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Laccase-mediated oxidation of phenolic derivatives

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ABSTRACT

The laccase-catalyzed oxidation of *para*-alkyl phenols (*p*-cresol, 3,4-dimethylphenol, tyrosol, 2′-*O*-acetyl-tyrosol) in biphasic systems has been investigated. With all the substrates compounds similar to the so-called "Pummerer's ketone" could be isolated in reasonable yields. Accumulation of this kind of products is due to the fact that these compounds, in contrast to other dimers, are "dead-end" products, as they cannot be further oxidized by the enzyme.

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1. Introduction

Biocatalyzed oxidation of phenols, particularly for technological applications, is a well-covered area of research. In fact, biooxidation of these compounds at the expense of molecular oxygen or H_2O_2 generates reactive radical intermediates that, under suitable conditions, can then undergo extensive polymerization. Accordingly, these biotransformations have been exploited in bioremediation processes [1] and for the production, under mild conditions, of new polyphenolic polymers [2].

The isolation of low molecular weight products is a much more difficult task, but it is a goal of synthetic relevance: for instance, a large number of dimeric napthol derivatives are widely used for the production of the famous and efficient Noyori's organometallic catalysts [3].

Oxygen-mediated oxidation of substituted phenols catalyzed by tyrosinase produces cathecols first, but then these intermediates are further oxidized to the corresponding quinones. The latter compounds easily polymerize in water, whereas in organic solvents it is possible either to trap the quinones with Diels–Alder condensations in a domino sequence [4], or to slow down the polymerization processes so that they can be reduced back to the stable catechols by extraction with an ascorbate solution [5].

H_2O_2-mediated oxidation of simple phenol derivatives catalyzed by peroxidases has been described by different authors [6–8]. Generally speaking, the yields of recovered dimers and trimers are quite low. For instance, oxidation of *p*-cresol (**1**) with horseradish peroxidase gave either the dimer **2** or the so-called "Pummerer's ketone" (**3**) as the main product, depending upon the reaction conditions used, in 27 or 12% yields, respectively [8].

Laccases are a third group of exocellular oxidoreductases [9], whose synthetic exploitation has been quite neglected until few years ago despite the fact that they are quite abundant in nature, particularly in fungi. Laccases are ideally "green" catalysts, as they can use air as oxidant and produce H_2O as the byproduct of the oxidation of four substrate molecules (phenols, aromatic or aliphatic amines) to the corresponding reactive radicals; the redox processes take place with the assistance of a cluster of copper atoms which is the catalytic site of the enzyme [10].

Synthetic exploitation of laccases can follow two different approaches: the direct oxidation of the substrate or the oxidation of an intermediate molecule (the so-called "mediator") that then oxidize the target substrate. The latter strategy is used in nature for the oxidation of lignin which, being a polymeric material, cannot reach the internal enzymatic copper cluster [11]. Examples of the *in vitro* exploitation of this approach are given by the laccase-mediated oxidative bleaching of indigo in the textile industry [12], or by the oxidation of a series of primary alcohols to the corresponding aldehydes or carboxylic acids mediated by TEMPO [13,14].

Few data are available, even today, on preparative scale laccase-catalyzed direct oxidation reactions of these substrates. Most reactions involve the modification of natural compounds, as, for instance, the synthesis of actinocine (the chromophoric component of the antibiotic actinomicin) from 3-hydroxy-4-methyl anthranilic acid [15], the oxidation of penicillin G [16], the stilbenic phytoalexin molecule resveratrol [17] or other hydroxy-stilbene derivatives [18], the plant antibiotic totarol [19] and the steroid 17β-estradiol [20], to give dimeric products *via* radical coupling reactions. The

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latter transformation, catalyzed by a laccase from *Polyporus versicolor*, was described more than 30 years ago and was one of the first examples, if not the very first, of the use of enzymes in biphasic systems [20a].

