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The Biomimetic Oxidation of β-1, β-0-4, β-5, and Biphenyl Lignin Model Compounds by Synthetic Iron Porphyrins

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Abstract—The degradation of four dimeric lignin model compounds by *meso*-tetra(2,6-dichloro-3-sulfonatophenyl)porphyrin iron chloride (TDCSPPFeCl) (**2**) are reported. 4-Ethoxy-3-methoxyphenylglycerol-β-guaiacyl ether (**3**) (a β-0-4 dimer) was cleaved to give 4-ethoxy-3-methoxybenzaldehyde (**4**) and guaiacol (**5**) as major products. The oxidation of 1-(4-ethoxy-3-methoxyphenyl)-2-(4-methoxyphenyl)-1,3-propandiol (**6**, a β-1 dimer) gave **4**, 4-methoxybenzaldehyde (**7**), and 4-methoxy-α-hydroxyacetophenone (**8**) as major products. Side chain oxidation and aromatic ring cleavage reactions were found to occur for the phenylcoumaran (α-5) model compound, ethyl dehydrodiisoeugenol (**12**). A biphenyl model compound, 4,4'-diethyldehydrodivanillin (**20**), was oxidized to give mono- and dicarboxy derivatives, as well as ring-cleaved products of the acid derivatives.

Introduction

Lignin is one of the most persistent natural polymers and its biodegradation might be a rate limiting step in the carbon–oxygen cycle on Earth.[1] The continued efforts made in recent years[2–4] have revealed, however, that under appropriate conditions, lignin can be metabolized very quickly. *Phanerochaete chrysosporium*, a white rot fungus, is the best lignin degrader found so far and has been found to be able to produce 21 isoenzymes[5] which can modify or degrade lignin or its model compounds. Fifteen of the isoenzymes belong to the group of lignin peroxidase[6,7] (ligninase) and the other six isoenzymes belong to the group of manganese-dependent peroxidases.[8]

Lignin peroxidases are heme proteins and an X-ray crystal structure of a lignin peroxidase has been solved by Poulos.[9] The active site (Figure 1) is completely

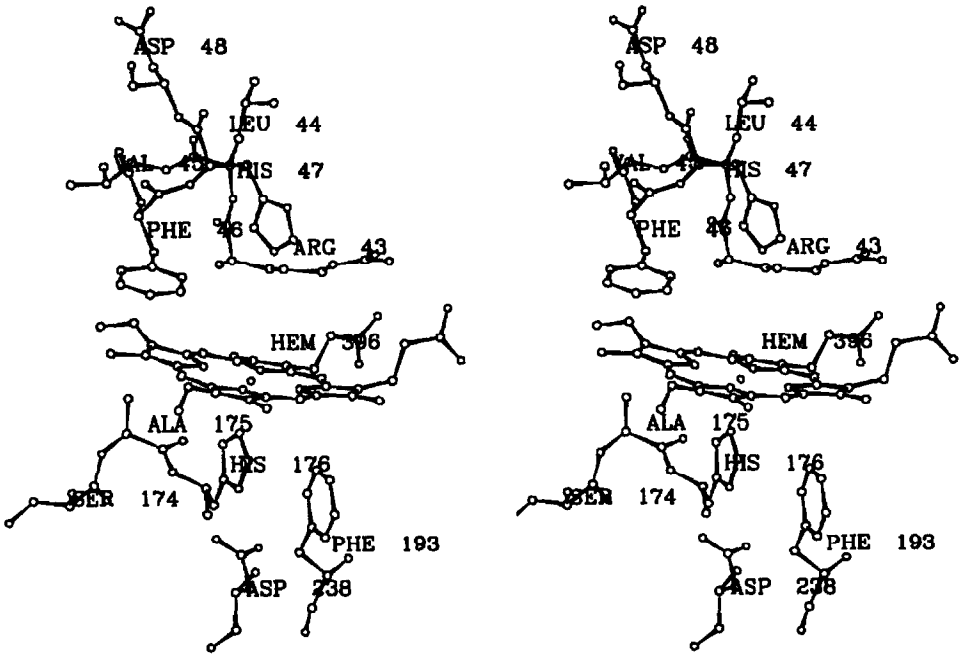

Figure 1. Stereotopic view of the active site of lignin peroxidase from *P. chrysosporium* (the coordinates were kindly provided by T. Poulos).

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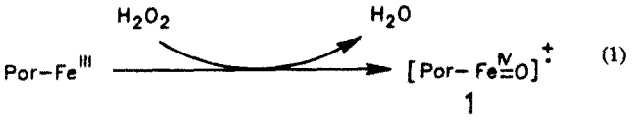
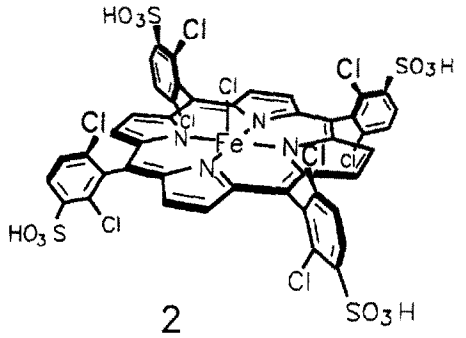
surrounded by protein and the iron center is not accessible to substrates. This is not surprising since the substrate is lignin which is a high molecular weight three dimensional polymer having a large number of structural features occurring in both enantiomeric forms, when chirality is possible, such that no single active site could accommodate these diverse structures.

