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Metallophthalocyanines as Possible Lignin Peroxidase Models

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Abstract—Several metalloporphyrins, particularly highly chlorinated water soluble *meso*-tetraphenylporphyrins, have been shown to be good biomimetics of the lignin peroxidases which degrade lignin *in vivo*. Metal complexes of the water soluble phthalocyaninetetrasulfonic acid have been examined as catalysts for the oxidation of lignin since the phthalocyanines are readily available and inexpensive. The copper(II), nickel(II) and cobalt(II) complexes showed little catalytic activity towards the oxidation of veratryl alcohol (a substrate of the lignin peroxidases). The iron(III) and manganese(III) complexes on the other hand were able to catalyze the oxidation of veratryl alcohol, 4-ethoxy-3-methoxyphenyl-glycerol-β-guaiacyl ether (a β-O-4-dimer) and 1-(4-ethoxy-3-methoxy)-2-(4-methoxyphenyl)-1,3-propanediol (a β-1 dimer). These catalysts are, however, much less stable than the halogenated *meso*-tetraphenylporphyrins and this lower stability, which is dependent upon pH and the oxidant, limits their use as catalysts.

Introduction

Lignin is the second most abundant biopolymer on Earth.[1] Billions of tons of lignin and lignin containing materials are produced annually. One of the ways of utilizing this valuable resource is to break the polymer down into small molecules which could be used as chemical raw materials. Microorganisms, especially the white-rot fungi, have been found to be able to degrade lignin. Two classes of enzymes, lignin peroxidases and manganese-dependent peroxidases, have been isolated[2–5] from the culture of a white-rot fungus, *Phanerochaete chrysosporium*. The lignin peroxidases and manganese-dependent peroxidases are believed to be two of the enzymes in *P. chrysosporium* responsible for lignin degradation. The identification of lignin peroxidases as nonspecific, heme-containing peroxidases led to a series of studies using metalloporphyrins as lignin peroxidase models. While simple metalloporphyrins such as protoporphyrin iron chloride and *meso*-tetraphenylporphyrin iron chloride (TPPFeCl) generally mimic the function of lignin peroxidase in degrading lignin model compounds, the metalloporphyrins themselves are rapidly and irreversibly oxidized in the presence of excess oxidants.[6] To increase the stability and catalytic efficiency of metalloporphyrins as biomimetic catalysts, we synthesized a number of sterically protected, water soluble metalloporphyrins including the iron(III) and manganese(III) complexes of *meso*-tetra(2,6-dichloro-3-sulfonatophenyl)porphyrin and *meso*-tetra(2,6-dichloro-3-sulfonatophenyl)-β-octachloroporphyrin.[7,8] These metalloporphyrins were shown to be stable towards a large excess of oxidant. *meso*-Tetra(2,6-dichloro-3-sulfonatophenyl)porphyrin iron(III) chloride (TDCSPPFeCl) was shown to be able to catalyze the degradation of several model compounds representing the major substructures of lignin.[8] Polycyclic aromatics and polychlorinated phenols were also oxidized by TDCSPPFeCl.[8] *meso*-Tetra(2,6-dichloro-3-sulfonatophenyl)-β-octachloroporphyrin (Cl_{16}TSPPFeCl) was shown to be an effective catalyst for pulp bleaching and pulp mill effluent decolorization.[9] These metalloporphyrins, therefore, have potential industrial applications in the pulp and paper industry as well as in the degradation of environmental pollutants. They are, however, expensive to prepare and this problem has to be solved before they can be used industrially.

The phthalocyanines belong to a class of synthetic compounds closely related to the naturally occurring porphyrins.[10] Metallophthalocyanines possess catalytic activities for a variety of reactions[11] including hydrocarbon autoxidation,[12,13] olefin epoxidation[14,15] and hydrocarbon hydroxylation.[16] Iron phthalocyanines have also been shown to have catalase- and peroxidase-like activities.[17,18] A number of metallophthalocyanines have been used as catalysts for pulping and pulp bleaching.[19] Their use as lignin peroxidase models, however, has not been reported. In this paper we report our primary studies using metallophthalocyanines as inexpensive substitutes of the metalloporphyrins as lignin peroxidase models. As metallophthalocyanines are insoluble in aqueous and most organic solvents, water soluble metal complexes of phthalocyaninetetrasulfonic acid were used in this study.

