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From Diols to Lactones under Aerobic Conditions using a Laccase/TEMPO Catalytic System in Aqueous Medium

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Abstract: An efficient catalytic system to oxidize quantitatively aliphatic diols using *Trametes versicolor* laccase and TEMPO has been developed in aqueous medium. Oxidations have occurred in a non-stereoselective fashion but with complete regio- and/or monoselectivity, obtaining lactones with excellent purity after simple extraction. This catalytic system has been demonstrated to be scalable, compatible with the presence of a variety of functionalities, and also allowed the successful enzyme recycling using a laccase-cross-linked enzyme aggregates (CLEA) preparation.

Keywords: aerobic oxidation; laccase; lactones; regioselectivity; TEMPO

The regioselective oxidation of alcohols to obtain aldehydes, ketones or carboxylic compounds is one of the most relevant and challenging transformations in organic chemistry, due to the utility of these derivatives as synthetic precursors for many drugs, fragrances and natural products. A large variety of stoichiometric reagents have been used for these oxidation reactions such as peroxides, hypervalent organoiodane, chromium oxides or sulfur-based reagents.[1] Particularly interesting are those catalytic oxidations using transition metal agents (Pd, Ru, Au, Rh) under aerobic conditions because oxygen generates water as the sole by-product.[2] To date, among all organometallic complexes employed to perform aerobic oxidations, probably copper-containing catalysts have emerged as the most efficient ones,[3] as recently reported by Hoover and Stahl in the chemoselective oxidation of a range of primary alcohols in the presence of other functional groups using (bpy)Cu(I) complexes, *N*-methylimidazole as base and acetonitrile as solvent.[4] While a variety of catalytic methods has been reported for the oxidation of alcohols in organic solvents, the development of more selective and environmentally friendly oxidation procedures remains nowadays as one of the major challenges for the production of fine chemicals.[5] Hence, there is a need for more efficient, greener and scalable catalytic oxidations in aqueous medium, biocatalytic processes being considered adequate tools for these types of transformations.[6]

Copper is a fundamental trace element involved in many redox reactions occurring in living systems and can be found in a number of proteins. Laccases are multicopper enzymes, which are present in many fungi, plants and bacteria.[7] Functionally, these oxidases reduce O_2 into H_2O at the expense of the corresponding substrate. Over the last decade, considerable improvements in protein isolation, expression and purification methods have provided access to these enzymes[8] making laccases ideal candidates for biooxidation processes.[9] Although phenolic derivatives are their natural substrates and have already found in some cases industrial applications,[10] laccases are not effective towards other non-phenolic substrates, so electron transfer mediators are then required. Recently, the laccase/TEMPO system has proven to be a suitable set-up for the oxidation of benzylic alcohols.[11] It is also noteworthy that laccases are accessible and inexpensive in comparison with other reported copper complexes and work in water efficiently.

As part of our interest in biocatalyzed processes, we have explored the potential application of a commercially available laccase from *Trametes versicolor* to the selective oxidation reactions of aliphatic diols in aqueous medium.

As a first attempt, the laccase/TEMPO system was used in a competition experiment for the selective oxidation of a 1-octanol and 2-octanol mixture. Reactions were conducted at room temperature, in NaOAc

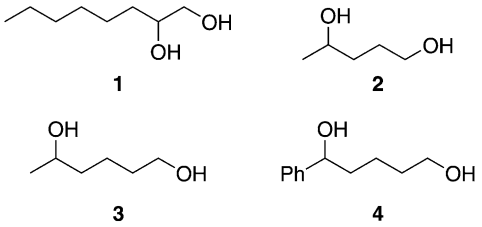


Figure 1. Chemical structure of selected diols used with the laccase/TEMPO system.

buffer (pH 4.8), open to ambient air, in the absence of exogenous bases, and with the commercial laccase from *Trametes versicolor* and TEMPO as catalytic system.[12] To our delight, GC analyses revealed a clear preference for the oxidation of the primary alcohol. This result led us to pursue the regioselective oxidation of a library of diols bearing both primary and secondary alcohols (Figure 1). Satisfyingly, the same tendency was observed for the reaction of 1,2-octanediol (**1**) using the laccase/TEMPO system, while the formation of the corresponding hydroxy aldehyde was observed at short reaction times, prolonged periods resulted in the oxidation of both primary and secondary alcohols. In order to improve the selectivity of our system and shift the equilibrium towards the formation of thermodynamically stable compounds, we decided to employ diols **2**–**4**, which can cyclize *in situ* leading to the corresponding lactones.