Instead, the degradation of lignin is initiated by a single electron oxidation either directly by the oxidized heme or by a mediator such as the manganese ion.[10] Lignin peroxidase contains a 10Å channel through which H_2O_2 approaches and oxidizes the heme. As with most heme proteins this oxidation occurs as follows to give the oxoferryl (Fe(IV)porphyrin π-cation radical[11] (**1**).



(1)



Complex **1** is known as compound I (found in many heme proteins) and it has an oxidation state two electrons less than the resting enzyme. When compound I brings about a one electron oxidation of lignin it is reduced to compound II which can also oxidize lignin by one electron. The catalytic cycle for lignin peroxidase is shown in Figure 2.

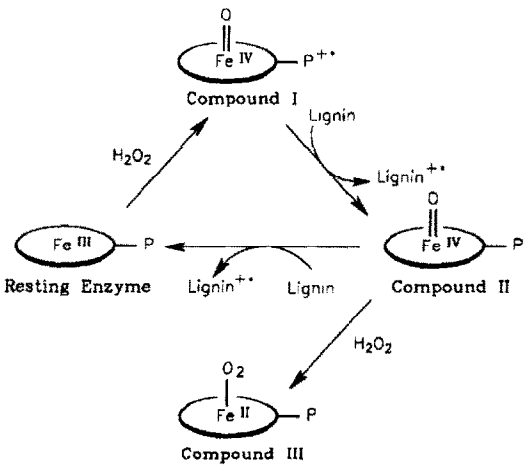


Figure 2. Proposed catalytic cycle of lignin peroxidase.

Compound I of all heme proteins are powerful oxidants and without the steric protection provided by the protein they would rapidly degrade, as do simple iron porphyrins, when reacted with hydrogen peroxide.

In order to overcome this lability of simple iron porphyrins we have prepared *meso*-tetra(2,6-dichloro-3-sulfonatophenyl)porphyrin iron chloride **2**. The eight chlorine atoms provide steric protection and at the same time the combined electronegativities raise the oxidation potential of the porphyrin bound iron.[12] These chlorinated porphyrins provide robust catalysts which closely mimic the lignin peroxidases in the way in which they react with lignin model compounds.

Lignin peroxidase can catalyze a variety of degradative reactions of lignin model compounds,[4,13] including Cα–Cβ cleavage, benzylic methylene group hydroxylation (Cα-hydroxylation), Cα-oxidation, Cα–Cβ double bond hydroxylation, demeth(ox)ylation and aromatic ring cleavage reactions. All the reactions follow the same mechanism of one-electron oxidation initiated by the highly reactive prosthetic group of lignin peroxidase, the protoporphyrin iron(IV) cation radical.[14] A number of sterically protected, water soluble porphyrins have been synthesized and used as lignin peroxidase models in our laboratory for the purpose of both mechanistic study and potential practical applications. The use of *meso*-tetra(2,6-dichloro-3-sulfonatophenyl)porphyrin iron chloride (**2**, TDCSPPFeCl) as a catalyst is reported here.

Being synthesized in plants through a peroxidase initiated free radical coupling of coniferyl, sinapyl and coumaryl alcohols, lignin is a random, heterogeneous polymer whose structure is represented by the different linkages and constituent units shown in Figure 3. The β-O-4 substructure (arylglycerol-β-aryl ether) is the most abundant (48 % for spruce lignin[15]) in lignin and β-O-4 dimers are the most often used dimeric model compounds in lignin biodegradation studies. The β-1 substructure (1,2-diaryl-1,3-propandiol), occurs in lignin in lesser amounts (7 % in spruce lignin,)[15] and is also often used in model studies. The phenylcoumaran substructure (β-5) constitutes 9–12 % of the substructures in spruce lignin. The biphenyl

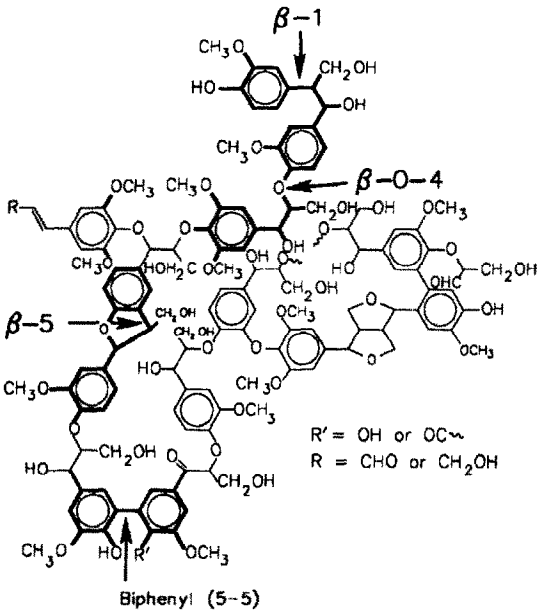


Figure 3. Partial structure of lignin. The substructure is discussed in this paper and highlighted with bold lines.

substructure is another one of the main substructures of lignin and 9.5–11 % of the linkages connecting the phenylpropanoid units of lignin are of the biphenyl type.

In this paper, we report our studies with dimeric model compounds which represent these four kinds of lignin substructures.

