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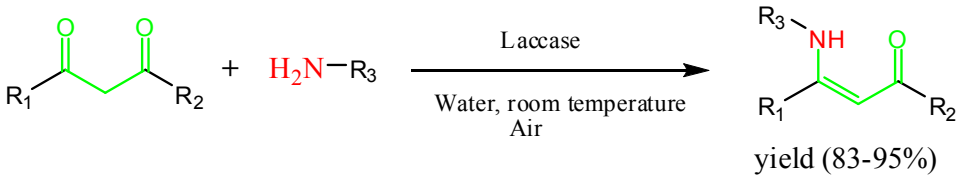

yield (83-95%)

Laccase-catalyzed enamination of 1,3-dicarbonyl compounds in water

Cite this: DOI: 10.1039/c0xx00000x

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ARTICLE TYPE

A new method for the enamination of 1,3-dicarbonyl compounds catalyzed by laccase in water

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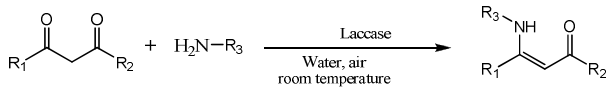
Received (in XXX, XXX) Xth XXXXXXXXX 20XX, Accepted Xth XXXXXXXXX 20XX

DOI: 10.1039/b000000x

A new method for the enamination of 1,3-dicarbonyl compounds catalyzed by laccase in water is described for the first time. Besides providing a green and efficient method for the synthesis of enaminones, this study extends the applicability of laccase in organic synthesis.

Enaminones are important intermediates in organic synthesis [1–2]. In particular, they have been employed as synthons of a wide variety of heterocycles and pharmaceutical compounds [3-4]. Generally, enaminones are prepared by direct condensation of β-dicarbonyl compounds with amines under reflux in an aromatic solvent with azeotropic removal of water [5]. In recent years, a variety of Lewis acid catalysts have been developed for the condensation [6-10], such as Yb(OTf)3, $Zn(ClO_4)_2{\cdot}6H_2O$, $InBr_3$, $CeCl_3{\cdot}7H_2O$, $NaAuCl_4$, *etc*. Additionally, several non-conventional techniques (such as microwave and ultrasound) have also been reported to carry out this kind of reactions [11–12]. However, most of the existing methods suffered from one or more limitations such as toxic metal catalysts, volatile organic solvents and complicated reaction process. Thus, searching for a convenient, environmentally friendly and efficient method is highly desirable.

Over the past three decades, enzymatic catalysis has emerged as an elegant synthetic method since it is more mild, efficient and highly selective, involves less energy consumption, and produces fewer side products [13-14]. Laccase (benzenediol : oxygen oxidoreductase, EC 1.10.3.2, copper containing phenol oxidases) is a widely used biocatalyst in organic synthesis because it has an excellent ability to oxidize a broad range of substrates (aromatic methyl groups, benzylic, allylic, and aliphatic alcohols, ethers, benzyl amines and hydroxylamines) [15-20]. Laccases have also been used to catalyze the oxidation of catechols and hydroquinones to the corresponding benzoquinones and related transformations [21-22]. Furthermore, the oxidative coupling of several natural phenolics using oxygen as the oxidant can also be catalyzed by laccases [23-24]. However, to the best of our knowledge, no attempts have been made to apply laccase in the synthesis of enaminones. Herein, we described a new method for the synthesis of enaminones catalyzed by laccase in water for the first time (**Scheme 1**).



Scheme 1. laccase-catalyzed enamination of 1,3-dicarbonyl compounds in water

Initially, three commercially available laccases were applied to catalyze the condensation of aniline and acetoacetone in water. As shown in **Table 1**, the condensation could be carried out by all the selected laccases. *Trametes versicolor* laccase and *Rhus vernicifera* laccase had moderate activities while *Trametes villosa* laccase presented excellent catalytic activity. Obviously, the catalytic activity of enzyme depended mainly on its type and origin. It could also be found that the reaction rate was nearly equal to the control reaction when the reaction was catalyzed with denatured enzyme or bovine serum albumin (BSA), respectively. These results were very encouraging and spurred us to further explore this reaction.

Table 1 Condensation of acetylacetone with aniline in the presence of various enzymes. [a]

Entry	Enzyme	Conversion[c] (%)	Yield [d] (%)
1	*Rhus vernicifera* laccase (60U [e])	21	19
2	*Trametes versicolor* laccase (60U [e])	55	52
3	*Trametes villosa* laccase (60U [e])	88	83
4	*Trametes villosa* laccase (denatured) [b]	16	14
5	Bovine serum albumin [f]	16	12
6	Control (no enzyme)	15	11

[a] Reaction condition: aniline 1mmol, acetoacetone 1mmol, room temperature, 60 U laccase, 2mL deionized water, 45 min, air.

