_{side\ chain}$ |
| XVIIIb | A | 1.36 (8) | 19.00 (2) |
| XX | A | 1.48 (8) | 4.88 (1) |
| Syringaldehyde | A | 1.55 (8) | |
| XVIIIc | B | 0.69 (8) | 5.60 (4) |
| Acetosyringone | B | 1.61 (8) | |
| Syringyl alcohol | A | 1.16 (8) | 18.64 (2) |
| α-Methyl syringyl alcohol | A | 0.70 (8) | 4.34 (4) |

*A. 22.5% Methanol--water.

B. 12.5% Cellosolve--water.

The low concentrations of radicals formed in these reactions made it impossible to separate the contributions of the ring and methoxy hydrogens to the coupling constants, and to assign accurate ratios for the spectral lines. Even the final products, which normally have quite prominent lines, gave rather stunted spectra. The hydroxy radicals produced by the enzyme from hydrogen peroxide were being quenched by the vanillyl moieties cleaved from the ethers during the oxidations, leaving fewer of them to produce the intermediates that were capable of showing in the spectra. The result was a weaker spectrum than is normally obtained. Addition of more peroxide to the reaction would probably result in stronger spectra but it would also speed the reaction to a point where intermediates could not be monitored by the machine.

Although phenoxy radicals exist in wood and ESR spectra of them resemble those of some model compounds (29), it is not presently known whether the model ethers generate spectra comparable to those of lignin. The visibility of radicals such as XVIIIb and XVIIIc only gets appreciable when both the substrate and the oxidation products are completely soluble in the reaction medium. With a polymeric substance such as lignin, solubility problems are acute even with the substrate and therefore only the most stable radical species (i.e., syringaldehyde and acetosyringone-type moieties) are found in resolvable concentrations.

The use of solvents such as cellosolve, which can be used in large concentrations without seriously inhibiting enzyme action (37), may result in the formation of radicals like those obtained from these model ethers.

# EXPERIMENTAL

## General

Starting materials were purchased from Aldrich Chemical Company, Milwaukee, Wisconsin. Horseradish peroxidase enzyme Type VI (M.W. 44,100) was purchased from Sigma Chemical Company, St. Louis, Missouri and a stock solution of $1.8 \times 10^{-6}$ M enzyme in glass-distilled water was used in the enzymatic oxidations.

Thin-layer chromatography was carried out on precoated plates of Silica Gel F-254, layer thickness 0.25 mm, purchased from E. Merck, Darmstadt, Germany. Column chromatography was done on Silica Gel Powder, 60-200 mesh, made by J. T. Baker Chemical Company, Phillipsburg, New Jersey.

Nuclear magnetic resonance (NMR) studies were done on a Varian T-60 spectrometer using tetramethylsilane as an internal standard. Chemical shift values are given in $\delta$ (ppm) units in the text. In addition, Figures 6-18 illustrate the spectra of the benzyl-aryl ethers synthesized. Carbon-13 NMR spectra were recorded on a Bruker WH-90 $^{13}C$ Resonance Spectrometer using dimethylsulfoxide-$d_6$ as the solvent and tetramethylsilane and deuterium oxide as internal standards.

Infrared spectra were recorded on a Perkin-Elmer 137 Spectrophotometer in chloroform solution, unless otherwise stated.

Visible spectra were monitored on a Bausch & Lomb UV-Visible Spectrophotometer, calibrated to 0 and 100% absorbance before each run.

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

Electron spin-resonance (ESR) spectra were recorded on a Varian E-3 ESR Spectrometer. Solutions were placed in the cavity of the machine in flat quartz cells and the spectra were monitored at 95.9 gHz with a frequency modulation of 100 kHz.

Melting points were taken on a Laboratory Devices Meltemp apparatus and are uncorrected.

Elemental analyses were performed by Huffman Laboratories of Wheatridge, Colorado and Scandanavian Microanalytical Laboratories, Herlev, Denmark.

## Syntheses of Monomeric Compounds

### 4-O-Acetylvanillin (Va) and 4-O-Acetylsyringaldehyde (Vb)

A solution was made in 30 ml of anhydrous pyridine of 10 gm of vanillin and 7 gm of acetic anhydride was added. The mixture was stirred and warmed for 1 hr, then poured into 400 ml of icewater. The crystals which formed

were collected by vacuum filtration, washed with water, dried and recrystallized from methanol--water. Yield 12.5 gm (98%); mp 71-72.5°; /76-77° (38)/.

In the same manner, 10 gm of syringaldehyde was treated in pyridine with 6 gm of acetic anhydride, worked up and recrystallized as before. Yield 11 gm (89%); mp 111-113.5°; /112-113° (39)/.

3-Methoxy-4-O-acetylbenzyl Alcohol (VIa) and
3,5-Dimethoxy-4-O-acetylbenzyl Alcohol (VIb)

A 12.5-gm sample of Va was suspended in 50 ml of 95% ethanol and to this mixture was added 0.3 gm of 10% Pd on activated charcoal. The resultant mixture was stirred overnight under 1 atmosphere of hydrogen. As the reaction progressed, the sparingly soluble starting material was comverted into the soluble alcohol and vanished into solution. The solution was filtered through sintered glass to remove the catalyst and the solvent was evaporated to yield a pale-yellow oil. This oil was generally used directly in further reactions, but a small amount was recrystallized from ether--pentane. Yield 10.7 gm (98%); mp 46-48.5°; /oil (18)/.

In the same manner, 11 gm of Vb was hydrogenated to yield 10.6 gm of a pale-yellow oil which crystallized on standing and was recrystallized as before, mp 93.5-95.5°; nmr ($CDCl_3$) $\delta$ 2.21 (s, 3), 3.68 (s, 6), 4.42 (s, 2), 6.54

(s, 2); mass spectrum m/e (rel intensity) 226 (14), 185 (18), 184 (100), 167 (20), 123 (16), 109 (14), 43 (23).

4-O-Acetylacetosyringone (Vc) and 1-(3,5-Dimethoxy-4-O-acetylphenyl)-ethanol (VIc)

Acetosyringone, 15 gm, was dissolved in 40 ml of anhydrous pyridine and the solution treated with 8 ml of acetic anhydride. The mixture was warmed for 1 hr with stirring, then cooled and poured into icewater. The crystals were collected by vacuum filtration, washed, dried and recrystallized from acetone--water. Yield 18.3 gm (100%); mp 154-157°; nmr ($CDCl_3$) $\delta$ 2.34 (s, 3), 2.58 (s, 3), 3.82 (s, 6), 7.19 (s, 2); mass spectrum m/e (rel intensity) 238 (15), 197 (43), 196 (100), 182 (43), 181 (100), 153 (21), 66 (21), 43 (82).

A 12-gm sample of Vc was suspended in 50 ml of 95% ethanol and 0.4 gm of 10% Pd on activated charcoal was added. The suspension was stirred under 1 atmosphere of hydrogen overnight. As in previous reactions, the solid ketone was converted to the alcohol, which dissolved as the reaction progressed. The catalyst was filtered off on sintered glass and the solvent was evaporated under vacuum. The yellow oil which resulted crystallized on standing and was used in subsequent preparations without further purification. A small amount was recrystallized from ether--pentane to obtain a melting point. Yield 12 gm (99%); mp 76-77.5°; nmr ($CDCl_3$) $\delta$ 2.01 (d, 3), 2.12 (s, 3),

3.76 (s, 6), 5.44 (q, 1), 6.88 (s, 2); mass spectrum m/e (rel intensity) 240 (20), 198 (85), 183 (100), 155 (80), 137 (30), 123 (80), 109 (40).

4-Carbethoxysyringaldehyde and 4-Carbethoxyacetosyringone

Syringaldehyde, 4 gm was dissolved in 15 ml of dry pyridine and 4 gm of ethyl chloroformate was added dropwise. The solution was stirred and warmed for 1 hr, then poured into icewater. The crystals were collected by vacuum filtration, washed with water, dried and recrystallized from acetone--water. Yield 5.5 gm (98.5%); mp 94.5-96.5°; nmr ($CDCl_3$) δ 1.39 (t, 3), 3.90 (s, 6), 4.31 (q, 2), 7.10 (s, 2), 9.86 (s, 1); mass spectrum m/e (rel intensity) 254 (9), 210 (12), 183 (24), 182 (100), 181 (85), 167 (23), 28 (37).

In the same manner, 10 gm of acetysyringone was dissolved in 40 ml of pyridine and treated with 6 gm of ethyl chloroformate. Workup followed the above procedure. Yield 13 gm (95%); mp 109-111°; nmr ($CDCl_3$) δ 1.45 (t, 3), 2.58 (s, 3), 3.84 (s, 6), 4.22 (q, 2), 7.20 (s, 2); mass spectrum m/e (rel intensity) 268 (19), 224 (17), 196 (93), 181 (100), 153 (17), 43 (50).

3,5-Dimethoxy-4-carbethoxybenzyl Alcohol and 1-(3,5-Dimethoxy-4-carbethoxyphenyl)-ethanol

A 6-gm sample of 4-carbethoxysyringaldehyde was suspended in 50 ml of 95% ethanol and 0.1 gm of 10% Pd on activated charcoal was added. The mixture was stirred

overnight under 1 atmosphere of hydrogen. The solid aldehyde reacted and went into solution as in the case of the analogous acetate Vb. The catalyst was filtered off through sintered glass and the solvent evaporated at low pressure. A pale-yellow oil resulted, which crystallized on standing. Recrystallization occurred from ether--pentane. Yield 6 gm (99%); mp 72-74°; nmr ($CDCl_3$) $\delta$ 1.34 (t, 3), 3.78 (s, 6), 4.27 (q, 2), 4.52 (s, 2), 6.55 (s, 2); mass spectrum m/e (rel intensity) 270 (26), 198 (87), 183 (100), 181 (52), 155 (86), 123 (88), 95 (51), 83 (57).

1-(3,5-Dimethoxy-4-hydroxyphenyl)-propanol (XII)

A solution was prepared of 9 gm of ethyl bromide in 19 ml of dry tetrahydrofuran (THF). This solution was added dropwise to a stirring mixture of 2 gm of magnesium turnings in 5 ml of THF. At the end of the reaction, the Grignard solution was transferred to a dropping funnel and added to a solution of 2.5 gm of syringaldehyde in 50 ml of THF. After addition was complete, the reaction was stirred and refluxed for 30 min and allowed to stand at room temperature overnight. The reaction mixture was then poured into very dilute hydrochloric acid solution and extracted twice with 40-ml portions of chloroform. The fractions were combined, washed once with water, dried ($Na_2SO_4$) and evaporated under low pressure. An oil resulted which quickly formed crystals

on standing. These were recrystallized from ether. Yield 1.6 gm (55%); mp 91-94°; /94-95° (40)/.

1-(3,5-Dimethoxy-4-hydroxyphenyl)-
3-methylpropanol (XIII)

Isopropyl magmesium bromide was prepared by reacting 13.6 gm of isopropyl bromide with 2.54 gm of magnesium turnings in 150 ml of THF as in the procedure above. After the reaction was complete, the solution was added as before to a solution of 10 gm of syringaldehyde in 150 ml of THF. The reaction mixture was stirred at room temperature overnight and poured into a saturated solution of ammonium chloride containing a few drops of dilute hydrochloric acid. The organic layer was removed and the aqueous layer was extracted twice with 50-ml portions of chloroform. The organic phases were combined, dried and evaporated as before. The product was recrystallized from benzene. Yield 7 gm (56.5%); mp 131-133°; nmr ($CDCl_3$) $\delta$ 0.78 (d, 3), 0.99 (d, 3), 1.95 (m, 2), 3.86 (s, 6), 4.21 (d, 1), 5.52 (s, 1), 6.54 (s, 2); mass spectrum m/e (rel intensity) 226 (50), 183 (100), 155 (42), 123 (80), 95 (35); ir 3518 (OH), 3000 ($CH_3$), 1120 ($OCH_3$).

2-(3,5-Dimethoxy-4-hydroxyphenyl)-
2-butanol (XIV)

A solution of ethyl magnesium bromide was prepared as before with 11.1 gm of ethyl bromide and 2.5 gm of magnesium turnings in 100 ml of THF. This solution was

added as before to a stirred THF solution of 10 gm of acetosyringone. The reaction was allowed to stir at room temperature overnight, then poured into a saturated solution of ammonium chloride. Workup and recrystallization followed the previous procedures. Yield 4 gm (34.7%); mp 119-122°; nmr ($CDCl_3$) $\delta$ 0.78 (t, 3), 1.49 (s, 3), 1.77 (q, 2), 3.84 (s, 6), 5.51 (s, 1), 6.74 (s, 2); mass spectrum m/e (rel intensity) 226 (42), 197 (88), 155 (83), 140 (34), 57 (59), 43 (100); ir 3507 (OH), 2950 ($CH_3$) 1110 ($OCH_3$).