Results and Discussion

The β-O-4 dimer, 4-ethoxy-3-methoxyphenylglycerol-β-guaiacyl ether (**3**), was successfully cleaved at the Cα–Cβ bond by TDCSPPFeCl and *t*-BuOOH to give 4-ethoxy-3-methoxybenzaldehyde (**4**) and guaiacol (**5**) as the major products (Figure 4). Other products were found in very small amounts and have not been identified. In the degradation of β-O-4 dimers by lignin peroxidase, Cα–Cβ cleavage is also the major reaction.[7,16]

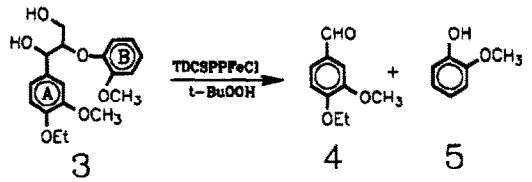


Figure 4. The oxidation of 4-ethoxy-3-methoxyphenylglycerol-β-guaiacyl ether (**3**) by TDCSPPFeCl and *t*-BuOOH.

Oxidation of 1-(4-ethoxy-3-methoxyphenyl)-2-(4-methoxyphenyl)-1,3-propandiol (**6**) by TDCSPPFeCl and *t*-BuOOH (Figure 5) give 4-ethoxy-3-methoxybenzaldehyde (**4**), 4-methoxybenzaldehyde (**7**), and 4'-methoxy-α-hydroxyacetophenone (**8**) as major products. Small amounts of 4-ethoxy-3-methoxybenzoic acid (**9**) and 4-methoxybenzoic acid (**10**) were also isolated by preparative TLC and detected by GC after methylation, with diazomethane, of the acidic products. 4-Methoxyphenylglycol (**11**), a possible intermediate of the reaction, was not seen on TLC. Careful GC and GC–MS analyses of the acetylated products detected only trace amount of 4-methoxyglycol (**11**) diacetate. Oxidation of synthetic **11** under the same condition gave **7**, the ketone **8**, and a small amount of **10**. A large amount of starting material, **11**, remained after the reaction. Competitive oxidation of a 1:1 (molar ratio) mixture of the β-1 dimer **6** and the glycol **11** indicated that the β-1 dimer **6** was preferentially oxidized over the glycol **11** under the condition of this experiment. Therefore, the glycol **11** was not an important intermediate in the reaction and ketone **8** was formed directly. In the oxidation of **6** by lignin peroxidase,[17–19] 4-ethoxy-3-methoxybenzaldehyde (**4**), 4-methoxybenzaldehyde (**7**), and the glycol **11** were major products and the ketone **8** was found only as a minor product under aerobic condition. Under anaerobic condition,[19] the ketone was not observed. The reason for the ketone **8** instead of glycol **11** being formed as the major product in this study is not clear. The greater oxidizing power of the electronically activated catalyst TDCSPPFeCl might be a factor. Mono-methoxylated aromatics such as 4-methoxybenzyl alcohol and 4-methoxyglycol are not readily oxidized by lignin peroxidase,[19–21] but can be readily oxidized by TDCSPPFeCl and *t*-BuOOH. Oxidation of the mono-methoxylated ring (B-ring) of the β- 1 dimer **6** to give the ketone directly as proposed by Renganathan *et al.*,[19] though not operative in the case of lignin peroxidase catalyzed reaction, might be a pathway of ketone **8** formation in the oxidation of **6** by TDCSPPFeCl. It was noted that when the β-1 dimers were oxidized using tetraphenylporphyrin iron chloride[22] and protohemin[23] as catalysts, the glycol was found to be produced in lesser amounts than the ketone, suggesting that common differences exist between lignin peroxidase catalyzed reaction and that catalyzed by the different model systems.

Degradation of phenylcoumaran (β-5) model compounds by lignin peroxidase has not been reported. In order to investigate the ability of TDCSPPFeCl in degrading the various substructures of lignin, we studied the oxidation of an easily available β-5 dimer, ethyl dehydrodiisoeugenol (**12**) (Figure 6). Oxidation of **12** by TDCSPPFeCl and *t*-BuOOH gave the sidechain cleaved product **13** as the initial product, further oxidation gave Cα–Cβ, cleaved products 4-ethoxy-3-methoxybenzaldehyde (**4**), **19**, the acids **14** and **15**, and the aromatic ring cleavage products **15** and **18**. Ring cleaved acid **16** was also found as a product. It is not clear if **16** was a product of aromatic cleavage of the acid **15** or was derived from the oxidation of the ring cleaved aldehyde **17**. The configuration of the c–d double bond of **17** was elucidated by comparing the coupling constant of H_c and H_d with the corresponding

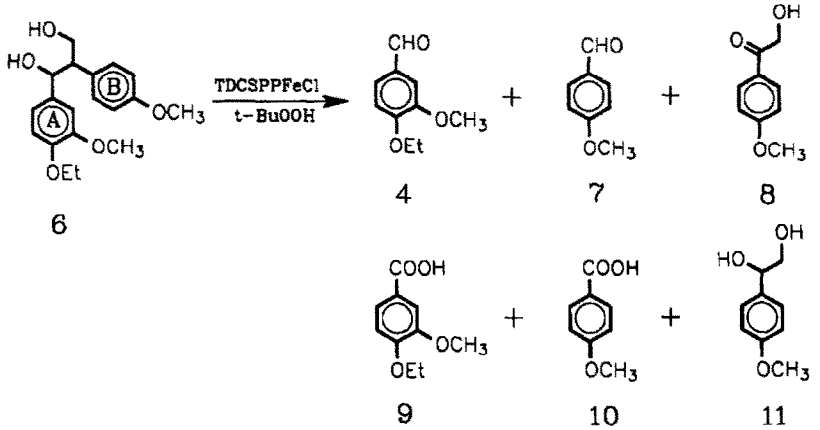


Figure 5. The oxidation of 1-(4-ethoxy-3-methoxyphenyl)-2-(4-methoxyphenyl)-1,3-propandiol (**6**) by TECSPPFeCl and *t*-BuOOH.

