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MUSHROOM TYROSINASE AS AN OXIDANT FOR THE SYNTHESIS OF 5,6-DIHYDROXYINDOLE DERIVATIVES

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Abstract: Several catecholamines have been converted to 5,6-diacetoxyindole derivatives by the use of mushroom tyrosinase as oxidant.

Melanin[1] is a biopolymer found throughout the animal and plant kingdoms. In animals, the black pigment eumelanin occurs in skin, hair, feathers, insect cuticles, malignant melanoma and the ink sac of the squid, while pheomelanin[2] covers a broad spectrum of colors ranging from violet to yellow. Biosynthesis[3] of melanin is initiated by the conversion of L-tyrosine to dihydroxyphenylalanine (DOPA), which is then oxidized by tyrosinase to yield phenylalanine-3,4-quinone (dopaquinone). The unstable quinone undergoes a series of spontaneous reactions that result in the formation of 5,6-dihydroxyindole (DHI), which then polymerizes to melanin.

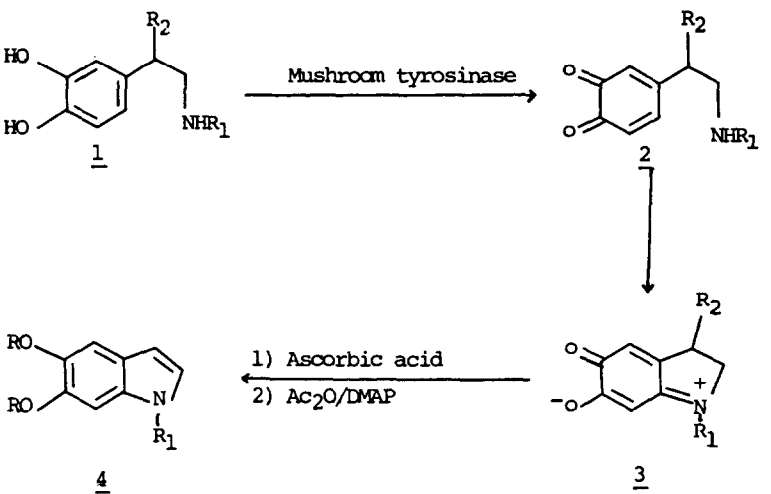
As part of a program directed towards *in vitro* melanin formation, we sought to use mushroom tyrosinase as an oxidant for the oxidation of catecholamines to produce *o*-quinones, which, upon reduction, will give 5,6-dihydroxyindole derivatives.

Over the past several years, the use of enzymes as catalysts in organic synthesis has markedly increased.[4] For example, pig liver esterase has found extensive use for the enantioselective hydrolysis of chiral and prochiral esters.[5] Lipase has been used for esterification[6], transesterification[6], and synthesis of macrocyclic lactones.[7] Oxidation of *meso*-diols with horse liver alcohol dehydrogenase proceeds with complete enantiotopic specificity to give chiral lactones.[8] Ketones are reduced by yeast to afford alcohols[9]. Recently, Klibanov[10] reported that mushroom tyrosinase can catalyze the oxidation of a number of phenols in chloroform to give *o*-quinones quantitatively.

Although mushroom tyrosinase has been used for the preparation of synthetic melanins[3b], the enzyme has never been utilized for the synthesis of 5,6-dihydroxyindole derivatives.

In an initial experiment, a mixture of D,L-epinephrine *1a* and mushroom tyrosinase in phosphate buffer at pH 6.8 was vigorously shaken in air for 20 min at room temperature. The solution rapidly became deep-red as oxidation proceeded. The resulting red aminochrome[11] *3a* was directly reduced with ascorbic acid in a biphasic water-ether system.

The intense red color of the solution was readily discharged by the reducing agent to give N-methyl-5,6-dihydroxyindole 4 (R=H, R_1=Me). Since this is known to be unstable, the ether layer was immediately treated with acetic anhydride and dimethylaminopyridine (DMAP) to afford the diacetate[12] 4a in 63% overall isolated yield. Similarly, N-isopropyl-5,6-diacetoxyindole[12] 4b and 5,6-diacetoxyindole[12] 4c were obtained from N-isopropyl-D,L-noradrenaline 1b and norepinephrine 1c in 70% and 34% overall yields, respectively. 5,6-Diacetoxyindole 4d (same as 4c) was also obtained from 3-hydroxytyramine 1d (dopamine) in less than 10% yield (Table).



Low yields observed in the case of 1c and 1d are not unexpected from mechanistic considerations. The transient o-quinone 2 undergoes intramolecular Michael reaction because of the proximity of the aminoalkyl side chain, to give the aminochrome 3. The slow cyclization of 2c and 2d, because of the weak nucleophilicity of the primary amino group, allows competing reactions to occur. It is known that o-quinone polymerizes rapidly in water to a melanin-like pigment and inactivates the enzyme.[13] This is the reason many attempts to use tyrosinase as a practical catalyst in organic synthesis have been unsuccessful.

In summary, mushroom tyrosinase can be used as an oxidant on a preparative scale for the conversion of aminoalkylcatechols to aminochromes which provide simple and fast access to N-alkylsubstituted 5,6-dihydroxyindoles. Immobilization of the enzyme on solid supports is under investigation to evaluate reusability.

Table: Oxidative-reductive cyclization of catecholamines to 5,6-diacetoxyindole derivatives.

	Substrate 1	Product 4 (R=Ac)	Overall Yield (%)
a	R_1=CH_3, R_2=OH	R_1=CH_3	63
b	R_1=$CH(CH_3)_2$, R_2=OH	R_1=$CH(CH_3)_2$	70
c	R_1=H, R_2=OH	R_1=H	34
d	R_1=R_2=H	R_1=H	<10

Typical experimental procedure: To a solution of D,L-epinephrine (100 mg, 0.55 mmole) in 50 M phosphate buffer (pH 6.8) (50 ml) was added mushroom tyrosinase[14] (5 mg). The mixture was vigorously shaken with exposure to air for 20 min at 23°C. Progress of the reaction was monitored by its absorption spectrum measured at 475 nm. After addition of diethyl ether (100 ml), ascorbic acid (1 g) was added to the two-phase reaction mixture and it was stirred for 15 min until the red color of the solution was completely discharged. The ether layer was separated and the aqueous layer was extracted with ether. The combined organic layers were treated with sodium sulfate (10 g) followed by Ac_2O (1 ml) and dimethylaminopyridine (100 mg). After work-up and purification on an SiO_2 column (eluent: CH_2Cl_2), N-methyl-5,6-diacetoxyindole (95 mg, 63%) was obtained as a white solid, mp 107-108°C.[15]

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14. Mushroom tyrosinase (EC 1.14.18.1) was purchased from the Sigma Chemical Co., as a solid with specific activity of 3300 units/mg.
15. Satisfactory spectroscopic data (^{1}HNMR and MS) were obtained for 4a, 4b and 4c.

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