4-O-Benzylsyringaldehyde

A solution of 5 gm of syringaldehyde in 15 ml of absolute methanol was prepared and stirred under nitrogen. A 10-ml methanolic solution of 1.1 gm of sodium hydroxide was added to the syringaldehyde solution and the mixture was brought up to reflux temperature. To this mixture was added dropwise a solution of 4.7 gm of benzyl bromide in 5 ml of methanol. Reflux was continued for 1 hr after addition was complete and the mixture was allowed to stir under nitrogen at room temperature overnight. The mixture was poured into dilute aqueous sodium bicarbonate solution and extracted twice with 75-ml portions of chloroform. The extracts were combined, washed quickly with water, dried ($Na_2SO_4$) and evaporated. The resulting oil crystallized on standing and was recrystallized from ether--pentane. Yield 6.6 gm (88%); mp 50-52.5°; nmr ($CDCl_3$) $\delta$ 3.71 (s, 6), 5.04 (s, 2),

7.00 (s, 2), 7.30 (m, 5), 10.33 (s, 1); mass spectrum m/e (rel intensity) 272 (46), 92 (42), 91 (100), 65 (34), 39 (19).

cis- and trans-2-(3,5-Dimethoxy-4-hydroxyphenyl)-2-butene

A stirred solution of 7.8 gm of triphenylphosphine in 50 ml of dimethylformamide was made and to this was added dropwise 3.5 gm of ethyl bromide. After addition was complete, the reaction mixture was heated to reflux for 1.5 hr, cooled and allowed to stir at room temperature overnight. The dimethylformamide solution was then poured into an excess of ether. After a few minutes, a grainy white precipitate of ethyl phosphonium bromide began to form. The precipitate was filtered, washed thoroughly with petroleum ether and dried. Yield 11 gm (100%); mp 208-209.5°; [203-205° (41)].

A slurry of 19.6 gm of ethyl phosphonium bromide in 175 ml of dry benzene was stirred under nitrogen. Into this slurry was dissolved 4 gm of benzoic acid. A 2.2 M hexane solution of n-butyllithium was added to the slurry until the red color of the ylid had reached maximum intensity. In all, 60 ml of the n-butyllithium solution were added. The solution was stirred and heated for 2 hr, but despite the heat and the excess of n-butyllithium, some of the ethyl phosphonium bromide remained unreacted. The reaction was then cooled to 0° and a solution of 5 gm of Vc in 200 ml of

dry benzene was added dropwise with stirring. When addition was complete, the mixture was allowed to sit for 1.5 days at room temperature. The reaction mixture was then poured into saturated sodium bicarbonate solution and the organic layer was washed with water, dried ($MgSO_4$) and evaporated. The resultant oil was distilled under reduced pressure to yield a clear, light yellow oil. The NMR spectrum showed the cis, trans composition of the mixture to be 57 and 43%, respectively. Yield 1.32 gm (30%); bp 129-155° (4 mm); nmr ($CDCl_3$) $\delta$ 1.56, 1.70 (d, 3), 1.91 (s, 3), 3.74 (s, 6), 5.39, 5.65 (d, 1), 6.31, 6.48 (s, 2); mass spectrum m/e (rel intensity) 208 (22), 123 (19), 121 (85), 119 (99), 117 (100), 94 (30), 84 (63), 82 (73), 47 (57), 35 (34); ir (smear) 3770 (OH), 1602 (C=C), 1235 ($OCH_3$).

3-(3,5-Dimethoxy-4-hydroxyphenyl)-1-propene

A solution of 30 gm of 2,6-dimethoxyphenol in 78 ml of dry acetone was prepared and to this was added 23.7 gm of allyl bromide and 35.3 gm of anhydrous potassium carbonate. This mixture was stirred and refluxed overnight and then the acetone was evaporated, 50 ml of water was added and the solution was extracted three times with 50-ml portions of ether. The extracts were combined, washed with water, dried ($K_2CO_3$) and evaporated. The product (crude 2,6-dimethoxyphenyl allyl ether) was refluxed at 100-150 mm for 3 hr, then the pressure was lowered to 35 mm and the product

distilled until the distillate no longer gave a positive ferric chloride test. Much of the phenyl allyl ether degraded to tar from the continuous heating; hence the low yield. Yield 17 gm (45%); bp 184° (35 mm); [123-125° (2 mm) (42)].

5-Hydroxyvanillyl Alcohol o-Quinone

A solution of 3 gm of 5-hydroxyvanillin was made in 25 ml of isopropyl alcohol and to this was added 3 gm of sodium borohydride. The solution was refluxed for 2 hr and allowed to stand overnight. The reaction mixture was then poured into saturated boric acid solution and extracted three times with 25-ml portions of chloroform. The extracts were combined, dried ($Na_2SO_4$) and evaporated. The product, a brown oil, was dissolved in methanol and poured into a solution of 4 gm of sodium periodate in 200 ml of water and shaken. When the red color of the o-quinone had reached a maximum in intensity, the solution was extracted with chloroform. The extract was dried ($K_2CO_3$) and evaporated. Column chromatography on silica gel with 50% ether--pentane separated a small amount of the o-quinone for mass spectral analysis. Mass spectrum m/e (rel intensity) 168 (4), 149 (16), 139 (5), 129 (24), 87 (25), 85 (93), 83 (100), 71 (30), 57 (50); vis max (95% ethanol) 950 nm (ε 25.0).

## Syntheses of Dimeric and Trimeric Compounds

### 3-Methoxy-4-O-acetylbenzyl-2'-methoxy-4'-formylphenyl Ether (VIIa) and 3,5-Dimethoxy-4-O-acetylbenzyl-2'-methoxy-4'-formylphenyl Ether (VIId)

A 20-ml chloroform solution of 10 gm of VIa was prepared and treated with a 10-ml chloroform solution of phosphorus tribromide. After addition was complete, the reaction mixture was boiled gently for 20 min. Chloroform was added occasionally to keep the volume constant.

An acetone solution of 7.8 gm of vanillin was stirred and refluxed under nitrogen with 7 gm of anhydrous potassium carbonate until a milky-yellow homogeneous slurry of potassium vanillate was formed. The chloroform solution of the benzyl bromide prepared above was washed once with water, dried ($MgSO_4$) and added dropwise to the acetone solution. The reaction was refluxed and stirred for 1 hr after addition was complete and let stir under nitrogen at room temperature overnight. The mixture was then poured into water and extracted twice with 75-ml portions of chloroform. The extracts were combined, washed twice with water, dried and evaporated, yielding a brown oil which crystallized out in the cold. The product was recrystallized from benzene--pentane. Yield 10.2 gm (61%); mp 121-123°; nmr ($CDCl_3$) $\delta$ 2.22 (s, 3), 3.68 (s, 3), 3.83 (s, 3), 5.07 (s, 2), 7.00 (m, 6), 9.97 (s, 1); mass spectrum m/e (rel intensity) 330 (1), 180 (71), 153 (33), 152 (21),

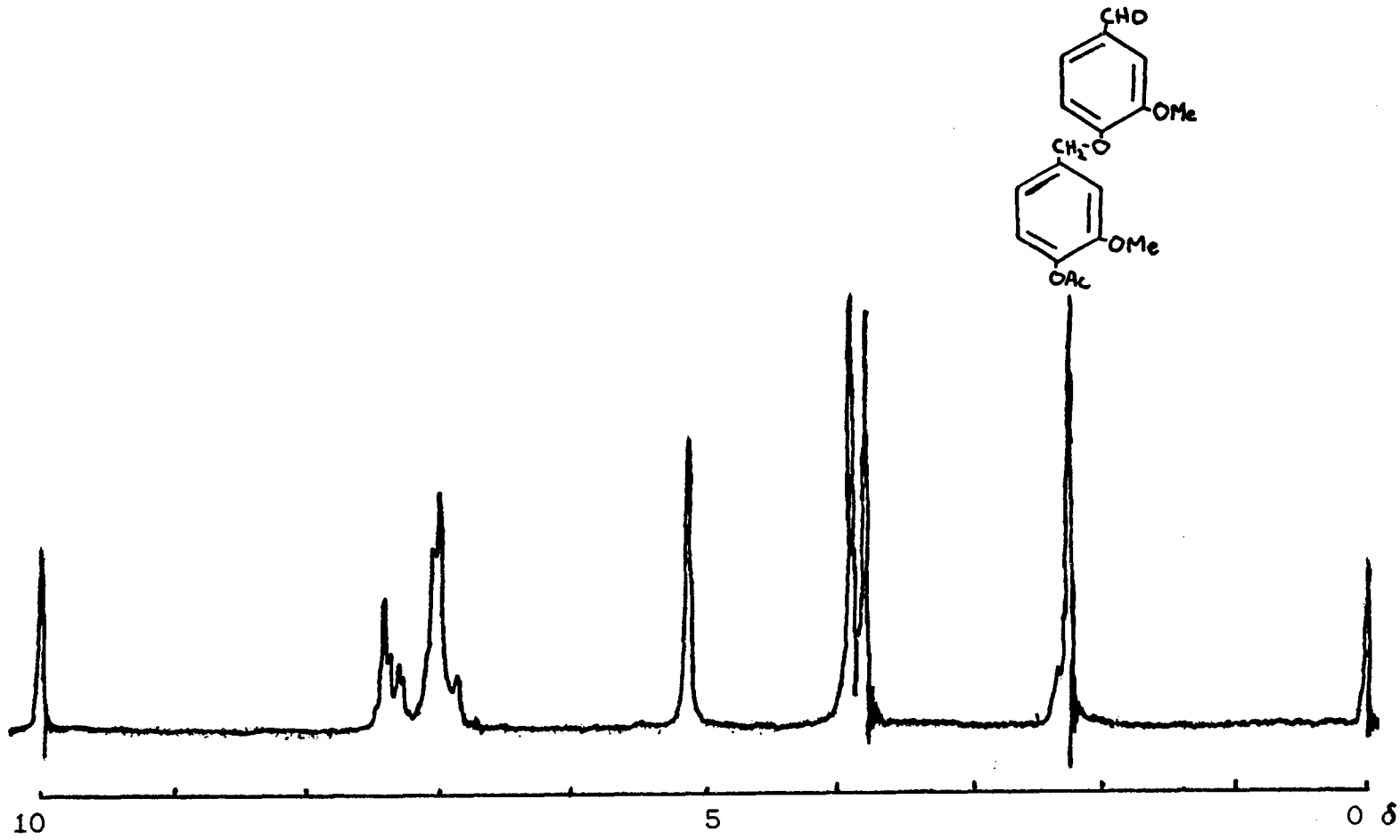



Figure 5. NMR spectrum of 3-Methoxy-4-O-acetylbenzyl-2'-methoxy-4'-formyl-phenyl Ether (VIIa).

139 (56), 138 (100), 123 (26), 108 (18); ir 2750 (CHO), 1745 (C=O), 1250 ($OCH_3$), 1112 (C-O-C).

In the same fashion, a 30-ml chloroform solution of 10.5 gm of VIb was treated with 12.5 gm of phosphorus tribromide previously dissolved in 10 ml of chloroform. This solution was treated as before and added to an acetone solution of 6.9 gm of vanillin refluxing under nitrogen with 2 gm of potassium hydroxide and 6 gm of potassium carbonate. The reaction was refluxed for 1 hr, let stir under nitrogen at room temperature overnight and worked up as before. The product was recrystallized from a small amount of methanol saturated with ether and pentane. Yield 10 gm (63%); mp 151.5-154°; nmr ($CDCl_3$) δ 2.30 (s, 3), 3.80 (s, 6), 3.93 (s, 3), 5.17 (s, 2), 6.71 (s, 2), 7.10 (m, 3), 10.08 (s, 1); mass spectrum m/e (rel intensity) 360 (4), 209 (82), 167 (100), 151 (40), 147 (49), 136 (22), 123 (30), 106 (18), 95 (25); ir 2850 (CHO), 1780 (C=O), 1190 ($OCH_3$), 1140 (C-O-C).

3,5-Dimethoxy-4-O-acetylbenzyl-2'-methoxyphenyl Ether (VIIc) and 3,5-Dimethoxy-4-O-acetylbenzyl-2'-methoxy-4'-(1-propenyl)-phenyl Ether (VIIe)

A solution of 0.5 gm of VIb in 10 ml of chloroform was reacted with 0.6 gm of phosphorus tribromide in 5 ml of chloroform as before. The solution was warmed for 15 min, washed and dried. An acetone solution of 0.56 gm of guaiacol was refluxed under nitrogen with 2 gm of potassium

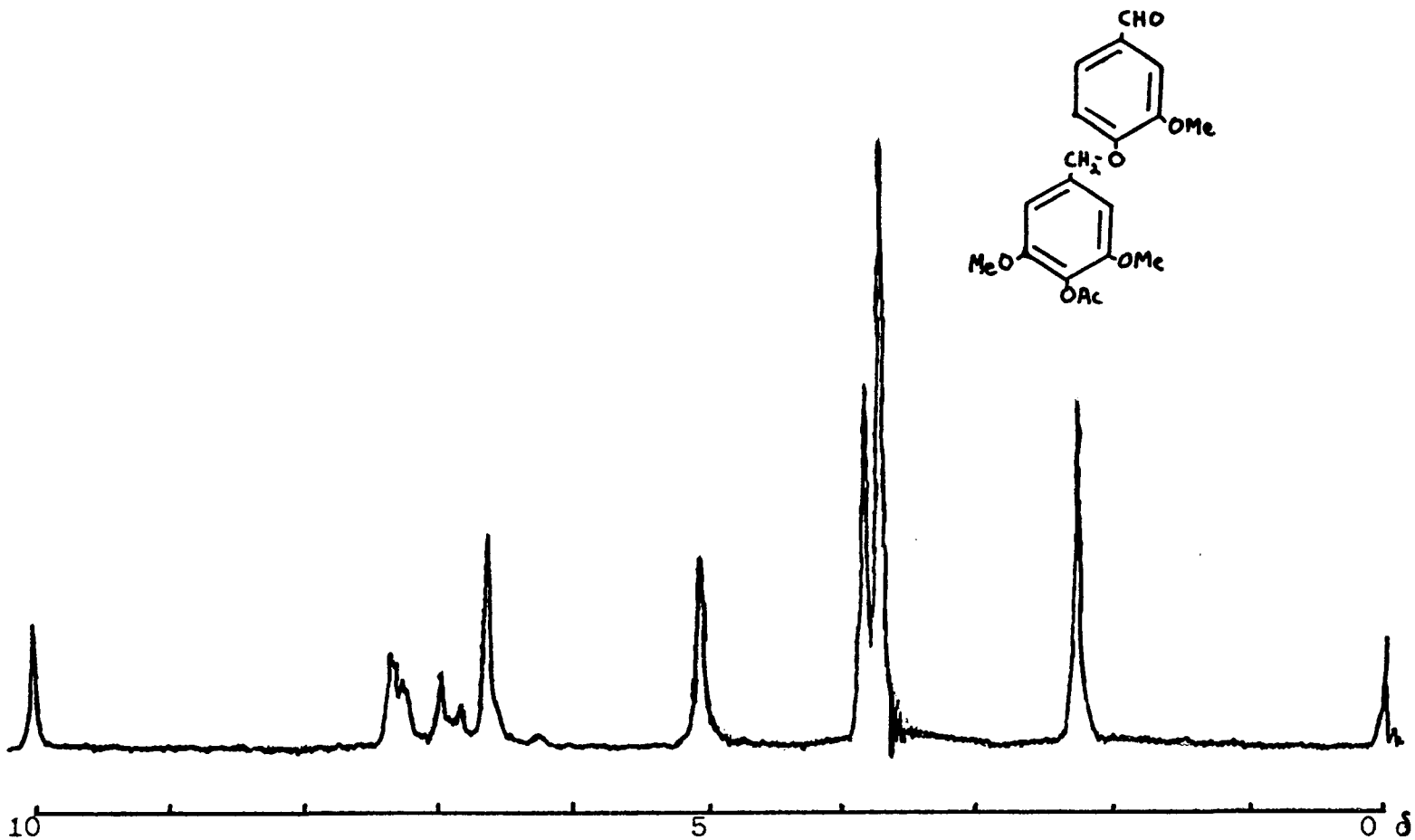



Figure 6. NMR spectrum of 3,5-Dimethoxy-4-O-acetylbenzyl-2'-methoxy-4'-formylphenyl Ether (VIId).