[b] 60U *Trametes villosa* laccase was pre-treated at 100 ºC for 1h.

[c] Conversion acetylacetone was monitored by HPLC.

[d] Yields are given for isolated products.

[e] Method of laccase activity determination was supplied in supporting information

[f] Bovine serum albumin (2mg) was usedin the reaction

It is important to point out that water was used as the only solvent in this condensation. To address the challenges of green chemistry, the possibility of using water to replace the hazardous organic solvents in enzyme-catalyzed reactions is another advantage. In addition to its environmental benefits, the use of water as a solvent is both inexpensive and safe. However, only

few examples of laccase-catalyzed reactions in pure water have been reported previously [25, 26]. In this study, laccase can exhibit a high activity in pure water, and give a satisfied yield of enaminone. Considering the potential effect of pH [27-28], we detected the initial and final pH values (7.3 and 6.7) in this condensation. The observed slight change of pH value should not influence the catalytic performance of laccase significantly.

The time course of the condensation of acetylacetone with aniline catalyzed by *Trametes villosa* laccase was illustrated. The reaction reached its equilibrium in approximately 45 min with a conversion of 88% of acetoacetone and isolated yield 83% of the product (**Fig. 1**).

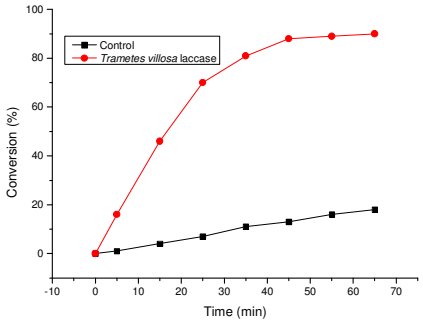


Fig. 1. The time course of the condensation of acetylacetone with aniline catalyzed by *Trametes villosa* laccase

Reaction condition: aniline 1mmol, acetoacetone 1mmol, room temperature, *Trametes villosa* laccase 60U, 2mL deionized water, air, conversion of acetylacetone was monitored by HPLC.

The effect of the amount of *Trametes villosa* laccase on the yield was investigated (data not shown here). It was found that the use of lower amount of laccase (15 U or 30 U) required a longer reaction time (> 1.5h) to afford a comparable result. By increasing the amount of enzyme, the number of active sites that took part in the condensation would increase [29]. Thus, more active sites would convert the substrates into products. However, there was no obvious difference between 60U and 90 U laccase in 2mL reaction system. After 60 U, the limiting step of this condensation may not be a catalytic phenomenon, but the rate of oxygen dissolution due to the oxygen limitation (the oxygen is consumed more rapidly than it goes inside the medium).So it was believed that 60U laccase was sufficient for the synthesis of enaminone.

The scope of the reaction was explored and the results are summarized in **Table 2**. Various amines were used in the condensations with an equivalent amount of 1,3-dicarbonyl compounds. In the case of 1,6-diaminlhexane, 2 equiv. of acetylacetone were used, giving products with two enaminone groups (Entry 5). It could be found that all the reactions were fast (15 min to 45 min) and mild (room temperature, in water) with high to excellent isolated yield (83–95%). Aliphatic amines and cyclic amine were shown to be more reactive to undergo the reaction, and a shorter reaction time was required compared to aromatic amines. The condensation also worked well when secondary amine (Entry 3) was utilized. It is noteworthy that *p*-hydroxyaniline has also been converted successfully into the corresponding enaminone (Entries 7 and 8) without any oxidation by-product of *p*-hydroxyaniline or similar product which was previously reported by Hajdok et al [30]. In addition, all the 1,3-dicarbonyl compounds such as methyl acetoacetate or ethyl acetoacetate underwent similar reactions, leading to the corresponding enaminones in high yields (Entries 8 and 9). As for the unsymmetrical diketones, such as 1-benzoylacetone (Entry 10), the regiochemistry was controlled by the more reactive carbonyl group, which underwent the attack by amine to give exclusively 1-phenyl-3- (butylamino)but-2-en-1-one.

