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Journal of Molecular Catalysis B: Enzymatic 35 (2005) 100–107
JOURNAL OF MOLECULAR CATALYSIS B: ENZYMATIC
www.elsevier.com/locate/molcatb

Product profiles in enzymic and non-enzymic oxidations of the lignin model compound *erythro*-1-(3,4-dimethoxyphenyl)-2-(2-methoxyphenoxy)-1,3-propanediol

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Received 12 January 2005; received in revised form 24 May 2005; accepted 1 June 2005
Available online 18 July 2005

Abstract

The *erythro* form of the lignin model compound 1-(3,4-dimethoxyphenyl)-2-(2-methoxyphenoxy)-1,3-propanediol (**1**) was oxidized with laccase/ABTS, lead(IV) tetraacetate (LTA), lignin peroxidase/H_2O_2, cerium(IV) ammonium nitrate (CAN) and Fenton's reagent. The product profiles obtained with the different oxidants were compared after separation, identification and quantification of the products using HPLC, UV-diode array detector and electrospray ionization mass spectrometry in positive ionization mode. The oxidants generated different product profiles that reflected their different properties. Oxidation with laccase/ABTS resulted almost exclusively in formation of 1-(3,4-dimethoxyphenyl)-3-hydroxy-2-(2-methoxyphenoxy)-1-propanone (**2**). Oxidation with LTA resulted in more 3,4-dimethoxybenzaldehyde (**3**) than ketone **2**. Lignin peroxidase and CAN gave similar product profiles and aldehyde **3** was the predominant product (only small amounts of ketone **2** were formed). Oxidation with Fenton's reagent resulted in the formation of more aldehyde **3** than ketone **2** but the yields were very low. CAN served as an excellent model for the lignin peroxidase-catalyzed oxidation, while the laccase-mediator system, LTA and Fenton's reagent provided distinctly different product profiles. *Erythro*-1-(3,4-dimethoxyphenyl)-1,2,3-propanetriol was present among the products obtained on oxidation with LTA, lignin peroxidase, CAN and Fenton's reagent. The differences in redox potential between the oxidants afford an explanation of the diverse product patterns but other factors may also be of importance. The reactions leading to cleavage of the β-ether bond with formation of 1-(3,4-dimethoxyphenyl)-1,2,3-propanetriol (veratrylglycerol) were found to proceed without affecting the configuration at the β-carbon atom.

Keywords: 1-(3,4-Dimethoxyphenyl)-2-(2-methoxyphenoxy)-1,3-propanediol; Lignin degradation; Laccase; Lignin peroxidase; Non-enzymic oxidants

1. Introduction

Lignin is an abundant renewable organic material and the constituent of woody plants that is most resistant to biological degradation. It is also the component that gives a dark colour to pulp in paper manufacture. Consequently, there are several reasons to investigate the molecular mechanisms behind the oxidation of lignin. Furthermore, there is currently much interest regarding selective and environmentally benign methods for oxidation of organic compounds, including alcohols [1].

Lignins are complex and irregular biopolymers consisting of phenylpropane units linked by a variety of C–C and C–O–C bonds [2]. 1-(3,4-Dimethoxyphenyl)-2-(2-methoxyphenoxy)-1,3-propanediol (**1** in Fig. 1) represents the most common substructure in lignin, the arylglycerol β-aryl ether structure, in which a β-O-4 linkage interconnects two phenylpropane units. The β-O-4 structure accounts for roughly one half of the inter-unit linkages in lignin [2]. Lignin degradation is to a large extent being elucidated by the use of model compounds, such as **1**, that allow

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doi:10.1016/j.molcatb.2005.06.003