Results and Discussion

Veratryl alcohol (3,4-dimethoxybenzyl alcohol) is a secondary metabolite of lignin biodegradation by *P. chrysosporium* and is often used as a lignin model

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Table 1. Percentage yield[a] for the oxidation of veratryl alcohol by TSPCMnCl and TSPCFeCl under various conditions[b]

		*m*CPBA	t-BuOOH	H_2O_2	NaClO	PhIO	$KHSO_5$
TSPCMnCl	pH 3[c]	32	3	1	3	14	0
	pH 7[c]	27	11	17	4	7	trace
	pH 10[c]	29	16	10	6	9	2
	CH_3CN	24	-	-	-	-	-
	CH_3OH	27	-	-	-	-	-
TsPCFeCl	pH 3[c]	12	3	trace	3	9	7
	pH 7[c]	7	1	1	1	6	trace
	pH 10[c]	16	3	1	1	4	trace

[a]Yield based on veratryl alcohol, reaction carried out at room temperature.
[b]The reaction mixture contained 1×10^{-4} mol of oxidant, 5×10^{-7} mol of metallophthalocyanine and 5×10^{-5} mol of veratryl alcohol in 5 mL of solvent.
[c]Phosphate buffer.

compound. The oxidation of veratryl alcohol to give veratraldehyde (3,4-dimethoxybenzaldehyde) has been used to measure the activity of lignin peroxidases[20] and is used in this study to compare the catalytic activities of the metallophthalocyanines.

With cobalt(II) phthalocyaninetetrasulfonic acid (TSPCCo) and the corresponding Cu(II) and Ni(II) analogs (TSPCCu and TSPCNi) as catalysts, the oxidation of veratryl alcohol by various oxidants gave only trace amounts of veratraldehyde as seen by thin layer chromatography (TLC). Iron(III) and manganese(III) phthalocyanine are better catalysts for the oxidation of veratryl alcohol and the yields of veratraldehyde under various conditions are listed in Table 1. However the yields are still too low for TSPCFeCl and TSPCMnCl to be effectively used as catalysts. In addition, the catalysts are unstable and in most cases are bleached within a few minutes. During the oxidation of veratryl alcohol by TSPCMnCl and *m*-chloroperbenzoic acid (*m*CPBA), for example, TSPCMnCl is completely bleached within 1 min. The initial substrate oxidation must be fast since the catalyst is destroyed within 1 min but the yield of veratraldehyde was still 30%. An experiment was carried out where *m*CPBA was added to a solution containing TSPCMnCl, veratryl alcohol was not added to the solution until the deep green color of TSPCMnCl disappeared. Little veratraldehyde was detected after 60 min, indicating that TSPCMnCl was the catalyst and its degradation products had no catalytic activity.

Heme peroxidases, including lignin peroxidases, contain an iron protoporphyrin IX coordinated to a proximal imidazole group of histidine at their active sites. Imidazole has been used to facilitate metalloporphyrin catalyzed oxidations.[21] We have shown that the presence of imidazole could increase the catalytic activity of a manganese porphyrin for the oxidation of veratryl alcohol by hydrogen peroxide.[8] As shown in Table 2, the presence of imidazole at certain concentrations also increased the yield of veratraldehyde using the present catalyst.

Table 2. The effect of imidazole on the oxidation of veratryl alcohol by TSPCMnCl and H_2O_2 at pH 7[a] at room temperature

imidazole/TSPCMnCl (molar ratio)	1	10	50	100
% yield of veratraldehyde (based on veratryl alcohol)	17	20	23	10

[a]The reaction mixture contained 5×10^{-7} mol of TSPCMnCl, 1×10^{-4} mol of H_2O_2 and 5×10^{-5} mol of veratryl alcohol in 5 mL of pH 7 phosphate buffer.

The yield of oxidation of veratryl alcohol by metalloporphyrins is dependent on the solvent[8] and thus solvent effects on the oxidation of veratryl alcohol by TSPCMnCl and *m*CPBA have been studied. TSPCMnCl was much more stable towards *m*CPBA in organic solvents such as acetonitrile and methanol (containing 100 μL of water in 5 mL solvent) than in aqueous solution. The yield of veratraldehyde (Table 1), however, stayed about the same.

