prepared by the same procedure described for the *erythro* isomer; yield 11.1 g (97%), mp 173–175°; ir (Nujol) 3.80 (OH), 3.95 (NH), 5.75 and 6.25 μ (C=O); nmr ($CDCl_3$) δ 3.22 (s, 3, $-OCH_3$) 3.80 (s, 3, $-OCH_3$), and 7.34 ppm [m, 15, $(C_6H_5)_3$]. Again, only half the AB quartet was visible at 4.27 ppm, $J_{\alpha\beta}$ = 2.2 cps.

Anal. Calcd for $C_{25}H_{25}NO_5$: C, 71.58; H, 6.01; N, 3.34. Found: C, 71.41; H, 5.89; N, 3.32.

Registry No.—**1** Me ester HCl, 5680-79-5; N-trityl **1** Me ester, 10065-71-1; **2** Me ester HCl, 13515-93-0; N-trityl **2** Me ester, 13515-73-6; **3** Me ester HCl, 2133-40-6; N-trityl **3** Me ester, 13515-74-7; **4** Me ester HCl, 18598-50-0; N-trityl **4** Me ester, 17267-82-2; **5** Me ester HCl, 5619-04-5; N-trityl **5** Me ester, 13515-76-9; **6** Me ester HCl, 14358-33-9; N-trityl **6** Me ester, 13515-78-1; **7** Me ester HCl, 2491-20-5; N-trityl **7** Me ester, 18598-57-7; **8** Me ester HCl, 13515-98-5; N-trityl **8** Me ester, 13515-79-2; **9** Me ester HCl, 18598-59-9; N-trityl **9** Me ester, 18598-60-2; **10** Me ester HCl, 2491-18-1; N-trityl **10** Me ester, 18598-62-4; **11** Me ester HCl, 18598-63-5; N-trityl **11** Me ester, 18598-64-6; **12** Me ester HCl, 7517-19-3; N-trityl **12** Me ester, 18598-66-8; **13** Me ester HCl, 13515-99-6; N-trityl **13** Me ester, 13515-80-5; **14** Me ester HCl, 13515-95-2; N-trityl **14** Me ester, 18598-70-4; **15** Me ester HCl, 18598-71-5; N-trityl **15** Me ester, 18598-72-6; **16** Me ester HCl, 6306-52-1; N-trityl **16** Me ester, 18598-73-7; **17** Me ester HCl, 18598-74-8; N trityl **17** Me ester, 18598-75-9; **18** Me ester HCl, 18684-16-7; **19** Me ester HCl, 7524-50-7; N-trityl **19** Me ester, 18598-80-6; **20** Me ester HCl, 7524-52-9; N-trityl **20** Me ester, 18598-78-2; **21** Me ester HCl, 3417-91-2; N-trityl **21** Me ester, 18621-06-2; **22** Me ester HCl, 3196-73-4; N-trityl **22** Me ester, 13515-81-6; **23** Me ester HCl, 13031-60-2; N-trityl **23** Me ester, 14470-68-9; **24** Me ester HCl, 13516-02-4; N-trityl **24** Me ester, 14357-95-0; dimethyl N-benzyl-DL-aspartate hydrochloride, 18598-81-7; dimethyl N-benzhydryl-DL-aspartate hydrochloride, 18598-82-8.

New Structures from the Enzymic Dehydrogenation of Lignin Model *p*-Hydroxy-α-carbinols

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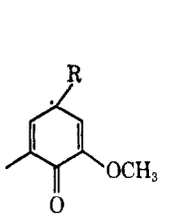
Received July 10, 1968

The dehydrogenation of α-ethylvanillyl alcohol (**2**) in aqueous solution with peroxide and peroxidase produced the novel dibenzo[*d*,*f*][1,3]dioxepin **3** with the side chain being expelled as propionaldehyde. The important lignin model guaiacylglycerol β-guaiacyl ether (**12**) undergoes the same type of reaction. Indications are that other *p*-hydroxybenzyl alcohols and some ethers react in a similar fashion. This reaction is significant in consideration of the structural features of the lignin macromolecule and also serves to indicate that the cyclohexadienone forms of phenoxy radicals **1** may be important contributors in the biosynthesis process.

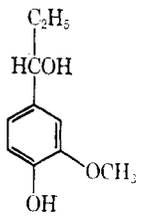
We have reported that on enzymic dehydrogenation *p*-hydroxypropiophenones give rise to the formation of novel *o*,*p*′-biphenyls as well as side chain transfer reactions with formation of esters of aliphatic acids or the free acids themselves. These reactions are thought to come about through coupling of *p*-cyclohexadienone radicals **1** with other mesomeric radicals which rearomatize through side chain transfer or expulsion to form biphenyl and polyphenyl compounds.[2]

This study has now been expanded to some *p*-hydroxy-α-carbinol compounds and preliminary results showing the involvement of radical **1** and side chain expulsion and dioxepin formation have been reported.[2a] Reviews on the importance and detection of α-carbinol groups in lignin with both free and etherified *p*-hydroxyl groups have been published.[3-5]

When α-ethylvanillyl alcohol (**2**) was dehydrogenated in aqueous solution using hydrogen peroxide and peroxidase, a distinctive odor of propionaldehyde could be detected after 2–3% of the peroxide had been added. By using 2.1 equiv (1.05 mol) of peroxide per mole of phenol, the novel dibenzo[*d*,*f*][1,3]dioxepin **3** was formed in a yield corresponding to 67% of the theoretical.