One of our present research directions is related to the exploitation of enzymes for the selective formation of carbon–carbon bonds. We reasoned that phenol-dimerization, catalyzed by laccases, might be of interest in this respect and in this report, we describe the results obtained using the laccases from *Mycelyopthora termophyla* (MtL) and from *Trametes versicolor* (TvL) in the oxidation of substituted phenol derivatives.

2. Experimental

2.1. Materials and methods

2.1.1. Enzymes and chemicals

The lipases from *Candida antarctica* (Novozyme 435®) and the laccase from *M. termophyla* (MtL) were from Novozymes. *T. versicolor* (TvL) laccase was purchased from Sigma–Aldrich. The enzymes were each used in quantities based on respective activities, as in previous investigations [19,21]. All chemicals and solvents were purchased from Sigma–Aldrich.

2.1.2. Laccase-catalyzed oxidation of p-cresol (**1**)

(a) *p*-Cresol (50 mg, 0.46 mmol) was dissolved in a mixture of ethanol (1.5 mL) and 50 mM TRIS-buffer, pH 7.5 (3.5 mL) and a lyophilized sample of laccase from *Myceliophtora thermophyla* (30 mg, ∼30 U evaluated using syringaldazine as a substrate) was added. The homogeneous solution was gently shaken at 30 °C for 2 h, until complete conversion of the substrate was achieved. The solution was extracted with AcOEt and the crude residue was purified by silica flash chromatography (petroleum ether–AcOEt, 8:2) to give **3** (10 mg, 20% isolated yields).

(b) *p*-Cresol (50 mg, 0.46 mmol) was dissolved in AcOEt (2 mL), while the MtL laccase (30 mg, ∼30 U) was dissolved in 50 mM TRIS-buffer, pH 7.5 (2 mL). The biphasic reaction was gently shaken at 30 °C for 24 h. The phases were separated, the water solution was extracted with AcOEt and the crude residue was purified by silica flash chromatography (petroleum ether–AcOEt, 8:2) to give 8 mg of a mixture of **2** and **3** (16% isolated yields).

TLC (petroleum ether–AcOEt, 8:2, R_f **1**, 0.50, R_f **2**, 0.35, R_f **3**, 0.35. RP-HPLC (eluent, MeOH–H_2O, 7:3; flow 0.5 mL/min, λ: 254 nm): t_r **1**: 3.63 min, t_r **2**: 8.41 min, t_r **3**: 6.08 min. **2** ^{1}H NMR δ (ppm, $CDCl_3 + D_2O$): 7.10 (2 H, dd, J_1 = 8.7 Hz, J_2 = 1.0 Hz, H-4 and H-4′); 7.05 (2 H, s, H-6 and H-6′); 6.90 (2 H, d, J = 8.2 Hz, H-3 and H-3′); 5.40 (2 H, s, phenolic OHs); 2.30 (6 H, s, CH_3 and CH_3′). **3** ^{1}H NMR ($CDCl_3 + D_2O$) δ (ppm): 6.95 (1 H, s, H-10); 6.94 (1 H, d, J = 10.2 Hz, H-3); 6.70 (1 H, d, J = 8.0 Hz, H-13); 6.45 (1 H, dd, J_1 = 8.0 Hz, J_2 = 1.8 Hz, H-12); 5.90 (1 H, d, J = 10.2 Hz, H-2); 4.70 (1 H, t, J = 3.2 Hz, H-5); 3.05 (1 H, dd, J_1 = 17.5 Hz, J_2 = 2.8 Hz, H-6a); 2.78 (1 H, dd, J_1 = 17.5 Hz, J_2 = 3.8 Hz, H-6b); 2.31 (3 H, s, CH_3-15); 1.62 (3 H, s, CH_3-14).

2.1.3. Laccase-catalyzed oxidation of 3,4-dimethylphenol (**4**)

(a) 3,4-Dimethylphenol (4, 60 mg, 0.50 mmol) was dissolved in a mixture of ethanol (1.5 mL) and 50 mM TRIS-buffer, pH 7.5 (3.5 mL) and a lyophilized sample of laccase from *M. thermophyla* (30 mg, ∼30 U evaluated using siringaldazine as a substrate) was added. The homogeneous solution was mildly shaken at 30 °C for 4 h, till complete conversion of the substrate. The solution was extracted with AcOEt and the crude residue was purified by silica flash chromatography (petroleum ether–AcOEt, 8:2) to give 5 and 6 (32 mg, 53% isolated yields).

