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Biomimetic Degradation of Lignin and Lignin Model Compounds by Synthetic Anionic and Cationic Water Soluble Manganese and Iron Porphyrins

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Abstract—The biomimetic oxidation of 5-5′ condensed and diphenylmethane lignin model compounds with several water soluble anionic and cationic iron and manganese porphyrins in the presence of hydrogen peroxide is reported. The oxidative efficiency of manganese and iron *meso*-tetra(2,6-dichloro-3-sulphonatophenyl) porphyrin chloride (TDCSPPMnCl and TDCSPPFeCl, respectively), *meso*-tetra-3-sulphonatophenyl porphyrin chloride (TSPPMnCl) and manganese *meso*-tetra(*N*-methylpyridinio)porphyrin pentaacetate (TPyMePMn($CH_3COO)_5$) was compared on the basis of the oxidation extent of the models tested. Manganese porphyrins were found more effective in degrading lignin substructures than iron ones. Among them the cationic TPyMePMn ($CH_3COO)_5$, never used before in lignin oxidation, showed to be the best catalyst. The catalytic activity of porphyrins in hydrogen peroxide oxidation of residual kraft lignin was also investigated. The use of quantitative ^{31}P NMR allowed the focusing on the occurrence of different degradative pathways depending on the catalyst used. TPyMePMn($CH_3COO)_5$ was able to perform the most extensive degradation of the lignin structure, as demonstrated by the decrease of aliphatic hydroxyl groups and carboxylic acids. Noteworthy, no significant condensation reactions occurred during manganese porphyrins catalyzed oxidations of residual kraft lignin, while in the presence of iron porphyrins a substantial increase of condensed substructures was detected. © 1999 Elsevier Science Ltd. All rights reserved.

Introduction

Lignin is a complex tridimensional biopolymer highly recalcitrant to biodegradation. In view of the renewed interest in alternative pulping and bleaching processes, the possibility of using enzymes as mild and environmentally benign agents is being considered by the pulp and paper industry.[1] In particular, the white-rot fungus *Phanerochaete chrysosporium* has been found so far the best lignin degrader. This fungus produces several isoenzymes which belong to the groups of lignin peroxidases (ligninases) and manganese dependent peroxidases.[2,3] The active center of such enzymes is constituted by a heme site, which in the presence of hydrogen peroxide performs a one electron oxidation of the lignin aromatic moieties.[4] The catalytic cycle consists in a two electron oxidation of Fe(III)protoporphyrin IX to give a highly reactive oxo-iron(IV) protoporphyrin IX π-cation radical which is than reduced to the initial state by two different one electron reductions by the substrates (Fig. 1).[5]

Biomimetic catalysts such as metalloporphyrins, which can yield highly oxidized metallo-oxo species, have been used as lignin peroxidase models, and their potentiality for lignin degradation has been a subject of several studies.[6] However, natural porphyrins are unstable under catalytic oxidation conditions due to their self-destruction or to the formation of inactive μ-oxo complexes. In addition their solubility in water is negligible. In order to overwhelm these problems, sulphonatophenyl groups were added to the porphyrin ring to increase both their water solubility and redox potential, i.e. the resistance to oxidants.[7–12] Furthermore sterically and/or electronically protected polychlorinated or polyfluorinated porphyrins were synthesized to increase their stability.[9,12]

Residual kraft lignin contains significant amounts of 5-5′ and diphenylmethane substructures.[13] To date there are no literature reports dealing with the behavior of porphyrins toward such lignin moieties despite the fact that

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Key words: Lignin; oxidation; porphyrins; ^{31}P NMR; biomimetic.

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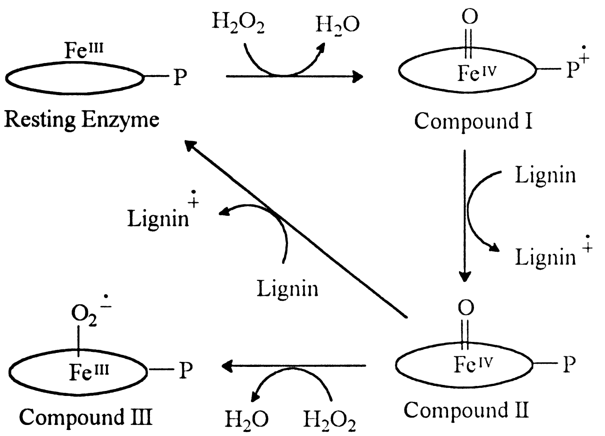
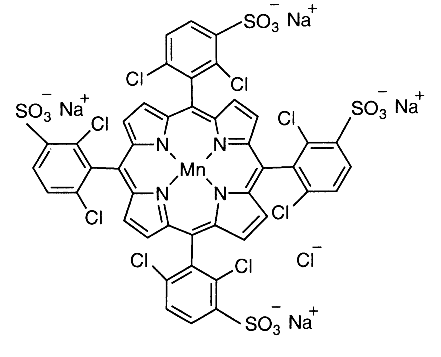


Figure 1. Catalytic cycle of lignin peroxidase. The resting enzyme is oxidized by H_2O_2 to π-cation radical known as compound **I**. Compound **I** undergoes a one electron oxidation of lignin and is reduced to compound **II** which can in turn perform an other one-electron oxidation of lignin thus regenerating the enzyme in its resting form.