In order to show that metallophthalocyanines oxidize lignin model compounds in the same way as lignin peroxidases and metalloporphyrins, the oxidation of two dimeric lignin model compounds by TSPCFeCl and TSPCMnCl was studied. The oxidation of the β-4 dimer, 4-ethoxy-3-methoxyphenylglycerol-β-guaiacyl ether (**1**), by *m*CPBA and either TSPCFeCl or TSPCMnCl was incomplete and gave only small

amounts of 4-ethoxy-3-methoxybenzaldehyde and the α-ketone **2** as shown in Figure 1. Similar products were found in the oxidation of the β-O-4 dimer by lignin peroxidases.[3]

The β-1 dimeric model compound, 1-(4-ethoxy-3-methoxyphenyl)-2-(4-methoxyphenyl)-1,3-propanediol (**3**), was more readily oxidized than the β-O-4 dimer. No starting material was detected by HPLC when it was oxidized by either TSPCFeCl or TSPCMnCl in the presence of two equivalents of *m*CPBA. The products were similar to those of the reaction catalyzed by lignin peroxidases[22] and metalloporphyrins[6,8] as shown in Figure 2.

Metallophthalocyanines have been used as catalysts for a variety of oxidative reactions including epoxidation of olefins, hydroxylation of hydrocarbons, and decomposition of hydrogen peroxide. The stability of these catalysts in the presence of excess oxidants, which may be a major limitation for their industrial applications, has been rarely discussed. Among the metallophthalocyanines used in this study, both effective catalysts TSPCFeCl and TSPCMnCl are unstable and are bleached within a few minutes under the experimental conditions. One exception was that of TSPCFeCl which is relatively stable in the presence of hydrogen peroxide; however, the yield of oxidation products was very low. The stability of TSPCFeCl and TSPCMnCl was dependent upon the pH of the solution as well as the type of oxidant used. Since the optical spectra of TSPCFeCl and TSPCMnCl change upon addition of oxidants, it is not possible to follow the destruction of TSPCFeCl and TSPCMnCl at a single wavelength and quantitative stability data are not available. Figures 3 and 4 show UV-vis spectral change of TSPCMnCl upon addition of 1 equivalent of KSO_5 and *t*-BuOOH at pH 3, 7 and 10. When KSO_5 was the oxidant, the stability order was pH 10 > pH 7 ≃ pH 3. When tBuOOH was the oxidant, however, the stability order was pH 3 > pH 7 ≡ pH 10. In the presence of a 100 fold excess of oxidant, TSPCFeCl and TSPCMnCl are rapidly destroyed. TSPCFeCl and TSPCMnCl are much less stable when compared to the sterically protected metalloporphyrins such as TDCSPPFeCl. They are also less stable than the non-sterically protected metalloporphyrins such as *meso*-tetra(4-sulfonatophenyl)porphyrin iron chloride (TSPPFeCl), which still retains about 30% of its activity 10 min after adding 100 fold excess of oxidant.[8]

Experimental

Chemicals

Veratryl alcohol, 4'-methoxyacetophenone, hydrogen peroxide, *t*-butylhydroperoxide, sodium hypochlorite, *m*CPBA and potassium hydrogen monopersulfate (Oxone) were all of reagent grade. Copper phthalocyanine-3,4',4",4"'-tetrasulfonic acid tetrasodium salt (TSPCCu) and nickel phthalocyaninetetrasulfonic acid tetrasodium salt (TSPCNi) were purchased from Aldrich. Iodosylbenzene,[23] 4-ethoxy-3-methoxyphenyl-

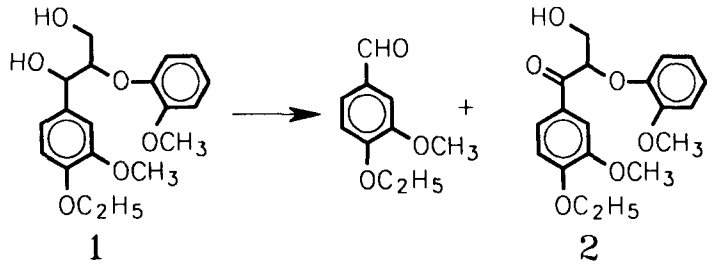

Figure 1. The oxidation of **1** by *m*CPBA in aqueous acetonitrile (pH 3) catalyzed by TSPCFeCl or TSPCMnCl.