carbonate until a homogeneous pale-green slurry resulted. The chloroform solution was added dropwise and the mixture was heated for 30 min after addition was complete and allowed to stand at room temperature under nitrogen overnight. Workup was as before, yielding a yellow-brown oil. The product was recrystallized from chloroform--pentane. Yield 0.1 gm (13.6%); mp 96-99°; nmr ($CDCl_3$) $\delta$ 2.30 (s, 3), 3.79 (s, 6), 3.89 (s, 3), 5.08 (s, 2), 6.70 (s, 2), 6.90 (s, 4); mass spectrum m/e (rel intensity) 332 (15), 209 (84), 168 (58), 167 (100), 151 (25), 136 (15), 123 (20), 95 (11).

A solution of 2.0 gm of VIb in 30 ml of chloroform was treated with 2.4 gm of phosphorus tribromide as before. An acetone solution of 1.4 gm of isoeugenol was refluxed under nitrogen with 2 gm of potassium carbonate until a greenish-yellow homogeneous slurry was formed. The chloroform solution, after washing and drying, was then added dropwise and the stirring and refluxing was continued overnight in the nitrogen atmosphere. Workup followed previous procedures. The product was recrystallized from chloroform--pentane. Yield 0.5 gm (15%); mp 109-111°; nmr ($CDCl_3$) $\delta$ 1.75 (d, 3), 2.17 (s, 3), 3.63 (s, 6), 3.72 (s, 3), 4.88 (s, 2), 6.38 (m, 7); mass spectrum m/e (rel intensity) 372 (31), 206 (91), 167 (100), 165 (77), 164 (100), 163 (42), 149 (20), 123 (17), 107 (21); ir 1725 (C=O), 1570 (C=C), 1245 (C-O-C), 1135 ($OCH_3$).

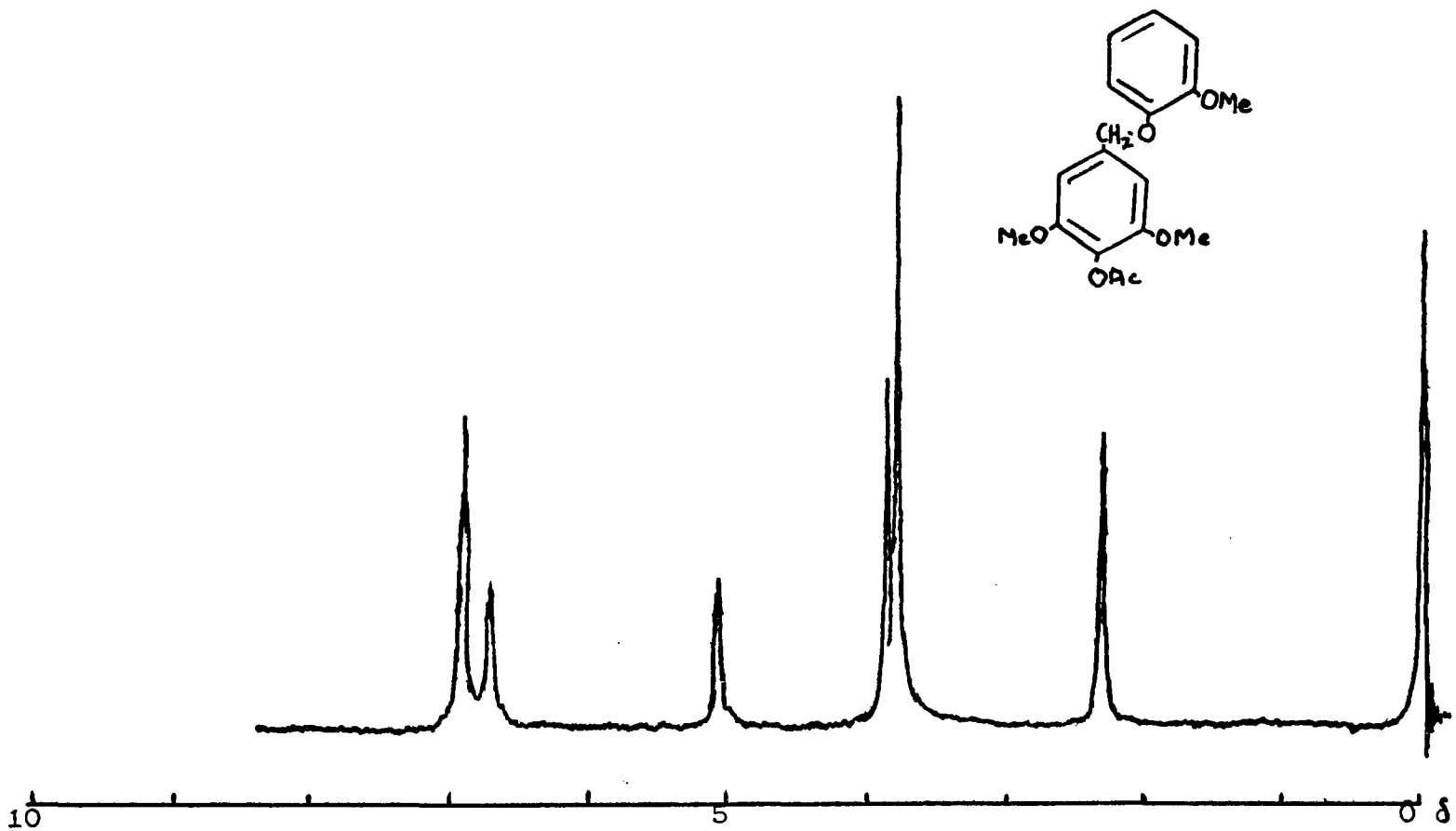



Figure 7. NMR spectrum of 3,5-Dimethoxy-4-O-acetylbenzyl-2'-methoxyphenyl Ether (VIIc).

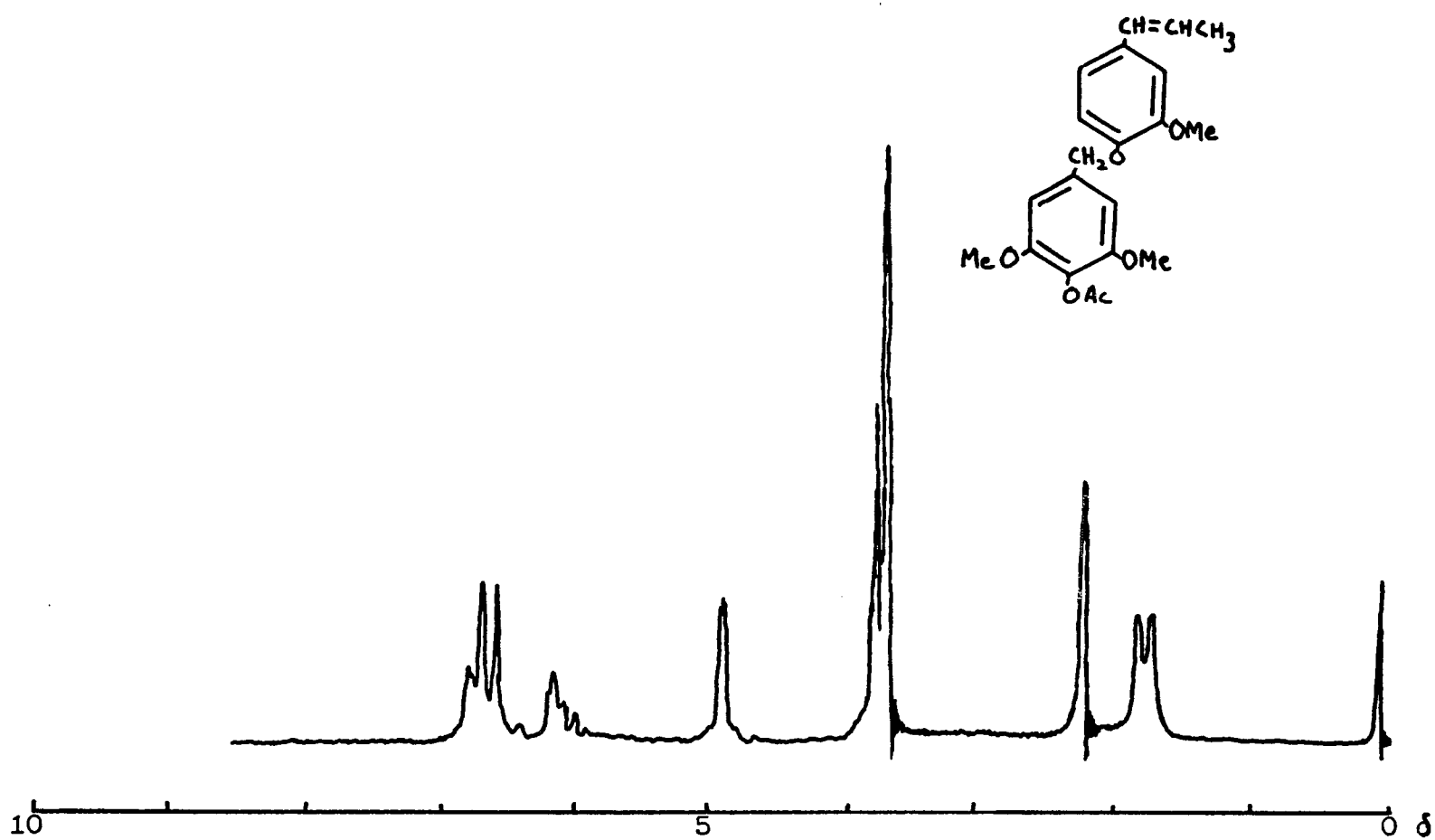



Figure 8. NMR spectrum of 3,5-Dimethoxy-4-O-acetylbenzyl-2'-methoxy-4'-(1-propenyl)-phenyl Ether (VIIe).

Anal. Calcd for $C_{21}H_{24}O_6$: C, 67.73; H, 6.50. Found: C, 67.16; H, 6.51.

3-Methoxy-4-O-acetylbenzyl-2'-methoxy-4'-hydroxymethylphenyl Ether (VIIb) and 3-Methoxy-4-O-acetylbenzyl-2'-methoxyphenyl-4'-benzyl-2"-methoxy-4"-formylphenyl Ether (IX)

A solution of 5 gm of VIa in 20 ml of chloroform was reacted with 7 gm of phosphorus tribromide as before. An acetone solution of 4 gm of vanillyl alcohol was refluxed under nitrogen with 4 gm of potassium carbonate. The washed and dried chloroform solution was added to the refluxing acetone slurry, refluxed for 2 hr after addition was complete and allowed to stir under nitrogen at room temperature overnight. Workup followed the previous procedures. The product was recrystallized from chloroform--ether. Yield 3.7 gm (44%); mp 132-137°; nmr (DMSO-$d_6$) $\delta$ 2.23 (s, 3), 3.78 (s, 6), 4.41 (d, 2), 5.03 (s, 2), 5.10 (m, 1), 7.02 (m, 6); mass spectrum m/e (rel intensity) 332 (5), 196 (24), 154 (81), 138 (22), 137 (100), 122 (12); ir 3610 (OH), 1760 (C=O), 1265 (C-O-C), 1155 ($OCH_3$).