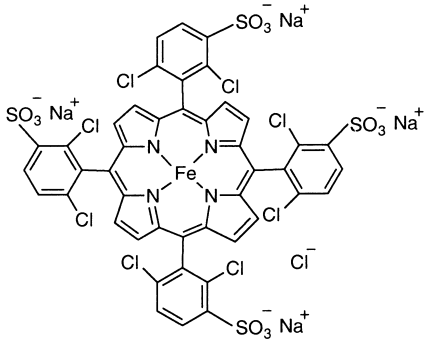
5-5′ models carrying electron withdrawing substituents instead of the electron donating groups typical of lignin substructures, and monomeric models have been widely studied.[6–11] The oxidative behavior of porphyrins on isolated lignins and residual kraft lignins has not been much explored.[12]

In this work we report the oxidation pathway of 5-5′ and diphenylmethane lignin models, compounds **1** and **5**, respectively, in the presence of cationic and anionic water soluble porphyrins, using hydrogen peroxide as oxidant. The oxidative efficiency of anionic manganese and iron *meso*-tetra(2,6-dichloro-3-sulphonatophenyl) porphyrin chloride (TDCSPPMnCl and TDCSPPFeCl, respectively) and *meso*-tetra-4-sulphonatophenyl porphyrin chloride (TSPPMnCl) was compared on the basis of the oxidation extent of the tested models (Fig. 2). The catalytic activity of the cationic manganese *meso*-tetra(*N*-methylpyridinio)-porphyrin pentaacetate (TPyMePMn($CH_3COO)_5$) on lignin model compounds was determined at two different pH values, 3 and 6 (Fig. 2).

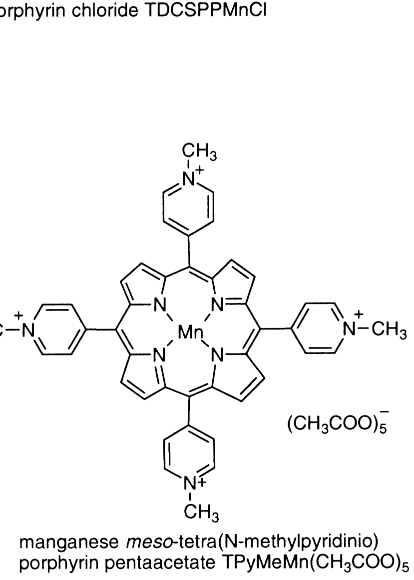
The catalytic efficiency of the previously reported anionic and cationic porphyrins, was also tested on residual kraft lignin in order to evaluate any possible difference in the transformations induced in the polymer substructure. The structure of residual kraft lignin before and after oxidation was determined by ^{31}P NMR spectroscopy. Advanced magnetic resonance techniques have proven to be excellent analytical tools for the structural elucidation of these complex biopolymers. In particular, Argyropoulos[14–19] developed ^{31}P based novel magnetic resonance methods for suitably phosphitylated lignins that allow the detection and quantitative



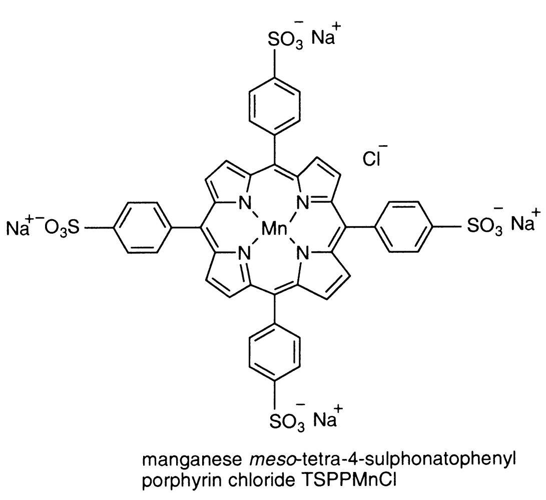
manganese *meso*-tetra(2,6-dichloro-3-sulphonatophenyl) porphyrin chloride TDCSPPMnCl



iron *meso*-tetra(2,6-dichloro-3-sulphonatophenyl) porphyrin chloride TDCSPPFeCl



manganese *meso*-tetra(N-methylpyridinio) porphyrin pentaacetate TPyMeMn($CH_3COO)_5$



manganese *meso*-tetra-4-sulphonatophenyl porphyrin chloride TSPPMnCl

Figure 2. Structures of porphyrins used as catalysts in the oxidation of lignin and lignin model compounds.

determination of all functional groups in lignin possessing reactive hydroxyl groups, that is, aliphatic OH, the various forms of phenolic OH and carboxylic acids.

The analysis of the structural modifications induced by porphyrins catalyzed oxidation of residual kraft lignin, performed by quantitative ^{31}P NMR, showed the occurrence of different degradative pathways depending on the catalyst used. This experimental protocol allowed to point out that the cationic porphyrin TPyMePMn(CH_3COO)$_5$ is able to perform the most extensive degradation of the lignin structure, as indicated by the decrease of aliphatic hydroxyl groups and carboxylic acids increase, with the occurrence of relatively few condensation reactions.