1



2

The proposed mechanism of formation of the dioxepin **3** is through the formation first of the *o*,*o*′-dihydroxybiphenyl **4** of α-ethylvanillyl alcohol followed by further dehydrogenation of **4** to give the phenoxy radical **5** of the biphenyl which couples with the cyclohexadienone mesomeric form of the same radical **6** to give the dienone tetramer **7**. This then rearomatizes through loss of propionaldehyde to **8**, which on further dehydrogenation gives phenoxy and *p*-cyclohexadienone radicals. These through intramolecular radical coupling give dioxepin **3** (Scheme I).

(1) Maintained at Madison, Wis., in cooperation with the University of Wisconsin.

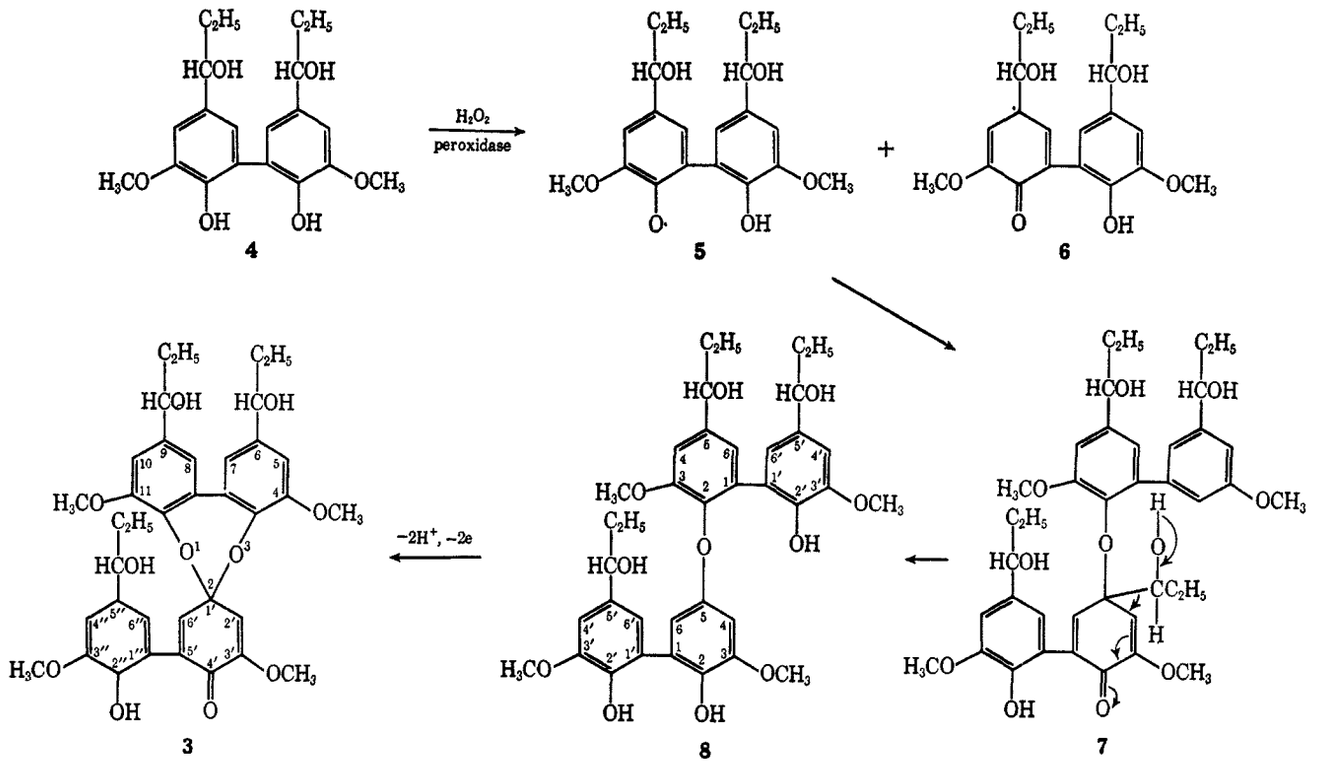
(2) (a) J. C. Pew and W. J. Connors, *Nature*, **215**, 623 (1967); (b) J. C. Pew and W. J. Connors, *J. Org. Chem.*, **34**, 585 (1969).

(3) E. Adler, *Paperi Puu*, **11**, 634 (1961).

(4) E. Adler, H. D. Becker, T. Ishihara, and A. Stamvick, *Holzforschung*, **20**, 3 (1966).

(5) J. Marton and E. Adler, *Tappi*, **46**, 92 (1963).