(b) 3,4-Dimethylphenol (**4**, 60 mg, 0.50 mmol) was dissolved in AcOEt (2 ml), while the MtL laccase (30 mg, ∼30 U) was dissolved in 50 mM TRIS-buffer, pH 7.5 (2 mL). The biphasic reaction was mildly shaken at 30 °C for 24 h. The phases were separated, the water solution was extracted with AcOEt and the crude residue was purified by silica flash chromatography (petroleum ether–AcOEt, 8:2) to give 14 mg di **6** (23%).

TLC (petroleum ether–AcOEt, 8:2, R_F **4**, 0.44, R_F **5**, 0.32, R_F **6**, 0.20. RP-HPLC (eluent, MeOH–H_2O, 7:3; flow 0.5 mL/min, λ: 254 nm): t_r **4**: 4.20 min, t_r **5**: 14.30 min, t_r **6**: 8.45 min. **5** ^{1}H NMR δ (ppm, $CDCl_3 + D_2O$): 7.02 (2 H, s, H-3 and H-3′); 6.85 (2 H, s, H-6 and H-6′); 2.22 and 2.20 (6 H each, s each, two CH_3 and two CH_3′); MS: m/z = 426 (M^+). **6** ^{1}H NMR ($CDCl_3 + D_2O$) δ (ppm): 7.04 (1 H, s, H-10); 6.65 (1 H, s, H-13); 5.88 (1 H, s, H-2); 4.63 (1 H, dd, J_1 = 4.0 Hz, J_2 = 3.0 Hz, H-5); 3.02 (1 H, ddd, J_1 = 17.5 Hz, J_2 = 2.5 Hz, J_3 = 1.0 Hz H-6a); 2.75 (1 H, dd, J_1 = 17.5 Hz, J_2 = 3.5 Hz, H-6b); 2.25 (3 H, s, CH3); 2.23 (3 H, s, CH3); 1.93 (3 H, d, J = 1.5 Hz CH3); 1.59 (3 H, s, CH_3); ^{13}C NMR δ (ppm, $CDCl_3$): δ = 194.72 (C-1); 158.65 (C-3); 157.30 (C-8); 137.81 (C-9); 129.06, 129.04 (C-11, C-12); 126.01 (C-2); 125.12 (C-10); 111.71 (C-13); 87.87 (C-5); 47.93 (C-4); 37.07 (C-6); 20.52, 20.16, 19.57, 19.54 (C-14, C-15, C-16, C-17). MS: m/z = 426 (M+).

2.1.4. Oxidation of tyrosol (**7**) by laccase from T. versicolor

Tyrosol (**7**, 500 mg) dissolved in 50 mL AcOEt was added to 50 mL acetate buffer 20 mM pH 3.5 in which the laccase from *T. versicolor* (500 U) had been previously dissolved. The solution was incubated at 30 °C under gentle shaking, and the conversions was followed by TLC (eluent: AcOEt). After 48 h the organic phase was separated and the water phase was extracted with AcOEt. Following drying over sodium sulfate, the solvent was evaporated under reduced pressure and the crude residue was purified by flash chromatography (eluent: AcOEt) to give the product **9** (43 mg, 8.7% yields).