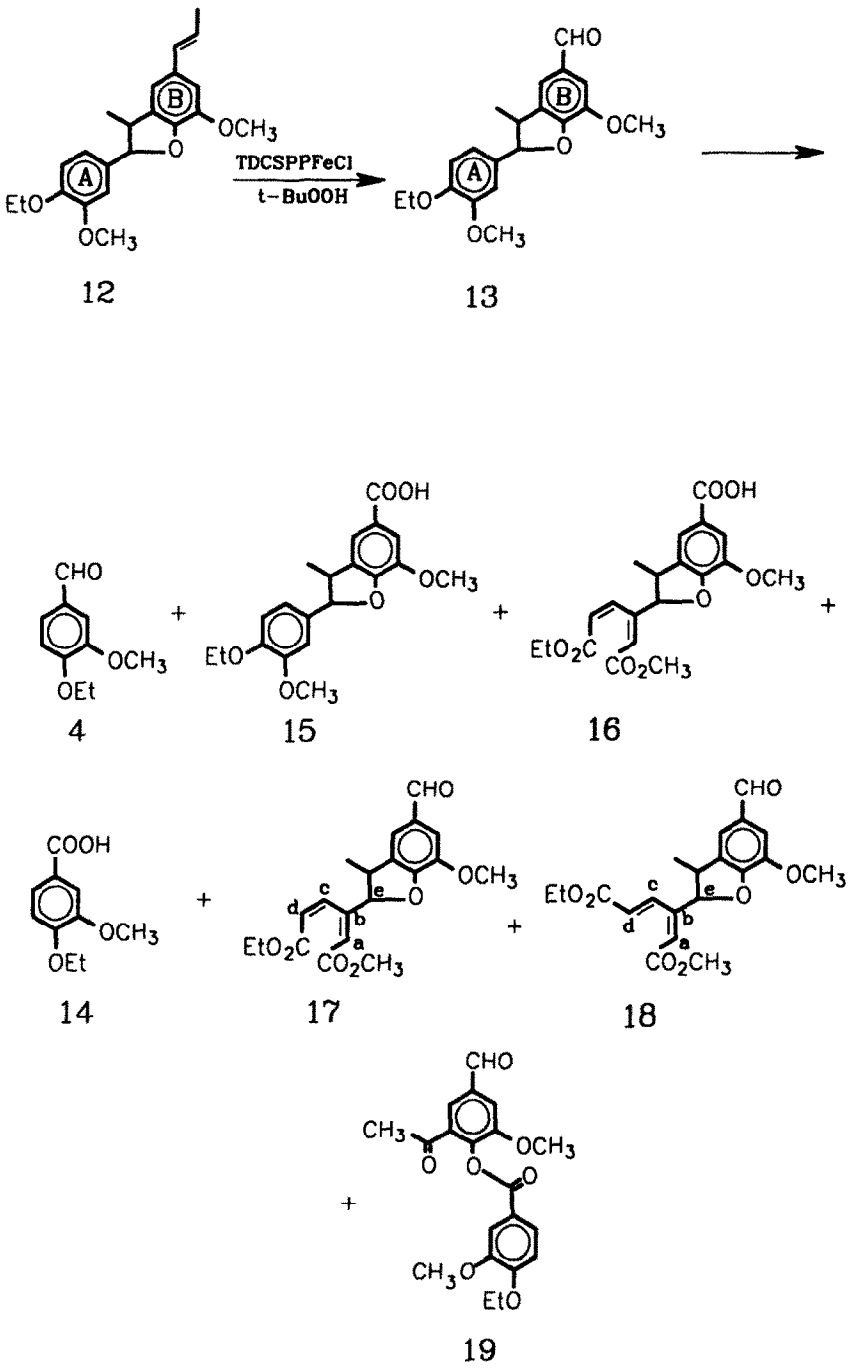


Figure 6. The oxidation of 4-*O*-ethyldehydrodiisocyenol (**12**) by TDCSPPFeCl and *t*-BuOOH.

coupling constant in compound **18**. The configuration of the a–b double bond was determined by NOE difference spectra. Positive signals appeared for H_a and H_c when H_e was irradiated.

Metabolism of, B-5 model compounds by a ligninolytic culture of *Phanerochaete chrysosporium* has been studied.[24,25] During the degradation of methyl dehydrodiconiferyl alcohol,[25] the double bond of the side-

chain was first hydroxylated to give the glycol, which was further cleaved to give the aldehyde. In the degradation of ethyl dehydrodiisoeugenol (**12**) by TDCSPPFeCl, however, the glycol was not isolated because of its further rapid reaction and the aldehyde **13** was isolated as the first product.