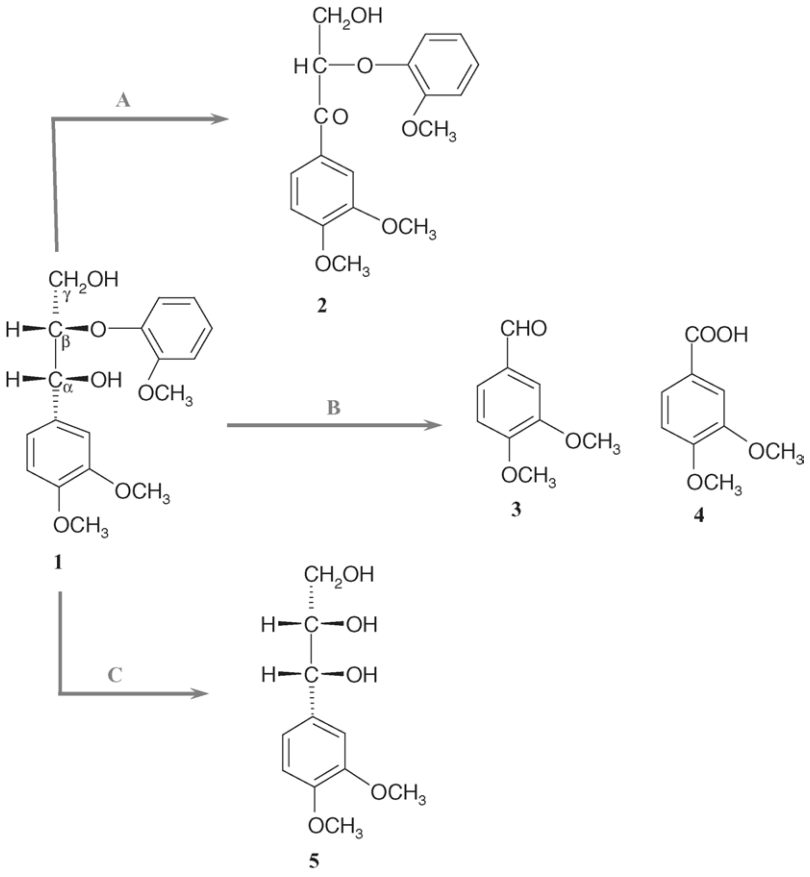

Fig. 1. Reaction routes observed in oxidation experiments with *erythro*-1-(3,4-dimethoxyphenyl)-2-(2-methoxyphenoxy)-1,3-propanediol (**1**): C_α oxidation (A), C_α/C_β cleavage (B) and β-ether cleavage (C).

detailed studies of specific reactions, which are difficult to characterize using lignin preparations or wood. The structures of both diastereoisomers of **1** are established by X-ray crystallography [3,4]. Analyses of lignin samples by ^{1}H NMR spectral methods have provided estimates of the proportions of the *erythro* and *threo* forms of arylglycerol β-aryl ether structures in lignin [5]. The distribution of the *erythro* and *threo* forms has been found to vary in different types of wood (e.g. [6]). Previous results demonstrate the importance of stereochemical considerations with respect to the susceptibility of β-O-4 lignin model compounds to enzymatic and non-enzymatic oxidation [7].

Among wood-degrading organisms, white-rot fungi, such as *Phanerochaete chrysosporium* and *Trametes versicolor*, are the most efficient and the best studied with regard to lignin degradation. White-rot fungi secrete laccases and peroxidases that directly or in conjunction with low-molecular mass mediators oxidize lignin substructures (reviewed in [8]). Laccases are multicopper oxidases that reduce molecular oxygen to water and oxidize a wide variety of substrates [9,10]. Laccases are known to oxidize phenolic compounds but in combination with mediators non-phenolic lignin substructures can be oxidized as well [11]. In the presence of mediators such as 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and 1-hydroxybenzotriazole (HBT), laccase was found to degrade lignin model compounds [12] and delignify kraft pulp [13]. However, the exact mechanism of action behind laccase-mediator systems remains to be elucidated [14]. Mediators can act as diffusible oxidizing agents that enter the fiber secondary wall to engage in close contact with lignin. In combination with manganese-dependent peroxidases, Mn(III) may play a role as a mediator in lignin biodegradation. Lignin peroxidase catalyzes the formation of radical intermediates from lignin model compounds, which may result in the formation of a variety of different oxidation products [15]. Lignin peroxidase is a ferric hemoprotein, which follows the classical peroxidase mechanism using H_2O_2 as the oxidizing substrate [16]. It catalyzes the oxidation of both non-phenolic and phenolic monomeric and dimeric lignin model compounds but also oxidizes larger substrates, such as phenolic lignin oligomers, synthetic polysaccharide-supported lignin model compounds and lignin [8,17,18].