^{1}H NMR δ (ppm, MeOD): δ = 7.21 (1H, d, J = 2.0 Hz, H-13); 7.07 (1H, dd, J_1 = 8.5 Hz, J_2 = 2.0 Hz, H-15); 6.73 (1H, d, J = 8.5 Hz, H-16); 4.80 (1H, dt, J_1 = 3.0 Hz, J_2 = 1.0 Hz, H-5); 4.15 (1H, dt, J_1 = 9.5 Hz, J_2 = 6.5 Hz, H-8_a); 4.06 (1H, dt, J_1 = 9.5 Hz, J_2 = 6.5 Hz, H-8_b); 3.95 (1H, m, H-3); 3.74 (2H, t, J = 7.0 Hz, CH_2-18); 2.88 (1H, dd, J_1 = 18.5 Hz, J_2 = 3.0 Hz, CH_2-6_a); 2.83 (1H, dd, J_1 = 18.5 Hz, J_2 = 3.0 Hz, CH_2-6_b); 2.82 (1H, m, H-9_a); 2.80 (2H, t, J = 7.0 Hz, CH_2-17); 2.59 (1H, dd, J_1 = 18.0 Hz, J_2 = 3.5 Hz, H-2_a); 2.23 (1H, m, H-9_b); 2.22 (1H, dd, J_1 = 18.0 Hz, J_2 = 2.5 Hz, H-2_b). ^{13}C NMR δ (ppm, MeOD): δ = 207.52 (C-1); 158.02 (C-11); 132.38 (C-14); 129.75 (C-12); 129.54 (C-15); 123.64 (C-13); 109.15 (C-16); 87.70 (C-5); 83.24 (C-3); 66.90 (C-8); 63.05 (C-18); 52.73 (C-4); 39.37 (C-6); 38.83 (C-2); 38.33 (C-17); 37.84 (C-9). MS, m/z = 274 Da. (The NMR data were in accordance to literature [23].)

2.1.5. Acetylation of tyrosol by lipase from C. antarctica (Novozym 435)

Tyrosol (**7**, 200 mg), vinyl acetate (5 ml) and the enzymatic preparation Novozym 435 (75 mg) were added to a vial. The solution was incubated at 45 °C, under vigorous shaking (200 rpm), and the conversion was followed by TLC (eluent: petroleum ether, AcOEt 3:7). After 3 h the enzyme was eliminated by filtration, the solvent was evaporated under reduced pressure and the crude material was purified by flash chromatography (eluent: petroleum ether–AcOEt, 9:1) to give the product **10** (210 mg, 80.5% yields): ^{1}H NMR δ (ppm, MeOD): δ = 7.05 (2H, d, J = 8.5 Hz, H-3, H-5); 6.72 (2H, d, J = 8.5 Hz, H-2, H-6); 4.21 (2H, t, J = 7.0 Hz, CH_2-8); 2.83 (2H, t, J = 7.0 Hz, CH_2-7); 2.01 (3H, s, CH_3).

2.1.6. Oxidation of acetyl-tyrosol (**10**) by laccase from T. versicolor

Tyrosyl acetate (**10**, 50 mg) dissolved in 5 mL AcOEt was added to 5 mL acetate buffer 20 mM pH 3.5 in which the laccase from *T.*

versicolor (50 U) had been previously dissolved. The solution was incubated at 30 °C under mild shaking, and the conversion was followed by TLC (eluent: petroleum ether, AcOEt 7:3). After 48 h the organic phase was separated and the water phase was extracted with AcOEt. Following drying over sodium sulfate, the solvent was evaporated under reduced pressure and the crude residue was purified by flash chromatography (eluent: petroleum ether, AcOEt 8:2, then petroleum ether, AcOEt 7:3) to give the products **11** (2.7 mg, 5.4% yields) and **12** (10 mg, 20.1% yields).

11: ^{1}H NMR δ (ppm, MeOD): δ = 7.19 (2H, d, J = 9.0 Hz, H-3, H-5); 6.88 (4H, m, H_{ar}); 6.77 (1H, d, J = 1.5 Hz, H-14); 4.25 and 4.17 (2H each, t each, J = 7.0 Hz, CH_2-8 and CH_2-16); 2.90 and 2.80 (2H each, t each, J = 7.0 Hz, CH_2-7 and CH_2-15); 2.01 (3H, s, CH_3); 1.96 (3H, s, CH_3). MS, m/z = 358, 298 due to $[M-AcOH]^+$, 238 due to [M-2 AcOH]$^+$.