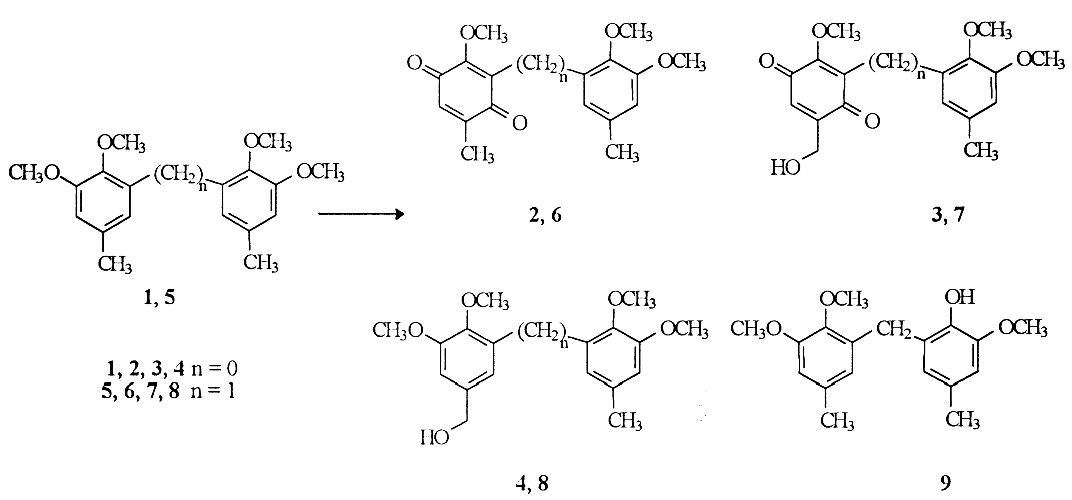
Results

Oxidation of 2,2′,3,3′-tetramethoxy-5,5′-dimethyl biphenyl 1

5-5′ Condensed model **1** was oxidized in the presence of hydrogen peroxide and several Mn or Fe porphyrins at pH 3 or 6. More specifically, TDCSPPMnCl and TSPPMnCl were used at pH 6; TDCSPPFeCl was used at pH 3 in accordance with the maximum activity pH previously reported.[10] TPyMePMn(CH_3COO)$_5$ was used at pH 3 and 6 in order to test the pH of its optimal catalytic activity. The same reactivity pattern was observed under all the tested experimental conditions, the main metabolites detected being in every case the *p*-quinones **2** and **3** (Scheme 1, Table 1). In analogy with the general oxidative mechanisms previously reported for porphyrin-catalyzed reactions,[9] the following pathway can be hypothesized. The formation of compound **2** can be rationalized on the basis of the electronic effect exerted by the methyl group. The intermediate radical cation $\mathbf{1}^{+\cdot}$, generated during the first one electron oxidation step, could be further oxidized by the highly electrophilic species (P)Me^{IV} = O. The site of oxidation is determined by the distribution of the electronic density on the aromatic ring, which is in turn modulated by the electronic effect of the substituent at the C-5 position.[9] The electron donating effect of the methyl group could direct the second oxidation step at the C-6 to yield the corresponding *p*-quinone **2** (Scheme 2). This behavior had been previously reported on monomeric compounds carrying electron-releasing substituents.[9] A reaction of side-chain oxidation yielded to the product of benzylic oxidation **4**. The occurrence of side chain oxidation reactions had been previously described.[8–10] The *p*-quinone **3** could be formed, in analogy with **2**, by the further oxidation of **4**.

In Table 1 are reported the yields of products **2**, **3**, **4** and the amount of conversion of **1** under the different experimental conditions examined. The latter data, showing the amounts of the starting product **1** after reaction, measured by quantitative GC analysis, can be considered as an index of the relative catalytic efficiency of the different porphyrins in the hydrogen peroxide oxidation.

A comparison between TDCSPPMnCl and TDCSPPFeCl showed that the manganese porphyrin was able to perform a more extensive oxidation than the iron one. This could be due to a higher stability under oxidative conditions rather than to an intrinsic higher reactivity.[10] Under such experimental conditions the homolytic cleavage of the peroxydic bond in a Fenton like fashion is in competition with the heterolytic reaction.[20–22] The anionic porphyrin TSPPMnCl showed to be the less active catalyst. In fact it lacks of any electron withdrawing substituent which can enhance the redox potential. In fact the corresponding TDCSPPMnCl in which two chlorine atoms have been added to each phenyl ring showed to be more active. TPyMePMn (CH_3COO)$_5$ showed a higher conversion rate at pH 3 than 6 (Table 1). However the amount of products **2**, **3**, and **4** were comparable. Probably when the reaction was performed at pH 3, the higher acidity of the medium underwent further radical coupling reactions yielding higher molecular weight condensed products.[23] Under

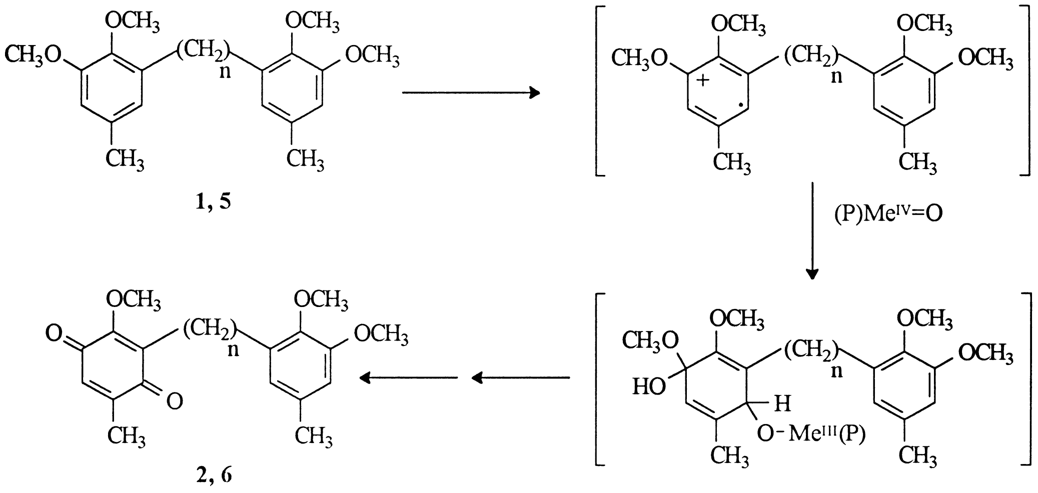


Scheme 1. Products obtained from the oxidation of lignin model compounds **1** and **5**.

