

## 196c Activity of Cinnamate 4-Hydroxylase Towards Un-Natural Substrates

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Cinnamate 4-hydroxylase (C4H) is the first cytochrome P450 in the plant phenylpropanoid biosynthesis, a key pathway leading to many important compounds such as lignins, flavonoids, and coumarins. In the presence of cytochrome P450 reductase (CPR), cofactor NADPH and oxygen, C4H catalyzes the *para*-hydroxylation of *trans*-cinnamic acid into *p*-coumaric acid. We tested 22 aromatic substituted analogues of *trans*-cinnamic acid as substrates and observed 7 that were metabolized by C4H expressed in *S. cerevisiae*. The kinetic parameters of these reactions were measured. The structures of the metabolized products were determined by UV/Vis spectroscopy, LC/MS, and NMR. In most cases the hydroxylation was *para* to the propenoic acid group, consistent with the natural product. Based upon the structural identification two of the products have not been previously reported in the literature. This demonstrates the utility of using enzymatic oxidative biotransformations to obtain novel compounds which have potential as pharmaceutical intermediates or monomers. Based on a homology model of C4H, we are currently pursuing site-directed mutagenesis to alter the substrate selectivity of C4H. We will report the kinetic and binding parameters of these mutants towards *trans*-cinnamic acid and analogues.