In a similar manner, 1.7 gm of VIIb was dissolved in 10 ml of chloroform and reacted with 1.4 gm of phosphorus tribromide. An acetone solution of 0.8 gm of vanillin was refluxed under nitrogen with 3 gm of potassium carbonate and the washed and dried chloroform solution was added to the

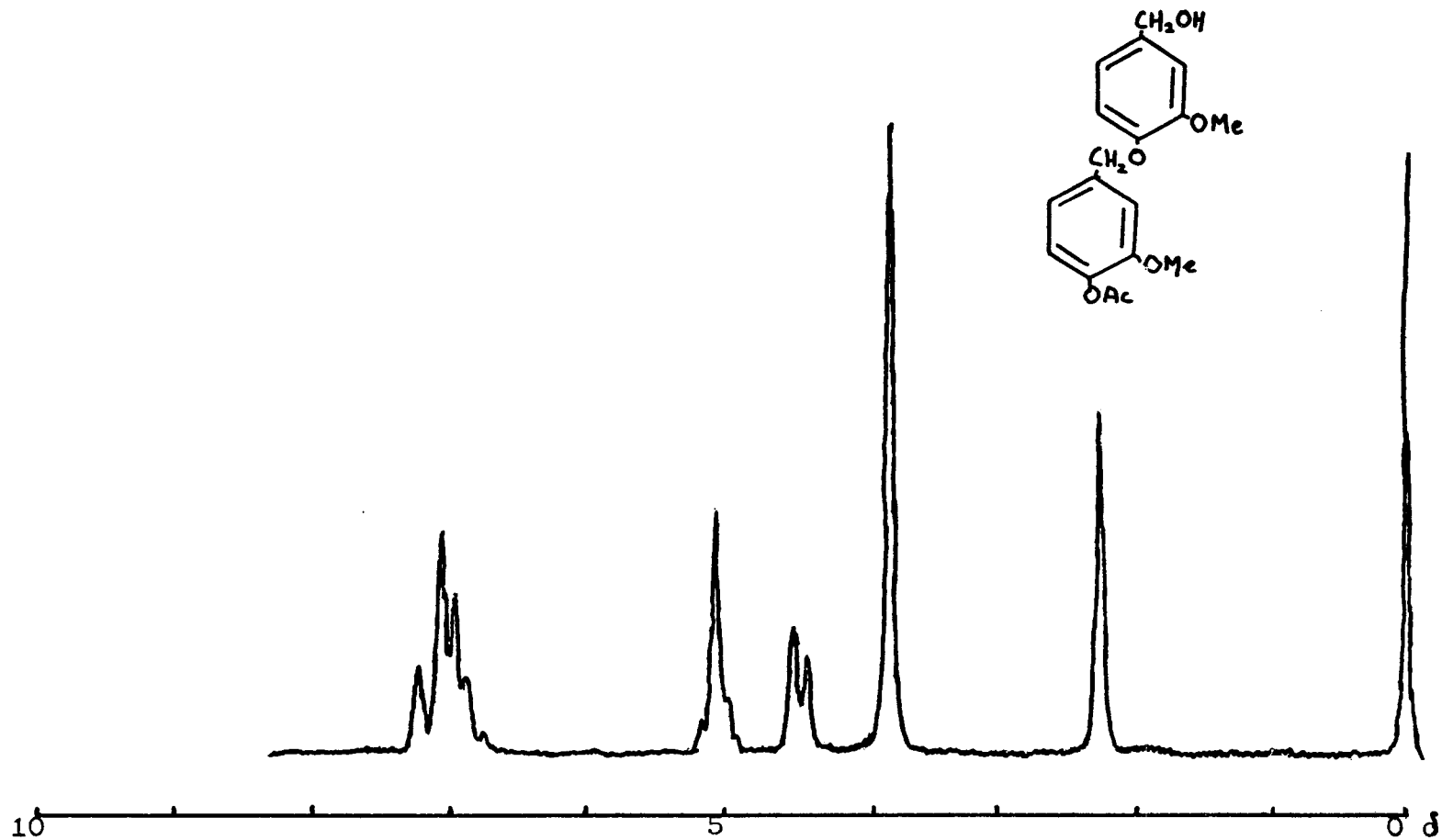



Figure 9. NMR spectrum of 3-Methoxy-4-O-acetylbenzyl-2'-methoxy-4'-hydroxymethylphenyl Ether (VIIb).

acetone slurry as before. The reaction mixture was refluxed for 2 hr after addition was complete and allowed to stir under nitrogen at room temperature overnight. Workup followed the same procedure as that of the dimers. The product was recrystallized with difficulty from chloroform--pentane; an amorphous white powder was obtained. Yield 1.5 gm (63%); mp 145-147°; nmr (DMSO-$d_6$) $\delta$ 2.25 (s, 3), 3.78 (s, 6), 3.81 (s, 3), 5.02 (s, 2), 5.09 (s, 2), 7.19 (m, 9), 10.12 (s, 1); mass spectrum m/e (rel intensity) 466 (1), 315 (39), 179 (62), 151 (17), 137 (100), 122 (13), 107 (19); ir 2790 (CHO), 1735 (C=O), 1255 (C-O-C), 1135 ($OCH_3$). Anal. Calcd for $C_{26}H_{26}O_8$: C, 66.95; H, 5.57. Found: C, 64.98; H, 5.85.

1-(3,5-Dimethoxy-4-O-acetylphenyl)-ethyl-2'-methoxy-4'-formylphenyl Ether (VIIf) and 1-(3,5-Dimethoxy-4-O-acetylphenyl)-ethyl-2'-methoxy-4'-(1-propenyl)-phenyl Ether (VIIg)

A 25-ml chloroform solution of 7 gm of VIc was reacted with 8 gm of neat phosphorus tribromide. The mixture was allowed to stand at room temperature for 10 min and then boiled gently for 15 min, cooled, washed and dried as in previous procedures. An acetone solution of 4.5 gm of vanillin was stirred and refluxed under nitrogen with 5 gm of potassium carbonate until the characteristic yellow slurry formed and to this was added the chloroform solution. The reaction was allowed to stir and reflux under nitrogen overnight. Workup followed previous procedures. The oil

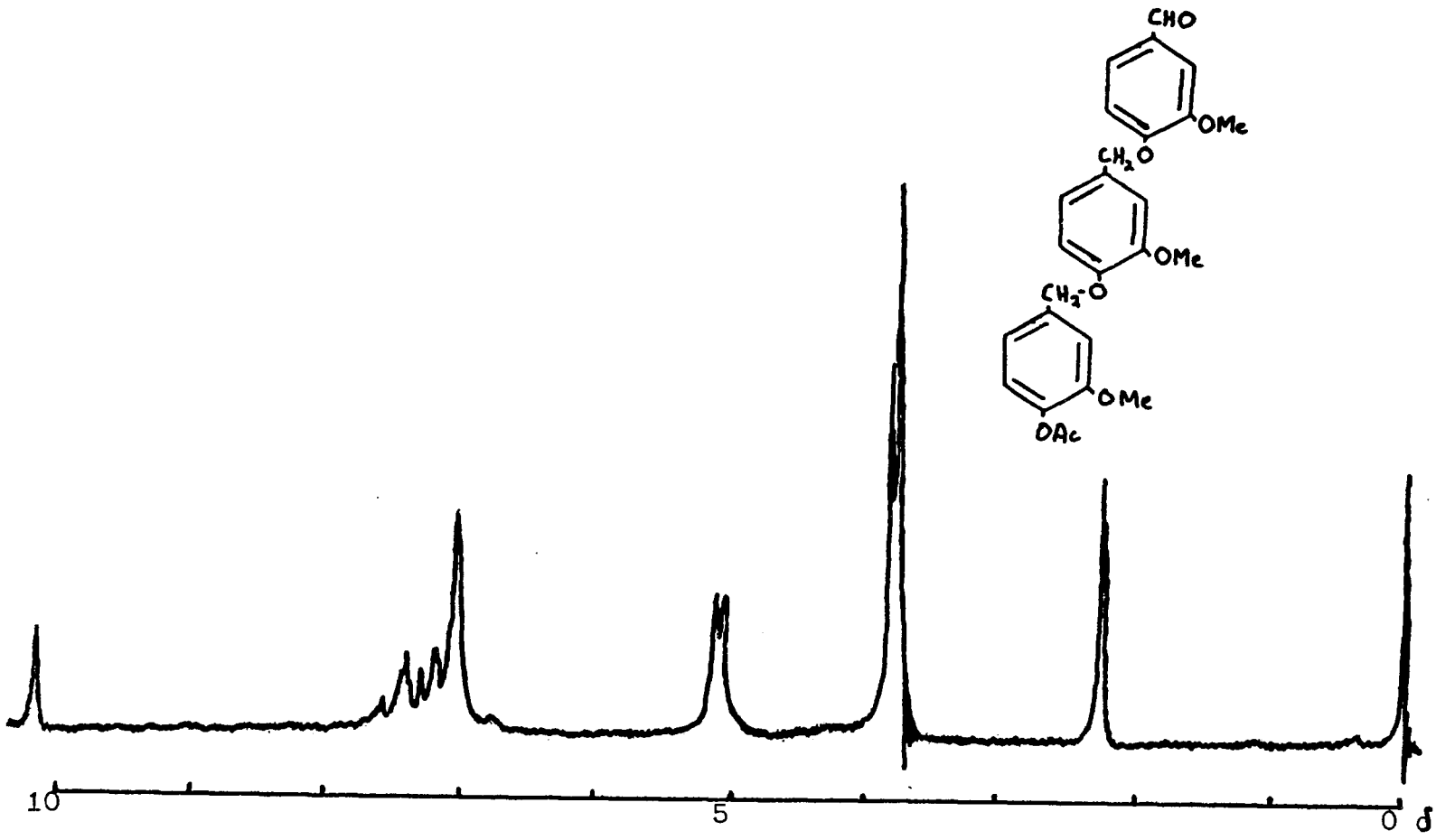



Figure 10. NMR spectrum of 3-Methoxy-4-O-acetylbenzyl-2'-methoxyphenyl-4'-benzyl-2"-methoxy-4"-formylphenyl Ether (IX).

which resulted crystallized on standing. Recrystallization from chloroform--pentane resulted in a yellow powder which melted at 111-118°. This powder was eluted through a silica gel column with 15-25% ether--pentane and a yield of 7.4 gm (68%) of white crystals melting at 118-119.5° was obtained; nmr ($CDCl_3$) $\delta$ 1.65 (d, 3), 2.22 (s, 3), 3.70 (s, 6), 3.84 (s, 3), 5.30 (q, 1), 7.05 (m, 5), 9.97 (s, 1); mass spectrum m/e (rel intensity) 374 (1), 223 (93), 197 (12), 182 (96), 181 (100), 162 (21), 151 (40), 137 (12), 121 (23); ir 2885 (CHO), 1760 (C=O), 1260 (C-O-C), 1140 ($OCH_3$).

Anal. Calcd for $C_{20}H_{22}O_7$: C, 64.16; H, 5.92. Found: C, 64.01; H, 6.14.

Using a procedure identical to that above, 6.3 gm of VIc was reacted with 7 gm of phosphorus tribromide. An acetone solution of 4 gm of isoeugenol was reacted under nitrogen with 5 gm of potassium carbonate. After washing and drying, the chloroform solution was added to the acetone slurry and the reaction mixture was refluxed for 2 hr after addition was complete and allowed to stir under nitrogen at room temperature overnight. Workup followed the above procedure. The product was recrystallized from chloroform--pentane. Yield 1 gm (10%); mp 114.5-117°; nmr ($CDCl_3$) $\delta$ 1.64 (d, 3), 1.81 (d, 3), 2.25 (s, 3), 3.72 (s, 6), 3.82 (s, 3), 5.20 (q, 1), 6.45 (m, 7); mass spectrum m/e (rel intensity) 386 (6), 223 (15), 206 (16), 181 (100), 165 (52),

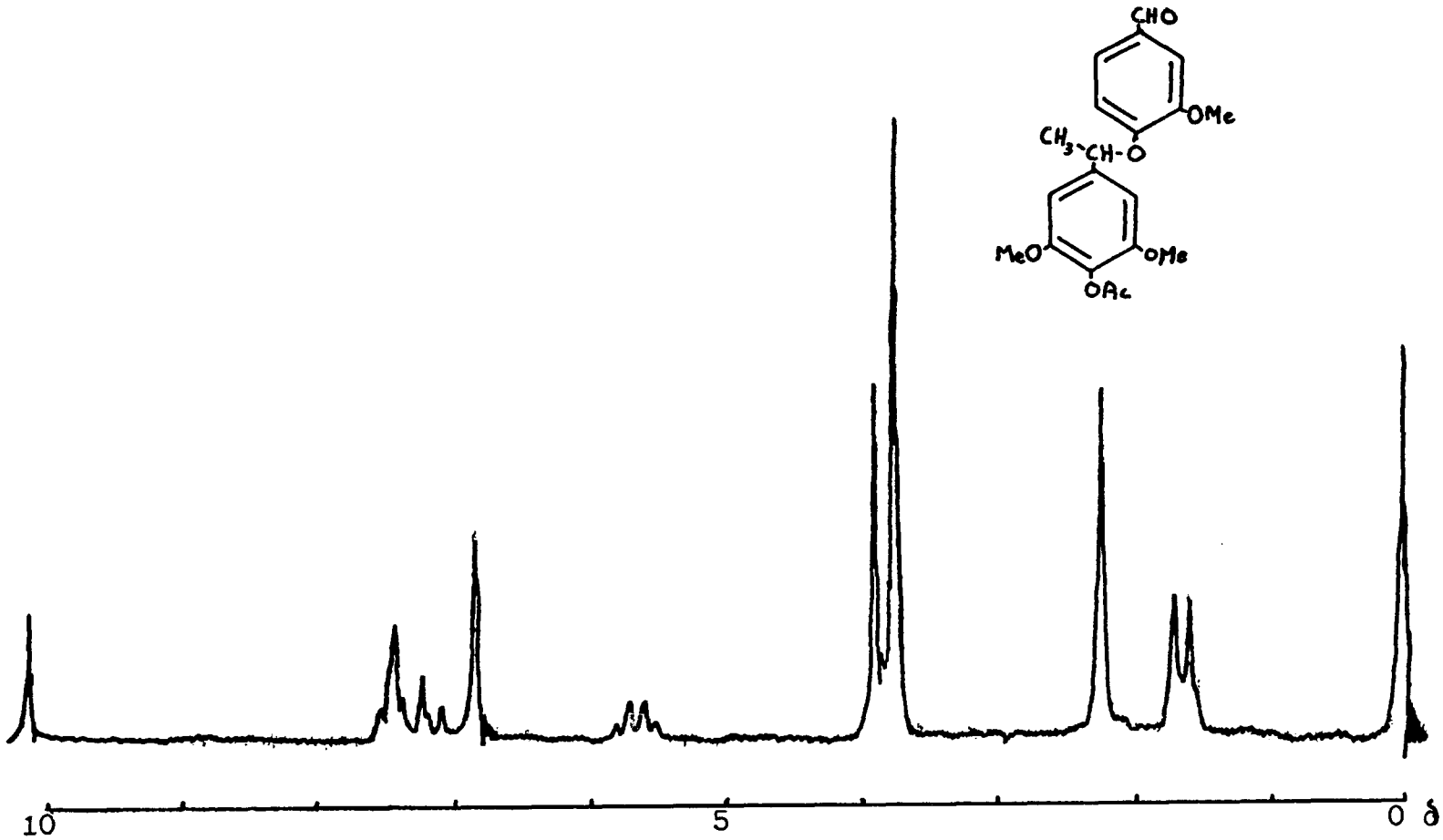



Figure 11. NMR spectrum of 1-(3,5-Dimethoxy-4-O-acetylphenyl)-ethyl-2'-methoxy-4'-formylphenyl Ether (VIIf).