Table 1. Conversion of lingin model compound **1** and product yields

Treatment (%)	TpyMePMnAc$_5$ pH 3[a]	TPyMePMnAc$_5$ pH 6[b]	TSPPMnCl[c]	TDCSPPMnCl[d]	TDCSPPFeCl[e]	H_2O_2 pH 3[f]	H_2O pH 6[g]
Conversion of **1**	72.9	58.8	27.3	50.1	44.7	27.4	18.4
2	51.2	53.9	21.6	43.9	Trace	Trace	15.2
3	2.04	2.22	3.14	2.55	3.14	2.22	1.63
4	1.14	1.19	1.57	1.90	2.17	1.41	0.87

[a] Oxidation in the presence of TPyMePMnAc$_5$ at pH 3.
[b] Oxidation in the presence of TPyMePMnAc$_5$ at pH 6.
[c] Oxidation in the presence of TSPPMnCl at pH 6.
[d] Oxidation in the presence of TDCSPPMnCl at pH 6.
[e] Oxidation in the presence of TDCSPPFeCl at pH 3.
[f] Control experiment at pH 3 in the absence of porphyrins.
[g] Control experiment at pH 6 in the absence of porphyrins.



1, 2: n = 0
5, 6: n = 1

Scheme 2. Proposed reaction pathway for the formation of *p*-quinones **2** and **6**.

both the experimental conditions TPyMePMn($CH_3COO)_5$ was found to be more effective in the degradation of **1** than the anionic catalysts.

Oxidation of 2,2′,3,3′-tetramethoxy-5,5′-dimethyl diphenylmethane 5

The oxidation of the diphenylmethane model compound **5** showed an analogous reaction pathway with *p*-quinone **6** being the main product recovered (about 10% yields) (Schemes 1 and 2). However, the proximity of the GC peaks of **5** and **6** did not allow the quantitative evaluation of the products obtained. In the oxidation mediated by TPyMePMn($CH_3COO)_5$, the percentage of conversion of **5** has been estimated on the basis of the substrate isolated after the reaction at about 15%. Besides quinone **6**, quinone **7**, a product of side chain oxidation **8**, and a product of demethoxylation **9** were also detected in low amount.

Oxidation of residual kraft lignin

The oxidation of residual kraft lignin with TDCSPPMnCl and TSPPMnCl was carried out at pH 6, while the oxidation with TDCSPPFeCl at pH 3 according to the previously reported values for the maximum activity of iron and manganese porphyrins found at different pH values.10 The oxidations with TPyMePMn($CH_3COO)_5$ were performed both at pH 3 and 6.

The structural modifications induced on the polymer were quantitatively determined by ^{31}P NMR spectroscopy. The lignin samples were phosphitylated with 2-chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholane in the presence of a known amount of cyclohexanol as internal standard and than submitted to quantitative ^{31}P NMR spectroscopy.[16] The spectra obtained showed well resolved signals for the different hydroxyl groups present within the various samples. Figure 3 shows a typical spectrum together with the detailed signals assignment based on earlier efforts.[17] In Table 2 are reported the quantitative data obtained from the spectra of lignin before and after the different treatments. In Figure 4 is reported the comparison of the efficiency of TDCSPPFeCl and TDCSPPMnCl in oxidations performed at pH 3 and 6, respectively. Under both the experimental conditions, a decrease of aliphatic OH groups was evident, showing the occurrence of side-chain

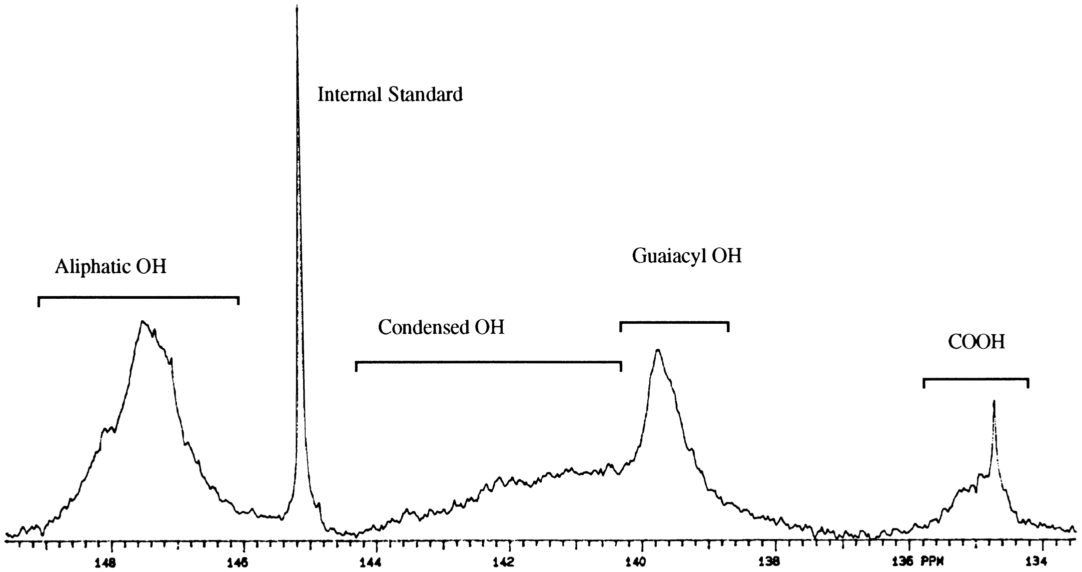


Figure 3. ^{31}P NMR spectrum of softwood residual kraft lignin after phosphitylation with 2-chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholane. The signal assignment is based on earlier efforts.[16,17]