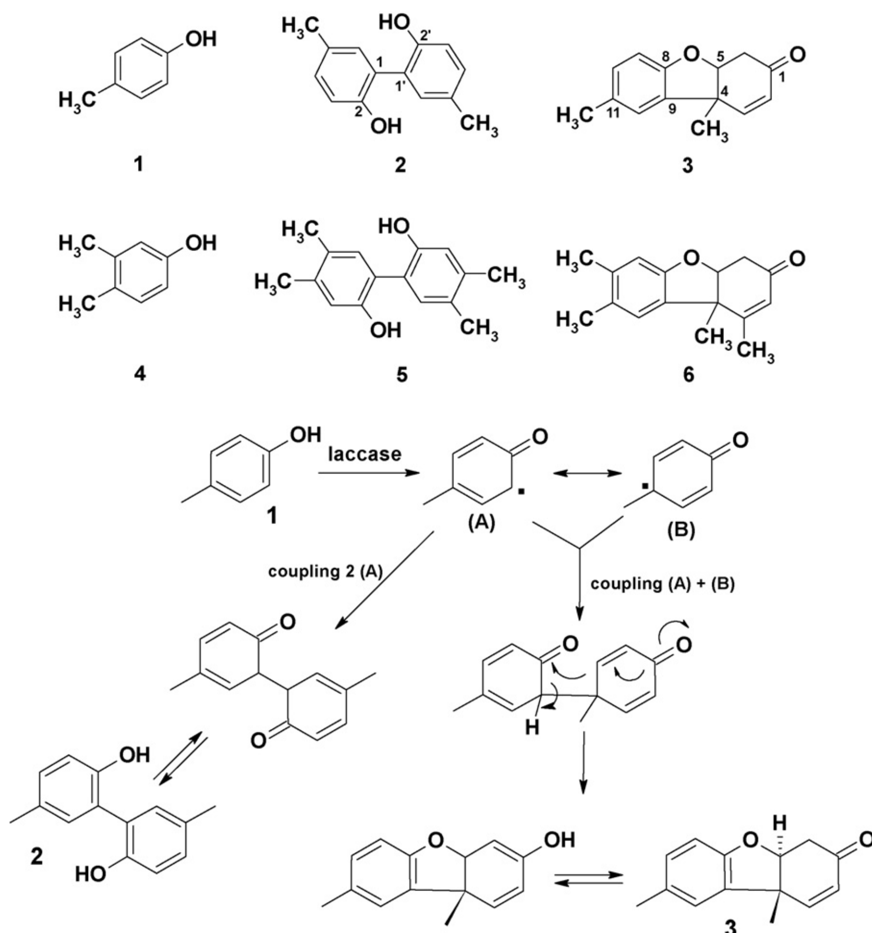
12: ^{1}H NMR δ (ppm, MeOD): δ = 7.23 (1H, dd, J_1 = 2.0 Hz, J_2 = 0.5 Hz, H-10); 7.08 (1H, dd, J_1 = 8.0 Hz, J_2 = 2.0 Hz, H-12); 6.73 (1H, d, J = 8.0 Hz, H-13); 6.65 (1H, dd, J_1 = 10.5 Hz, J_2 = 2.0 Hz, H-3); 5.97 (1H, dd, J_1 = 10.5 Hz, J_2 = 1.0 Hz, H-2); 5.00 (1H, m, H-5); 4.32 (1H, dt, J_1 = 11.5 Hz, J_2 = 7.0 Hz, H-17$_a$); 4.25 (2H, t, J = 7.0 Hz, CH_2-15); 4.24 (1H, dt, J_1 = 11.5 Hz, J_2 = 7.0 Hz, H-17$_b$); 3.01 (1H, dd, J_1 = 17.5 Hz, J_2 = 4.0 Hz, H-6$_a$); 2.94 (1H, ddd, J_1 = 17.5 Hz, J_2 = 3.0 Hz, J_3 = 0.5 Hz, H-6$_b$); 2.91 (2H, t, J = 7.0 Hz, CH_2-14); 2.47 (1H, dt, J_1 = 15.0 Hz, J_2 = 7.0 Hz, H-16$_a$); 2.32 (1H, dt, J_1 = 15.0 Hz, J_2 = 7.0 Hz, H-16$_b$); 2.01 (6H, s, 2 CH_3). ^{13}C NMR δ (ppm, MeOD): δ = 150.35 (C-3); 130.76 (C-12); 127.12 (C-2); 124.91 (C-10); 110.75 (C-13); 85.73 (C-5); 66.25 (C-15); 61.75 (C-17); 38.98 (C-6); 35.42 (C-14); 35.01 (C-16); 20.70 (2 CH_3). MS, m/z = 298 due to $[M-AcOH]^+$.

