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Laccase-catalyzed synthesis of 2,3-ethylenedithio-1,4-quinones

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ABSTRACT

Laccases (benzenediol:oxygen oxidoreductase EC 1.10.3.2) belong to the family of multicopper oxidases. These environmentally friendly enzymes require O_2 as their only co-substrate and produce H_2O as their sole by-product. As a result, they have acquired increasing use in biotechnological applications, particularly in the field of organic synthesis. In the current study, laccases have been employed to successfully couple 1,2-ethanedithiol to various substituted hydroquinones to produce novel 2,3-ethylenedithio-1,4-quinones in good yields via an oxidation–addition–oxidation–addition–oxidation mechanism. The reactions proceeded in one-pot under mild conditions (room temperature, pH 5.0). This study further supports the use of laccases as green tools in organic chemistry. Furthermore, it provides evidence that laccase-catalyzed cross-coupling reactions involving small thiols are possible, in spite of research that suggests small thiols are potent inhibitors of laccases.

1. Introduction

The use of enzymes in organic synthesis has increased in previous decades [1] and, as part of the principles of green chemistry, is one of the main strategies geared toward providing a more sustainable industry. Biocatalysts demonstrate many advantages over conventional chemical catalysts in that they are renewable, biodegradable, relatively inexpensive, highly selective, and are active in aqueous solvents under mild conditions.

Laccases (benzenediol:oxygen oxidoreductase EC 1.10.3.2) are a type of blue multicopper oxidase that are present in many species of fungi, bacteria, algae, insects, and plants [2]. They selectively oxidize phenolic compounds, such as hydroquinones and catechols, as well as aromatic and polyamines, whilst concomitantly reducing O_2 to H_2O. Due to their environmentally benign nature and selectivity, these biocatalysts have received increased attention over the past few decades in a variety of biotechnological applications, including organic synthesis both on the lab and industrial scale [3]. The ability of laccases to oxidize both hydroquinones and catechols to produce in situ *para*- and *ortho*-quinones, respectively, has been exploited in a vast array of coupling reactions involving both carbon and nitrogen derived nucleophiles. Similar reactions involving sulfur based nucleophiles, however, have been few [4].

The focus of the current study was to employ laccases to perform the cross-coupling reaction of various substituted hydroquinones with a small dithiol, namely 1,2-ethanedithiol **1**. This study leverages the recent study conducted by Kidwai et al. whereby a successful laccase-catalyzed addition of a diamine to both hydroquinones and catechols was achieved for the synthesis of novel quinoxalines [5]. The current study was conducted in spite of research that suggests some small sulfhydryl compounds (e.g. cysteine) are potent inhibitors of laccases from particular fungal species [6].

The reaction products of the laccase-catalyzed addition of **1** with substituted hydroquinones **2** contain the 2,3-ethylenedithio-1,4-quinone substructure (Fig. 1). Compounds containing quinones are present all throughout nature and in many biologically active natural products [7]. The compounds synthesized in this study are no different. For example, 3′,4′-(ethylenedithio)avarone (Fig. 2), a synthetic derivative of the marine sponge sesquiterpene quinone avarone, is a compound that contains the 2,3-ethylenedithio-1,4-quinone structural moiety and has shown to exhibit antiproliferative activity toward tumor cells [8]. Another compound with similar structural features, dithianon (Fig. 2), has also been shown to possess cancerostatic properties [9] as well as fungicidal activity [10].

Traditional syntheses of sulfide-substituted 1,4-quinones involve the nucleophilic addition of thiol to 2,3-dichloro-1,4-quinone derivatives [11]. These reactions are usually conducted in ethanol and require heat. In addition, alkylthiols only undergo substitution to the quinone once; thus, sodium salts of the alkyl

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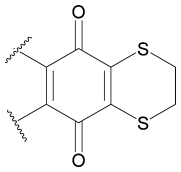

Fig. 1. 2,3-Ethylenedithio-1,4-quinone substructure.