Cα–Cβ cleavages are important reactions for both β-1 and β-O-4 dimers catalyzed by lignin peroxidase and iron porphyrins. We have shown that TDCSPPFeCl can also catalyze the Cα–Cβ cleavage of β-5 model compounds. Aromatic ring cleavage of veratryl alcohol and derivatives,[26–28] and β-O-4 dimers[29–33] has been reported. It is interesting to note that in the case of β-O-4 dimers, aromatic ring cleavage occurred only for the B-ring (the β-phenoxy ring) in all the reactions[29–33] and ring cleavage on the A-ring (the phenylglycerol ring) of β-O-4 dimers was not found. In addition, no ring cleavage reaction has been found to occur for β-1 dimers. It is most probable that the Cα–Cβ cleavage reaction of β-1 and β-O-4 dimers occurs so rapidly and the A-ring cation radical is too short lived to be attacked by water and then by oxygen, which normally leads to the ring cleavage. The cation radicals of methoxybenzenes[34] and of the B-ring of an α-oxo β-O-4 dimer[35] have been detected by ESR. Spectral detection of the A-ring cation radical of β-1 and β-O-4 dimers, however, has not been reported.

The isolation of A-ring cleaved products **16–18** suggests that the Cα–Cβ cleavage of the β-5 dimer **13** was not the preferred route of degradation compared to that of β-1 and β-O-4 model compounds. The reason that ring cleavage on the B-ring of the β-5 dimer 13 was not found was probably due to the deactivation of the B-ring by the carbonyl group of the acid or aldehyde.

Guaiacyl substructures of lignin are easily condensed by the phenol oxidizing enzymes of white-rot fungi to give 5-5' biphenyl substructures,[36,37] which are substantially more resistant to microbial attack.[38] The degradation of the biphenyl substructure, therefore, is very important. The metabolism of dehydrodivanillic acid by bacteria[39] and by the whiterot fungus, *P. chrysosporium*,[40] have been reported and 5-carboxyvanillic acid was found as a product in both cases. However, the pathway of the reaction and the enzyme(s) responsible for the reactions remain unclear. Degradation of tetrameric model compounds containing biphenyl structure by bacteria,[41,42] and by *P chrysosporium*[43] has also been studied and the major reactions were side-chain cleavage. Aromatic ring cleavage or biphenyl bond cleavage of the biphenyl structure were not found.

We selected 4,4'-diethyldehydrodivanillin (**20**) as our biphenyl model compound because of solubility problems associated with dehydrodivanillin itself and possible side-chain reactions previously reported for the tetramers[37–39] would make the reactions too complex. Compound **20** was oxidized by TDCSPPFeCl and *t*-BuOOH at its side-chain to give mono- and dicarboxy derivatives **21** and **22** (Figure 7). Two direct ring cleavage products **23** and **24** were also isolated. Initially the structure of **23** was somewhat uncertain as to where ring cleavage had occurred, i.e. whether **23** was derived from **21** by cleavage of the carboxy- or formyl-bearing ring was not clear. Comparison of the ^{1}H NMR and mass spectra of **23** with those of **20** and compounds **22–24** supports the structure of **23** shown in Figure 7. In addition, it seems that ring cleavage takes place only on the ring having a carboxy group as no ring cleavage products derived directly from **20** have been isolated.

The isolation of **23** and **24** shows clearly that the aromatic ring of biphenyl structure can be cleaved directly without prior demeth(ox)ylation, most probably through the same one-electron oxidation mechanism of lignin model compound oxidation. Shimada *et al.*[44] have proposed a cation-radical pathway for aromatic ring cleavage of veratryl alcohol based on isotope labelling experiments. Similar direct ring opening products have been identified from the oxidation of a β-O-4 dimer,[45] veratryl acetate,[28] 1-(4-ethoxy-3-methoxyphenyl)propane,[46] veratryl methyl ether,[27] and the phenylcoumarin dimer described above. Studies with veratryl alcohol as a model compound showed[27] that it was metabolized to carbon dioxide by *P. chrysosporium* through quinone and aromatic ring cleavage products as intermediates. Aromatic ring cleavage of biphenyl structures is also probably one of the first and most critical steps for their complete degradation.

The oxidation of biphenyl type model compounds by purified lignin peroxidase or other lignin degrading enzymes has not been reported; it can be predicted from this study that lignin peroxidase can oxidize biphenyl

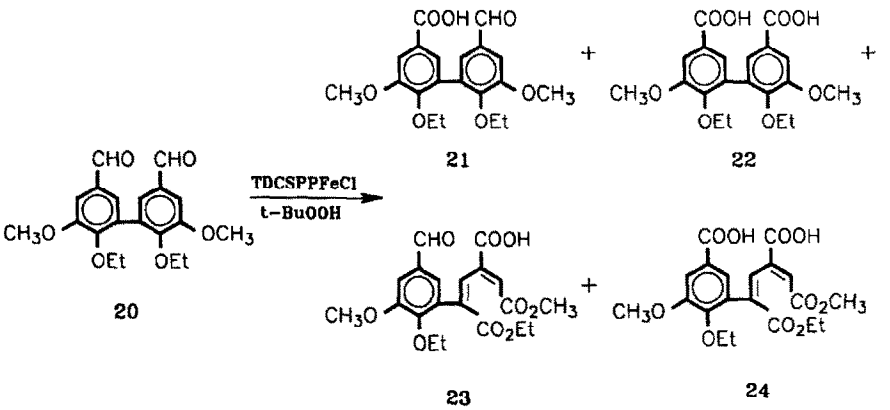


Figure 7. The oxidation of 4,4'-diethyldehydrodivanillin by TDCSPPFeCl and *t*-BuOOH.