2.1.7. Oxidation of 4-vinylphenol (13) by laccase from T. versicolor

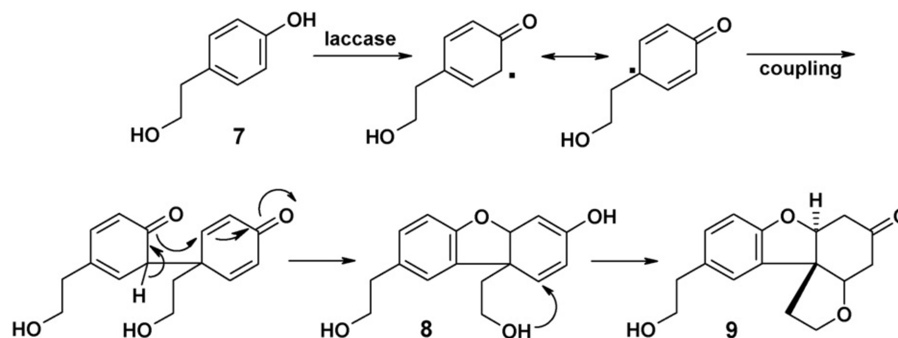
4-Vinylphenol (**13**, 1000 mg) dissolved in 100 mL of AcOEt was added to 100 mL acetate buffer 20 mM pH 3.5 in which the laccase from *T. versicolor* (1430 U) had been previously dissolved. The solution was incubated at 30 °C under mild shaking, following the conversion by TLC (eluent: petroleum ether–AcOEt, 9:2). After 48 h the organic phase was separated and the water phase was extracted with AcOEt. Following anhydrification over sodium sulfate, the solvent was evaporated under reduced pressure and the crude residue was purified by flash chromatography (eluent: petroleum ether–AcOEt, 9:2) to give the product **15** (114 mg, 11.4% yields). Then the eluent was changed to $CHCl_3$/MeOH 95:5 to isolate the product **14** (29 mg, 2.7% yields).

15: ^{1}H NMR δ (ppm, $CDCl_3$): 7.31 (1H, s, H-4); 7.30 (2H, d, J = 8.4 Hz, H-2′, H-6′); 7.22 (1H, d, J = 8.0 Hz, H-6); 6.85 (2H, d, J = 8.4 Hz, H-3′, H-5′); 6.81 (1H, d, J = 8.0 Hz, H-7); 6.69 (1H, dd, J_1 = 17.6 Hz, J_2 = 10.8 Hz, H-1″); 5.73 (1H, t, J = 8.8 Hz, H-2); 5.61 (1H, d, J = 17.6 Hz, H-2″$_{trans}$); 5.12 (1H, d, J = 10.8 Hz, H-2″$_{cis}$); 3.60 (1H, dd, J_1 = 15.6 Hz, J_2 = 9.2 Hz, H-3$_a$); 3.22 (1H, dd, J_1 = 15.6 Hz, J_2 = 8.0 Hz, H-3$_b$). ^{13}C NMR δ (ppm, $CDCl_3$): 159.46 (C-8); 155.53 (C-4′); 136.61 (C-1″); 133.94 (C-5); 130.87 (C-9); 127.61 (C-2′, C-6′); 127.00 (C-6); 122.37 (C-4); 115.52 (C-3′, C-5′); 111.09 (C-2″); 109.22 (C-7); 84.46 (C-2); 38.06 (C-3). MS, m/z = 238 Da.