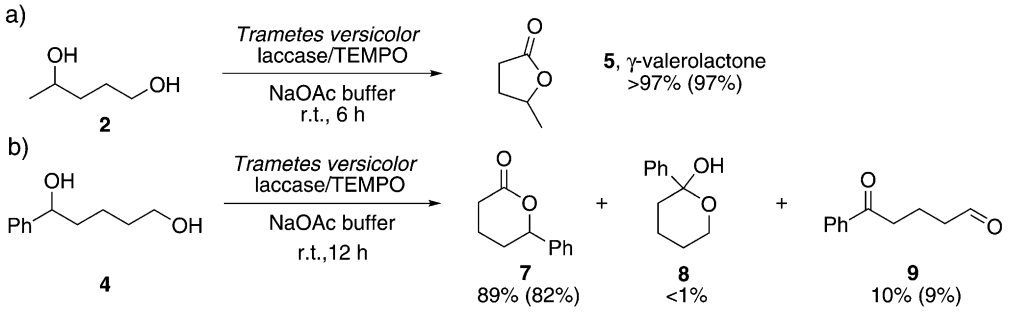
Remarkably when the reaction was carried out with 1,4-pentanediol (**2**), oxidation of the primary alcohol took place selectively generating a hydroxy aldehyde intermediate, which immediately cyclized affording a hemiacetal. The subsequent oxidation of the latter gave access to the stable γ-valerolactone in quantitative yield (Scheme 1, a). Excellent regioselectivity was also observed when 1,5-hexanediol (**3**) was treated with the laccase/TEMPO system delivering δ-caprolactone (**6**) in 92% isolated yield. It is noteworthy to highlight the relevance of these monomers for industry,[13] and that herein excellent yields can be achieved in aqueous medium after a simple extraction purification step. Additionally, the use of 1-phenyl-1,5-pentanediol as substrate (**4**, Scheme 1, b) led also to interesting results. When containing an aliphatic primary and a benzylic alcohol (known substrates for laccases),[11] the primary position was selectively oxidized providing lactone **7** in 89% conversion after the chemical cyclization process, while 10% of ketoaldehyde **9** was also formed. Only traces of the hemiketal **8** were detected in the crude material coming from the benzylic oxidation.[14]

Based on the laccase/TEMPO-catalyzed oxidation mechanism,[15] where the oxoammonium ion acts as the actual oxidant, lactone **7** was obtained as a racemic mixture. However, when enantioenriched diol (*S*)-**4** was used (>95% *ee*), lactone (*S*)-**7** was obtained with the same enantiomeric excess as oxidation takes place with high selectivity for the primary alcohol (see the Supporting Information).

Application of this methodology to 3-substituted 1,5-pentanediols (**11**–**16**, Table 1)[16,17] enabled the facile and efficient preparation of functionalized tetrahydro-2*H*-pyran-2-ones in water with potential interest for industry.[13] Gratifyingly, monoselective oxidation of diols **10**–**16** was achieved in all cases, and subsequent *in situ* cyclization led to lactones **17**–**23** in very high isolated yields (91–97%, Table 1). Bubbling O_2 through the solution speeded up the reactions achieving complete conversion in 2.5–8 h,[18] isolating the lactones with excellent purity after a simple extraction, since this oxidative method produces water as the only by-product.

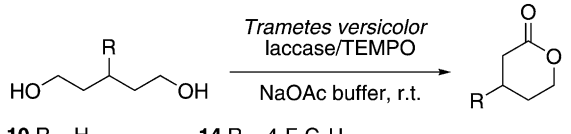
To further explore the scope of this system, aromatic 1,4-diol **24** was employed as a substrate (Scheme 2). Interestingly, treatment of benzenemethanediol with the laccase/TEMPO catalytic system afforded isobenzofuranone **25** in excellent yields (93%) and without the need of further purification, demonstrating the potential of this catalytic system for the production of five-membered ring lactones, interesting building blocks for the preparation of bioactive molecules.[19]

Some of the reactions were effectively scaled up, for instance, 600 mg of **11** and 200 mg of **16** were treated with the laccase/TEMPO catalytic system, obtaining the corresponding 3-methyl-1,5-lactone (**18**) and 3-(2-methoxyphenyl)-1,5-lactone (**23**) in 90% and



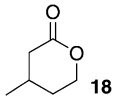
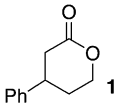
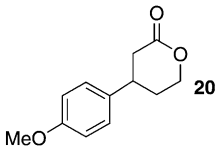
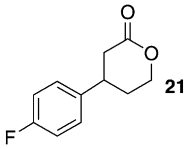
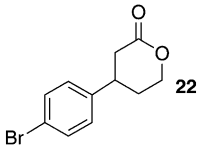
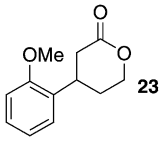
Scheme 1. Catalytic oxidation using the laccase/TEMPO system. Isolated yields appear in brackets: a) 1,4-pentanediol; b) 1-phenyl-1,5-pentanediol.