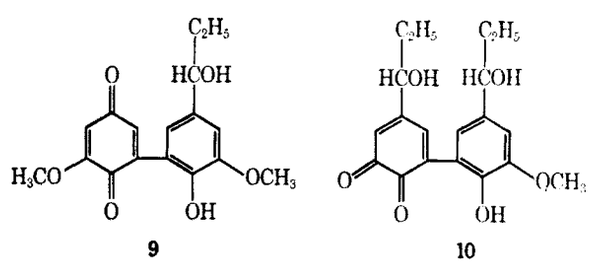
SCHEME I



If **8** is formed in the aqueous dehydrogenation reaction, it must be immediately dehydrogenated to form **3**. When **3** was reduced with $NaBH_4$, a compound was isolated for which the nmr spectrum of its acetate was consistent with the hexaacetate of **8**. Compound **8** could be separated from **3** and **4** in prepared mixtures by tlc on silica GF with 100:10, $CHCl_3$–EtOH but no conclusive evidence for the presence of **8** in the dehydrogenation mixture could be obtained. On enzymic dehydrogenation of **8** in aqueous solution, or by Ag_2O oxidation in ether, **3** was regenerated. Propionaldehyde was detected during the former experiment so inter- as well as intramolecular coupling must take place on dehydrogenation of this compound. The isolated biphenyl **4** can also be converted directly into **3** through enzymic dehydrogenation in aqueous solution. It is interesting to note that neither **4** nor **8** can be converted into **3** through enzymic dehydrogenation in aqueous alcohol or acetone; apparently due to hydrogen bonding of the biphenyl with the organic solvent.

It is important to note that carbonyl functions of the dioxepin structure and the aldehyde from side chain cleavage could make up a significant part in the estimation of carbonyl content in lignin, one-half of which had previously been assigned to β-C of the side chain of the guaiacylpropane unit because of lack of better information.[3]

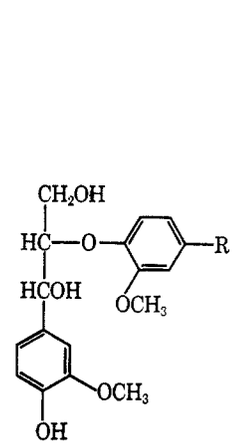
Trimeric dioxepin formations without loss of side chains have been reported in the oxidative coupling of alkoxy or alkyl phenols,[6,7] and the spiro ketals on treatment with mineral acids gave rise to quinones. Dioxepin **3** on treatment with mineral or acetic acid at room temperature also gives rise to colored compounds, and the uv spectrum of this reaction mixture in the 600–400-mμ region is similar to that of extracted wood meal under the same conditions. The main quinone from the acidolysis of **3** with HOAc–H_2O has been identified as **9**. From Hewgill's work[7] quinone **10**



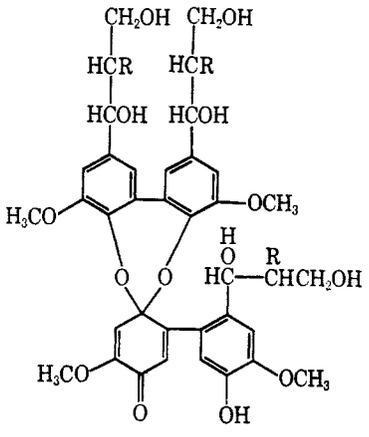
would also be expected in the acidolysis mixture but this has not been identified. However, to form an *o*-quinone, methyl alcohol must be split from the molecule, and this has been identified in the steam distillate of the reaction mixture by oxidation to formaldehyde and detection by chromotropic acid.[8]

In the initial stages of the biosynthesis of lignin, guaiacylglycerol β-coniferyl ether (**11**) is believed to be prominent among the dimers first formed.[9] For this reason it is of particular interest to investigate the further dehydrogenation of compounds of this type. Since **11** is difficult to synthesize in quantity, the often-used substitute, guaiacylglycerol β-guaiacyl ether (**12**), rather than **11**, was used in this work. This was dehydrogenated in aqueous solution under conditions similar to those used with the α-ethylvanillyl alcohol and the precipitate subjected to chromatographic separation. An amorphous, chromatographically pure substance **13** was isolated with the expected molecular weight as determined by vapor pressure osmometry, correct analytical values and having the characteristic

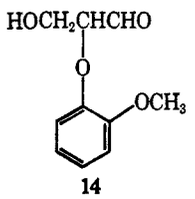
(6) F. R. Hewgill, *J. Chem. Soc.*, 4987 (1962).
(7) F. R. Hewgill and B. S. Middleton, *J. Chem. Soc., C*, 2316 (1967).
(8) W. Horwitz, "Official Methods of Analysis of the Official Association of Agricultural Chemists," 9th ed, Association of Official Agricultural Chemists, Washington, D. C., 1960, p 109.
(9) K. Freudenberg, Advances in Chemistry Series, No. 59, American Chemical Society, Washington, D. C., 1966, p 1.



11, R = CH=CHCH$_2$OH
12, R = H



13, R = —O— (OCH$_3$)



14

dienone triplet in the ir spectrum which was previously noted with the crystalline dioxepin from α-ethylvanillyl alcohol. The ultraviolet spectrum of the substance was very close to that of the crystalline dioxepin **3** mixed with an appropriate quantity of guaiacol isopropyl ether. The substance was readily reduced with $NaBH_4$ to give a product with a uv spectrum resembling in general shape that of **8**. A close match was achieved by mixing the calculated amount of guaiacyl isopropyl ether with **8**, both with the neutral and alkaline spectra.