Table 2. Distribution of aliphatic, phenolic, and carboxylic hydroxy groups in residual kraft ligins before and after porphyrin catalyzed oxidations[a]

Lignin/treatment	Aliphatic OH	Condensed phenolic OH	Guaiacyl OH	COOH
	(mmol/g)			
TPyMePMnAc$_5$ pH 3	1.78	0.97	1.04	0.35
TPyMePMnAc$_5$ pH 6	1.64	0.91	1.09	0.65
TSPPMnCl pH 6	1.79	0.93	1.07	0.30
TDCSPPMnCl pH 6	1.76	0.90	0.98	0.48
TDCSPPFeCl pH 3	1.75	1.01	1.14	0.41
Kraft lignin[b]	1.94	0.92	1.27	0.27
H_2O_2 pH 3[c]	1.78	0.94	1.02	0.36
H_2O_2 pH 6[d]	1.80	0.89	1.08	0.34

[a] Data obtained from quantitative ^{31}P NMR spectra of samples phosphylated with 2-chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholane.

[b] Starting reference sample.

[c] Control experiment in the absence of porphyrins at pH 3.

[d] Control experiment in the absence of porphyrins at pH 6.

oxidation reactions. The increase of COOH units further confirms this behavior. However such an increase, in the case of TDCSPPMnCl was found too high to be explained only on the basis of side chain oxidation reactions. Thus the presence of aromatic ring cleavage reactions cannot be ruled out.

The effect of H_2O_2 porphyrin mediated oxidation on lignins was also evident on the phenolic groups modifications. In the presence of TDCSPPMnCl only a decrease of guaiacyl groups was observed, while in the presence of TDCSPPFeCl an increase in the NMR chemical shift region of condensed structures was evident (Fig. 4). Such structures have been previously assigned to β-5, 4-O-5 and 5-5′ condensed units.[24] This seems to indicate a different reaction pathway for iron and manganese porphyrins, with the latter being able to carry out an oxidative process with a low amount of coupling reactions. The coupling reactions occurring during oxidations catalysed by iron porphyrins could be due to the formation of hydroxyl radicals in the homolytic cleavage of hydrogen peroxide. Furthermore, the overall oxidation was found more extended on lignins treated with TDCSPPMnCl than with TDCSPPFeCl. This behavior is in accord with the previously examined extent of oxidation of lignin model **1**.

A comparison of the catalytic activity of the cationic porphyrin TPyMePMn(CH_3COO)$_5$ at different pH values showed that its performance was improved at pH 6 rather than pH 3 (Table 2, Fig. 5). In fact the treatments performed at pH 6 resulted in a net increase of the carboxylic units and decrease of aliphatic hydroxyl groups. Since the COOH increase was found to be more than the aliphatic OH decrease, the carboxylic units cannot be formed only by side-chain oxidation reactions, but also aromatic ring cleavage processes could occur.

The comparison of anionic and cationic water soluble Mn porphyrins TSPPMnCl and TPyMePMn(CH_3COO)$_5$, respectively, showed an increased efficiency in the cationic TPyMePMn(CH_3COO)$_5$ (Table 2, Fig. 6). The efficiency was evaluated on the basis of the aliphatic OH decrease and COOH increase. To our knowledge this is the first example of the use of a cationic porphyrin for lignin oxidation. It is worth noting that in all the oxidations carried out at pH 6 the amount of condensed phenolic substructures did not vary significantly. When the oxidations were performed at pH 3, processes occurring with condensation reactions were found active. In particular the iron porphyrin TDCSPPFeCl was more prone to undergo condensation than TPyMePMn(CH_3COO)$_5$.

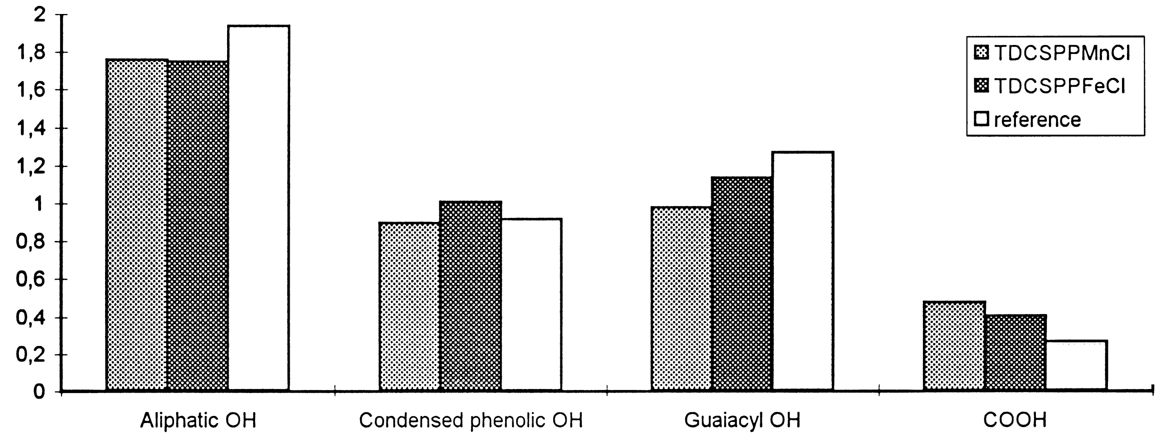


Figure 4. Comparison of the efficiency of TDCSPPFeCl at pH 3 and TDCSPPMnCl at pH 6 in the oxidation of residual kraft lignin (mmol/g).