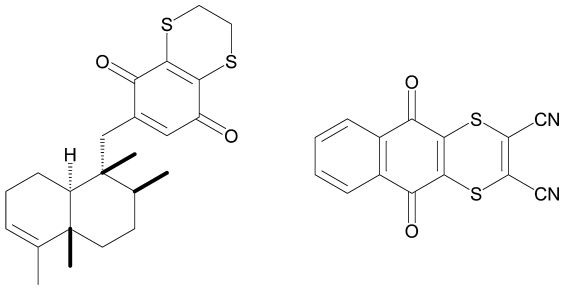

Fig. 2. Compounds containing the 2,3-ethylenedithio-1,4-quinone substructure.

thiols must be prepared and used for the substitution reaction to occur at both position 2 and 3 of 2,3-dichloro-1,4-quinone derivatives. Furthermore, prior synthetic steps must be performed to arrive at the 2,3-dichloro-1,4-quinone intermediate that require harsh conditions and a chemical oxidant (cerium ammonium nitrate) [4d]. Thus, the laccase-catalyzed addition of 1,2-ethanedithiol to substituted hydroquinones conducted in this study is a simple, one-step, green alternative to the synthesis of 2,3-ethylenedithio-1,4-quinones.

2. Experimental

2.1. Materials and methods

Laccase from *Trametes villosa* was donated by Novo Nordisk Biochem, NC. All compounds were purchased from Aldrich except *tert*-butylhydroquinone, which was a product of Acros Organics. All compounds, solvents, and enzyme were used as received without further purification. TLC experiments were carried out on aluminum sheets pre-coated with silica gel 60 (EMD Chemicals). Column chromatography was carried out using silica gel 60 (EM Separations Technology) as the stationary phase. Melting points for the products were obtained using a Barnstead International Mel-Temp® apparatus and are uncorrected. The enzyme assay was conducted using a PerkinElmer Lambda 35 UV-vis spectrophotometer. GC–MS experiments were run using an Agilent Technologies 7890A GC system equipped with a HP-5MS column coupled with a 5975C inert MSD with triple-axis detector. FTIR data was obtained via a PerkinElmer Spectrum 100 FTIR spectrometer using the ATR method. All NMR experiments were carried out on a Bruker 400 MHz spectrometer (^{1}H at 400 MHz and ^{13}C at 100 MHz) at room temperature (25 °C) using $CDCl_3$ as the solvent unless stated otherwise. All chemical shifts are given in ppm relative to TMS and multiplicities are designated as s (singlet) and m (multiplet). Accurate mass analyses were performed by the Georgia Institute of Technology Bioanalytical Mass Spectrometry Facility on a Micromass AutoSpec M spectrometer.

2.2. Enzyme assay

Laccase activity was determined according to standard literature procedures which involve the oxidation of 2,2′-azino-bis-(3-ethylbenzthiazoline-6-sulfonate) (ABTS) [12]. The oxidation of 3.50 mL of solution consisting of 50 μM ABTS in 0.10 M sodium acetate buffer (pH 5.0) by 8.0×10^{-5} mL laccase was observed spectrophotometrically at room temperature (22 °C) via a UV–vis spectrophotometer by following the absorbance increase at 420 nm ($\varepsilon_{420} = 3.6 \times 10^4\ M^{-1}\ cm^{-1}$). Laccase activity is expressed in units (U) where U = μmol ABTS oxidized per minute. The laccase activity was measured to be 1510 U per mL enzyme stock solution.

2.3. General procedure for the laccase-catalyzed reaction of 1,2-ethanedithiol **1** with substituted hydroquinones **2**

The hydroquinone **2** (0.50 mmol) was added to a 50 mL round bottom flask equipped with a stir bar followed by 15 mL of solvent and the mixture was stirred. Once the solid had dissolved, 1,2-ethanedithiol **1** (2.50 mmol) was introduced, followed by 50 U of laccase. The reaction mixture was stirred at room temperature (22 °C) for 16 h whilst O_2 was bubbled through. The reaction progress was monitored by TLC using silica gel coated on aluminum sheets as the stationary phase, 1:1 EtOAc/hexane (v:v) mixture as the mobile phase, and iodine vapor as the staining agent. Once the reaction was complete, the reaction mixture was extracted with EtOAc (3× 20 mL), dried over $MgSO_4$, and the solvent removed via rotary evaporation. The crude extract was purified via column chromatography using silica gel as the stationary phase and 1:1 EtOAc/hexane (v:v) mixture as the mobile phase to obtain the desired products. The products were characterized using HRMS, ^{1}H NMR, ^{13}C NMR, and FTIR.