Inorganic oxidants may serve as models for the enzyme-catalyzed oxidation of non-phenolic lignin substructures. The strong one-electron oxidant cerium(IV) ammonium nitrate (CAN) has been used as a model oxidant [19]. CAN has also been used to mimic lignin peroxidase-catalyzed oxidation of

dimeric lignin model compounds [7,20] and veratryl alcohol [21,22]. Lead(IV) tetraacetate (LTA) effects oxidative cleavage of 1,2-glycols, a reaction that has been suggested to occur after an initial formation of a bidentate complex [23]. To our knowledge, LTA has not been studied previously as an oxidant of lignin model compounds. Fenton's reagent (ferrous ions combined with hydrogen peroxide) is commonly believed to generate a strong, non-specific oxidant, namely the hydroxyl radical. Activated species of oxygen, notably hydroxyl radicals, have been suggested to play a role in wood decay by different types of fungi, including white-rot and brown-rot fungi [24]. However, the exact mechanisms of action by which different types of wood-degrading fungi operate remain to be clarified. Considering the fact that white-rot and brown-rot fungi cause distinctly different types of wood decay, it would be surprising if these two types of fungal degradation relied on the same catalyst. Therefore, comparisons of the product patterns exhibited by non-enzymic oxidants and the oxidative enzymes secreted by white-rot fungi are of interest.

In this study, a stereochemically defined, non-phenolic lignin model compound, *erythro*-1-(3,4-dimethoxyphenyl)-2-(2-methoxyphenoxy)-1,3-propanediol (**1**), was oxidized with enzymic and non-enzymic oxidants to elucidate the differences in product profiles. The product patterns were analyzed by HPLC followed by detection, identification and quantification with UV-diode array detector (UV-DAD) and mass spectrometry (MS). Advantages of HPLC compared with GC–MS include that extraction and derivatization can be avoided and that direct quantification of the products can be achieved. The results show the differences in product profiles exhibited on enzymic and non-enzymic oxidations. The relationship between the results obtained with the oxidants and the degradation of the arylglycerol β-aryl ether **1** in fungal cultures is discussed.

2. Experimental

2.1. Chemicals

Reagent grade chemicals were used unless otherwise stated. Synthetic methods for the preparation of *erythro*-1-(3,4-dimethoxyphenyl)-2-(2-methoxyphenoxy)-1,3-propanediol (**1**) (Fig. 1) [25,26], 1-(3,4-dimethoxyphenyl)-3-hydroxy-2-(2-methoxyphenoxy)-1-propanone (**2**) [27] and the diastereomers of veratrylglycerol (the *erythro* isomer is designated **5**) (Fig. 1) [28] have been described in the literature. Veratraldehyde (**3**) and veratric acid (**4**) are commercially available.

2.2. Enzymes

Laccase: A solution containing laccase from *Trametes* (*Coriolus*) *versicolor* (Jülich Fine Chemicals GmbH, Jülich, Germany) was diluted five times. Ammonium sulfate was added to a concentration of 750 g/l and the solution was kept at 8 °C overnight. Precipitated material was collected by centrifugation at 6000 × *g* and 4 °C for 30 min. The ammonium sulfate precipitation was performed a second time and the pellet was dissolved in a minimal volume of distilled water. After dialysis (membrane cut-off 12–14,000) against 100 mM phosphate buffer at 8 °C overnight, the enzyme solution was applied to a DEAE Sepharose column equilibrated with 0.1 M phosphate buffer (pH 6.0). A blue band became visible and the corresponding fractions were collected. The purified laccase was dialyzed against distilled water at 8 °C, frozen in liquid nitrogen, and stored at −80 °C.

Lignin peroxidase: Lignin peroxidase was prepared from *T. versicolor* PRL572 as previously described [29].

2.3. Enzyme assays

Laccase: Determination of laccase activity was performed using a UV-1601PC spectrophotometer (Shimadzu, Kyoto, Japan). The reaction mixture contained 0.4 mM ABTS, 50 mM acetate buffer (pH 5.2) and laccase. The change in absorbance at 414 nm was recorded for 5 min at 21 °C. One unit was defined as the amount of laccase that forms 1 μmol ABTS radical cation ($\varepsilon = 3.6 \times 10^4\ M^{-1}\,cm^{-1}$ at 414 nm [30]) per min.

Lignin peroxidase: Lignin peroxidase activity was also determined spectrophotometrically. The reaction mixture contained 100 mM tartrate buffer (pH 3), 2 mM 3,4-dimethoxybenzyl alcohol (veratryl alcohol) and 0.4 mM hydrogen peroxide. The change in absorbance at 310 nm was monitored for 1 min at 21 °C. One unit was defined as the amount of enzyme that forms 1 μmol of veratraldehyde ($\varepsilon = 9.3 \times 10^3\ M^{-1}\,cm^{-1}$ [31]) per min.