Table 2 *Trametes villosa* laccase catalyzed synthesis of enaminones. [a]

Entry	1,3-Dicarbonyl compound	Amine	Time (min)	Conversion (%)[b]	Yield (%)[d]
1	Acetoacetone	n-Butylamine	15	99	95
2	Acetoacetone	Aniline	45	88	83
3	Acetoacetone	Dibutylamine	30	96	93
4	Acetoacetone	Cyclohexylamine	25	94	90
5	Acetoacetone[d]	1,6-Diaminlhexane	20	96	92
6	Acetoacetone	tert-Butylamine	40	92	87
7	Acetoacetone	p-Hydroxyaniline	35	95	89
8	Ethyl acetoacetate	p-Hydroxyaniline	40	91	87
9	Ethyl acetoacetate	n-Butylamine	25	94	91
10	Methyl acetoacetate	n-Butylamine	20	95	93
11	1-Benzoylacetone	n-Butylamine	25	91[c]	89

[a] Reaction condition: amine 1mmol, 1,3-dicarbonyl compound 1mmol, room temperature, *Trametes villosa* laccase 60U, 2mL deionized water, air.

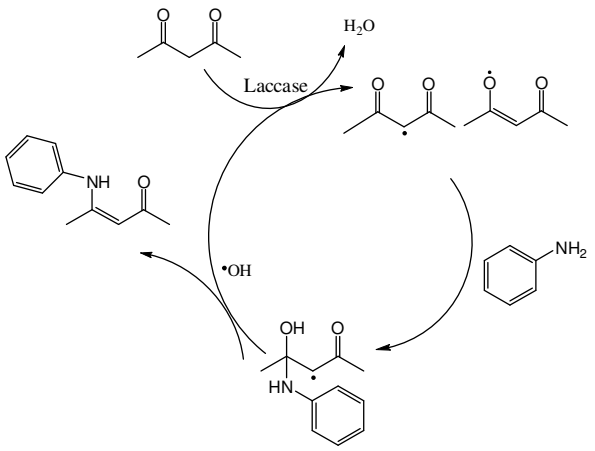
[b] Conversions of 1,3-Dicarbonyl compounds were monitored by HPLC.

[c] Conversion of 1-Benzoylacetone was monitored by GC

[d] Yields are given for isolated products.

[e] Entry 5: Acetoacetone 2mmol.

It is widely accepted that laccase can remove a single electron from the substrate and generate a free radical, with the concomitant reduction of oxygen to water during its catalytic process [31, 32]. Based on this viewpoint and experiment results, we attempted to propose a reaction pathway of this catalytic system (**Scheme 2**). Firstly, the laccase abstract an electron from 1,3-dicarbonyl compound to produce a free redical, and then undergoes an 1,4-addition with the amine as an nucleophile. Finally, the product is formed by intramolecular dehydration. Further experiments are currently in progress to fully prove this hypothesis and will be reported in due course.



Scheme 2. Proposed mechanism of laccase-catalyzed synthesis of enaminone.

Conclusions

In conclusion, the laccase-catalyzed synthesis of enaminones in water with high yields was reported for the first time in this study. Compared with other "green" methods [33-35], this method has several advantages including shorter reaction time (< 45 min), higher yield (>83%), mild reaction conditions (r. t.), environmental friendly catalyst, operational and experimental simplicity. Besides, it provides a new case of laccase-catalyzed reactions and extends the utility of laccase in organic synthesis. Furthermore, water used as solvent makes this method environmentally friendly and applicable for the large-scale synthesis of enaminones considering the global interest in the field of green synthesis. It's well known that immobilization is a powerful tool to improve enzyme features (activity, specificity, reusability, stability, *et al*) in modern biotechnology [36-41]. In order to improve the performance of laccase and cut the costs, a study adopting the technique of immobilization is currently in progress and will be reported in due course.

We gratefully acknowledge the National Natural Science Foundation of China (No. 21172093, 31070708, 21072075) and the Natural Science Foundation of Jilin Province of China (No. 201115039, 20140101141JC) for the financial support.

Notes and references

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† Electronic Supplementary Information (ESI) available: [details of any supplementary information available should be included here]. See DOI: 10.1039/b000000x/
‡**Laccase catalyzed synthesis of enaminones**
Laccase (60U) was added to a 10 ml round-bottom flask containing amine (1 mmol), 1,3-dicarbonyl compound (1 mmol) and deionized water (2 ml). The mixture was stirred with a magnetic stirrer (200 rpm) at room temperature for the specified time. HPLC were used to monitor the conversion of 1,3-dicarbonyl compounds in the reaction. GC was used to monitor the conversion of 1-benzoylacetone after extracting the reaction system with ethyl acetate. When the conversion was not changed obviously (<3%) in 10min, the reaction was stopped by extracting the reaction system with ethyl acetate (3*10 mL) and washed with brine solution. The combined organic phases were dried over anhydrous sodium sulfate and concentrated under vacuum. The crude product thus was purified by column chromatography on silica gel using ethyl acetate-petroleum ether. All the isolated products were well characterized by their ^{1}H-NMR spectral analysis.

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