structures since TDCSPPFeCl mimics lignin peroxidase in oxidizing most lignin model compounds. In conclusion, TDCSPPFeCl was shown to be a good model for lignin peroxidase in oxidizing β-1 and β-O-4 dimers. This biomimetic lignin peroxidase can catalyze Cα–Cβ cleavage and side-chain oxidation (from aryl aldehyde to aryl acid). A direct aromatic ring opened product derived from cleavage of the A-ring of a β-5 dimer was isolated for the first time. Direct aromatic ring cleavage of a biphenyl dimer was demonstrated to occur, probably through the well documented one electron oxidation pathway.

Experimental

NMR Spectra were recorded on a Bruker WH 400 spectrometer using tetramethylsilane as an internal standard. Low resolution mass spectra were recorded on a AEI MS9 spectrometer and high resolution mass spectra (HRMS) were recorded on a Kratos MS50 spectrometer. An HP 5890A Gas Chromatograph equipped with a 25 meter HP17 capillary column was used for the GC analyses. Delsi Nermag R10-10C/Kratos MS80RFA were used for the GC–MS analyses. Preparative thin layer chromatography (TLC) were performed on a Model 7924T Chromatotron (Harrison Research) using silica gel 60 PF_{254} as absorbent. Merck precoated silica gel 60 PF_{254} TLC plates (0.2 mm) was used for analytical purpose. *t*-BuOOH, and isoeugenol were from Aldrich.

Syntheses of model compounds

4-Ethoxy-3-methoxyphenylglycerol-β-guaiacyl ether[47] (**3**), 1-(4-ethoxy-3-methoxyphenyl)-2-(4-methoxyphenyl)-1,3-propandiol[48] (**6**), ethyl dehydrodiisoeugenol[49] (**12**) and 4,4'-diethyldehydrodivanillin[50] (**20**) were synthesized following reported procedures. Compounds **12** and **20** were characterized by ^{1}H NMR and MS.

Compound **12**: ^{1}H NMR ($CDCl_3$) δ: 1.39 (3H, d, J = 7 Hz), 1.46 (3H, t, J = 8 Hz), 2.07 (3H, dd, J_1 = 7 Hz, J_2 = 0–1 Hz), 3.43–3.52 (lH, m), 3.87 (3H, s), 3.90 (3H, s), 4.10 (2H, q, J = 8 Hz), 5.12 (lH, d, J = 9 Hz), 6.07–6.17 (lH, m), 6.34–6.42 (1H, m), 6.77–7.01 (5H, m). MS m/z (%): 355 (24.1, M+l), 354 (100.0, M), 137 (14.1), 91 (16.7), 77 (21.0), 65 (14.6), 55 (19.2).

Compound **20**: ^{1}H NMR ($CDCl_3$) δ: 1.12 (6H, t, J = 7 Hz), 3.98 (6H, s), 4.02 (4H, q, J = 7 Hz), 7.46 (2H, d, J = 1–2 Hz), 7.51 (2H, d, J = 1–2 Hz), 9.91 (2H, s). MS m/z (%) 359 (M+l, 10.4), 358 (M, 52.2), 302 (20.8), 241 (10.7), 91 (16.2), 32 (15.9), 28 (100.0).

4-Methoxyphenylglycol (**11**) was prepared by sodium borohydride reduction of 4-methoxy-α-hydroxyacetophenone acetate, which was prepared following reported procedures.[51]

Oxidation of model compounds and analysis of products

All the reactions were carried out at room temperature in air, at the end of the reaction excess oxidant was removed and the products were separated by preparative TLC as previously described[28] after derivatization. The isolated products were characterized by NMR and MS. 4-Ethoxy-3-methoxyphenylglycerol-β-guaiacyl ether (**3**) (0.5 mmol) was oxidized by TDCSPPFeCl (1 μmol) and either 1.5 mmol of *m*-chloroperbenzoic acid or 2.5 mmol of *t*-BuOOH in 50 mL of 1:1 acetonitrile/ pH 3 buffer (0.1 M phosphate) for 3 h. The products were extracted with ethyl acetate, dried and acetylated with excess 1: 1 acetic anhydride/pyridine for 24 h. The oxidation of **6** and derivatization of the products were the same except the reaction time was 6 h. Compound **12** (0.5 mmol) was oxidized by TDCSPPFeCl (0.5 μmol) and *t*-BuOOH (2.5 mmol) in 60 mL of 2:1 acetonitrile/pH3 buffer for 2 h to give **13** as the major product with small amounts of **4**. Compound **13** was separated and oxidized under the same condition for 3 h. The oxidation of **13** gave acidic products which were methylated with diazomethane before separation.

NMR and MS characterization of products

Compound **8**[52]: ^{1}H NMR ($CDCl_3$) δ: 3.58 (1H, t, J = 5 Hz, exchangeable), 3.90 (3H, s), 4.83 (2H, d, J = 5 Hz), 6.98 (2H, d, J = 9 Hz), 7.90 (2H, d, J = 9 Hz). MS m/z (%): 166 (10.0), 136 (30.7), 135 (100.0), 107 (35.5), 92 (46.2), 77 (64.4), 64 (21.0).