14: ^{1}H NMR δ (ppm, $CDCl_3$): 7.19 (2H, d, J = 8.4 Hz, H-2′, H-6′); 6.81 (2H, d, J = 8.8 Hz, H-3′, H-5′); 6.65 (1H, dd, J_1 = 10.4 Hz, J_2 = 2.4 Hz, H-4); 6.27 (1H, d, J = 10.4 Hz, H-5); 5.90 (1H, dd, J_1 = 17.6 Hz, J_2 = 10.4 Hz, H-1″); 5.29 (1H, d, J = 10.4 Hz, H-2″$_{cis}$); 5.22 (1H, d, J = 17.6 Hz, H-2″$_{trans}$); 4.86 (1H, dd, J_1 = 11.2 Hz, J_2 = 6.0 Hz, H-



Scheme 1. Laccase-catalyzed oxidation of *p*-cresol (**1**).



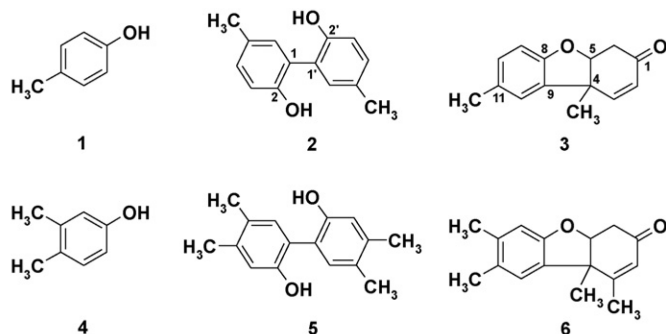
Scheme 2. Laccase-catalyzed oxidation of tyrosol (**7**).

2); 4.49 (1H, q, J = 2.8 Hz, H-8); 2.86 (1H, dd, J_1 = 17.2 Hz, J_2 = 3.2 Hz, H-7$_a$); 2.62 (1H, dd, J_1 = 17.2 Hz, J_2 = 3.2 Hz, H-7$_b$); 2.43 (1H, dd, J_1 = 12.8 Hz, J_2 = 5.6 Hz, H-3$_a$); 2.22 (1H, dd, J_1 = 12.8 Hz, J_2 = 10.8 Hz, H-3$_b$). ^{13}C NMR δ (ppm, CDCl$_3$): 197.66 (C-6); 155.58 (C-4′); 149.26 (C-4); 137.59 (C-1″); 133.94 (C-1′); 130.43 (C-5); 127.22 (C-2′, C-6′); 117.82 (C-2″); 115.49 (C-3′, C-5′); 80.79 (C-8); 80.03 (C-2); 51.26 (C-9); 48.13 (C-3); 38.32 (C-7). MS, m/z 256 Da.

3. Results and discussion

As starting model substrates, we chose the already mentioned *p*-cresol (**1**) and its analogue 3,4-dimethylphenol (**4**). The commercially available laccases MtL or TvL were used as the catalysts in two different reaction systems: (a) a homogeneous solution of **1** or **4** in TRIS buffer containing EtOH (10%, v/v) as a cosolvent; (b) a biphasic system TRIS buffer–AcOEt (1:1, v/v). Reactions were much faster in the homogeneous solutions and complete conversions were obtained after few hours, whereas reactions in biphasic systems were stopped after 24 h. These first experiments indicated that even with laccases it is not easy to exploit these oxidative reactions to produce low molecular weight compounds, the main products being brown-red polymeric materials.

However, oxidized derivatives could be isolated by chromatography and fully characterized by NMR and mass spectrometry. Specifically, the dimers **2** and **3** (from **1**, see Scheme 1) and the corresponding analogues **5** and **6**, (from **4**) were clearly identified.