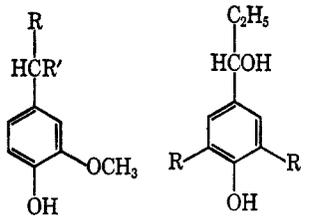
Acidolysis with aqueous acetic acid gave an orange color which was spectrally almost identical with that given by the crystalline compound **3**. Dioxepin **13** was also prepared by dehydrogenation of the *o,o'*-biphenyl of guaiacylglycerol β-guaiacyl ether.

The aqueous phase of the dehydrogenation mixture was examined for the expected side chain cleavage product, glyceraldehyde α-guaiacyl ether (**14**). This could not be isolated as such because of probable dimerization. The crystalline 2,4-dinitrophenylhydrazone derivative of aldehyde **14** was, however, readily obtained when the isolated side chain cleavage product was treated with the reagent. The acidic reagent solution apparently hydrolyzed this dimer, liberating **14** which then formed the derivative.

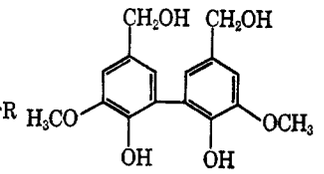
The ready dehydrogenation of these two compounds to dioxepins raises the question as to whether other *p*-hydroxybenzyl alcohols couple with side chain elimination or formation of dioxepins. Several compounds were screened without attempting to isolate the reaction products. With vanillyl alcohol (**15**), formaldehyde was readily detected in the reaction mixture and with apocynol (**16**), acetaldehyde was liberated. In both instances, the total product gave, on treatment with cold aqueous acetic acid, an orange color which was spectrally almost identical with that observed with dioxepin **3**. In the case of α-ethyl-*p*-hydroxybenzyl alcohol (**17**), propionaldehyde was evolved, and the product gave on acidolysis an orange color similar to that observed with the known dioxepins.

The dehydrogenation reaction proceeded very slowly, and care was necessary to avoid deactivating the peroxidase by an excess of hydrogen peroxide. Dehydrogenation of α-ethylsyringyl alcohol (**18**), either alone or in admixture with 4-propylguaiacol, yielded no propionaldehyde, but the former compound in admixture with dehydrodivanillyl alcohol (**19**) yielded both formaldehyde and propionaldehyde. Thus, the *p*-C in the syringyl compound probably serves as the spiro atom in dioxepin formation since the product gives an orange color with aqueous acetic acid.

Ethers of *p*-hydroxybenzyl alcohols are also of interest since the cyclic ether dimers dehydrodiconiferyl alcohol (**20**) and pinoresinol (**21**) are considered to be

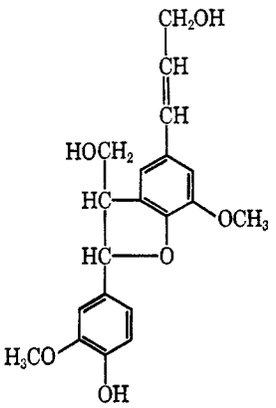


15, R = H; R' = OH
16, R = CH$_3$; R' = OH
22, R = H; R' = OC$_2$H$_5$

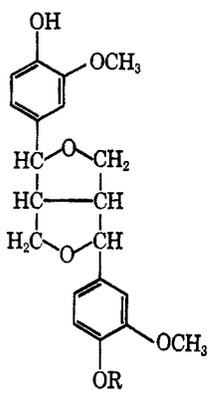


17, R = H
18, R = OCH$_3$

19



20



21, R = H
23, R = CH$_3$

intermediates in lignin formation. When vanillyl ethyl ether (**22**) was dehydrogenated in aqueous solution, the product gave substantial color on acidolysis with aqueous acetic acid indicating a dioxepin was formed. With dehydrodiconiferyl alcohol (**20**), on the other hand, no color was observed, and a dioxepin was not therefore indicated under the conditions used. Pinoresinol (**21**), being bifunctional, tends to form polyphenyl chains when dehydrogenated alone. For this reason, pinoresinol monomethyl ether (**23**) was used. The dehydrogenation product was light orange in color before acidolysis, but the intensity of color increased about 50% when treated with acid. Thus there is a suggestion of dioxepin formation, but the dioxepin appears to be hydrolyzed, in part, during the dehydrogenation (or some of the quinoidal substance is formed directly without intermediary dioxepin formation). Products from the dehydrogenation of coniferyl alcohol (biosynthetic lignin, DHP) are frequently orange in color and with some preparations gave additional color on acidolysis.

It is concluded that in the biosynthesis of lignin by the coupling of phenoxy radicals, resonance forms of

cyclohexadienone type **1** play an important role. This is especially true when the position *ortho* to the phenolic group is blocked as with a biphenyl linkage, a diphenyl ether linkage, or a methoxyl group. The coupling is generally accompanied by the expulsion of a side chain, and when biphenyl groups are involved, a dioxepin type of structure may be formed. These may persist as such, or may become hydrolyzed to quinoidal compounds, and thus contribute to the color of the woody tissue.

Experimental Section

α-Ethylvanillyl alcohol (**2**) was prepared by the Grignard reaction of vanillin and EtMgI[10] or by $NaBH_4$ reduction of 4-hydroxy-3-methoxypropiophenone, mp 83–84° (lit.[10] mp 84–85°).

Anal. Calcd for $C_{10}H_{14}O_3$: C, 65.92; H, 7.57. Found: C, 66.10; H, 7.71.