Table 1. Oxidation of 1,5-diols by the *Trametes versicolor* laccase/TEMPO catalytic system in aqueous medium.[a]

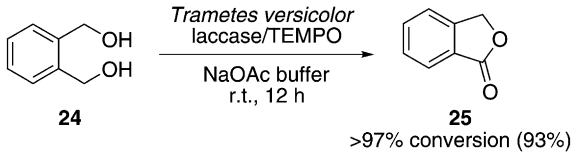


10 R = H
11 R = Me
12 R = C_6H_5
13 R = 4-OMe-C_6H_5
14 R = 4-F-C_6H_5
15 R = 4-Br-C_6H_5
16 R = 2-OMe-C_6H_5

17–23

Entry	Product	Time [h]	Conversion[b] (Yield [%])[c]
1	 **17**	2.5	>97% (92)
2	 **18**	2.5	>97% (92)
3	 **19**	8	>97% (91)
4	 **20**	5.5	>97% (97)
5	 **21**	7	>97% (92)
6	 **22**	7	>97% (92)
7	 **23**	3	>97% (93)

[a] Small scale reactions were performed in NaOAc buffer 50 mM, pH 4.8 at room temperature, total volume: 5 mL, [substrate]: 25–30 mM, [TEMPO]: 4–6 mM, 10 U/mL of laccase and bubbling O_2.
[b] Followed by GC.
[c] Isolated yields.



Scheme 2. Synthesis of isobenzofuranone **25** using the laccase/TEMPO catalytic system.

92% yield, respectively. The scalability of this process is critical for the development of an economic process for industry. In this sense, immobilization of enzymes makes recycling possible while improving stability.[20] To this end, we used a laccase immobilized as cross-linked enzyme aggregates (CLEAs)[21] in our system with diols **11** and **16**. To our delight, the laccase-CLEA resulted in similar product yields (89–92%). Additionally, this reaction occurred without significant loss of enzyme activity after three reuse cycles, isolating the final products in similar yields compared to the free-laccase experiments.

In conclusion, we have described the use of the *Trametes versicolor* laccase/TEMPO catalytic system to efficiently oxidize interesting aliphatic diols in a regio- and/or monoselective manner, obtaining highly valuable lactones with excellent purity after a simple extraction. Due to the oxidation mechanism of this system, lactones were obtained as a racemic mixture, but starting from an enantioenriched diol, no racemization was observed. This is the first report of an aerobic oxidation of 1,4- and 1,5-diols in aqueous medium using laccases and in the absence of a base. This catalytic system is compatible with the presence of unprotected secondary alcohols, activated benzylic hydroxy groups and other functionalities such as methoxy, fluorine or bromine substituents. Furthermore, scalability of the process has been demonstrated using an inexpensive commercially available laccase, which makes this methodology a direct competitor of Cu-organometallic complexes under aerobic oxidative conditions and other TEMPO systems. Finally, the possibility of using immobilized laccases has also been demonstrated, rendering this chemoenzymatic methodology as a very attractive tool for industrial purposes.

Experimental Section

General Procedure for the Preparation of 1,5-Lactones using Laccase from *Trametes versicolor*

A solution of the corresponding diol **2–4**, **10–16** or **24** (25–30 mM) in an NaOAc buffer pH 4.8 (5 mL), was treated with TEMPO (4–6 mM) and this mixture was stirred until complete dissolution. Then, laccase was added (10 U/mL) and the solution stirred vigorously in an open-to-air tube at room temperature overnight (oxygen can be bubbled through the solution to minimize reaction times). The reaction time courses were followed by GC analysis until no starting material remained. Dichloromethane was added (2×5 mL), the layers were separated, the organic phase washed with brine (10 mL), dried over Na_2SO_4 and evaporated under reduced pressure to give the corresponding lactone without further purification. Conversion values were determined by GC analysis, and enantiomeric excess by HPLC (see Supporting Information).

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