2.4. Reactions with erythro-1-(3,4-dimethoxyphenyl)-2-(2-methoxyphenoxy)-1,3-propanediol (*1*)

Oxidants were added in three stages. After the initial addition of the oxidant, the sample was incubated for 30 min and a sample for HPLC analysis was taken (Stage 1). Subsequently, a second addition of oxidant was made, the sample was incubated for 2.5 h, and a sample for analysis was taken (Stage 2). Finally, a third addition of oxidant was made, the sample was incubated for 21 h, and a sample for analysis was taken (Stage 3). The concentrations resulting from each addition of oxidant (with all dilutions caused by the additions and changes in volume caused by sample withdrawal taken into account) are given within parentheses in the text below. The initial reaction medium (Stage 1) was always 20 mM with respect to acetate buffer (pH 4.0) and, unless otherwise stated, 1 mM with respect to the substrate, arylglycerol β-aryl ether **1** [3]. Additions of oxidants in Stages 2 and 3 were made with small volumes to minimize dilution of the buffer and the substrate. The temperature was 21 °C.

Negative control: The negative control contained only acetate buffer and **1** but no oxidant.

Laccase control: Laccase was added initially (0.35 U/ml), after Stage 1 (0.15 U/ml) and after Stage 2 (0.23 U/ml).

Laccase/ABTS control: The concentration of ABTS was 1 mM. The reaction mixture contained acetate buffer but no compound **1**. Laccase was added as described above.

Laccase/ABTS: The concentration of ABTS was 1 mM. Laccase was added as described above.

Lead(IV) tetraacetate: Lead(IV) tetraacetate (LTA) ($C_8H_{12}O_8Pb$) was added initially (0.5 mM), after Stage 1 (0.66 mM) and after Stage 2 (0.96 mM).

Hydrogen peroxide control: Hydrogen peroxide was added initially (60 mM), after Stage 1 (26 mM) and after Stage 2 (38 mM).

Lignin peroxidase: Lignin peroxidase was added initially (0.12 U/ml), after Stage 1 (0.12 U/ml) and after Stage 2 (0.18 U/ml). Hydrogen peroxide was also added initially (0.4 mM), after Stage 1 (0.52 mM) and after Stage 2 (0.77 mM).

Cerium(IV) ammonium nitrate: $Ce(NO_3)_4{\cdot}NH_4NO_3$ (CAN) was added initially (0.5 mM), after Stage 1 (0.66 mM) and after Stage 2 (0.96 mM).

Fenton's reagent: The concentration of iron(II) chloride was 2 mM. Hydrogen peroxide additions were made in the same way as for the hydrogen peroxide control.

2.5. Analysis of reaction mixtures

The substrate and products in the reaction mixtures were separated using an HPLC system. Detection and quantification was performed using a diode-array detector (DAD). The identification of products **2**–**5** (Fig. 1) was based on comparison with data for the corresponding reference compounds (HPLC retention times, UV–vis spectra from the DAD and MS).

HPLC: An HP 1100 Series HPLC system equipped with a binary pump (Hewlett-Packard, Palo Alto, CA, USA) was used together with an XTerra™ MS C_{18} (5 μm, 2.1 mm × 150 mm) analytical column equipped with an XTerra™ MS C_{18} (5 μm, 2.1 mm × 10 mm) guard column (Waters, Milford, MA, USA). The eluent consisted of a gradient of Milli-Q water (Millipore, Bedford, MA, USA) and acetonitrile, both of which contained 2 mM formic acid. The flow rate was 0.2 ml/min. The gradient started with 5% acetonitrile for 5 min after which the acetonitrile content increased linearly to 10% (after 10 min), 30% (after 20 min) and 50% (after 40 min). Finally, the acetonitrile content was kept at 50% until 60 min. Detection was performed with an HP 1050 Series diode-array detector (Hewlett-Packard). For quantification, external calibration curves (four points) for all individual analytes were used. All analytes were quantified at 280 nm. The samples had to be diluted to concentrations suitable for the analytical system when ketone **2** was quantified. The retention times (min) were: *erythro*-1-(3,4-dimethoxyphenyl)-2-(2-methoxyphenoxy)-1,3-propanediol (**1**), 29.2; *threo*-1-(3,4-dimethoxyphenyl)-2-(2-methoxyphenoxy)-1,3-propanediol, 29.3; 1-(3,4-dimethoxy-phenyl)-3-hydroxy-2-(2-methoxyphenoxy)-1-propanone (**2**), 33.4; veratraldehyde (**3**), 26.5; veratric acid (**4**), 24.5; *erythro*-veratrylglycerol (**5**), 7.4; *threo*-veratrylglycerol, 10.5; ABTS, variable.