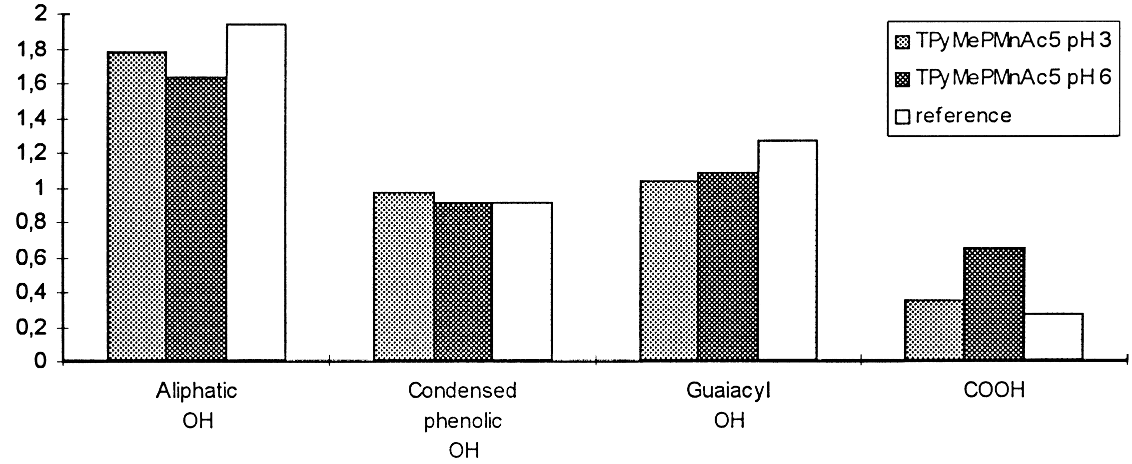


Figure 5. Comparison of the efficiency of TPyMePMn($CH_3COO)_5$ at pH 3 and 6 in the oxidation of residual kraft lignin (mmol/g).

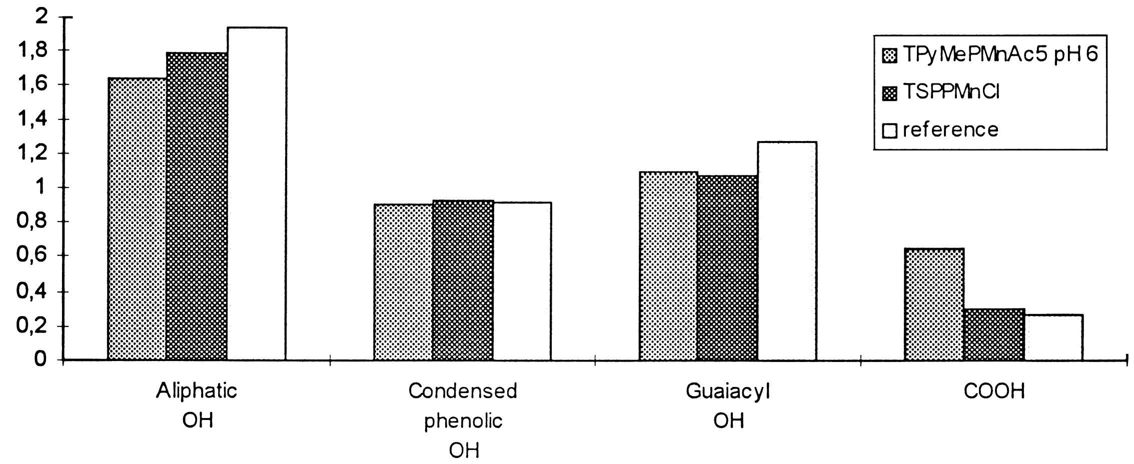


Figure 6. Comparison of the efficiency of anionic and cationic Mn porphyrins TSPPMnCl and TPyMePMn($CH_3COO)_5$ in the oxidation of residual kraft lignin (mmol/g).

Conclusions

This study shows that anionic and cationic water soluble manganese porphyrins are more effective in degrading residual kraft lignin and lignin substructures than iron porphyrins. This effect could be due to a higher stability of Mn porphyrins under oxidizing conditions. Among these the cationic TPyMePMn($CH_3COO)_5$, never used before in lignin oxidation, showed to be the best catalyst.

Both Fe and Mn porphyrins are able to oxidize such recalcitrant condensed lignin substructures as those represented in models **1** and **5**, yielding to *p*-quinones that in turn can be easily cleaved. However, during hydrogen peroxide oxidation of residual kraft lignin in the presence of TDCSPPFeCl further condensation reactions are likely to occur thus giving as final products modified lignins with increased amounts of condensed substructures. On the other hand residual kraft lignin treated with Mn porphyrins at pH 6 did not show any significative increase in the condensed units. This finding suggests that the overall lignin oxidation by H_2O_2 in the presence of Fe and Mn porphyrins follows different reaction pathways. This different behavior can be due to the formation of hydroxyl radicals by the homolytic cleavage of the peroxydic bond of H_2O_2 which, especially in the case of iron porphyrins, is in competition with the heterolytic reaction. The suppression of condensation reactions occurring during Mn porphyrins catalyzed oxidations of residual kraft lignin prompts at a further evaluation of their applicability in lignin degradation processes.

Experimental

Quantitative ^{31}P NMR spectra were obtained on a Varian XL-300 spectrometer by using methods identical to those described by Argyropoulos.[12,15] The chemical shifts were referenced to phosphoric acid. The ^{31}P NMR data reported in this effort are averages of three phosphitylation experiments followed by quantitative ^{31}P NMR acquisitions. The maximum standard deviation of the reported data was 2×10^{-2} mmol/g, while the maximum standard error was 1×10^{-2} mmol/g.