Compound **13**: ^{1}H NMR ($CDCl_3$): 1.44–1.49 (6H, overlap of a doublet and a triplet), 3.53–3.62 (1H, m), 3.87 (3H, s), 3.96 (3H, s), 4.12 (2H, q, J = 8 Hz), 5.27 (1H, d, J = 9 Hz), 6.85–6.98 (3H, m, aromatic), 7.35–7.42 (2H, aromatic), 9.86 (1H, s). MS m/z (%): 343 (21.9, M+1), 342 (100.0, M), 314 (11.9), 313 (21.3), 157 (11.4), 149 (12.7), 137 (33.7), 103 (10.3), 95 (19.1), 91 (29.9), 89 (16.0), 79 (18.2), 78 (17.6), 77 (58.2), 76 (18.0), 65 (33.2), 64 (12.5), 63 (19.5), 55 (31.6), 53 (16.1), 52 (10.6), 51 (28.6), 44 (22.0), 43 (15.0), 41 (12.9), 40 (24.3).

Compound **15**, (isolated as its methyl ester): ^{1}H NMR ($CDCl_3$) δ: 1.42 (3H, d, J = 7 Hz), 1.46 (3H, t, J = 8 Hz), 3.49–3.58 (1H, m), 3.87 (3H, s), 3.91 (3H, s), 3.94 (3H, s), 4.11 (2H, q, J = 8 Hz), 5.22 (1H, d, J = 9 Hz), 6.84–6.97 (3H, aromatic), 7.51 (2H, broad s, aromatic). MS m/z (%): 372 (18.3, M), 167 (21.0), 149 (58.5), 111 (10.4), 83 (11.9), 77 (10.7), 76 (10.9), 71 (49.4), 70 (50.5), 69 (18.8), 65 (10.9), 59 (16.6), 57 (100.0), 56 (24.7), 55 (47.7), 44 (32.1), 43 (69.4), 42 (13.3), 41 (61.0), 40 (61.2).

Compound **16**, (isolated as its methyl ester): ^{1}H NMR ($CDCl_3$) δ: 1.27 (3H, t, J = 8 Hz), 1.39 (3H, d, J = 7 Hz), 3.42–3.47 (1H, m), 3.69 (3H, s), 3.90 (3H, s), 3.95 (3H, s), 4.17 (2H, q, J = 8Hz),5.18 (1H, d, J = 8Hz),6.07 (1H, d, J = 12–13Hz), 6.14 (1H, d, J = 0–lHz), 6.94 (1H, dd, J_1 = 12–13 Hz, J_2 = 0–1 Hz), 7.49–7.54 (2H, aromatic). MS m/z (%): 404 (23.8), 373 (18.3), 358 (35.3), 345 (17.3), 344 (37.7), 331 (27.9), 330 (27.7), 315 (15.3), 314 (17.2), 299 (40.0), 298 (19.6), 285 (20.0), 271 (27.7), 269 (17.1), 267 (22.6), 179 (100.0), 167 (15.5), 151 (23.4), 150 (22.3), 149 (33.3), 123 (26.0), 59 (17.1), 57 (28.5), 41

(20.5). Exact mass calcd for $C_{21}H_{24}O_8$: 404.1472; found (HRMS): 404.1479.

Compound **17**: ^{1}H NMR ($CDCl_3$) δ: 1.26 (3H, t, J = 8 Hz), 1.41 (3H, d, J = 8 Hz), 3.45–3.54 (1H, m), 3.69 (3H, s), 3.96 (3H, s), 4.18 (2H, q, J = 8 Hz), 5.23 (1H, d, J = 8 Hz), 6.09 (1H, d, J = 12Hz), 6.13 (1H,m), 6.91–6.97 (1H,m), 7.30 (1H, broad s), 7.35 (1H, broad s), 9.83 (1H, s). MS m/z (%): 374 (31.0), 342 (25.9), 329 (24.8), 328 (72.2), 315 (29.1), 314 (42.0), 301 (72.7), 300 (44.1), 296 (29.3), 270 (23.2), 269 (100.0), 241 (61.3), 240 (21.3), 213 (54.3), 209 (21.1), 181 (20.9), 155 (40.0), 150 (35.9), 149 (23.3), 135 (20.2), 123 (67.4), 115 (24.2), 111 (26.6), 91 (28.8), 79 (27.0), 77 (37.5), 69 (26.8), 65 (24.1), 59 (69.1), 57 (25.8), 55 (32.6), 53 (21.5), 51 (25.1), 44 (37.8), 43 (51.1), 41 (33.9). Exact mass calcd for $C_{20}H_{22}O_7$: 374.1366; found (HRMS): 374.1369.