4,4′-α-Hydroxypropyl-6,6′-biguaiacol (**4**) was prepared by the $NaBH_4$ reduction of the *o,o′*-biphenyl of 4-hydroxy-3-methoxypropiophenone. It crystallized with 0.5 mol of water, which could not be removed without decomposition taking place, mp 106–108°.

Anal. Calcd for $C_{20}H_{26}O_6 \cdot 0.5H_2O$: C, 64.67; H, 7.33. Found: C, 64.61; H, 7.36.

Enzymic Dehydrogenation of 2 and Isolation of 6,9-Di(α-hydroxypropyl)-3′,4,11-trimethoxy-5′-[2′′-hydroxy-5′′-(α-hydroxypropyl)-3′′-methoxyphenyl]dibenzo[*d,f*][1,3]dioxepin-2-spiro-4′-cyclohexa-2′,5′-dienone (**3**).—**2** (10 g, 0.0275 mol) was dissolved in 4 l. of H_2O by heating and after cooling the solution to room temperature 40 mg of peroxidase was added to this rapidly stirred solution. 1% H_2O_2 (93.5 ml, 0.0275 mol, 0.055 equiv) was added with rapid stirring over 1 hr. Stirring was continued overnight and the mixture then filtered. The filter cake was air dried and dissolved in EtOAc and allowed to stand overnight. The crystals of **3** were filtered (29% yield) and recrystallized from EtOAc, mp 200–202°.

Tlc examination of the mother liquors showed much **3** still in solution, but this would not crystallize apparently because of impurities. In another experiment the precipitate showed 61% of **3** (or a conversion of 67% allowing for loss of side chain) by quantitative tlc analysis on silica GF with $CHCl_3$–EtOH, 100:15 as solvent. The dioxepin was also prepared by dehydrogenation of the biphenyl compound **4**: uv max 285.1 mμ (ε 1.02 × 10⁴) and 254.5 (1.82 × 10⁴); ir (KBr disk) 1640 (C=C, conjugated), 1665 (C=O), 1675 cm^{-1} (C=O); nmr (CD_6CO) δ 0.92 (t, 3, *J* = 7 Hz, C-5′′ **CH_3**), 0.97 (t, 6, *J* = 7 Hz, C-6, C-9 **CH_3**), 1.72 (m, 2, *J* = 7 Hz, C-5′′ **CH_2**), 1.78 (m, 4, *J* = 7 Hz, C-6, C-9 **CH_2**), 3.68 (s, 3, **CH_3O**), 3.86 (s, 3, **CH_3O**), 3.92 (s, 6, **CH_3O**), 3.99 (s, 1, CHOH), 4.14 (s, 1, CHOH), 4.20 (s. 1, CHOH), 4.48 (t, 1, C-5′′ CHOH), 4.61 (t, 2, C-6, C-9 CHOH), 5.92 (d, 1, *J* = 3 Hz, –C=CH), 6.75 (d, 1, *J* = 3 Hz, CH_3OC=CH), 6.80 (d, 1, *J* = 2 Hz, aromatic), 7.0 (d, 1, *J* = 2 Hz, aromatic), 7.18 (s, 4, aromatic), 7.32 (s, 1, phenolic OH).

Anal. Calcd for $C_{37}H_{42}O_{11}$: C, 67.06; H, 6.34; OCH_3, 18.73; mol wt, 662. Found: C, 66.78; H, 6.28; OCH_3, 18.65; mol wt, 702 (with Machrolab 301A).

Propionaldehyde was isolated by steam distilling the dehydrogenation mixture of **2** into a 2,4-dinitrophenylhydrazine solution and isolating the crystalline 2,4-dinitrophenylhydrazone, recrystallized from EtOH, mp 154–155° (lit.[11] mp 154°). Using 2.1 equiv (1.05 mol) of peroxide per mole of phenol and measuring the propionaldehyde liberated, a conversion of 82% was calculated, assuming all the side chain was formed by the indicated mechanism. Comparison with the 67% above suggests then some side chain is expelled through dimerization or polymerization.

Hexaacetate of 5,5′-Di(α-hydroxypropyl-3,3′-dimethoxy-2′-hydroxybiphenyl-2-yl 2,2′-Dihydroxy-3,3′-dimethoxy-5′-α-hydroxypropylbiphenyl-5-yl Ether (**8**).—After reduction of **3** with $NaBH_4$, the isolated powder was acetylated in pyridine–acetic anhydride and the acetate recovered as a powder. In the nmr spectra of the hexaacetate **8**, there was now no distinction between various **CH_3**, **CH_2**, **HCOAc** protons at the resonance center for each group: nmr ($CDCl_3$) δ 0.90 (t, 9, *J* = 6.5 Hz, **CH_3**), 1.88 (m, 6, *J* = 6.5 Hz, **CH_2**), 2.0 (s, 12, CH_3C=O), 2.07 (s, 3, CH_3C=O), 2.11 (s, 3, CH_3C=O), 3.70 (s, 3, OCH_3), 3.83 (s, 9, **OCH_3**), 5.63 (t, 3, *J* = 6.5 Hz benzylic **HC**), 6.12 (d, 1, *J* = 3 Hz, aromatic), 6.42 (d, 1, *J* = 3 Hz, aromatic), 6.73 (d, 1, *J* = 2 Hz, aromatic), 6.85 (s, 4, aromatic), 6.93 (d, 1, aromatic).

