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Laccase-generated quinones in naphthoquinone synthesis via Diels–Alder reaction

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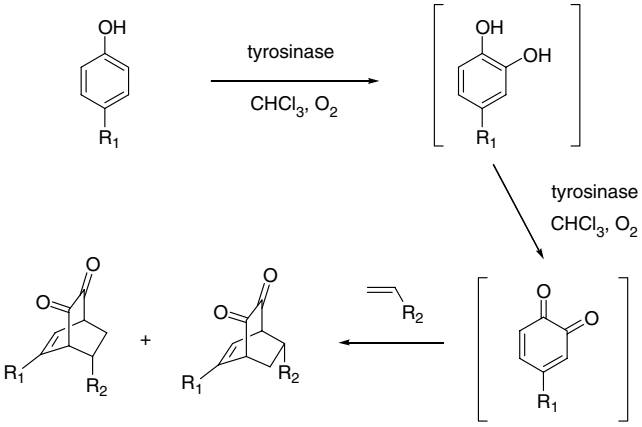
Abstract—The tandem synthesis of naphthoquinones was conducted from the reaction of laccase-generated quinones and acyclic dienes via Diels–Alder reaction. This reaction was carried out under mild condition in aqueous medium and yielded naphthoquinones up to 80%. In addition, the effect of solvent was also investigated and water was shown to be optimal for this reaction.

Laccases (benzenediol:oxygen oxidoreductase, EC 1.10.3.2) are copper-containing glycoprotein oxidoreductase enzymes found in plants and fungi.[1] They catalyze the oxidation of a broad range substrates,[2] such as methoxyphenols, phenols, *o*-, and *p*-diphenols, aminophenols, polyphenols, polyamines, and lignin-related molecules, by reducing dioxygen in a four-electron reduction to water. Moreover, laccases frequently exhibit high oxidative selectivity in an aqueous solution and provide a unique green chemistry solution for a variety of oxidations. These properties make laccase an attractive biocatalysts in organic synthetic chemistry. Recently, studies have investigated the use of laccase in organic synthesis.[2a,3] For example, utilizing their well known propensity to oxidize phenolics, Lalk and co-workers reported laccase catalyzed a nuclear animation tandem reaction.[3a] These studies have demonstrated the synthetic research capabilities of this oxidative enzyme.

This study summarizes our interests in the use of laccase for the synthesis of substituted napthoquinones. Naphthoquinones are naturally occurring compounds which have attracted interest in total synthesis because of their wide range of biological activity including antitumor,[4] wound healing,[5] anti-inflammatory,[5] and antimicrobial[6] and antiparasitic activities.[7]

The combination of enzymatic with nonenzymatic transformations for tandem reactions was first reported by Waldmann and co-workers in 1998.[8] They reported the synthesis of highly functionalized bicycle[2.2.2]octenes by a tyrosinase-initiated hydroxylation–oxidation of phenols followed by a Diels–Alder (DA) reaction with electron rich dienophiles (see Scheme 1). These studies, conducted in chloroform, provided a unique three-step one-pot reaction of bicyclic DA products in high yields with the key intermediate being reactive *ortho*-quinones. The applicability of enzyme catalyzed domino reactions in green chemistry has only recently been fully appreciated.



Scheme 1. The example of enzyme-initiated reaction cascade reported by Waldmann and co-workers.[8]