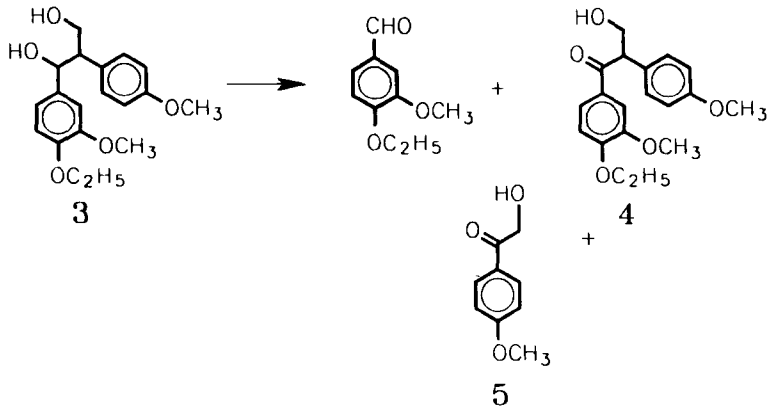

Figure 2. The oxidation of **3** by *m*CPBA in aqueous acetonitrile (pH 3) catalyzed by TSPCFeCl or TSPCMnCl.

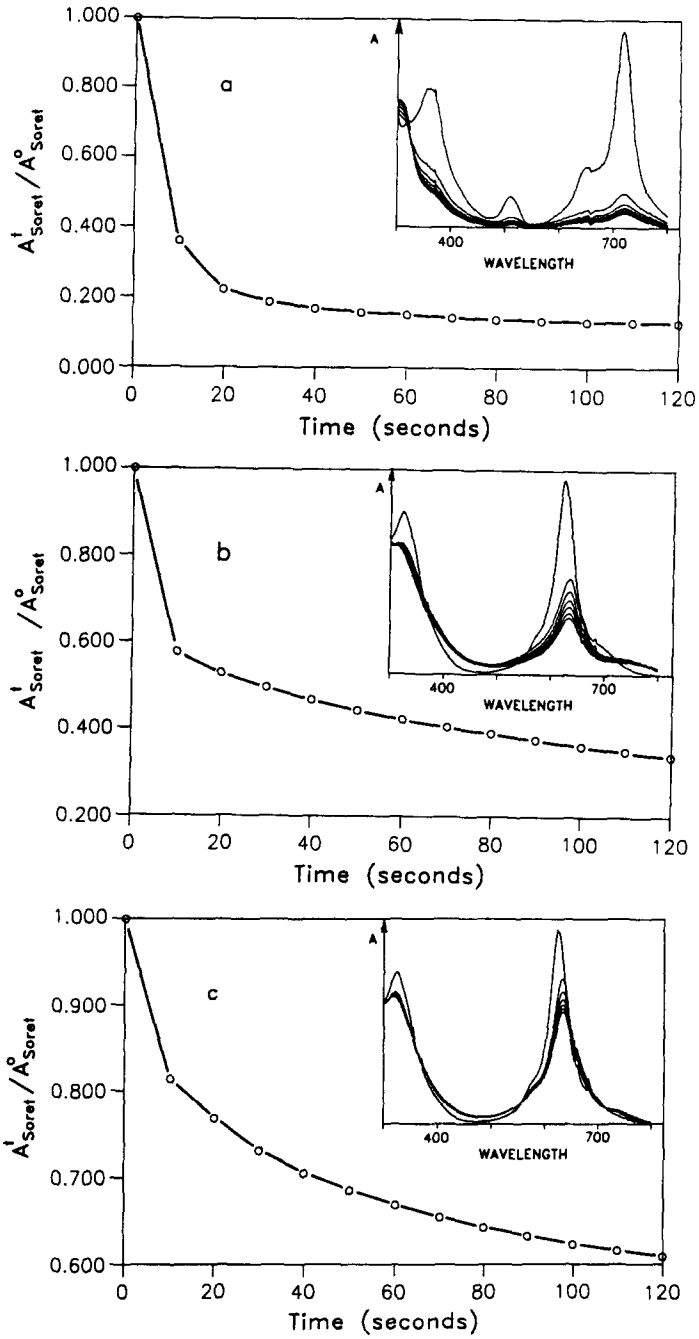

Figure 3. The UV-vis spectral changes of TSPCMnCl (1×10^{-7} mol in 3 mL 0.1 M phosphate buffer, a, pH 3.0; b, pH 7.0; c, pH 10.0) upon addition of one equivalent of *m*CPBA (1×10^{-7} mol) in the presence of veratryl alcohol (5×10^{-6} mol) at room temperature over a period of 2 min; 20 s between each scan.

glycerol-β-guaiacyl ether (**1**, a β-O-4 dimer)[24] and its α-ketone analog **2**,[25] 1-(4-ethoxy-3-methoxy)-2-(4-methoxyphenyl)-1,3-propanediol (**3**, a β-1 dimer)[26] and its α-ketone analog **4**[25] were prepared as previously reported.

