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Tetrahedron Letters 49 (2008) 2476–2478
Tetrahedron Letters

Trimerisation of indole through laccase catalysis

Chrystelle Ganachaud, Vanessa Garfagnoli, Thierry Tron, Gilles Iacazio *

Laboratoire BiosCiences FRE CNRS 3005, Case 432, Faculté des Sciences et Techniques de Saint Jérôme, Aix-Marseille Université, Avenue Escadrille Normandie-Niemen, 133397 Marseille Cedex 20, France

Received 15 November 2007; revised 3 February 2008; accepted 5 February 2008
Available online 9 February 2008

Abstract

Indole is cleanly bioconverted into a trimer upon laccase action. The formed compound has been identified as 2,2-bis(3′-indolyl)-indoxyl (**1**) by X-ray diffraction study. Optimisation of the reaction through the use of dioxygen overpressure and TEMPO as mediator allowed a yield of more than 50% of isolated product for **1**. 2,2-Bis(3′-indolyl)indoxyl is a natural compound that has been isolated from bacterial sources. However, this is the first report on a clearly identified enzyme that could be involved in its (bio)synthesis. A mechanism, based on the initial formation of a transient indole hydroperoxide, is proposed to account for the laccase catalysed synthesis of **1**.
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Keywords: Laccase; Indole; TEMPO; Biocatalysis; Oxidation; Green chemistry

Laccases are blue copper oxidases that catalyse a single electron oxidation of various phenolic and aromatic compounds with a concomitant four-electron reduction of dioxygen to water.[1,2] The technological potential of laccases has long been recognised and numerous studies devoted to their use in various bioprocesses (delignification, ethanol production, bioremediation, etc.) have been conducted.[3,4] Laccases have also been used as catalyst for fine chemistry and recent achievements in the field have been reviewed.[5,6] In our continuous effort to extend the potential application of laccases in bioconversion[7] we were interested in the capacity of laccases to oxidise the bicyclic aromatic amine indole into coloured products.[8]

In a first trial, the reaction has been tested in 96-microtiter well plates.[9,10] The development of a yellow colour was indicative of a transformation as compared to a blank (without enzyme). TLC analysis of the diethyl ether extract of the reaction mixture revealed a clean but incomplete transformation of indole into a major yellow compound. As a characterisation of the formed product required more material, the reaction was scaled up. When conducted in an open vessel, the reaction was quite long and the yield in yellow compound was poor (Fig. 1). In order to improve these reaction parameters, the biotransformation was then performed in a screw-cappped Schlenk tube under 2 bar of dioxygen overpressure.[11] The reaction was followed by HPLC[12] and stopped after 48 h. The formed yellow compound was then easily purified on a RP-18 open silica

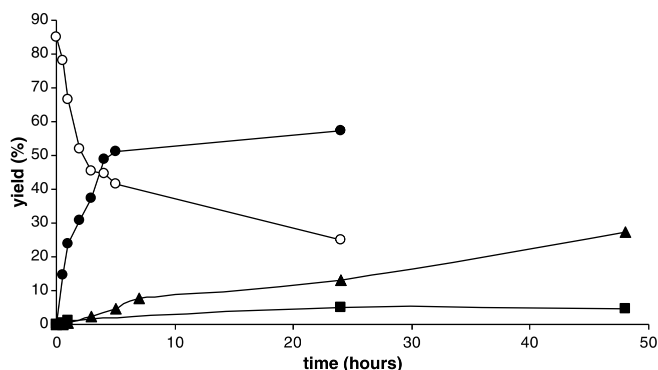

Fig. 1. Evolution of trimer **1** synthesis through laccase catalysed indole oxidation. ■: open vessel. ▲: closed vessel, oxygen overpressure 2 bar. ●: closed vessel, oxygen overpressure 2 bar, TEMPO 30% (mol/mol). ○: consumption of indole substrate, experimental conditions as for ●.

* Corresponding author. Tel.: +33 491 282 856; fax: +33 491 832 208.
E-mail address: gilles.iacazio@univ-cezanne.fr (G. Iacazio).