Anal. Calcd for $C_{49}H_{56}O_{17}$: CH_3CO, 28.2. Found: CH_3CO, 28.5.

Acidolysis of 3.—**3** (1 g) was dissolved in 100 ml of hot HOAc, 100 ml of H_2O was added, and the solution was allowed to stand overnight at room temperature. The solvent was removed at reduced pressure to give an orange acidolysis resin. Tlc of the resin on silica GF with 100:15 $CHCl_3$–EtOH as solvent showed the major components to be **9** and **4** as well as minor amounts of several other compounds.

2-[2′-Hydroxy-5′-α-hydroxypropyl-3′-methoxyphenyl]-6-methoxy-1,4-benzoquinone (**9**) crystallized from benzene: mp 132–133°; uv max (95% EtOH) 328 mμ (ε 3.6 × 10³), 286 (4.4 × 10³), 258 (1.2 × 10⁴); ir (KBr disk) 1600 cm^{-1} (C=C conjugated), 1650 (C=O), 1685 cm^{-1} (C=O); nmr ($CDCl_3$) δ 0.91 (t, 3, *J* = 7 Hz, **CH_3**), 1.75 (m, 2, *J* = 7 Hz, **CH_2**), 2.42 (s, 1, **HCOH**), 3.80 (s, 3, **CH_3O**), 3.86 (s, 3, **CH_3O**), 4.50 (t, 1, *J* = 7 Hz, **HCOH**), 5.93 (d, 1, *J* = 2.4 Hz, HC=C), 6.12 (s, 1, phenolic **OH**), 6.72 (d, 1, *J* = 1.8 Hz, aromatic), 6.77 (d, 1, *J* = 2.4 Hz, CH_3OC=**CH**), 6.95 (d, 1, *J* = 1.8 Hz, aromatic).

Anal. Calcd for $C_{17}H_{18}O_6$: C, 64.14; H, 5.70. Found: C, 64.28; H, 5.61.

1-(4-Hydroxy-3-methoxyphenyl)-2-(*o*-methoxyphenoxy)propane-1,3-diol (guaiacylglycerol β-guaiacyl ether) (**12**) was prepared by $NaBH_4$ reduction of the corresponding α-keto compound or by direct hydrogenolysis of the benzyl ether of the α-keto compound in EtOH over 10% Pd/C, and was isolated as a viscous oil. The ir and uv spectra showed absence of the α-carbonyl function, uv max (95% EtOH) 278 mμ (ε 5.28 × 10³).

Anal. Calcd for $C_{17}H_{20}O_6$: C, 63.73; H, 6.29. Found: C, 63.54; H, 6.48.

Enzymic Dehydrogenation of 12 and Isolation of the *o,o′*-Dihydroxybiphenyl.—**12** (1 g, 0.0031 mol) was dissolved in 10 ml of 50% aqueous ethanol and 5 mg of peroxidase was added. To this solution was added 4.8 ml (0.9 equiv of H_2O_2/mol of phenol) of 1% H_2O_2 with rapid stirring over 1 hr. The mixture was stirred 30 min longer and then allowed to stand overnight. The solvent was evaporated, and the biphenyl was separated out on a silicic acid column with 100:3.5 $CHCl_3$–EtOH solvent, and was isolated as a solid froth after solvent evaporation and drying in a vacuum oven, uv max (95% C_2H_5OH) 277.5 mμ (ε 4.24 × 10³).

Anal. Calcd for $C_{34}H_{38}O_{12}$: C, 64.08; H, 6.02. Found: C, 64.0; H, 5.95.

Enzymic Dehydrogenation of 12 and Isolation of Dioxepin 13.—**12** (2 g, 0.00625 mol) was dissolved in 2 l. of warm H_2O, and after the solution cooled to room temperature, 20 mg of peroxidase was added. To this solution was added with rapid stirring 22.3 ml (2.1 equiv of H_2O_2/mol of phenol) of 1% H_2O_2 during 1 hr and the mixture was allowed to stir overnight when it was filtered. Dioxepin **13** was purified by column chromatography on silicic acid with 100:7 $CHCl_3$–EtOH:solvent. It was also prepared from the *o,o′*-dihydroxybiphenyl compound of **12**. The uv spectrum of **13** was compared with that of **3** mixed with the appropriate quantity of guaiacyl isopropyl ether and **13** reduced with $NaBH_4$ was compared with **8** also mixed with the guaiacyl compound. Dioxepin **13** was subjected to acidolysis with aqueous acetic acid as described for **3** and the spectrum of the orange product compared with that from **3**: uv max (95% EtOH) 272 mμ (ε 1.55 × 10⁴); ir (KBr) 1640 (C=C, conjugated), 1660 (C=O, conjugated), and 1680 cm^{-1} (C=O).

Anal. Calcd for $C_{58}H_{60}O_{20}$: C, 51.06; H, 4.29; N, 14.90; mol wt, 1077. Found: C, 51.11; H, 4.42; N, 14.94; mol wt, 1090 (with Machrolab 301A).