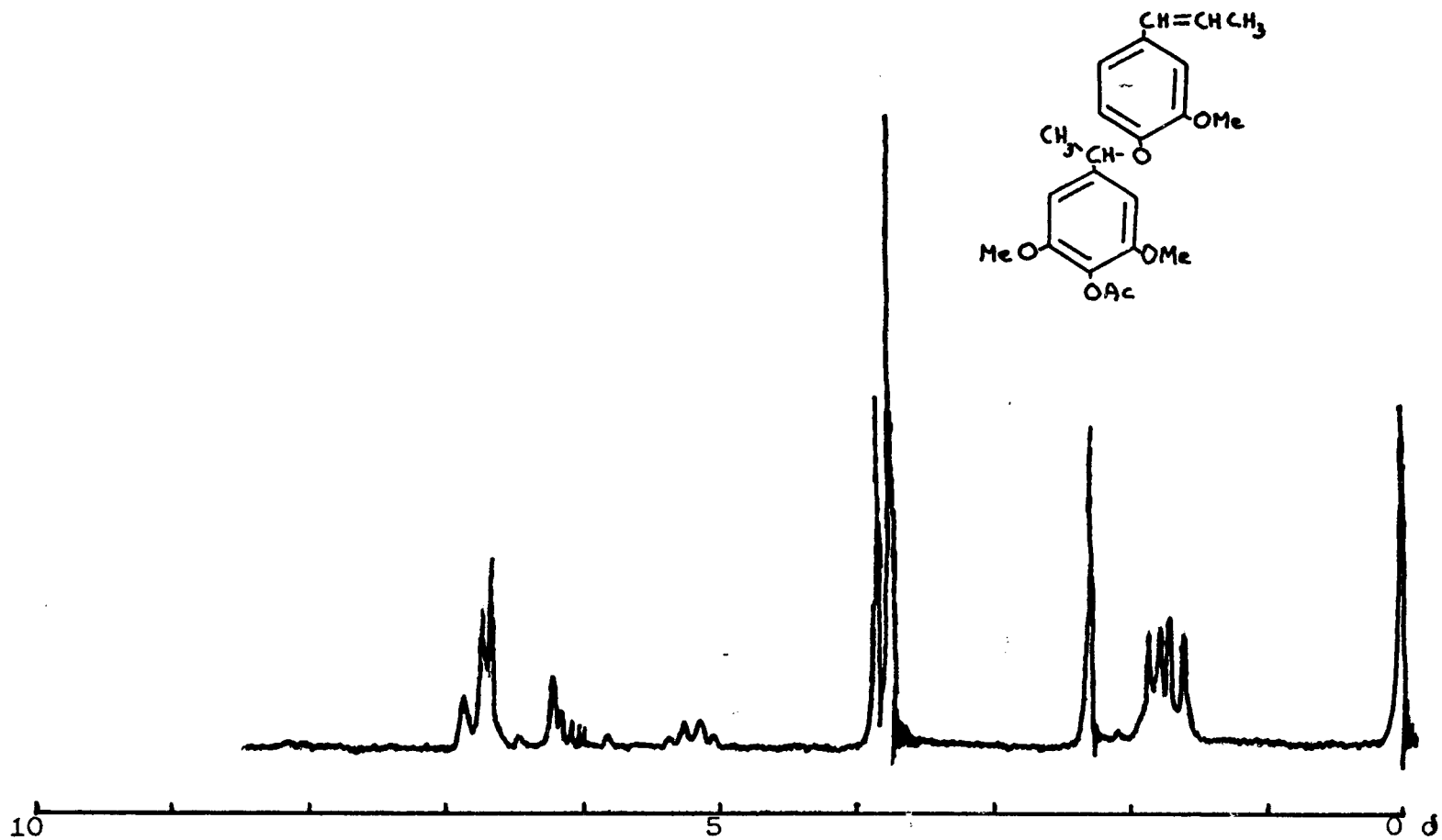



Figure 12. NMR spectrum of 1-(3,5-Dimethoxy-4-O-acetylphenyl)-ethyl-2'-methoxy-4'-(1-propenyl)-phenyl Ether (VIIg).

163 (100), 149 (14), 121 (16), 91 (28); ir 1750 (C=O), 1600 (C=C), 1210 (C-O-C), 1135 ($OCH_3$).

Anal. Calcd for $C_{22}H_{26}O_6$: C, 68.38; H, 6.78. Found: C, 68.51; H, 6.70.

3,5-Dimethoxy-4-carbethoxybenzyl-2'-methoxy-4'-formylphenyl Ether (Carbethoxy-VIId)

A 50-ml chloroform solution of 5.7 gm of 3,5-dimethoxy-4-carbethoxybenzyl alcohol was prepared and treated with a 20-ml chloroform solution of 6.2 gm of phosphorus tribromide. The solution was heated for 15 min, then cooled, washed and dried as in previous preparations and finally added dropwise to a refluxing acetone slurry of 3.4 gm of vanillin and 5 gm of potassium carbonate. The mixture was allowed to reflux and stir overnight in a nitrogen atmosphere. Workup procedure was the same as that for the acetate derivatives. The product was recrystallized from chloroform--pentane. Yield 7 gm (80%); mp 138.5-141.5°; nmr ($CDCl_3$) δ 1.33 (t, 3), 3.79 (s, 6), 3.89 (s, 3), 4.28 (q, 2), 5.14 (s, 2), 6.67 (s, 2), 7.18 (m, 3), 9.90 (s, 1); mass spectrum m/e (rel intensity) 390 (4), 239 (81), 195 (34), 168 (64), 167 (100), 151 (32), 136 (15), 123 (18); ir 2820 (CHO), 1760 (C=O), 1270 (C-O-C), 1140 ($OCH_3$).

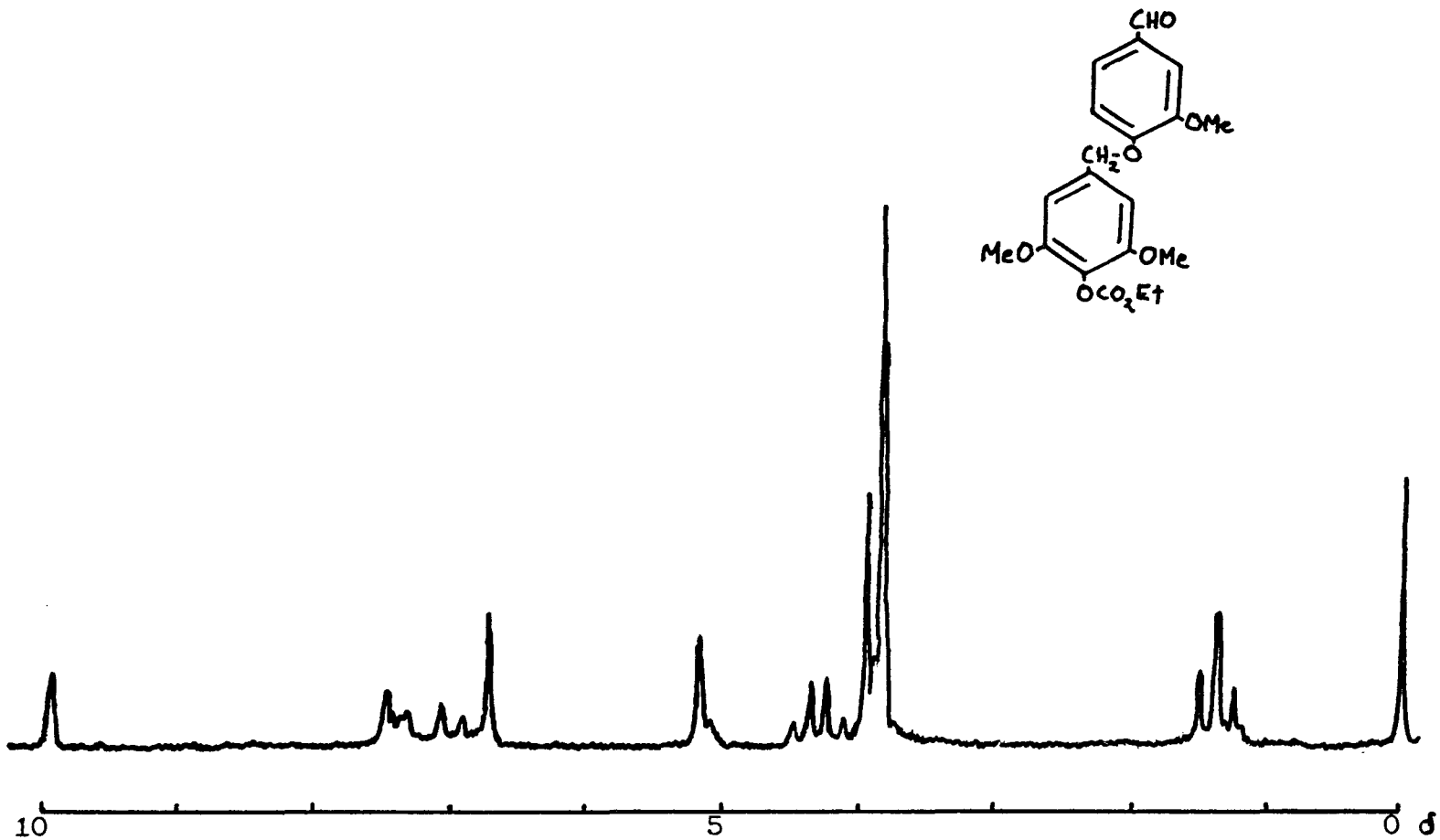



Figure 13. NMR spectrum of 3,5-Dimethoxy-4-carbethoxybenzyl-2'-methoxy-4'-formylphenyl Ether (Carbethoxy-VIId).

3,5-Dimethoxy-4-carbethoxybenzyl-2'-methoxy-4'-hydroxymethylphenyl Ether (X)

A 20-ml solution in dry monoglyme was made of 1 gm of carbethoxy-VIId and to this solution was added 15 ml of isopropanol. Sodium borohydride, 0.25 gm, was added to the solution and the mixture was stirred and refluxed overnight. The reaction was then cooled, poured into saturated boric acid solution and extracted twice with 75-ml portions of chloroform. The extracts were combined, washed once with water, dried ($Na_2SO_4$) and evaporated to leave a yellow oil. Crystallization was carried out from ether. Yield 0.5 gm (49%); mp 120.5-122°; nmr (DMSO-$d_6$) $\delta$ 1.24 (t, 3), 3.76 (s, 9), 4.20 (q, 2), 4.39 (d, 2), 5.02 (s, 2), 5.08 (m, 1), 6.86 (m, 5); mass spectrum m/e (rel intensity) 392 (16), 239 (52), 195 (40), 168 (66), 167 (100), 154 (22), 148 (18), 123 (17); ir 3690 (OH), 1760 (C=O), 1260 (C-O-C), 1140 ($OCH_3$).

Anal. Calcd for $C_{20}H_{24}O_8$: C, 61.22; H, 6.16. Found: C, 61.48; H, 6.15.