Compound **18**: ^{1}H NMR ($CDCl_3$) δ: 1.33 (3H, t, J = 8 Hz), 1.49 (3H, d, J = 8 Hz), 3.40–3.46 (1H, m), 3.76 (3H, s), 3.98 (3H, s), 4.16 (2H, q, J = 8 Hz), 5.25 (1H, d, J = 7 Hz), 6.16 (lH, d, J = 16 Hz), 6.16 (1H, s, broad), 7.33 (1H, s, broad), 7.40 (1H, s, broad), 8.50 (1H, d, broad, J = 16 Hz), 9.85 (1H, s). MS m/z (%): 374 (28.7), 342 (11.8), 328 (37.2), 314 (22.8), 301 (100.0), 300 (23.4), 269 (61.9), 268 (29.9), 241 (38.6), 213 (26.1), 150 (25.3), 123 (25.1), 111 (27.8), 77 (20.2), 59 (25.4), 57 (20.9), 55 (24.1), 44 (33.3), 43 (33.9), 41 (22.0). Exact mass calcd for $C_{20}H_{22}O_7$: 374.1366; found (HRMS): 374.1371.

Compound **19**: ^{1}H NMR ($CDCl_3$) δ: 1.53 (3H, t, J = 8 Hz), 2.59 (3H, s), 3.92 (3H, s), 3.97 (3H, s), 4.22 (2H, q, J = 8 Hz), 6.99 (1H, d, J = 8 Hz), 7.68–7.72 (2H, m), 7.90 (1H, dd, J_1 = 8 Hz, J_2 = 2 Hz), 7.96 (1H, d, J = 2 Hz), 10.03 (1H, s). MS m/z (%): 372 (2.4), 180 (36.8), 179 (100.0), 123 (37.3), 65 (20.7), 59 (31.5), 57 (26.4), 55 (22.6), 51 (20.1), 43 (55.9), 41 (36.9), 31 (20.7).

Compound **21** (isolated as its methyl ester): ^{1}H NMR ($CDCl_3$) δ: 1.09 (3H, t, J = 7 Hz), 1.12 (3H, t, J = 7 Hz), 3.91 (3H, s), 3.96 (3H, s), 3.97 (3H, s), 3.94–4.04 (4H, m), 7.43 (1H, d, J = 1–2 Hz), 7.49 (1H, d, J = 1–2 Hz), 7.65 (2H, s, broad), 9.90 (1H, s). MS m/z (%): 389 (M+1, 22.5), 388 (M, 100.0), 360 (20.7), 332 (18.6), 301 (15.1), 300 (56.9), 272 (15.5), 271 (15.8). Exact mass calcd for $C_{2l}H_{24}O_7$: 388.1523; found (HRMS): 388.1529.

Compound **22** (isolated as its dimethyl diester): ^{1}H NMR ($CDCl_3$) δ: 1.10 (6H, t, J = 7 Hz), 3.90 (6H, s), 3.95 (6H, s), 3.96 (4H, q, J = 7Hz), 7.62 (4H, s). MS m/z (%): 419 (M+1, 22.8), 418 (M, 100.0), 390 (22.6), 358 (21.2), 330 (82.6), 313 (26.8), 299 (20.5), 284 (21.6), 242 (29.3), 209 (21.3), 59 (25.3), 57 (20.7), 43 (21.5), 41 (22.9). Exact mass calcd for $C_{22}H_{26}O_8$: 418.1628; found (HRMS): 418.1632.

Compound **23** (isolated as its methyl ester): ^{1}H NMR ($CDCl_3$) δ: 1.21 (3H, t, J = 7 Hz), 1.27 (3H, t, J = 7 Hz) 3.76 (3H, s), 3.82 (3H, s), 3.94 (3H, s), 4.10 (2H, q, J = 7 Hz), 4.16 (2H, q, J = 7 Hz), 6.80 (1H, d, J = 1–2 Hz), 7.26 (1H, d, J = 1–2 Hz), 7.50 (lH, d, J = 1–2 Hz), 7.54 (1H, d, J = 1–2 Hz), 9.93 (1H, s). MS m/z (%): 420 (M, 4.6), 361 (5.6), 347 (14.8), 315 (7.1), 287 (27.7), 259 (13.5), 149 (12.7), 115 (18.1), 91 (14.2), 77 (20.8), 69 (13.6), 63 (13.9), 57 (17.3), 55 (20.1), 51 (18.7), 50 (15.0), 45 (19), 44 (100.0). Exact mass calcd for $C_{21}H_{24}O_9$: 420.1421; found (HRMS): 420.1429.

Compound **24** (isolated as its dimethyl diester): ^{1}H NMR ($CDCl_3$) δ: 1.21 (3H, t, J = 7 Hz), 1.25 (3H, t, J = 7 Hz), 3.76 (3H, s), 3.80 (3H, s), 3.92 (6H, s), 4.05 (2H, q, J = 7 Hz), 4.14 (2H, q, J = 7 Hz), 6.77 (1H, d, J = 1–2 Hz), 7.24 (lH, d, J = 1–2 Hz), 7.63 (1H, d, J = 1–2 Hz), 7.73 (1H, d, J = 1–2 Hz). MS m/z (%): 450 (m, 18.2), 391 (46.3), 377 (58.5), 359 (21.2), 345 (48.1), 331 (40.8), 318 (23.7), 317 (100.0), 303 (39.2), 287 (23.0), 285 (34.9), 273 (24.7), 262 (42.6), 259 (26.8), 258 (31.9), 257 (22.0), 234 (33.3), 233 (22.9), 203 (67.2), 175 (20.5), 149 (42.0), 115 (25.6), 91 (31.5), 77 (32.4), 71 (20.9), 69 (25.2). Exact mass calcd for $C_{22}H_{26}O_{10}$: 450.1526; found (HRMS): 450.1524.

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