Keywords: Laccase; Diels–Alder reaction; Naphthoquinone; Quinone; Green chemistry; Tandem reaction.
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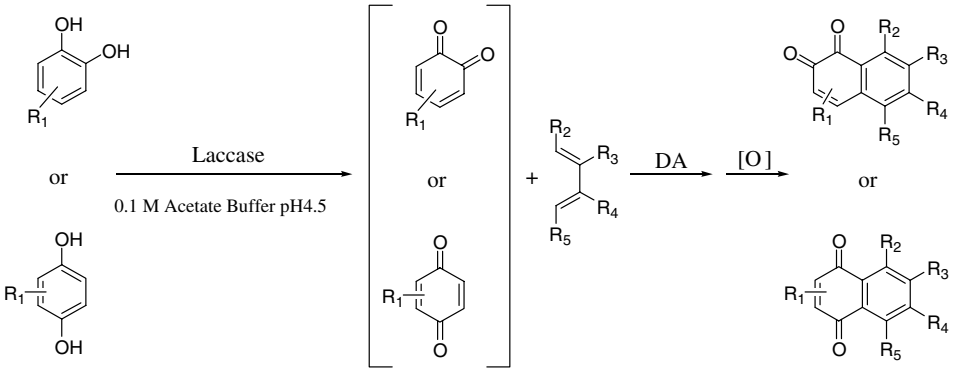


Scheme 2. Laccase-initiated cascade synthesis of substitute naphthoquinones via aqueous Diels–Alder reaction.

In this study, a series of substituted naphthoquinones were synthesized via an aqueous cascade reaction between acyclic dienes and in situ-generated quinones. The *ortho*- and *para*-quinones were synthesized in situ by the oxidation of the corresponding *o*- or *p*-benzohydroquinone by laccase. The initial Diels–Alder adduct was shown to undergo further oxidization by laccase and/or quinone to yield the desired naphthoquinones (see Scheme 2).

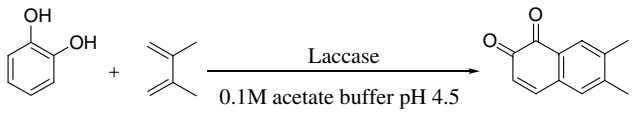
Initially, we focused our attention on the reaction of laccase with 1,2-catechols yielding the corresponding *o*-quinones which have an interesting reactivity profile in cycloaddition reactions,[9] and have been used in *o*-naphthoquinone synthesis.

In a preliminary study, the reaction of catechol (**1**) and 2,3-dimethyl-1,3-butadiene (**2**) in the presence of laccase was investigated. As summarized in Table 1, optimal yields of 6,7-dimethyl-1,2-naphthoquinone (**3**) was achieved when the reaction was conducted with 1 equiv of **1** and 10 equiv of **2** in the presence of laccase[10] in 0.1 M acetate buffer pH 4.5 at 3 °C for the first 2 h of the reaction. The reaction mixture was then warmed to room temperature and stirred for another 22 h.[11]

An excess of the diene was required to overcome the intrinsic instability of the *o*-benzoquinone as it will undergo competing decomposition, dimerization, and polymerization if insufficient diene is present for the Diels–Alder reaction.[12] In addition, the reaction temperature and medium were shown to have an effect on the reaction outcome. For example, if the reaction was preformed at room temperature or 60 °C the yield of **3** was diminished to only 10% and 0%, respectively. This result was attributed an increase in the rate of decomposition and polymerization of the in situ-generated *o*-quinone. Therefore, we retarded the rate of decomposition and polymerization by maintaining the initial reaction temperature to 3 °C for the first 2 h and then allowing the reaction mixture warm to room temperature. This cascade reaction system provided 47% of **3**. The reaction was performed in an aqueous acetate buffer at pH 4.5, generally known to be the optimum pH for laccase activity in the formation of quinone,[13] these conditions provided the best result (see Table 2). The lower percent yield in other solvent systems was due to a decrease of laccase activity in organic and aqueous–organic mixed solvent.[14] Moreover, the Diels–Alder reaction has shown to exhibit higher reactivity and selectivity in aqueous medium than in organic solvent.[15] Interestingly, the use of a 1:1 acetate buffer/chloroform medium, provided the aromatized DA adduct (5,8-dihydro-6,7-dimethyl-1,2-naphthoquinone) instead of fully oxidized product (**3**).