1-(3,5-Dimethoxy-4-hydroxyphenyl)-1-methoxypropane (XV) and 1-(3,5-Dimethoxy-4-hydroxyphenyl)-1-t-butoxypropane (XVI)

A 75-ml dichloromethane solution of XII was prepared and into this was bubbled dry hydrogen bromide gas. When the solution was saturated, as indicated by a piece of litmus paper at the gas exit tube, anhydrous magnesium

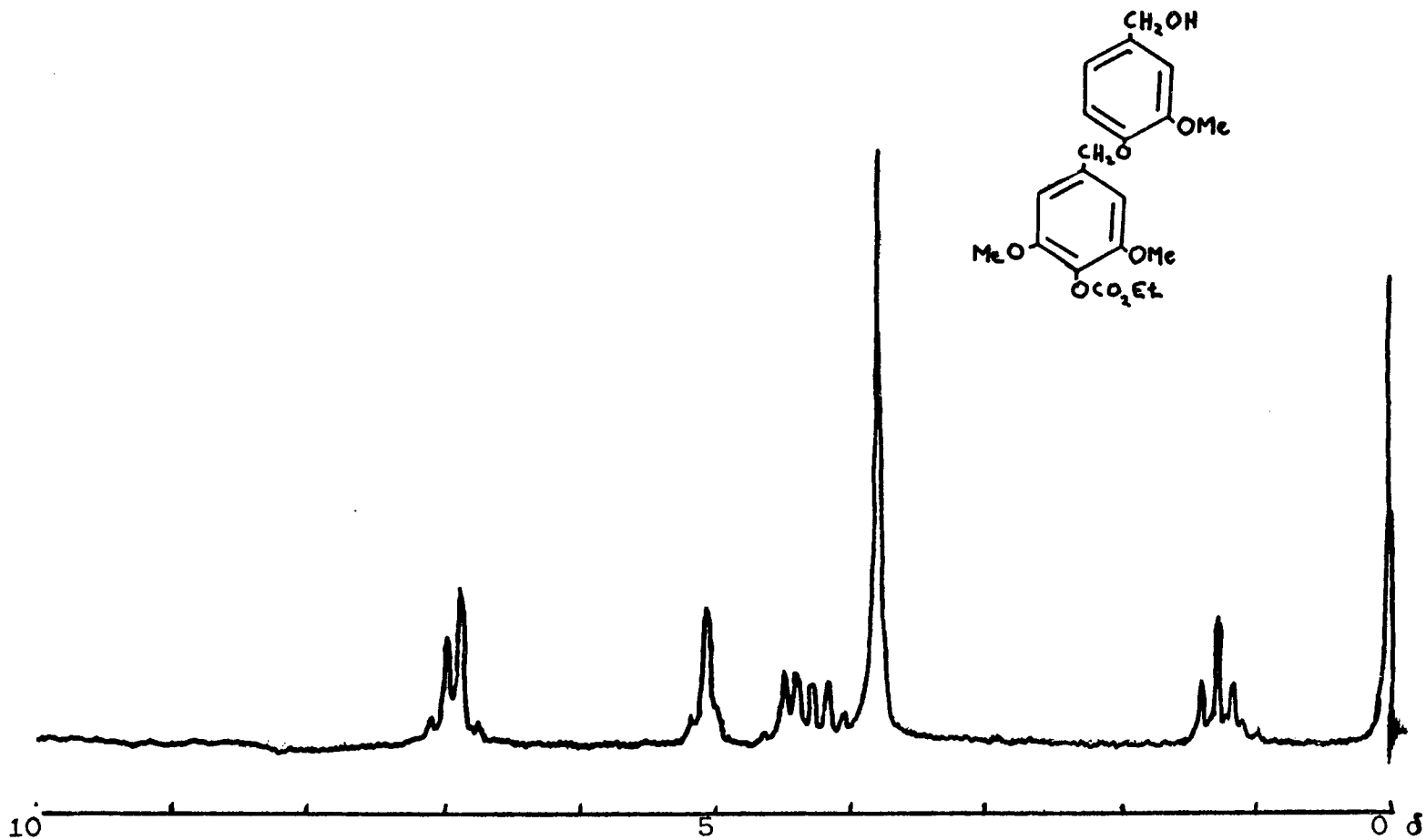



Figure 14. NMR spectrum of 3,5-Dimethoxy-4-carbethoxybenzyl-2'-methoxy-4'-hydroxymethylphenyl Ether (X).

sulfate was added to the solution to remove water released by the reaction. The drying agent was removed by vacuum filtration and the filtrate was added dropwise, with stirring, to a refluxing suspension of sodium bicarbonate in methanol. After addition was complete, the reaction was allowed to cool and stir at room temperature overnight. The solids were removed by filtration and the solvents were evaporated. The residual oil was chromatographed on a silica gel column. The product, a yellow oil, eluted with 10-20% ether--pentane. Yield 0.3 gm (27%); bp 214°; nmr ($CDCl_3$) $\delta$ 0.86 (t, 3), 1.74 (m, 2), 3.20 (s, 3), 3.82 (s, 6), 3.91 (m, 1), 6.56 (s, 2); mass spectrum m/e (rel intensity) 226 (15), 197 (100), 182 (18), 77 (8), 65 (9), 39 (14).

A 75-ml dichloromethane solution of XII was prepared, reacted with hydrogen bromide, dried and added as before to a refluxing suspension of sodium bicarbonate in t-butanol. After addition was complete, the reaction was allowed to stir at room temperature overnight and was worked up and chromatographed as before. Yield 0.3 gm (21%); bp 227°; nmr ($CDCl_3$) $\delta$ 0.99 (t, 3), 1.15 (s, 9), 1.54 (q, 2) 3.83 (s, 6), 4.27 (t, 1), 6.54 (s, 2); mass spectrum m/e (rel intensity) 268 (3), 239 (100), 211 (4), 183 (11), 123 (13), 95 (11).

3-Methoxy-4-hydroxybenzyl-2'-methoxy-4'-hydroxymethylphenyl Ether (VIIIa), 3,5-Dimethoxy-4-hydroxybenzyl-2'-methoxy-4'-hydroxymethylphenyl Ether (VIIIb) and 1-(3,5-Dimethoxy-4-hydroxyphenyl)-ethyl-2'-methoxy-4'-hydroxymethylphenyl Ether (VIIIc)

A stirred suspension of 1 gm of VIIa was made in 50 ml of anhydrous ether. To this suspension was added 0.3 gm of finely crushed lithium aluminum hydride. The mixture was stirred and refluxed overnight. Excess hydride was destroyed by the addition of wet ether to the reaction, which was then poured into saturated boric acid solution. The ether layer was drawn off and the aqueous layer was extracted twice with 25-ml portions of chloroform. All organic phases were combined, dried ($Na_2SO_4$) and evaporated. A yellow-brown oil was obtained which crystallized on standing. The product was recrystallized from chloroform--ether. Yield 0.1 gm (11%); mp 173-176°; nmr (DMSO-$d_6$) $\delta$ 3.63 (s, 6), 4.26 (d, 2), 4.57 (m, 1), 4.70 (s, 2), 6.52 (m, 6), 8.21 (s, 1); mass spectrum m/e (rel intensity) 290 (4), 154 (71), 138 (77), 137 (100), 123 (68), 107 (11), 95 (25); ir (nujol) 3480 (OH), 1280 (C-O-C), 1140 ($OCH_3$).

A 50-ml solution in anhydrous THF was made of 5 gm of VIId and to this solution was added 100 ml of dry ether. To the stirring mixture was added 0.8 gm of finely crushed lithium aluminum hydride. The reaction was stirred and refluxed for 2 hr after addition was complete, then cooled. Unreacted hydride was destroyed by the addition of wet ether

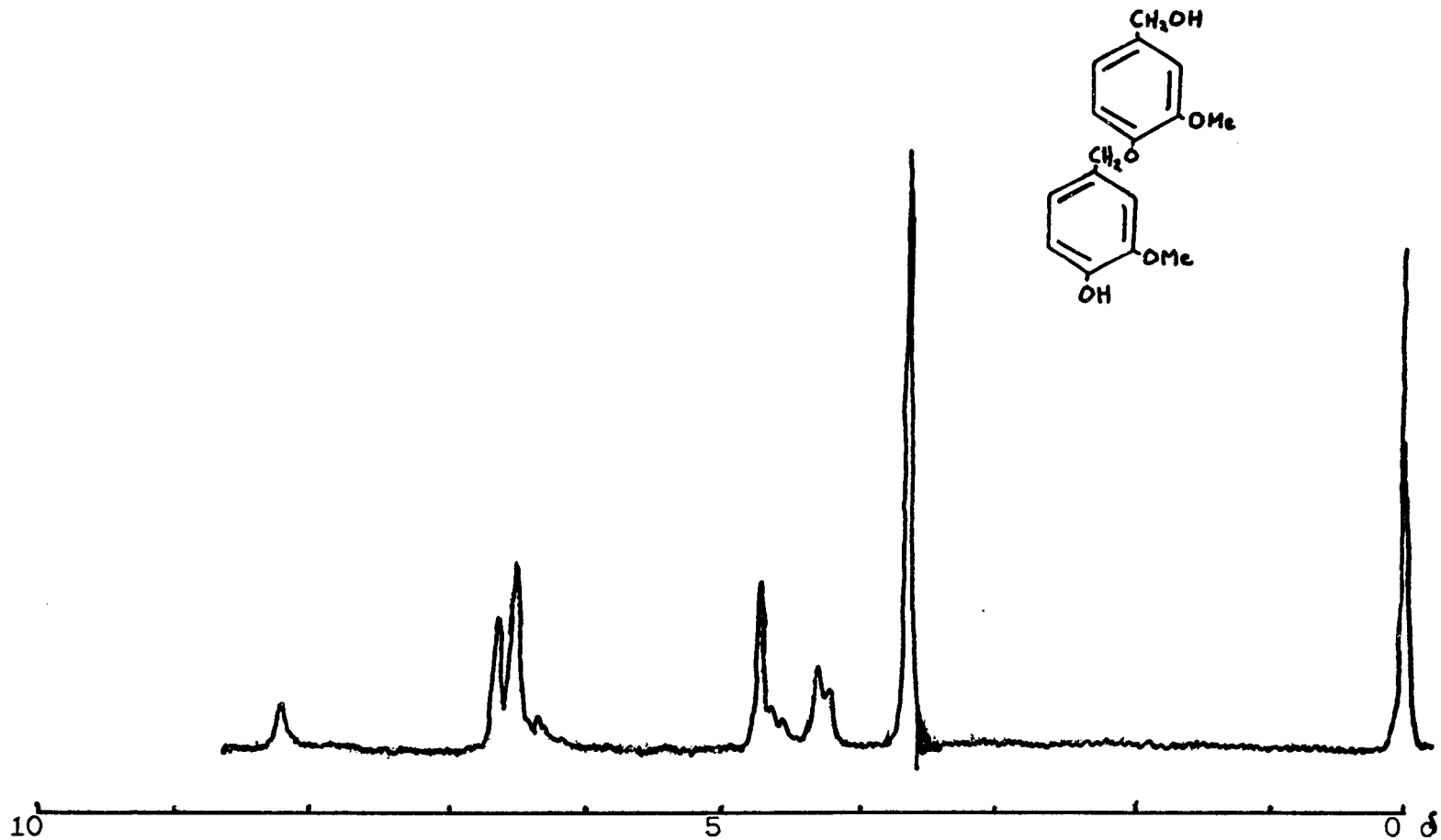



Figure 15. NMR spectrum of 3-Methoxy-4-hydroxybenzyl-2'-methoxy-4'-hydroxymethylphenyl Ether (VIIIa).

and the reaction was poured into saturated ammonium chloride solution. The organic phase was drawn off and the aqueous layer was extracted twice with 50-ml portions of chloroform. All organic layers were combined, dried and evaporated as before, leaving a pink oil. The oil was dissolved in methanol--ether and the solution saturated with pentane to effect crystallization. A faintly pink powder melting at 88.5-92.5° was obtained. A small amount of this powder was dissolved in methanol and the solution saturated with water. This technique produced feathery white crystals melting at 100-105°. Yield 3.0 gm (68%); nmr (DMSO-$d_6$) $\delta$ 3.72 (s, 9), 4.43 (d, 2), 4.92 (s, 2), 5.10 (d, 1), 6.84 (m, 5), 8.30 (s, 1); mass spectrum m/e (rel intensity) 334 (30), 320 (11), 167 (100), 154 (4), 137 (4), 122 (5), 106 (3); $^{13}C$ nmr (DMSO-$d_6$) $\delta$ 55.2, 56.6, 62.2, 70.0, 105.4, 110.0, 113.3, 117.9, 126.4, 134.6, 135.0, 145.6, 147.0, 148.4; ir (nujol) 3650 (OH), 1225 ($OCH_3$), 1120 (C-O-C).

Anal. Calcd for $C_{17}H_{19}O_6$: C, 63.74; H, 6.29. Found: C, 63.07; H, 6.27.