2.4. Spectroscopic data

2.4.1. 6-Methoxy-2,3-dihydrobenzo[b][1,4]dithiine-5,8-dione (**3a**)

Yield: 64%; purple solid; mp: 203–208 °C; IR: ṽ 1616 (C=O) cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 3.26 (s, 4H, $(CH_2)_2$), 3.77 (s, 3H, OCH_3), 6.09 (s, 1H, C=CH) ppm; ^{13}C NMR (DMSO-d_6): δ 26.08, 26.65, 56.76, 106.90, 127.34, 134.32, 158.94, 175.12, 181.36 ppm; HRMS (ESI): $C_9H_8O_3S_2$ calculated 227.9915, found 227.9922.

2.4.2. 6-Methyl-2,3-dihydrobenzo[b][1,4]dithiine-5,8-dione (**3b**)

Yield: 51%; purple solid; mp: 120–125 °C; IR: ṽ 1636 (C=O) cm^{-1}; ^{1}H NMR: δ 2.07 (s, 3H, CH_3), 3.25 (s, 4H, $(CH_2)_2$), 6.59 (s, 1H, C=CH) ppm; ^{13}C NMR: δ 15.65, 26.33, 26.51, 132.78, 137.36, 137.69, 145.70, 180.38, 180.71 ppm; HRMS (ESI): $C_9H_8O_2S_2$ calculated 211.9966, found 211.9960.

2.4.3. 6-(tert-Butyl)-2,3-dihydrobenzo[b][1,4]dithiine-5,8-dione (**3c**)

Yield: 68%; dark purple solid; mp: 112–117 °C; IR: ṽ 1635 (C=O) cm^{-1}; ^{1}H NMR: δ 1.29 (s, 9H, $C(CH_3)_3$), 3.24 (s, 4H, $(CH_2)_2$), 6.58 (s, 1H, C=CH) ppm; ^{13}C NMR: δ 26.20, 26.76, 28.80, 35.18, 123.96, 131.19, 132.03, 155.93, 180.20, 181.08 ppm; HRMS (ESI): $C_{12}H_{14}O_2S_2$ calculated 254.0435, found 254.0439.

2.4.4. 6-Phenyl-2,3-dihydrobenzo[b][1,4]dithiine-5,8-dione (**3d**)

Yield: 71%; black solid; mp: 72–77 °C; IR: ṽ 1633 (C=O) cm^{-1}; ^{1}H NMR: δ 3.29 (s, 4H, $(CH_2)_2$), 6.84 (s, 1H, C=CH), 7.46 (m, 5H, Ar-H) ppm; ^{13}C NMR: δ 26.36, 26.69, 128.09, 128.83, 129.79, 130.45, 132.16, 133.11, 135.35, 145.72, 179.67, 180.45 ppm; HRMS (ESI): $C_{14}H_{10}O_2S_2$ calculated 274.0122, found 274.0130.

2.4.5. 2,3-Dihydronaphtho[2,3-b][1,4]dithiine-5,10-dione (**3e**)

Yield: 74%; purple solid; mp: 215–220 °C; IR: ṽ 1643 (C=O) cm^{-1}; ^{1}H NMR: δ 3.31 (s, 4H, $(CH_2)_2$), 7.69 (m, 2H, Ar-H), 8.08 (m, 2H, Ar-H) ppm; ^{13}C NMR: δ 26.63, 126.50, 131.20, 133.33, 140.38, 178.18 ppm; HRMS (ESI): $C_{12}H_8O_2S_2$ calculated 247.9966, found 247.9966.