^{1}H and ^{13}C NMR spectra were recorded on a Varian XL 300 spectrometer. Mass Spectroscopy (MS) was performed with Hewlett–Packard 5971 mass-selective detector on a Hewlett–Packard 5890 gas chromatograph. All solvents were ACS reagent grade and were redistilled and dried according to standard procedures. Chromatographic purifications were performed on columns packed with Merck silica gel 60, 230–400 mesh for flash technique. Thin layer chromatography was carried out using Merck platten Kieselgel 60 F254. Porphyrins free bases and their metalllo derivatives were synthesized according to literature procedures.[20,25,26]

Synthesis of residual kraft lignin model compounds. Dihydromethyl veratrole **1** and (3,3′4,4′-tetramethoxy-6,6′-dimethyl) diphenylmethane **5** were synthesized starting from the corresponding phenols dihydrocreosol and (3,3′-dihydroxy-4,4′-dimethoxy-6,6′-dimethyl) diphenylmethane by alkylation with dimethylsulfate (Me_2SO_4) under alkaline conditions. Phenols (0.1 mol) were dissolved in 10 mL of NaOH 30% (aq solution) under magnetic stirring, and Me_2SO_4 (0.2 mol) were added at 60°C during 1 h. After 2 h the reaction mixtures were cooled, neutralized with ammonium chloride, and extracted with ethyl acetate. The organic solvent was evaporated under reduced pressure, and the crude products were crystallized from hexane–ethyl acetate to give **1** and **5** in quantitative yield.

Dihydromethyl veratrole 1. ^{1}H NMR ($CDCl_3$) (2.33 (s, 6H, CH_3), 3.64 (s, 6H, OCH_3), 3.88 (s, 6H, OCH_3), 6.64–6.74 (m, 4H, CH); ^{13}C NMR ($CDCl_3$) (21.26 (CH_3), 55.79 (OCH_3), 60.64 (OCH3), 112.58 (CH), 123.53 (CH), 132.59 (C) 132.81 (C), 144.60 (C), 152.30 (C). Mass Spectrometry data are reported in Table 3.

(3,3′4,4′-Tetramethoxy-6,6′-dimethyl) diphenylmethane 5. ^{1}H NMR ($CDCl_3$) (2.24 (s, 6H, CH_3), 3.76 (s, 6H, OCH_3), 3.85 (s, 6H, OCH_3), 3.96 (s, 2H, CH_2), 6.52–6.60 (m, 4H, CH); ^{13}C NMR ($CDCl_3$) (21.30 (CH_3), 29.07 (CH_2), 55.62 (OCH3), 60.37 (OCH_3), 111.21 (CH), 122.87 (CH), 133.29 (C) 134.46 (C), 144.88 (C), 152.66 (C). Mass Spectrometry data are reported in Table 3.

Oxidation of residual kraft lignin model compounds. General procedure. Hydrogen peroxide (30%) was added with stirring at 250°C at 4 µL aliquots every 5 min to a solution of the porphyrin catalyst (0.2 mM, 0.2 (mol) in 1 mL of dioxane/buffer citrate phosphate 100 mM pH 3 or 6 containing 10 mM of substrate (10 µmol). After 4 h the reaction mixtures were extracted in CH_2Cl_2 in the presence of saturated aqueous NaCl solution. The organic layer was dried over $MgSO_4$ and evaporated under reduced pressure. The residues were dissolved in 500 µL of pyridine in the presence of 4-propylbenzoic acid (0.5 equiv) as an internal standard. The mixture was than silylated by addition of bis-trimethylsilyl trifluoroacetamide (BSTFA) for GC–MS analysis.

Characterization of products. Gas chromatography and gas chromatography–mass spectrometry of the reaction

Table 3. Mass spectrometric data

Product	Derivative[a]	MS (*m*/*z*) data (%)
1	–	303 ($M^+ + 1$, 14), 302 (M^+, 100), 287 (16), 272 (21), 256 (93), 241 (25), 213 (23), 128 (28)
2	–	303 ($M^+ + 1$, 15), 302 (M^+, 96), 271 (100), 256 (34), 243 (41), 228 (98), 204 (43), 115 (45)
3	-$Si(CH_3)_3$	391 ($M^+ + 1$, 27), 390 (M^+, 100), 360 (24), 344 (28), 314 (16), 286 (20), 73 (98)
4	-$Si(CH_3)_3$	391 ($M^+ + 1$, 20), 390 (M^+, 100), 360 (93), 344 (14), 314 (23), 211 (18), 73 (42)
5	–	317 ($M^+ + 1$, 12), 316 (M^+, 100), 301 (11), 269 (23), 255 (21), 165 (26), 151 (99), 135 (78)
6	–	317 ($M^+ + 1$, 14), 316 (M^+, 100), 301 (5), 269 (15), 255 (14), 165 (18), 151 (56), 135 (48)
7	-$Si(CH_3)_3$	404 (M^+, 68), 315 (21), 299 (20), 283 (93), 239 (32), 225 (18), 165 (92), 150 (78), 135 (20), 73 (100)
8	-$Si(CH_3)_3$	405 ($M^+ + 1$, 12), 404 (M^+, 82), 357 (8), 223 (31), 165 (24), 73 (100)
9	-$Si(CH_3)_3$	374 (M^+, 92), 344 (100, 313 (20), 193 (36), 162 (28), 151 (32), 73 (81)

[a] Underivatized; -$Si(CH)_3$: trimethylsilylated with *N*,*O*-bis(trimethylilyl)-acetamide.

products were performed using a DB1 column (30 m ×0.25 mm and 0.25 mm film thickness), and an isothermal temperature profile of 100°C for the first 2 min, followed by a 20°C/min temperature gradient to 300°C and finally an isothermal period at 300°C for 10 min. The injector temperature was 280°C. Chromatography grade helium was used as the carrier gas. The fragmentation patterns are shown in Table 3. In order to fully characterize the recovered products, some of the reactions were performed using 0.1 mmol of substrates **1** and **5**. The oxidation products were isolated by TLC using hexane/ethyl acetate as eluant mixture and characterized by ^{1}H NMR spectroscopy.