A 50-ml solution in dry THF was made of 0.94 gm of VIIf and 50 ml of dry ether was added to keep the reflux temperature down. To this stirring solution was added 0.17 gm of finely crushed lithium aluminum hydride. After addition was complete, the mixture was stirred vigorously and refluxed for 1 hr, then cooled, quenched with wet ether and poured into saturated boric acid solution. The ether

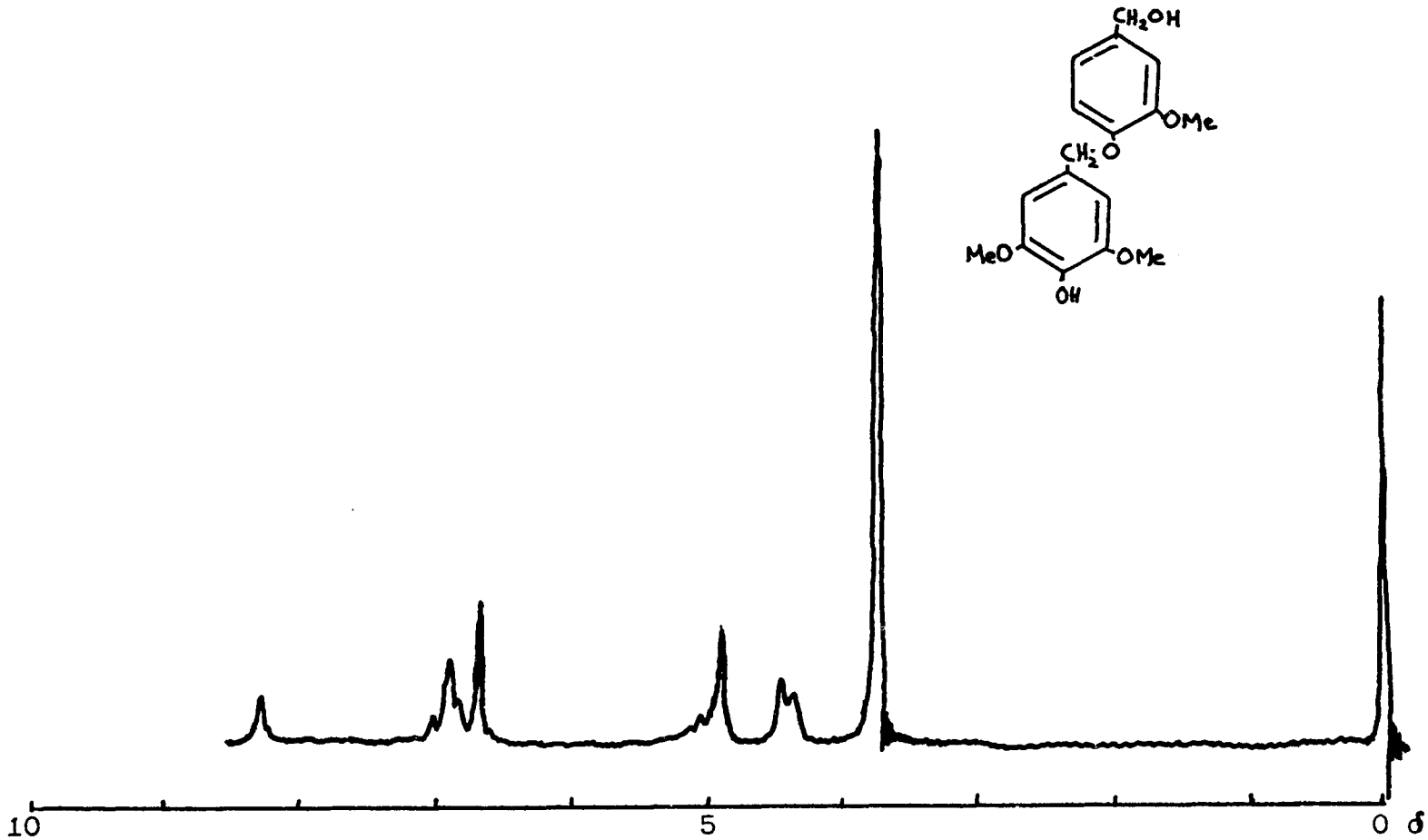



Figure 16. NMR spectrum of 3,5-Dimethoxy-4-hydroxybenzyl-2'-methoxy-4'-hydroxymethylphenyl Ether (VIIIb).

layer was removed and saved and the aqueous phase was extracted twice with 30-ml portions of chloroform. All organic phases were combined, dried and evaporated to yield an oil. The oil was dissolved in ether and pentane was added to the solution until it was faintly cloudy. Crystallization occurred very slowly in the cold, yielding faintly pink pellets. Yield 0.3 gm (35.8%); mp 109-111°; nmr (DMSO-$d_6$) $\delta$ 1.58 (d, 3), 3.69 (s, 6), 3.78 (s, 3), 4.37 (d, 2), 4.95 (d, 1), 5.29 (q, 1), 6.70 (m, 5), 8.14 (s, 1); mass spectrum m/e (rel intensity) 362 (9), 334 (4), 181 (100), 162 (70), 154 (99), 137 (94), 125 (87), 107 (69), 93 (94), 77 (80), 65 (92); ir (KBr) 3350 (OH), 1250 (C-O-C), 1140 ($OCH_3$).

Anal. Calcd for $C_{18}H_{22}O_6$: C, 64.66; H, 6.33. Found: C, 64.98; H, 5.85.

It is to be noted that the mass spectra of both VIIIb and VIIIc have parent peaks which are greater than the molecular weights of the compounds: VIIIb is 14 mass units less in weight than the m/e 334 peak obtained and VIIIc is 28 units less than the m/e 362 parent peak. This anomaly caused enough concern that the $^{13}C$ NMR spectrum of VIIIb was run in an effort to see if there was indeed an extra methylene group that had eluded analysis. The spectrum obtained shows clearly that there is not. Unfortunately, there was not enough VIIIc to run a similar spectrum, but the proton NMR spectrum (see Figure 17) and the elemental

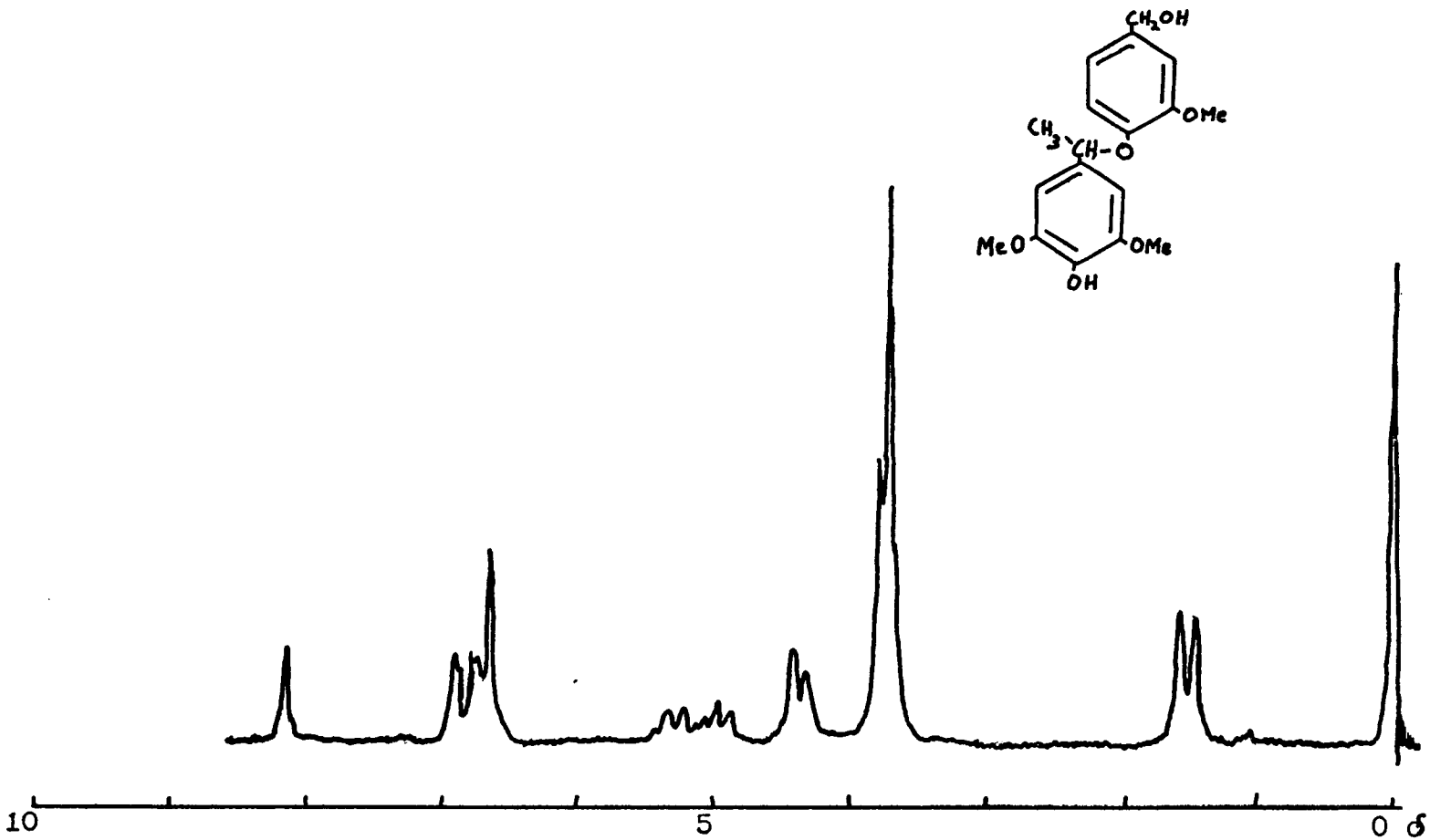
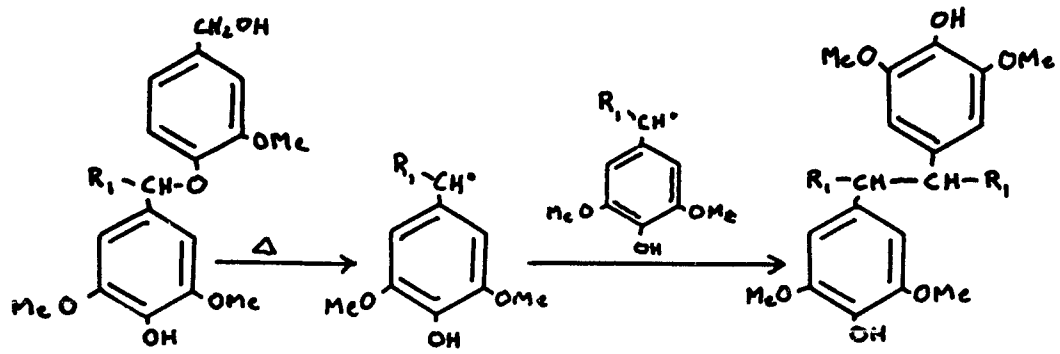



Figure 17. NMR spectrum of 1-(3,5-Dimethoxy-4-hydroxyphenyl)-ethyl-2'-methoxy-4'-hydroxymethylphenyl Ether (VIIIc).

analysis show no possibility of two extra methylene groups, so a $^{13}C$ NMR spectrum is not really necessary in this case.

What may possibly be happening in these instances is a thermal methyl transfer of the sort that Biemann and Thomas (43) discovered in the mass spectra of certain alkaloids. If a molecule is of low volatility, necessitating high temperatures for vaporization, and has both a methylating and an alkylatable group, a methyl transfer will take place, giving a parent ion of M + 14 or M + 15, depending upon the nature of the alkylatable group. The reactivity of the 5-position on the vanillyl moieties of these ethers has been remarked upon in the discussion above and the methoxyl and, in the case of VIIIc, the α-methyl group may be donating methyl fragments to the 5-positions of other molecules. These artificially enlarged molecules then show as false parent peaks.

Another explanation of this phenomenon has been offered by Dr. Ward Scott (44). The temperatures involved in volatilizing the samples of VIIIb and VIIIc may be homolytically cracking these molecules. The syringyl and α-methyl syringyl radicals may then be combining to form 1,2-diphenylethane structures with molecular weights higher than those of the original ethers. Since these structures are more stable to heat than the ethers, a larger proportion of them arrive whole at the ion collector, giving large M + 14 and M + 28 peaks.



VIII

MW: 334 (VIIIb), 362 (VIIIc)

Synthesis of diphenylethane molecules with unsymmetric α-substituents for mass-spectral experiments would show the prevalence of this type of cleavage and recombination in the volatilizing chamber.

3-Methoxy-4-hydroxybenzyl-2'-methoxy-4'-formylphenyl Ether (XI)

A 20-ml solution of 2.68 gm of N-bromosuccinimide was stirred under nitrogen at 0°. To this solution was added a solution of 1 gm of dimethyl sulfide in 10 ml of dichloromethane. After addition was complete, it was observed that the solution had turned opaque yellow. Stirring was continued, the temperature was lowered to -25°, and a solution of 1.54 gm of vanillyl alcohol in 300 ml of dichloromethane was added quickly to the reaction mixture. The -25° temperature was maintained for 4 hr and the stirring continued. After about 15 min, the opaque yellow of the reaction mixture changed to a clear, faint yellow

solution with white particles floating through it. At the end of 4 hr, the solution was washed twice with cold brine and dried ($MgSO_4$).

An acetone solution of 1.52 gm of vanillin was refluxed under nitrogen with 1.38 gm of potassium carbonate until the characteristic milky-yellow slurry formed. The dichloromethane solution prepared above was quickly added with vigorous stirring. When addition was complete, the heat was shut off and the reaction allowed to stir under nitrogen at room temperature overnight. The reaction mixture was then washed twice with water, dried ($Na_2SO_4$) and evaporated to yield a brown oil which crystallized on standing. The product was recrystallized from ether--pentane. Yield 1 gm (35%); mp 154-155.5°; nmr ($CDCl_3$) $\delta$ 3.81 (s, 3), 3.88 (s, 3), 5.04 (s, 2), 7.08 (m, 6), 10.09 (s, 1); mass spectrum m/e (rel intensity) 288 (1), 152 (56), 151 (58), 137 (100), 123 (20), 109 (21); ir 3450 (OH), 2770 (CHO), 1260 (C-O-C), 1140 ($OCH_3$).