After developing the optimum reaction conditions, the reaction of a variety of catechol substrates with diene **2** were examined[11] and these results are summarized in

Table 1. Preliminary study of the reaction of catechol (**1**) and 2,3-dimethyl-1,3-butadiene (**2**)

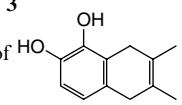


1 **2** **3**

Entry	**1**:**2** (equiv)	Temperature	Yield[a] of **3** (%)
1	1:10	3 °C (2 h), rt	47
2	1:10	rt	10
3	1:10	60 °C	No product formed
4	1:5	3 °C (2 h), rt	8
5	1:15	3 °C (2 h), rt	32

[a] Isolated yield.

Table 2. Solvent effect on the reaction of **1** and **2**[a]

Entry	Solvent	Yield[b] of **3** (%)
1	0.1 M Acetate buffer pH 4.5	47
2	Water	18
3	5% Aqueous PEG 2000	25
4	*p*-Dioxane	0
5	1:1 *p*-Dioxane/acetate buffer	8
6	1:1 Ethylene glycol/ acetate buffer	15
7	1:1 MeOH/acetate buffer	18
8	1:1 Chloroform/acetate buffer	0% of **3** 27% of 

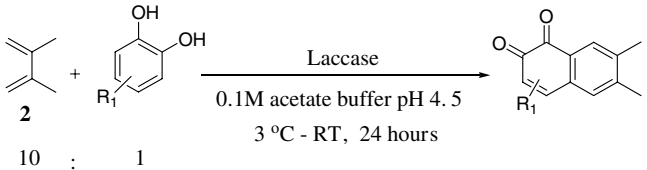
[a] Reaction conditions: **1** (1 equiv) and **2** (10 equiv) was stirred with laccase (4000 U/1g substrate) in solvent at 3 °C for 2 h and then stirred at room temperature for another 22 h.
[b] Isolated yield.

Table 3. The results show that the reaction depended on the reactivity of the in situ-generated *o*-quinones. The very high reactivity quinones, such as 3-methoxy-1,2-benzoquinone and 4-chloro-1,2-benzoquinone, which have rich electron donating group (OMe) and strong electron withdrawing group (Cl), respectively, did not provide good yields of the *o*-naphthoquinone product (entries 4 and 5). These quinones preferently underwent dimerization and polymerization. For example, in situ synthesis 3-methoxy-1,2-benzoquinone by laccase from the corresponding hydroquinone yielded 32% of the undesired product, which was converted from the dimerization intermediate, and only 11% of naphthoquinone product. Besides the reactivity of the in situ-generated quinones, steric factor also affected the formation of the product. Quinones with bulky groups provided very low yield of the products such as 4-*tert*-butyl-1,2-benzoquinone yielded only 14% product for a 4 day reaction, and 3,5-di-*tert*-butyl-1,2-benzoquinone gave no product but 97% of it remaining in the reaction solution (entries 6 and 7). From Table 3, the in situ-generated *o*-quinones with moderate reactivity clearly exhibited higher yields of the *o*-naphthoquinone adduct (entries 1–3), and 4-methyl-1,2-benzoquinone provided the highest yield (57%) in this reaction system (entry 2).

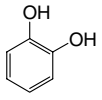
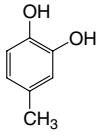
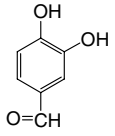
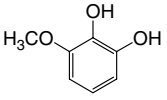
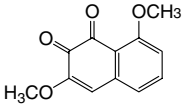
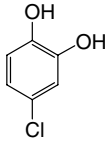
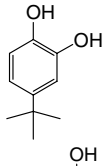
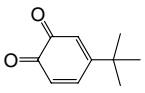
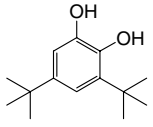
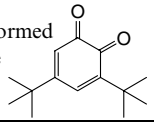
The versatility of this system for a variety of dienes was investigated by using 4-methylcatechol as starting material to generate 4-methyl-1,2-benzoquinone in situ. Table 4 demonstrates that many dienes can be used to react with 4-methyl-1,2-benzoquinone to generate *o*-naphthoquinone products in moderate to high yield. Optimal results were achieved when 1-methoxy-1,3-butadiene and 1-acetoxy-1,3-butadiene were used as diene reagent (entries 4 and 5). Both dienes provided very high yields of the product, and only 2 equiv of 1-acetoxy-1,3-butadiene was needed. This high yielding reaction can be attributed to the elimination of the methoxy or acetoxy group that 'pushed' the reaction forward to the product.