MS: The HPLC system was connected to an Esquire-LC ion-trap mass spectrometer with an API-electrospray interface operated in positive ionization mode (Bruker Daltonics, Bremen, Germany). The mass spectrometer was set to scan between *m*/*z* 50 and 400. The drying gas, nitrogen, was pumped into the interface at a rate of 7 l/min and at a temperature of 350 °C. Nitrogen was also employed as the nebuliser gas and was kept at 30 psi. The following software settings were used: nebuliser capillary tip 4500 V, endplate at the sampling orifice 4000 V, sampling capillary exit 100 V, $skimmer_1$ 33 V and $skimmer_2$ 6 V.

3. Results and discussion

The results of the oxidation experiments with the arylglycerol β-aryl ether **1** are summarized in Table 1 and Fig. 2. The reaction routes observed are shown in Fig. 1. The oxidations were performed in three stages to detect trends in the product formation patterns as the reactions were pushed further towards exhaustion. The results revealed significant differences in the product profiles (Table 1; Fig. 2) and this reflects the different properties of the oxidants.

Table 1
Yields of products (% of the theoretical yield) and proportion (%) of remaining starting material after oxidation of 1-(3,4-dimethoxyphenyl)-2-(2-methoxyphenoxy)-1,3-propanediol (**1**) (Fig. 1)

Oxidant	Yield (%) after Stage 1				
	1	**2**	**3**	**4**	**5**
Laccase/ABTS	n.d.[a]	26	–	<1	–
LTA	75	6	8	–	<1
Lignin peroxidase	97	<1	6	2	<1
CAN	99	–	2	–	<1
Fenton's reagent	87	1	4	1	1
Oxidant	**Yield (%) after Stage 2**				
	1	**2**	**3**	**4**	**5**
Laccase/ABTS	n.d.[a]	49	–	<1	–
LTA	65	7	15	2	<1
Lignin peroxidase	83	<1	12	3	2
CAN	97	<1	3	<1	<1
Fenton's reagent	78	2	4	1	2
Oxidant	**Yield (%) after Stage 3**				
	1	**2**	**3**	**4**	**5**
Laccase/ABTS	n.d.[a]	80	–	<1	–
LTA	40	10	30	3	<1
Lignin peroxidase	60	1	19	5	3
CAN	66	3	19	6	1
Fenton's reagent	67	2	3	2	2

[a] Not determined since ABTS interfered with the analysis.