Isolation of the Side-Chain Cleavage Product from the Dehydrogenation of 12.—The mother liquors from the aqueous enzymic dehydrogenation of **12** were saturated with salt and extracted with $CHCl_3$. The side chain cleavage product was isolated by preparative tlc on silica GF plates with 100:5 $CHCl_3$–EtOH solvent. The ir spectra showed no carbonyl, and the nmr spectra, while not fully interpreted, suggested a dimer. The compound was treated with 2,4-dinitrophenylhydrazine solution, and yielded a crystalline product, mp 145–146° from EtOH,

(10) P. C. Roberti, R. F. York, and W. S. Macgregor, *J. Amer. Chem. Soc.*, **72**, 5760 (1950).

(11) R. L. Shriner, R. C. Fuson, and D. Y. Curtin, "The Systematic Identification of Organic Compounds," 4th ed, John Wiley & Sons, Inc., New York, N. Y., 1959, p 283.

whose structure was consistent with the 2,4-dinitrophenylhydrazone of **14**: nmr ($CDCl_3$) δ 3.88 (s, 3, OCH_3), 4.03 (d, 2, J = 5.6 Hz, $HOCH_2$–), 4.98 (m, 1, J = 5.6 Hz, $HOCH_2CH$), 7.0 (m, 4, aromatic with OCH_3 on ring), 7.97 (d, 1, J = 5.6 Hz, HC=N). The following values are for the protons on the ring with NO_2 substitution: δ 7.94 (d, 1, J = 10.1 Hz, H_6), 8.35 (m, 1, J = 3.0 Hz, J = 10.1 Hz, H_5), 8.93 (d, 1, J = 3.0 Hz, H_3).

Anal. Calcd for $C_{16}H_{16}O_7N_4$: C, 64.67; H, 5.62; OCH_3, 20.15. Found: C, 64.71; H, 5.75; OCH_3, 20.32.

Acidolysis of Dioxepin 13.—The compound (16.8 mg, 0.0157 mmol) was dissolved in 1 ml of AcOH, 1 ml of H_2O was added, the mixture was allowed to stand overnight, then diluted to 50 ml with alcohol, and the visible portion of the spectrum was determined. This matched almost exactly the spectrum of a similar preparation using the crystalline dioxepin from α-ethylvanillyl alcohol **3** but in about half the above molar concentration. The spectrum was also very similar in shape to that obtained from benzoquinone **9** previously isolated from the acidolysis products of crystalline dioxepin **3**.

Vanillyl alcohol (15) was prepared by $NaBH_4$ reduction of vanillin, mp 113–114° (lit.[12] mp 113–114°).

Anal. Calcd for $C_8H_{10}O_3$: C, 62.32; H, 6.48. Found: C, 62.43; H, 6.60.

The compound was dehydrogenated in a manner similar to that used for **2**. Formaldehyde was detected in the solution by the chromotropic acid test.[13] The residue from the evaporation of the reaction mixture was treated with aqueous acetic acid to produce an orange color essentially as described for **3**.

α-Methylvanillyl alcohol (apocyanol) (16) was prepared by $NaBH_4$ reduction of acetovanillone:[14] mp 100–102° (lit.[14] mp 101–102°).

Anal. Calcd for $C_9H_{12}O_3$: C, 64.27; H, 7.19. Found: C, 64.15; H, 7.06.

This was dehydrogenated and subjected to acidolysis with acetic acid as above. Acetaldehyde was detected during the dehydrogenation by exposing sodium nitroprusside paper to the air above the mixture.[15]

α-Ethyl-*p*-hydroxybenzyl Alcohol (17).—To a solution of 15 g of 4′-hydroxypropiophenone in 102 ml of 1 *N* NaOH, 5 g of $NaBH_4$ dissolved in 50 ml of H_2O was added, and the mixture was allowed to stand overnight. The solution was acidified with 12 ml of AcOH, allowed to stand 16 hr, and the crystals were filtered and washed. Recrystallized twice from C_6H_6 gave 11.5 g of product, mp 75–76° (lit.[16] mp 73–75°).

Anal. Calcd for $C_9H_{12}O_2$: C, 71.03; H, 7.95. Found: C, 71.12; H, 8.08.

Dehydrogenation of 17 and Acidolysis of Product.—17 (1 g) was dissolved in 190 ml of hot H_2O, the solution cooled to 30°, 10 mg of proxidase dissolved in 10 ml of H_2O added, and then with vigorous stirring, 22.5 ml of H_2O_2 were added dropwise over a period of 2 hr. The odor of propionaldehyde was noted soon after the addition of peroxide was begun, and became fairly pronounced at the end of the operation. The mixture was allowed to stir 30 min longer, and then to stand 2 hr. The precipitate was filtered and dried. It amounted to 0.348 g, indicating that the peroxidase had probably been deactivated before completion of the reaction.

The precipitate (10 mg) was dissolved in 1.5 ml of warm AcOH, 0.5 ml of H_2O was added, and the solution was allowed to stand overnight. The orange solution was diluted to 25 ml with methyl Cellosolve and the visible spectrum was determined. It was similar to that observed with acidolysis of the dioxepin obtained from α-ethylvanillyl alcohol.

α-Ethyl-4-hydroxy-3,5-dimethoxybenzyl alcohol (18) was prepared by the action of EtMgBr on syringaldehyde in a manner similar to that described by Semechkina and Shorygina,[16] mp 96–97° (lit.[16] 94–95°).