In this study, we also conducted *p*-naphthoquinone synthesis by using a variety of 1,4-benzohydroquinones as a substrate for laccase to generate 1,4-benzoquinone in situ. As the result of *o*-quinone reaction above, the reactive 1-acetoxy-1,3-butadiene was chosen for this study. However, we found that the reaction of these less

Table 3. Reaction of **2** with a variety of catechol substrates

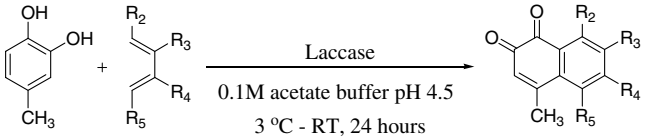


10 : 1

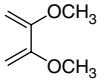
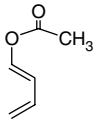
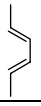
Entry	Catechol	Yield[a] (%)
1		47
2		57
3		28 ($R_1 = H$)
4		11 and 32 of 
5		No product formed
6[b]		14 and 15 of 
7		No product formed 97 of quinone 

[a] Isolated yield.
[b] 96 h reaction.

Table 4. Reaction of 4-methylcatechol with a variety of dienes

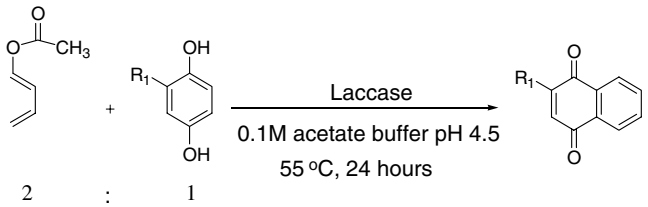


1 : 10

Entry	Diene	Yield[a] (%)
1		57
2		71
3		10
4		77 ($R_2 = H$)
5[b]		76 ($R_2 = H$)
6		No product formed

[a] Isolated yield.
[b] Only 2 equiv of 1-acetoxy-1,3-butadiene was used.

Table 5. Reaction of 1-acetoxy-1,3-butadiene with a variety of 1,4-benzohydroquinone



2 : 1

Entry	R_1	Yield[a] (%)
1	H	67
2	CH_3	75
3	OCH_3	81
4	Br	67
5	Cl	69

[a] Isolated yield.

reactive *p*-benzoquinones gave very low yield of the desired product at low temperature. Therefore, the reaction was conducted at 55 °C for *p*-naphthoquinone synthesis, and 1 equiv of 1,4-benzohydroquinone and 2 equiv of diene were used (Table 5).[16] The results in Table 5 show that this reaction system can be used for a one-pot synthesis of *p*-naphthoquinones in excellent overall yield.

In summary, an efficient green chemistry synthesis of naphthoquinone using laccase as an oxidant in aqueous medium was developed. In this reaction, laccase was used to oxidized *o*- and *p*-diphenols to generate *o*- and *p*-quinones in situ which further underwent Diels–Alder reaction and oxidation to form napthoquinone product. This reaction system can yield naphthoquinones up to 80% depending on the exact structure of the starting hydroquinone and diene.