## Acid Hydrolysis Reactions

### 3-Methoxy-4-hydroxybenzyl-2'-methoxy-4'-hydroxymethylphenyl Ether (VIIIa)

A 2-ml solution in 9:1 dioxane--water which was 0.2 M in hydrochloric acid was made of 50 mg of VIIIa. The mixture was placed in an oil bath heated at 50° and left for 8 hr. The mixture was then cooled and poured into dilute

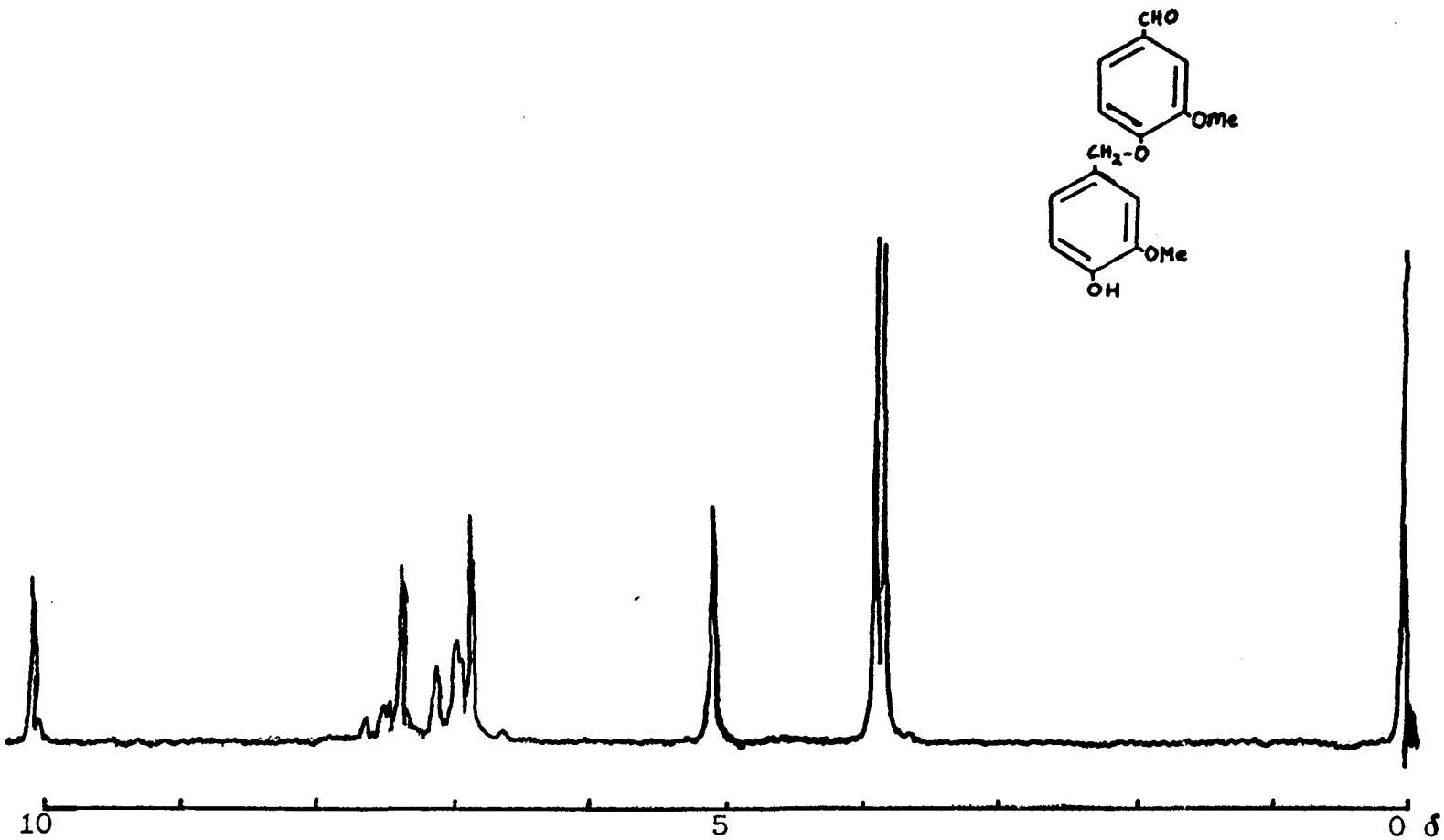



Figure 18. NMR spectrum of 3-Methoxy-4-hydroxybenzyl-2'-methoxy-4'-formylphenyl Ether (XI).

aqueous sodium bicarbonate solution. This solution was extracted twice with 15-ml portions of chloroform and the extracts were combined, dried ($Na_2SO_4$) and evaporated. The product mixture was chromatographed against standards on a 0.25 mm layer of Silica Gel F-254. Found: vanillyl alcohol (Rf 0.27).

3,5-Dimethoxy-4-hydroxybenzyl-2'-methoxy-4'-hydroxymethylphenyl Ether (VIIIb)

A 4-ml solution of 100 mg of VIIIb was made in 9:1 dioxane--water which was 0.2 M in hydrochloric acid. This solution was divided into two portions and placed in the 50° oil bath. One portion was allowed to stand in the bath for 8 hr and the other remained in the bath 24 hr. Workup for both reactions was the same as in the above procedure. The product mixtures were chromatographed against standards. Found: after 8 hr, VIIIb (Rf 0.24), syringyl alcohol (Rf 0.25), vanillyl alcohol (Rf 0.27); after 24 hr, syringyl alcohol (Rf 0.25), vanillyl alcohol (Rf 0.27).

1-(3,5-Dimethoxy-4-hydroxyphenyl)-ethyl-2'-methoxy-4'-hydroxymethylphenyl Ether (VIIIc)

A 2-ml solution in the acidic dioxane--water solution described above was made of 10 mg of VIIIc. This solution was heated at 50° for 8 hr, worked up and chromatographed as before. The silica gel plate was developed in iodine. Found: 1-(syringyl)-ethylene (Rf 0.64), α-methyl syringyl alcohol (Rf 0.20), vanillyl alcohol (Rf 0.27).

3-Methoxy-4-hydroxybenzyl-2'-methoxy-
4'-formylphenyl Ether (XI)

A 2-ml solution of 40 mg of XI was made in the dioxane--water acid solvent described above. This solution was reacted at 50° for 8 hr and worked up and chromatographed as before. Found: vanillin (Rf 0.57), vanillyl alcohol (Rf 0.25).

3-Methoxy-4-O-acetylbenzyl-2'-methoxy-
4'-hydroxymethylphenyl Ether (VIIb)

A solution of 100 mg of VIIb in 4 ml of dioxane--water acid solvent was made and divided into two portions. The portions were kept at 50° for 8 and 24 hr, respectively, and worked up and chromatographed as before. Found: after 8 hr, 4-O-acetylvanillyl alcohol (Rf 0.40), vanillyl alcohol (Rf 0.27); after 24 hr, vanillyl alcohol (Rf 0.27).

3,5-Dimethoxy-4-O-acetylbenzyl-2'-
methoxy-4'-formylphenyl Ether (VIId)

A solution of 40 mg of VIId was made in 4 ml of the acid solvent; this was divided into two parts, which were reacted at 50° for 8 and 24 hr, worked up and chromatographed as before. Found: after 8 hr, VIId (Rf 0.72), vanillin (Rf 0.57), 4-O-acetylsyringyl alcohol (Rf 0.44), syringyl alcohol (Rf 0.25); after 24 hr, VIId (Rf 0.72), vanillin (Rf 0.57), syringyl alcohol (Rf 0.25).

3-Methoxy-4-O-acetylbenzyl-2'-methoxyphenyl-4'-benzyl-2"-methoxy-4"-formylphenyl Ether (IX)

A solution of 50 mg of IX in 4 ml of the acid solvent was made, divided into halves, reacted at 50° for 8 and 24 hr and worked up as before. The reaction mixture was chromatographed against standards. Found: after 8 hr, IX (Rf 0.72), vanillin (Rf 0.57), 4-O-acetylvanillyl alcohol (Rf 0.40), vanillyl alcohol (Rf 0.27); after 24 hr, IX (Rf 0.72), vanillin (Rf 0.57), 3-methoxy-4-hydroxybenzyl-2'-methoxy-4'-formylphenyl ether (XI) (Rf 0.62), vanillyl alcohol (Rf 0.27).

## Enzymatic Oxidations

### Vanillyl and Syringyl Alcohols

A 10-ml methanol solution of 92 mg of vanillyl alcohol was prepared, and to this was added 110 mg of syringyl alcohol. This solution was raised in volume to 100 ml by the addition of distilled water and 0.7 ml (two equivalents) of 3% hydrogen peroxide and 24 ml of horseradish peroxidase stock solution (1 mg of enzyme) were added to the methanol--water solution. The mixture was swirled to mix the components (at this time it turned yellow), allowed to stand at room temperature overnight and worked up as in the acid hydrolysis experiments above. The product mixture was chromatographed against standards on Silica Gel F-254 as

before. Found: 2,6-dimethoxy-p-benzoquinone (Rf 0.63), vanillin (Rf 0.57), syringaldehyde (Rf 0.49), vanillyl alcohol (Rf 0.27), syringyl alcohol (Rf 0.25).

3-Methoxy-4-hydroxybenzyl-2'-methoxy-4'-hydroxymethylphenyl Ether (VIIIa)

A solution was made of 50 mg of VIIIa in 20 ml of methanol. The solution was diluted to 100 ml by the addition of distilled water and to this was added 0.31 ml (two equivalents) of hydrogen peroxide solution and 12.5 ml of enzyme stock solution. The solution was swirled to mix the contents, allowed to stand at room temperature overnight and worked up and chromatographed as before. Found: vanillin (Rf 0.57), vanillyl alcohol (Rf 0.27).

3-Methoxy-4-hydroxybenzyl-2'-methoxy-4'-formylphenyl Ether (XI)

A solution of 50 mg of XI was made in 20 ml of methanol. This solution was diluted to 100 ml as before, reacted with 0.31 ml of hydrogen peroxide and 12.5 ml of enzyme stock solution, worked up and chromatographed as before. Found: 2-methoxy-p-benzoquinone (Rf 0.76), vanillin (Rf 0.57), vanillyl alcohol (Rf 0.27).

3,5-Dimethoxy-4-hydroxybenzyl-2'-methoxy-4'-hydroxymethylphenyl Ether (VIIIb)

A 10-ml solution of 200 mg of VIIIb was made in methanol, and this solution was diluted to 100 ml by the addition of distilled water. The solution was divided into

four portions. The first portion was treated with 0.2 ml of 3% hydrogen peroxide and 12.5 ml of enzyme stock solution. The second portion received 5 ml of peroxide and 25 ml of enzyme solution. The third and fourth portions were treated with 0.2 ml of peroxide, but no enzyme solution. The first and second portions were reacted for 24 hr and worked up and chromatographed as before. The third portion was allowed to react for 1 week and the fourth reacted for 4 weeks. These portions were worked up and chromatographed as before. Samples for mass spectral analysis were column chromatographed with ether--pentane. Occasionally, enough of a high-Rf product could be obtained for a melting or boiling point or a NMR spectrum. Found: first portion, 2,6-dimethoxy-p-benzoquinone (Rf 0.63), mp 232° (dec), mass spectrum m/e (rel intensity) 168 (100), 69 (95), syringaldehyde (Rf 0.49) mp 114-116°, mass spectrum m/e (rel intensity) 182 (100), 181 (81), vanillin (Rf 0.57), mass spectrum m/e (rel intensity) 152 (100), 151 (100), 123 (43), vanillyl alcohol (Rf 0.27), mass spectrum m/e (rel intensity) 154 (100), 137 (55), syringyl alcohol (Rf 0.25); second portion, 2,6-dimethoxy-p-benzoquinone (Rf 0.63), 2-methoxy-p-benzoquinone (Rf 0.76), vanillin (Rf 0.57), syringaldehyde (Rf 0.49), vanillyl alcohol (Rf 0.27), syringyl alcohol (Rf 0.25); third portion, 3,5-dimethoxy-4-hydroxybenzylmethyl ether (Rf 0.47), bp 189°, nmr ($CDCl_3$) $\delta$ 3.37 (s, 3), 3.74 (s, 6), 4.35 (s, 2), 6.57 (s, 2), mass

spectrum m/e (rel intensity) 198 (19), 168 (88), 167 (57), 137 (100), 122 (23), 71 (27), VIIIb (Rf 0.24), syringyl alcohol (Rf 0.25), mass spectrum m/e (rel intensity) 184 (100), 167 (38), vanillyl alcohol (Rf 0.27), mass spectrum m/e (rel intensity) 154 (100), 137 (50); fourth portion, 3,5-dimethoxy-4-hydroxybenzylmethyl ether (Rf 0.47), VIIIb (Rf 0.24), 2,6-dimethoxy-p-benzoquinone (Rf 0.63), syringaldehyde (Rf o.49), vanillyl alcohol (Rf 0.27), syringyl alcohol (Rf 0.25). Mass spectra for the products of the fourth portion were the same as those above.

For ESR studies, a $10^{-2}$ M solution of VIIIb was made in 20% methanol--water and a 3 x $10^{-2}$ M solution of hydrogen peroxide was made. Into a test tube were placed 0.5 ml of VIIIb solution, 0.7 ml of hydrogen peroxide solution, 0.35 ml of methanol and 0.35 ml of water. At the start of the reaction, 200 µl of horseradish peroxidase stock solution was added, the mixture shaken and the solution poured into a flat quartz cell and placed in the cavity of the ESR spectrometer.

1-(3,5-Dimethoxy-4-hydroxyphenyl)-ethyl-2'-methoxy-4'-hydroxymethylphenyl Ether (VIIIc)

A 10-ml solution of 35 mg of VIIIc was made in methanol and this was diluted to 100 ml as before by the addition of distilled water. To this solution was added 0.19 ml (two equivalents) of 3% hydrogen peroxide and 12.5 ml of enzyme stock solution. The mixture was swirled,

allowed to stand at room temperature overnight, worked up and chromatographed as before. Mass spectral samples were obtained by column chromatography as previously described. Found: 1-(syringyl)-ethylene (Rf 0.64), mass spectrum m/e (rel intensity) 180 (100), 165 (47), 137 (40), 109 (30), 81 (44), acetosyringone (Rf 0.45), mass spectrum m/e (rel intensity) 196 (87), 181 (100), vanillin (Rf 0.57), mass spectrum m/e (rel intensity) 152 (94), 151 (100), vanillyl alcohol (Rf 0.27), mass spectrum m/e (rel intensity) 154 (100), 137 (55), α-methyl syringyl alcohol (Rf 0.20), mass spectrum m/e (rel intensity) 198 (2), 181 (100).

For ESR studies, a $10^{-2}$ M solution of VIIIc was made in 50% cellosolve--water. The reaction mixture consisted of 0.5 ml of VIIIc solution, 0.7 ml of 3 x $10^{-2}$ M hydrogen peroxide solution, 0.7 ml of water and 200 µl of enzyme stock solution. These components were mixed in a test tube and poured into a quartz cell as before.

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