4,4′-Dihydroxy-5,5′-dimethoxy-3,3′-biphenyldimethanol (dehydrodivanillyl alcohol) (19) was prepared by the reduction of dehydrodivanillin substantially as reported by Adler,[14] mp 196–197° (lit.[14] mp 187–190°).

Dehydrogenation of 19, a Mixture of 18 and 19 and Acidolysis of the Products.—A quantity of **19** (0.306 g, 1 mmol) was dissolved in 90 ml of hot H_2O, the solution was cooled to 30°, 10 ml of H_2O containing 5 mg of peroxidase was added, and the solution was stirred vigorously. H_2O_2 (1%, 3.06 ml) was added dropwise over a period of 1 hr. No odor of formaldehyde was apparent, but its presence was detected by the chromotropic acid reaction on a small portion of the mixture. Stirring was continued 1 hr, and the mixture was allowed to stand 1 hr. The precipitate was removed by filtration (mostly dehydrodivanillin), the filtrate was extracted with ethyl acetate, and the ethyl acetate was evaporated to give a resin.

This process was repeated adding, in addition to **19**, 0.212 g (1 mmol) of **18** and increasing the H_2O_2 to 5.10 ml. Propionaldehyde was soon in evidence.

Each of the resins (10 mg) was dissolved in 1 ml of AcOH, 1 ml of H_2O was added, and the solution was allowed to stand overnight. They were then diluted to 10 ml with EtOH, and the visible spectra determined. Both were similar to the colors previously obtained from the acidolysis of the dioxepins, but the spectrum from the mixed dehydrogenation showed a bulge in the 400-mμ region suggesting the presence of 2,6-dimethoxyquinone.

Dehydrogenation of Vanillyl Ethyl Ether (22).—The compound (1.0 g) was dissolved in 90 ml of H_2O, and 10 ml of H_2O containing 5 mg of peroxidase was added. The hydrogen peroxide (6.53 ml) was added in the usual manner, and the product separated. Formaldehyde was evolved and the product gave the typical quinoidal color on acidolysis with aqueous AcOH.

Pinoresinol (21) was isolated from spruce gum[17] and was purified by column chromatography on silicic acid, mp 121–122° (lit.[17] mp 121–122°).

Pinoresinol Monomethyl Ether (23).—To 17.9 g (0.05 mol) of pinoresinol in 75 ml of acetone was added 8.52 g (0.06 mol) of CH_3I and 6.90 g (0.05 mol) of K_2CO_3 and the mixture was refluxed and stirred for 24 hr when the acetone was evaporated. The mixture was taken up in H_2O and acidified, and the aqueous solution was extracted with $CHCl_3$. Product **23** was separated from the starting compound and the dimethyl ether by chromatography on silicic acid with 20:100 cyclohexane–$CHCl_3$ solvent. The product was isolated as an oil.

Anal. Calcd for $C_{21}H_{24}O_6$: C, 67.72; H, 6.50; OCH_3, 24.84. Found: C, 67.72; H, 6.69; OCH_3, 24.91.

Dehydrogenation of Pinoresinol Monomethyl Ether (23) and Acidolysis of Product.—The compound (0.200 g) was dissolved in 490 ml of boiling water, the solution was cooled, 10 ml of H_2O containing 10 mg of peroxidase was added and then dropwise, with stirring, 1.82 ml of 1% H_2O_2 was added over a period of 30 min. Stirring was continued 30 min more, the mixture was allowed to stand for 2 hr, filtered, and the precipitate was dried in a desiccator.

A solution of 10 mg of this precipitate in 5 ml of acetone was orange, similar to that exhibited by the dioxepins after acidolysis. The intensity of the color was increased by about 50% when 10 mg of the precipitate was dissolved in 0.4 ml of 50% aqueous AcOH, and the mixture was allowed to stand 2 hr before dilution with acetone.

Registry No.—**3**, 18588-26-6; **4**, 18588-27-7; **8** hexaacetate, 18588-28-8; **9**, 18588-29-9; **12**, 7382-59-4; *o,o′*-dihydroxybiphenyl of **12**, 18181-73-2; **13**, 18588-32-4; 2,4-dinitrophenylhydrazone of **14**, 18588-33-5; **23**, 487-39-8.

Acknowledgments.—Thanks are extended to Dr. John M. Harkin for supplying the dehydrodiconiferyl alcohol and samples of "biosynthetic lignins."

(12) V. C. Farmer, M. E. K. Henderson, and J. D. Russel, *Biochem. J.*, **74**, 257 (1960).

(13) W. Horwitz, "Official Methods of Analysis of the Official Association of Agricultural Chemists," 9th ed, Association of Official Chemists, Washington, D. C., 1960, p 389.

(14) E. Adler and S. Hernestam, *Acta Chem. Scand.*, **9**, 319 (1955).

(15) F. Feigl, "Spot Tests in Organic Analysis," 6th ed, Elsevier Publishing Co., New York, N. Y., 1960, p 352.

(16) A. F. Semechkina and N. N. Shorygina, *Izv. Akad. Nauk SSSR, Otd. Khim. Nauk*, 715 (1963).

(17) H. Erdtman in "Moderne Methoden der Pflanzenanalyse," Vol. 3, K. Paeche and M. V. Tracey, Ed., Springer-Verlag, Berlin, 1955, p 428.