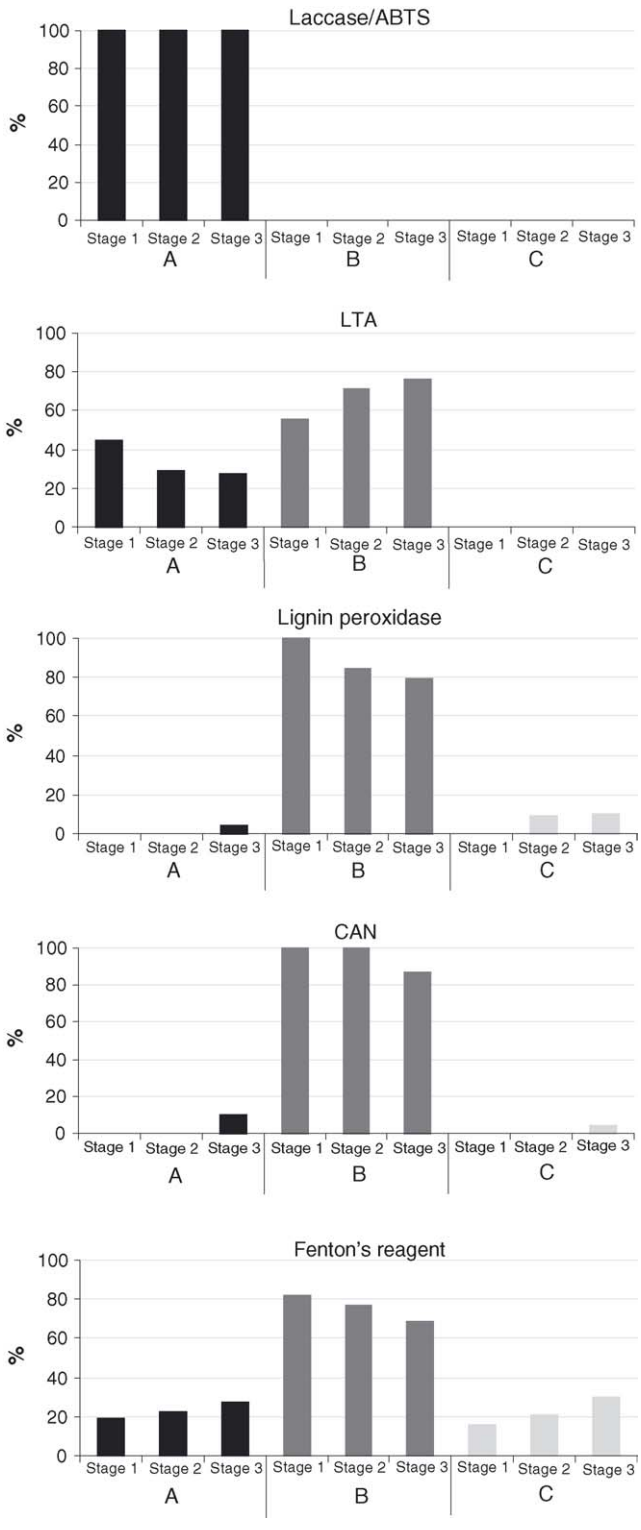

Fig. 2. Product distribution (in mol % of the total amount of identified products) after treatment of *erythro*-1-(3,4-dimethoxyphenyl)-2-(2-methoxyphenoxy)-1,3-propanediol (**1**) with different oxidants. (A), (B) and (C) refer to the reaction routes shown in Fig. 1.

Only very small amounts of ketone **2** were detected in the laccase control reaction (no ABTS added). Ketone **2** arises when the benzylic alcohol group of **1** is oxidized (C_α oxidation, cf. Fig. 1).

The predominant product formed in the laccase/ABTS reaction was ketone **2** (Table 1). Very small amounts of veratric acid (**4**) were also detected. That ketone **2** is the predominant oxidation product of **1** agrees with previous findings [11,13,32]. It has been shown that ketone **2** is the main product also when other laccase mediators are used [13,14,32]. Product formation in oxidations with laccase-mediator systems has been discussed in terms of ET (electron transfer) versus HAT (hydrogen atom transfer) oxidation routes. C_α–C_β cleavage is considered to proceed via an initial electron transfer reaction [32,33]. With regard to the laccase/ABTS system, an ET oxidation route appears feasible [32,33]. This suggests that the differences in product profiles (Table 1, Fig. 2) are not a result of different initial mechanistic steps in the reactions.

The main product identified in the reaction mixtures obtained on oxidation with LTA was aldehyde **3** (Table 1), which is formed after C_α–C_β cleavage (Fig. 1). Other products were **2** and **4**. Very small amounts of *erythro*-veratrylglycerol (**5**) were detected. LTA gave a more heterogeneous product pattern than the laccase-mediator systems. When the reaction proceeded, the C_α–C_β cleavage fraction increased while the C_α oxidation fraction decreased (Fig. 2).

A variety of products were detected in the lignin peroxidase reaction mixture (Fig. 3). The main product was aldehyde **3**. Small amounts of **2**, **4** and **5** were also detected

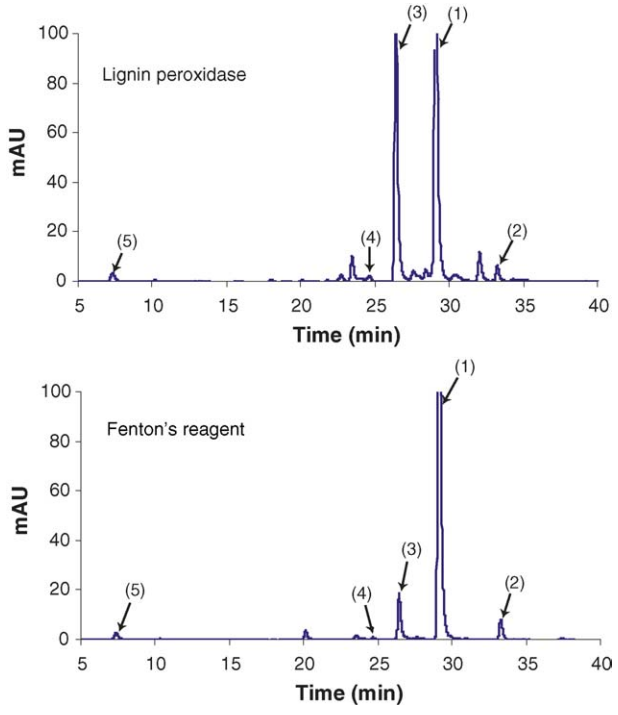

Fig. 3. HPLC chromatograms from analyses of reaction mixtures obtained on oxidation of *erythro*-1-(3,4-dimethoxyphenyl)-2-(2-methoxyphenoxy)-1,3-propanediol (**1**) with lignin peroxidase and Fenton's reagent (Stage 3).