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doi:10.1016/j.tetlet.2008.02.021

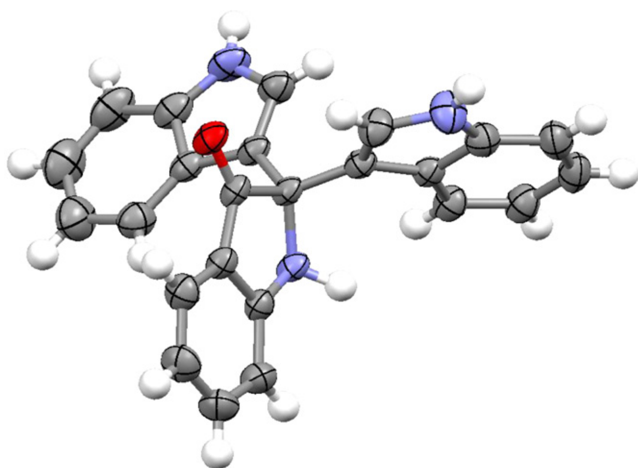

Fig. 2. Perspective view of **1**. A molecule of co-crystallised acetonitrile is omitted for clarity.

column, by loading the whole reaction mixture and performing a stepwise elution with a gradient of acetonitrile in water (0%, 20%, 40%, 50% and 60% acetonitrile). The structure of the formed compound (**1**) was solved by X-ray diffraction study (Fig. 2), the crystals[13] being obtained by the slow evaporation of a concentrated acetonitrile solution of **1**.

As shown in Figure 2, compound **1** is the result of indole trimerisation through the formation of a quaternary carbon centre. The chemical synthesis of **1** has already been described through indole oxidation.[14] This compound has also been characterised from natural (bacterial) sources such as *Vibrio parahaemolyticus*[15] and *Haemophilus influenzae*.[16] It should be stressed that our NMR data[17] are fully consistent with those obtained in the latter case but did not match those reported for the former case. It should also be noted that a closely related molecule, arising from the bismuth triflate catalysed reaction between indole and isatin (Fig. 3), was very recently described.[18]

The structural determination of **1** allowed the calculation of an isolated yield of 23% for the biotransformation when conducted under dioxygen overpressure. It is known

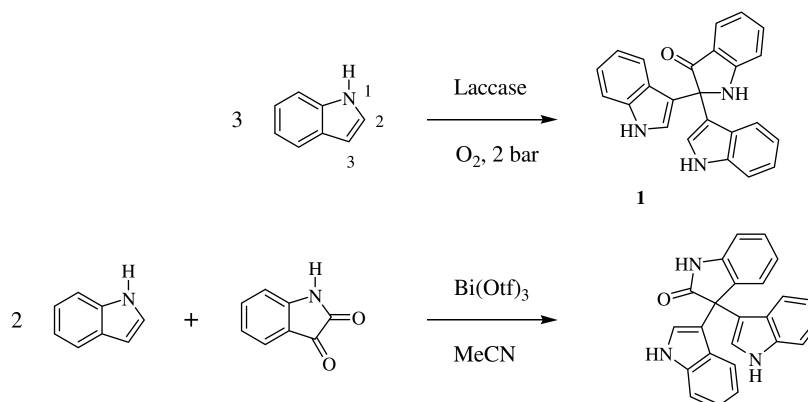

Fig. 3. Comparison of the laccase catalysed biotransformation of indole and the bismuth triflate catalysed reaction between indole and isatin.[18]

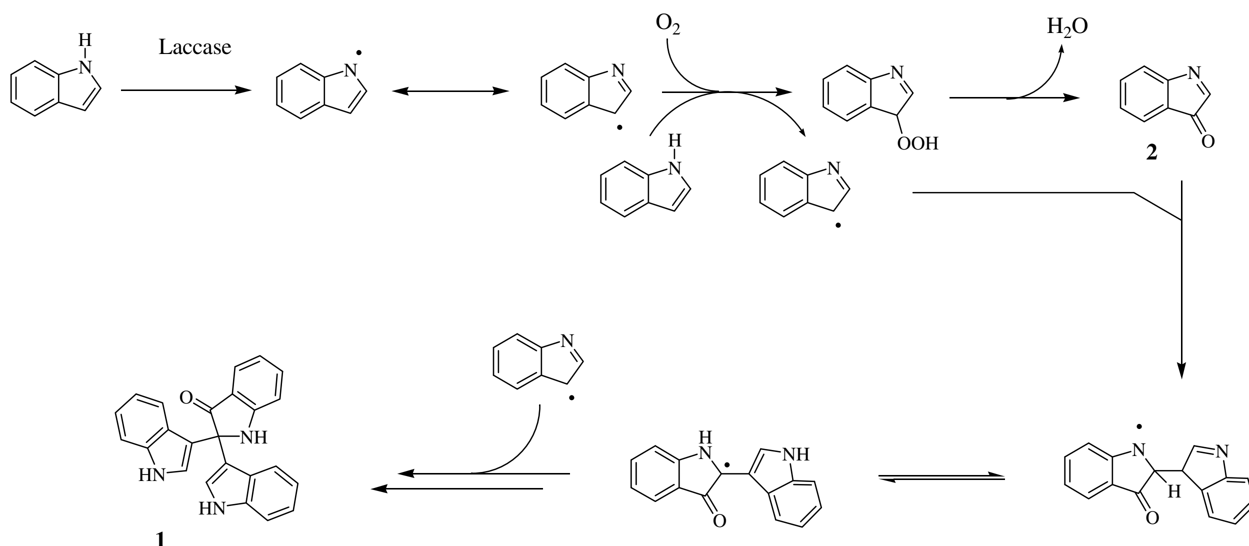

Fig. 4. Proposed mechanism to explain the laccase catalysed trimerisation of indole into **1**.

