

Out of the blue: Oxidative enzymatic coupling of *Psilocybe* indole alkaloids

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Upon injury of carpophores, psychotropic psilocybin (**1**)-producing "magic" mushrooms instantly develop an intense blue color. Purification of two enzymes in native form from *Psilocybe cubensis* was accomplished. They represent the two-step cascade of psilocybin deprotection and subsequent oxidative oligomerization. The phosphatase PsiP removes the 4-O-phosphate group to yield psilocin (**2**), while PsiL oxidizes its 4-hydroxy group enabling subsequent radical coupling reactions. These led to the formation of melanin-like, protein binding pigments. Mass spectrometry showed formation of a heterogeneous mixture of oligomers with up to 13 psilocin monomers. Reaction monitoring by *in situ* NMR spectroscopy indicated that the 5-position plays an important role in psilocin oxidation. Comparison with autoxidation of psilocin led to a comprehensive mechanistic concept of psilocin oxidation pathways under varying conditions.

Our results suggest that the phosphate ester of psilocybin represents a reversible natural protecting group, whose removal after wounding triggers pigment-on-demand formation. This implies that *Psilocybe* signature indole alkaloids may exert their ecological function also as oligomers.

To further expand our understanding of the underlying chemistry, oxidation studies of **2** analogs were carried out. Selective blocking of coupling sites by methylation at C-5 or C-7 stops the oligomerization cascade in oxidation reactions, thus leading to isolable dimeric and monomeric quinoid structures of varying colors. By substituting the amine-N of **2** for a C, a strong upfield shift and sharpening of the OH ¹H NMR signal strongly supports the hypothesis of an existing O-H-N hydrogen bond within **2**, that might impact the oxidation properties of the molecule.