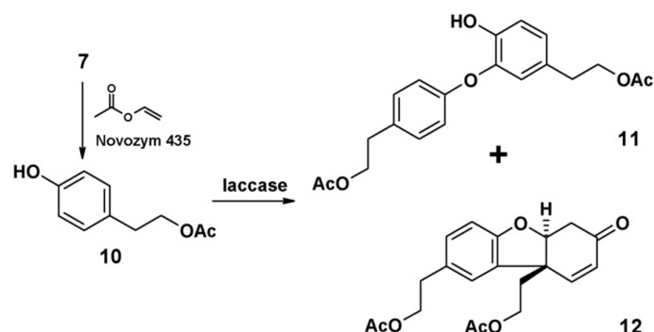
In a next step we turned our attention to the more interesting phenol tyrosol (**7**). This is one of the natural phenols which are present in olive oil and wine and are believed to contribute to their beneficial effects (cardioprotection and anti-atherogenic activity, antioxidant skin photoprotection and anti-inflammatory activity, . . .) [22]. Laccase-catalyzed oxidation of **7** was performed under varied conditions and the best results were obtained using the laccase from *Trametes* in a biphasic system. In this system, the compound **9** could be isolated and characterized. Its mass and NMR data were fully in accordance with those reported by Delle Monache and coworkers [23], who investigated the oxidation of **7** catalyzed by a laccase from the basidyomicete *Lentinus edodes*. Scheme 2 shows the plausible reaction mechanism (as proposed in [23]), which implies the coupling of two radicals, followed by two intramolecular Michael additions (compound **9** was a racemic mixture of the two *trans* enantiomers, only one of which is shown in Scheme 2).

A more polar intermediate (likely to be the dimer **8**) could be detected by TLC, but any attempt to isolate it failed, as it was converted into the final product **9** during the chromatography step.

In order to unambiguously confirm the reaction mechanism hypothesis, we prepared the monoacetylated derivative **10** by lipase-catalyzed acylation in organic solvent [24]. TvL-catalyzed oxidation of **10** worked smoothly and two compounds could be isolated in 5.4 and 20.1% yields, respectively. The minor product could be easily identified as the C–O dimer **11** (Scheme 3) by mass spectrometry (m/z at 358 Dalton) and its very simple ^{1}H NMR spectrum.

Fig. 1 shows the ^{1}H NMR spectrum of the major product. The presence of an olefinic double bond was clearly shown by the two doublets at 6.65 and 5.97 p.p.m. (J = 10.5 Hz), while the other signals between 7.2 and 6.7 p.p.m. were diagnostic for a three-substituted aromatic ring. COSY and HMQC analysis allowed the complete assignments of all the other signals of the ^{1}H- and ^{13}C NMR spectra to the corresponding proton and carbon atoms, thus confirming the proposed structure **12** (compound **12** was a racemic mixture of the two *trans* enantiomers, only one of which is shown in the scheme).

In a further investigation we have synthesized and submitted to laccase-catalyzed oxidation a series of substituted *p*-vinyl phenols, i.e. **13**. Due to the more extensive possible delocalization of the radical intermediate, the coupling pathways were much more complex. “Pummerer’s ketone” structures (such as **14**) could be isolated, but as very minor byproducts, whereas the main products



Scheme 3. Laccase-catalyzed oxidation of acetyl-tyrosol (**10**).

Fig. 1. ^{1}H NMR spectrum of compound **12**.

Scheme 4. Laccase-catalyzed oxidation of 4-vinyl-phenol (**13**).

were dimers such as **15** (Scheme 4). These results will be discussed in more details in due course.

4. Conclusions

Compounds similar to the so-called "Pummerer's ketone" could be isolated in reasonable yields by the laccase-catalyzed oxidation of *para*-alkyl phenols in biphasic systems. Relative accumulation of this type of product is not surprising as these compounds, in contrast to other dimers such as **2**, **5**, and **11** are "dead-end" in that they cannot be further oxidized by the laccase.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.molcatb.2009.12.016.

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