that the simple use of a redox mediator can both improve the yield and reduce reaction times in laccase biocatalysed reactions.[19] We thus tested three different common laccase mediators: ABTS, HBT and TEMPO at approximately one-third of the substrate concentration in microtiter plate. Of the three tested mediators only TEMPO proved to be really efficient. Indeed, by using both dioxygen overpressure and TEMPO, the reaction time was reduced from 48 to 24 h (Fig. 2) and the isolated yield improved from 23% to 51%.

From a mechanistic point of view this biotransformation could result from the initial formation of a C3-located indole hydroperoxide followed by its fractionation to indenone (**2**). Then, two indole radicals centred on C3 could add sequentially to **2**, leading finally to trimer **1** (Fig. 4).

We described the biocatalytic trimerisation of indole with the formation of a new quaternary carbon centre. The reaction was optimised in a closed reaction vessel pressurised with dioxygen, with the addition of TEMPO as a redox mediator. This is a novel example of the use of laccases in biotransformation, which expands their potential applications in fine chemistry. This work also provides an enzyme candidate for the biosynthesis of **1**. In *Haemophilus influenzae*, its biosynthesis could be linked to nitrate reduction.[16]

Acknowledgements

We are indebted to M. Giorgi for performing X-ray structure analysis and Marius Reglier for very helpful discussions. This work was partly supported by the European Commission, Sixth Framework Programme (NMP2-CT2004-505899, SOPHIED).

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8. This work has been conducted in the frame of the European Sophied project aiming to develop new ecofriendly synthesis of dyes.
9. For all experiments, isoenzyme Lac 3 from *Trametes* C30, heterogenously expressed in *Saccharomyces cerevisiae*, was used.[20] Laccase activity is expressed in U/L using syringaldazine as the substrate.
10. Preliminary experiments were initially conducted in 96-microtiter well plates to test the influence of the substrate concentration (5–100 mM), laccase activity (1750–70,000 U/L) and reaction times (24 vs 48 h). The substrate was added dissolved in dioxan (maximum dioxane concentration 20%), the reaction being conducted in 0.05 M acetate buffer pH 5, at 30 °C on an orbital shaker (120 rpm).
11. Bioconversions were conducted in a screw-capped Schlenk tube (100 mL) either left open or pressurised with 2 bar of dioxygen overpressure at a controlled temperature of 30 °C. The reaction mixture was composed of 9 mL of 0.05 M acetate buffer pH 5, 50 mM (final concentration) of indole (dissolved in dioxane, final dioxane concentration 10%) and the reaction was started by adding the laccase solution (final activity 17,500 U/L). Aliquots were periodically withdrawn and analysed by HPLC. When TEMPO was used, it was added dissolved in dioxane (final TEMPO concentration 15 mM, final dioxane concentration 11.5%).
12. Indole bioconversions were followed by HPLC using a RP-18 column associated with a diode array multi-pump Hewlett Packard apparatus. The substrate and product of the reaction were eluted with a linear gradient of acetonitrile in water (from 0% to 100% acetonitrile in 30 min) delivered at 1.3 mL/min. Detection was set at 254 nm. Acetophenone was used as internal standard. Retention times: indole (17.4 min), **1** (20.4 min), acetophenone (15.7 min).
13. Crystallographic data for **1** have been deposited at the Cambridge Crystallographic Data Centre under the access number CCDC 666843.
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17. Compound **1**: yellow solid. Mp = 244–245 °C. $\lambda_{max} = 400$ nm. ^{1}H NMR (DMSO-d_6) δ (ppm): 11.0 (br s, 2H), 8.1 (s, 1H), 7.5 (m, 2H), 7.3 (m, 4H), 7.1 (d, 2H), 7.0 (m, 2H), 6.9 (d, 1H), 6.8 (m, 2H), 6.7 (m, 1H). ^{13}C NMR (DMSO-d_6): δ (ppm) 200.8, 160.5, 137.4, 136.8, 125.5, 124.4, 123.9, 121.0, 120.5, 118.3, 117.7, 117.0, 113.9, 111.7, 111.5. HRMS m/z calcd for $C_{24}H_{17}N_3O$ 364.1444 $[M+H]^+$, found: 364.1442.
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