All UV-vis spectra were measured on an HP 8452A diode array spectrometer. The HPLC was a Waters 600E pump with a Waters 994 photo diode array detector equipped with a 3.9 mm 15 cm C-18 column. The mobile phase was 1:1 methanol:water.

The synthesis of cobalt phthalocyaninetetrasulfonic acid (TSPCCo)

Phthalocyaninetetrasulfonic acid (0.5 g, purchased from Porphyrin Products) was dissolved in DMF (30 mL). Cobalt(II) chloride (0.5 g) was added and the solution stirred at 16 °C. The reaction was complete in 60 min (followed by UV-vis spectroscopy). The reaction mixture was then cooled to room temperature and the product was precipitated with acetone (200 mL). The precipitate was collected, washed with acetone,

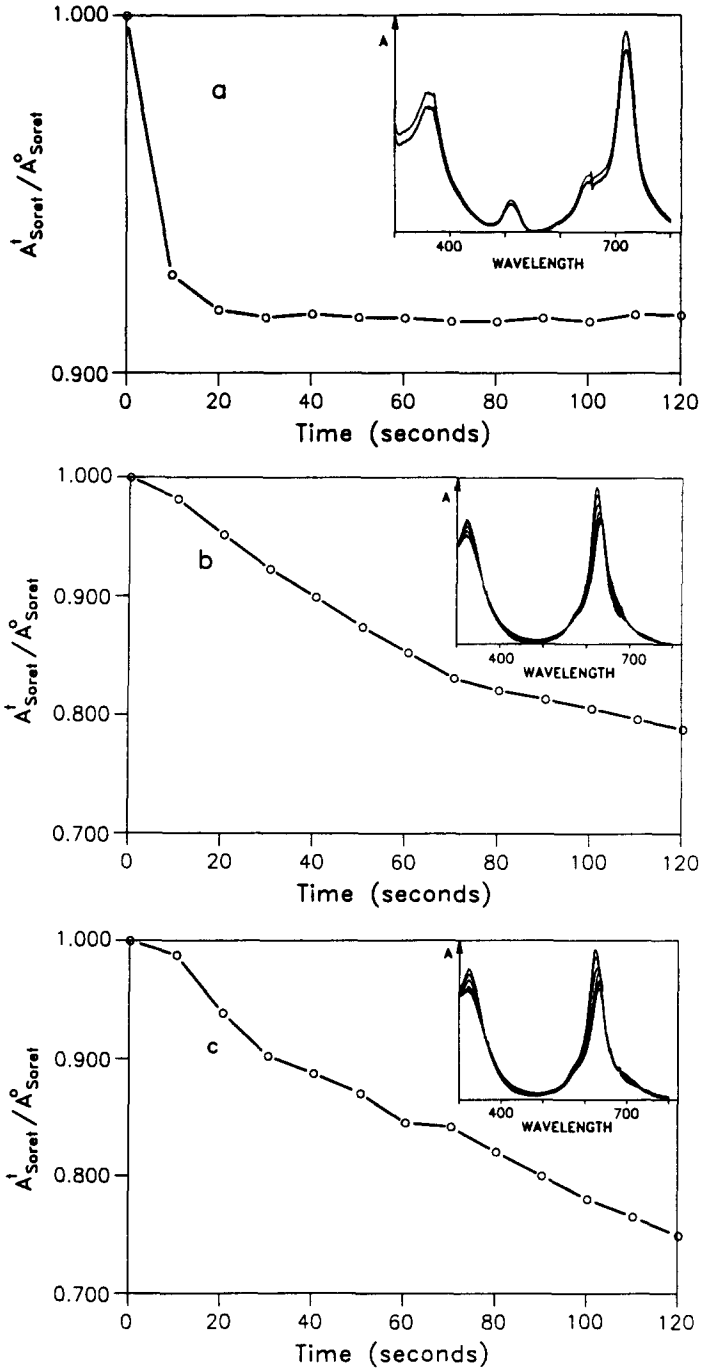

Figure 4. The UV-vis spectral changes of TSPCMnCl (1×10^{-7} mol in 3 mL 0.1 M phosphate buffer, a, pH 3.0; b, pH 7.0 c, pH 10.0) upon addition of one equivalent of *t*-BuOOH (1×10^{-7} mol) in the presence of veratryl alcohol (5×10^{-6} mol) at room temperature over a period of 2 min; 20 s between each scan.