(Table 1). No products were detected in the hydrogen peroxide control (no enzyme added). Previous results with lignin peroxidase as oxidant also suggest that the major consequence of oxidation of **1** is C_α/C_β cleavage, while C_α oxidation is less frequent [15,34].

The main product on oxidation of **1** with CAN was aldehyde **3** (Table 1). Compounds **2**, **4** and **5** were also detected. The product patterns obtained with lignin peroxidase and CAN were strikingly similar. Both oxidants predominantly resulted in the formation of products arising from C_α/C_β cleavage (Figs. 1 and 2). In both cases, veratraldehyde (**3**) and veratric acid (**4**) were formed, **3** being the main product. CAN represents an oxidant with a typical outer sphere electron transfer mechanism [35]. In contrast, the ABTS-mediated oxidation by laccase has been proposed to involve coupling products between ABTS and substrates [36–38] implying that the ABTS substrate interaction may be of the inner sphere type [35]. Although the reports regarding coupling products concern phenolic substrates [36–38], it seems conceivable that such interactions also are of importance for the differences in product profiles between CAN and ABTS-laccase observed in this study (Table 1; Fig. 2). The product profile obtained with lignin peroxidase/CAN was also clearly different from that obtained with LTA. The product pattern of LTA oxidation represents an intermediate between those of lignin peroxidase/CAN and the laccase-mediator system. Interestingly, Pb(IV) is an established inner sphere oxidant although an outer sphere electron transfer mechanism has been considered in some cases [35].

Another inorganic oxidant, 12-tungstocobalt(III)ate ($Co^{III}W$), has been used in comparisons with lignin peroxidase in the oxidation of monomeric, dimeric and trimeric lignin model compounds [39]. The catalytic efficiency obtained with lignin peroxidase decreased with the size of the substrate, while a very similar reactivity was observed when $Co^{III}W$ was used as the oxidant.

Oxidation with Fenton's reagent resulted in formation of **2**, **3**, **4** and **5** (Table 1; Fig. 3). This was the only one of the oxidants examined that did not provide a clearly dominant product (Table 1). Among the products identified, aldehyde **3** was found in highest yield. Comparisons of the relative proportions of identified products resulting from C_α oxidation, C_α/C_β cleavage and β-ether cleavage after different oxidation stages showed that Fenton's reagent gives a characteristic product pattern (Fig. 2).

Only one of the diastereomers of veratrylglycerol (the *erythro* form; **5**) was detected among the products. Since the HPLC method used provided good separation of *erythro*- and *threo*-veratrylglycerol (retention time 7.4 and 10.5 min, respectively), the results clearly demonstrate the formation of the *erythro* form. In analogous experiments with the *threo* form of 1-(3,4-dimethoxyphenyl)-2-(2-methoxyphenoxy)-1,3-propanediol, *threo*-veratrylglycerol was formed upon oxidation with LTA, lignin peroxidase, CAN or Fenton's reagent. Obviously, the reactions leading to cleavage of the β-ether bond proceed without affecting the configuration at the β-carbon atom.

There is a possibility that some of the products also serve as substrates for the oxidants. This is a motive for analyzing several stages of oxidation to examine whether the product distribution patterns (Fig. 2) remain stable. Kirk et al. [15] reported that ketone **2** was a poor substrate for lignin peroxidase. Preliminary experiments with **2** as substrate instead of **1** indicated that neither lignin peroxidase nor CAN oxidized **2**. In contrast, Fenton's reagent oxidized **2** at a notable rate.

The oxidants differ with regard to their redox potentials ($E^{\circ\prime}$). The redox potential of the T1-copper site of *Trametes versicolor* laccase has been determined to 785 mV versus NHE [9], corresponding to approximately 585 mV versus the Ag/AgCl reference electrode. Even though laccases are multicopper oxidases with a mononuclear T1 site and a trinuclear T2/T3 site with different $E^{\circ\prime}$-values, it is believed that the oxidation of the electron donor occurs at the T1 site and that the reduction of oxygen to water occurs at the T2/T3 cluster [40]. The redox potential of the laccase mediator ABTS was studied by Bourbonnais et al. [37]. The $E^{\circ\prime}$ versus Ag/AgCl reference electrode for $ABTS/ABTS^{\bullet+}$ and $ABTS^{\bullet+}/ABTS^{2+}$ were determined to 472 and 885 mV, respectively. Lignin peroxidase is able to oxidize substrates with a redox potential of at least 1.36 V versus NHE [41]. Standard redox potentials of 1760 and 1690 mV have been given for Ce^{4+}/Ce^{3+} and Pb^{4+}/Pb^{2+}, respectively [42]. Paulenova et al. [43] recently reported values close to 1500 mV versus NHE for the redox potential of cerium(IV) sulfate solutions. Berka et al. [44] reported that the redox potential for LTA in diluted acetic acid was 1450 mV versus NHE. The redox potential of the hydroxyl radical can be estimated to around 2.8 V [45,46]. The redox potential for the laccase-mediator system may therefore be relatively low compared with the other oxidants, a property that tentatively could be associated with a more specific product formation pattern, in which fewer products are generated. Similarly, the lack of dominant products observed in reactions with Fenton's reagent can be attributed to a high redox potential in comparison with the other oxidants.