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10. *Enzyme assay*: Laccase from *Trametes Villosa* (EC 1.10.3.2) was obtained from Novozymes, North Carloina. Laccase activity was determined by oxidation of 2,2′-azinobis-(3-ethylbenzyl thiozoline-6-sulfonate) (ABTS).[17] The assay mixture contained 25 μM ABTS, 0.1 M sodium acetate (pH 5.0), and a suitable amount of enzyme. The oxidation of ABTS was followed by an absorbance increase at 420 nm ($\varepsilon_{420} = 3.6 \times 10^4\ M^{-1}\ cm^{-1}$). Enzyme activity was expressed in units (U = μmol of ABTS oxidized per minute).
11. *Typical experimental procedure for o-naphthoquinone synthesis*: In a 250-mL round-bottom flask, 20 ml of cold 0.10 M acetate buffer pH 4.5 and 2,3-dimethyl-1,3-butadiene (**2**) (0.7 g, 9 mmol) were mixed together. The flask was then placed in an ice bath over a stirring plate. Next, 0.1 g (0.9 mmol) of catechol (**1**) dissolved in 20 mL of 0.10 M acetate buffer, and laccase (100 U) were added to the flask drop-wise. In the next 3 h of the reaction, 100 U of laccase was added each per hour.The reaction was then stirred under room temperature. After 24 h of the reaction, the reaction mixture was extracted by EtOAc. The organic phase was combined, dried over $MgSO_4$, and evaporated. The resulting crude products were purified by silica column chromatography, using ethyl acetate and petroleum ether as an eluent to obtain the products **3** (47%). Most compounds have been previously reported and characterized except the two compounds below. 4,7,8-Trimethyl-1,2-naphthoquinone: orange solid; mp 118 °C

(decomp.); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.36 (s, 3H, CH_3), 2.37 (s, 3H, CH_3) 2.59 (s, 3H, CH_3), 6.32 (s, 1H, CH), 7.29 (d, $J = 8$ Hz, 1H, Ar), 7.41 (d, $J = 8$ Hz, 1H, Ar); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 17.6, 20.9, 21.2, 124.5, 126.3, 129.8, 134.8, 135.6, 141.8, 144.1, 154.9, 181.6, 183.5; MS (EI) m/z 200 (M^+, 31%), 172 (100), 157 (22), 141(11), 129 (38), 115 (12), 102 (4), 77 (7), 63 (7), 51 (8), 44 (27); HRMS (EI) calcd for $C_{13}H_{12}O_2$ requires 200.0837, found: 200.0840. 4-Methyl-6,7-dimethoxy-1,2-naphthoquinone: red solid; mp 124 °C (decomp.); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.37 (s, 3H, CH_3), 3.97 (s, 3H, OCH_3), 4.03 (s, 3H, OCH_3), 6.25 (s, 1H, CH), 6.92 (s, 1H, Ar), 7.61 (s, 1H, Ar); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 20.7, 56.3, 108.7, 112.0, 125.2, 125.9, 130.8, 150.5, 153.2, 154.6, 178.3, 181.0; MS (EI) m/z 232 (M^+, 54%), 204 (100), 189 (37), 175 (4), 161 (9), 133 (9), 118 (8), 105 (12), 77 (5), 63 (7), 39 (6); HRMS (EI) calcd for $C_{13}H_{12}O_4$ requires 232.0735, found: 232.0734.

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16. *Typical experimental procedure for p-naphthoquinone synthesis*: *p*-Hydroquinone (0.1 g, 0.9 mmol), 1-acetoxy-1,3-butadiene (0.2 g, 1.8 mmol), and laccase (100 U) were stirred in 40 ml of 0.1 M acetate buffer pH 4.5 under air at 55 °C. In the next 3 h of the reaction, 100 U of laccase was added each per hour. After 24 h of the reaction, the reaction mixture was extracted by EtOAc. The organic phase was combined, dried over $MgSO_4$, and evaporated. The resulting crude products were purified by silica column chromatography, using ethyl acetate and petroleum ether as an eluent to obtain the products.
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