2-Methoxy-5-methyl-3(2,3-dimethoxy-5-methylphenyl)-1,4-benzoquinone 2. ^{1}H NMR (CDCl$_3$) (2.33 (s, 3H, CH$_3$), 2.33 (d, J = 1 Hz, 3H, CH$_3$) 3.64 (s, 3H, OCH$_3$), 3.84 (s, 3H, OCH$_3$), 3.88 (s, 3H, OCH$_3$), 6.65 (s, 1H, CH), 6.74 (s, 1H, CH), 6.74 (q J = 1 Hz, 1H, CH).

2-Methoxy-5-hydroxymethyl-3(2,3-dimethoxy-5-methylphenyl)1,4-benzoquinone 3. ^{1}H NMR (CDCl$_3$) (2.33 (s, 3H, CH$_3$), 3.64 (s, 3H, OCH$_3$), 3.84 (s, 3H, OCH$_3$), 3.88 (s, 3H, OCH$_3$), 4.56 (d, J = 1 Hz, 2H, CH$_2$OH), 6.65 (s, 1H, CH), 6.74 (s, 1H, CH), 6.74 (t, J = 1 Hz, 1H, CH).

3,4-Dimethoxy-5(2,3-dimethoxy-5-methylphenyl)benzyl alcohol 4. ^{1}H NMR (CDCl$_3$) (2.33 (s, 3H, CH$_3$), 3.64 (s, 3H, OCH$_3$), 3.83 (s, 6H, OCH$_3$), 3.88 (s, 3H, OCH$_3$), 4.56 (d, J = 1 Hz, 2H, CH$_2$OH), 6.6–6.9 (m, 4H, CH).

2-Methoxy-5-methyl-3(2,3-dimethoxy-5-methylbenzyl)-1,4-benzoquinone 6. ^{1}H NMR (CDCl$_3$) (2.24 (s, 3H, CH$_3$), 2.24 (d, J = 1 Hz, 3H, CH$_3$), 3.76 (s, 3H, OCH$_3$), 3.85 (s, 6H, OCH$_3$), 3.98 (s, 2H, CH$_2$), 6.52 (s, 1H, CH), 6.59 (s, 1H, CH), 6.72 (q, J = 1 Hz, 1H, CH).

2-Methoxy-5-hydroxymethyl-3(2,3-dimethoxy-5-methylbenzyl)1,4-benzoquinone 7. ^{1}H NMR (CDCl$_3$) (2.24 (s, 3H, CH$_3$), 3.76 (s, 3H, OCH$_3$), 3.83 (s, 3H, OCH$_3$), 3.85 (s, 3H, OCH$_3$), 3.98 (s, 2H, CH$_2$), 4.56 (d, J = 1 Hz, 2H, CH$_2$OH), 6.52 (s, 1H, CH), 6.59 (s, 1H, CH), 6.72 (t, J = 1 Hz, 1H, CH).

3,4-Dimethoxy-5(2,3-dimethoxy-5-methylbenzyl)benzyl alcohol 8. ^{1}H NMR (CDCl$_3$) (2.24 (s, 3H, CH$_3$), 3.76 (s, 3H, OCH$_3$), 3.83 (s, 6H, OCH$_3$), 3.85 (s, 3H, OCH$_3$), 3.98 (s, 2H, CH$_2$), 4.56 (s, 2H, CH$_2$OH), 6.5-6.8 (m, 4H, CH).

2-Methoxy-4-methyl-6(2,3-dimethoxy-5-methylbenzyl)-phenol 9. ^{1}H NMR (CDCl$_3$) (2.24 (s, 3H, CH$_3$), 2.28 (s, 3H, CH$_3$), 3.76 (s, 6H, OCH$_3$), 3.83 (s, 3H, OCH$_3$), 3.85 (s, 3H, OCH$_3$), 3.96 (s, 2H, CH$_2$), 6.5-6.8 (m, 4H, CH).

Isolation of residual kraft lignin. Residual kraft lignin was isolated from kraft pulp (*picea mariana*, kappa no. 31.5) using a slightly modified acidolysis procedure.[27,28] The yield was 38%, and the purity was confirmed by UV and klason lignin content measurements.

Oxidation of residual kraft residual lignin. General procedure. Oxidations of residual kraft lignins were carried out in buffer citrate phosphate 100 mM pH 3 or 6 at 50°C. To a suspension of lignin (60 mg/30 mL) was added the porphyrin (10 mg/30 mL) under stirring. The addition of hydrogen peroxide (30%, 20 μL) initiated the reaction. Hydrogen peroxide was further supplied every 20 min to a final amount of 100 μL. After 6 h the reaction mixture was centrifuged, washed thoroughly with water, centrifuged again and freeze-dried.

Quantitative ^{31}P NMR. Derivatization of the lignin samples with 2-chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholane were performed as previously described. Samples of lignin, (30 mg) accurately weighed, were dissolved in a solvent mixture composed of pyridine and deuterated chloroform, 1.6/1 v/v ratio (0.5 mL). The phospholane (100 μL) was then added, followed by the internal standard and the relaxation reagent solution (100 μL each).

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