Kirk et al. studied the degradation of veratrylglycerol-β-guaiacyl ether (mixture of **1** and the *threo* form) in fungal cultures of *Polyporus* (*Trametes*) *versicolor* and *Stereum frustulatum* [47]. Ketone **2** was found as a product in wood-meal cultures of both *P. versicolor* and *S. frustulatum*. Interestingly, purified *P. versicolor* laccase, which would not normally oxidize veratrylglycerol-β-guaiacyl ether to **2** at any notable rate, contributed to this reaction in the presence of a preparation of spruce milled wood lignin [47]. This suggests that the lignin preparation could act as a mediator of laccase-catalyzed oxidation.

Incubation of veratrylglycerol-β-guaiacyl ether in *P. chrysosporium* cultures resulted mainly (55.5% yield based on consumed substrate) in formation of veratryl alcohol (produced by reduction of **3** in the fungal cultures) and ketone **2** (7.6% yield based on consumed substrate) [34]. With lignin

peroxidase, compounds **2** and **3** accounted for a substantial part of the products both in our study [∼50% yield based on consumed substrate in Stage 3 (cf. Table 1)] and in the study by Kirk et al. (71% yield based on consumed substrate) [34]. Kirk et al. [34] reported formation of traces of **2** and **3** (<0.1 and <0.01% yield based on consumed substrate) on oxidation with Fenton's reagent (83.1% of the substrate had been consumed). A larger yield of products could be accounted for in our reactions with Fenton's reagent (up to ∼30% of the substrate was consumed) (Table 1). In both cases, most of the consumed substrate had been converted to unidentified products. The combined yields of **2** and **3** were much lower for Fenton's reagent than for lignin peroxidase (Table 1, [34]). Interestingly, the yield of products observed in a fungal culture (63.1% yield of **2** and **3** based on consumed substrate in a culture in which 97% of the substrate had been consumed [34]) agrees well with the yield obtained with lignin peroxidase but disagrees with that obtained with Fenton's reagent.

Thiol-mediated oxidation of veratrylglycerol-β-guaiacyl ether (or related compounds) resulted in formation of **2** (or related compounds) [48,49]. β-O-4 ether cleavage products were observed, but no C_α/C_β cleavage products were reported [48]. Oxidation of an *erythro/threo* mixture of the β-ether model 1-(4-ethoxy-3-methoxyphenyl)-2-(2-methoxyphenoxy)-1,3-propanediol with a system consisting of manganese peroxidase, Mn(II) and linoleic acid resulted in higher yields of the corresponding α-ketone than of C_α/C_β cleavage products [50].

The product distribution pattern from **1** offered a characteristic fingerprint for the different oxidants studied, which could be related to differences in redox potential but, as pointed out above, other factors may also be of importance. Lignin peroxidase and CAN gave a similar product pattern, while the laccase-mediator system, LTA and Fenton's reagent gave rise to distinctly different product profiles. The results demonstrate that oxidation with CAN serves as an excellent model for lignin peroxidase, the product formation pattern of which is in agreement with results from fungal cultures. Studies with both the *erythro* and the *threo* forms of the arylglycerol β-aryl ether **1** showed that the configuration at the C_β carbon atom was retained upon oxidation leading to liberation of veratrylglycerol. Evidently the reaction mechanisms involved in the formation of this compound have features in common in the sense that the 2-methoxyphenyl group is degraded and released without a change of the configuration of the β-carbon atom. Further studies regarding stereochemical aspects of degradation of lignin model compounds with different oxidants are underway.

Acknowledgements

The authors thank M.Sc. P.-O. Andersson for the performance of some of the oxidation experiments. This work was supported by grants from the Swedish Research Council.

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