dissolved in water and filtered. The filtrate was passed through an ion exchange column (3 × 15 cm glass column packed with Amberlite IR-120 ion exchanger, H^+ type) and eluted with deionized water to remove free Co^{2+}. The aqueous solution was dried by rotary evaporation to give cobalt phthalocyaninetetrasulfonic acid (0.5 g) as a black crystalline powder. The UV-vis spectrum (in water) of the TSPCCo so obtained showed that it was a mixture of the oxygen-free and oxygen-bound cobalt complexes.[27,28]

The synthesis of manganese(III) phthalocyanine-tetrasulfonic acid (TSPCMnCl)

Phthalocyaninetetrasulfonic acid (0.5 g) and $Mn(OAc)_2 4{\cdot}H_2O$ (1.7 g) were dissolved in water (30 mL) and refluxed for 60 min. A black solid formed when the reaction mixture was cooled down. Acetone (200 mL) was added to further precipitate the product. The precipitate was collected and washed with acetone. The manganese phthalocyaninetetrasulfonic acid so

obtained was probably a polymer and had a very low solubility in water. It was dissolved in dilute sodium hydroxide, filtered, and carefully neutralized to neutral pH with dilute hydrochloric acid. The solution was then passed through an ion exchange column and dried as described above to give 0.45 g of the product. The manganese phthalocyaninetetrasulfonic acid is a black crystalline powder and dissolves in water to give a green solution. Its UV-vis spectrum was the same as that previously reported for manganese(III) phthalocyaninetetrasulfonic acid.[27,29]

Iron(III) phthalocyaninetetrasulfonic acid (TSPCFeCl) was synthesized in 80% yield following the method described above for the synthesis of cobalt phthalocyaninetetrasulfonic acid except $FeCl_2 4 \cdot H_2O$ (0.25 g) was used. It had the same UV-vis spectrum as that reported for Iron(III) phthalocyaninetetrasulfonic acid.[27,30]

The oxidation of veratryl alcohol

The reaction was initiated by adding the oxidant (1×10^{-4} mol) to 5 mL of aqueous phosphate buffer (0.1 M) containing 5×10^{-7} mol of metallophthalocyanine and 5×10^{-5} mol of veratryl alcohol. The mixture was stirred at room temperature for 60 min and any remaining oxidant was decomposed by adding solid sodium bisulfite. When the reaction was carried out at pH 3, saturated sodium bicarbonate was added to neutralize the solution before adding sodium bisulfite. Saturated sodium bicarbonate was also added to the solution when mCPBA was the oxidant to remove the *m*-chlorobenzoic acid. 4'-Methoxyacetophenone (5×10^{-5} mol) was added at the end of the reaction (as an internal standard for HPLC analysis) and the reaction mixture was then extracted with ethyl acetate (3 × 3 mL). The extracts were combined and analyzed by HPLC for the formation of veratraldehyde.

The oxidation of β-O-4 and β-1 dimers

The β-O-4 or the β-1 dimer (5×10^{-6} mol) was dissolved in 4 mL 1:1 acetonitrile:pH 3 aqueous buffer containing 5×10^{-7} mol of either iron(III) or manganese(III) phthalocyaninetetrasulfonic acid, 1×10^{-5} mol of *m*CPBA was added as a powder and the mixture was stirred at room temperature for 60 min. Saturated sodium bicarbonate was added to pH 7 and solid sodium bisulfite was added to decompose any remaining *m*CPBA. The solution was extracted with ethyl acetate (3 × 3 mL), the organic extracts were combined, reduced in volume to 2 mL and subjected to HPLC analysis. The products were identified by comparing their retention times on HPLC, their UV-vis spectra, and their R_f values on TLC with those of known standards.

Conclusions

The copper(II), nickel(II) and cobalt(II) complexes of phthalocyaninetetrasulfonic acid were found to have little catalytic activity towards the oxidation of veratryl alcohol under the conditions used. TSPCFeCl and TSPCMnCl could generally mimic the function of lignin peroxidase in the oxidation of veratryl alcohol and β-1 and β-O-4 lignin model compounds. The presence of imidazole slightly increased the yield of the oxidation of veratryl alcohol by TSPCMnCl and hydrogen peroxide. The major problem and limitation of these two catalysts was their low stability, which was dependent on the pH of the solvent and the nature of the oxidant used. The use of organic solvents instead of aqueous solvents increased their stability but did not change their overall catalytic activity. The structure of the metallophthalocyanines has to be modified to increase their stability before they can be used as effective biomimetic catalysts.

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