Biocatalytic oxidative cleavage of alkenes using novel metal-dependent aromatic dioxygenases

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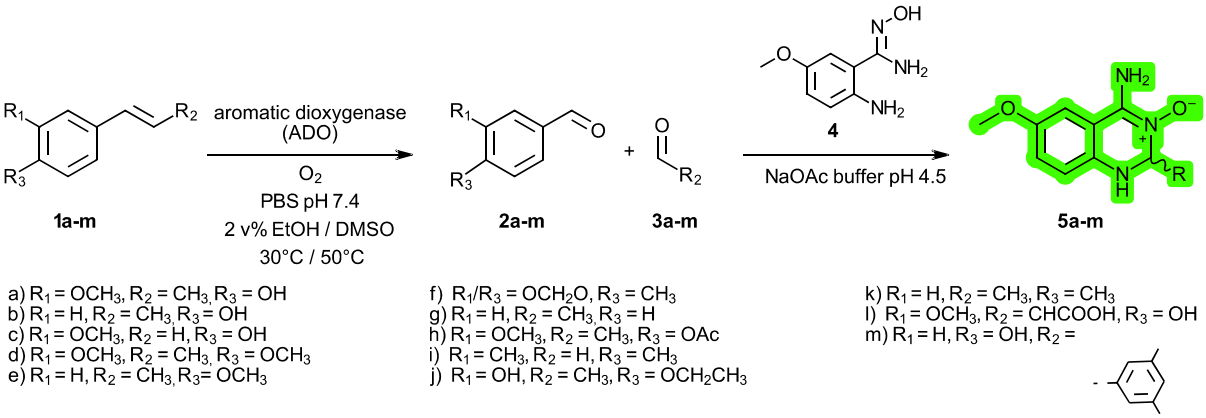

*Scheme 1: Left: Oxidative cleavage of **1** by aromatic dioxygenases (ADOs) to the corresponding aldehydes (**2, 3**). Right: Detection of the formed aldehydes via ABAO-assay using 2-aminobenzamidoxime derivate **4**. The resulting quinazolines **5** exhibit UV absorption and fluorescence properties.*

Oxidative cleavage of alkenes can be used to obtain carbonyl compounds, valuable building blocks in various areas, including food, flavor, and the pharmaceutical industry.[1] While this can be done *via* chemical approaches, like ozonolysis[2], we are interested in milder and safer enzymatic approaches using novel metal-dependent aromatic dioxygenases (ADOs). In the presence of oxygen, these enzymes enable the oxidative cleavage of substrates such as isoeugenol (**1a**) to the corresponding aldehydes (Scheme 1). For the substrate screening, HPLC, GC, or a pooling approach based on the reported ABAO-assay[3] are used. The latter is a photometric/ fluorometric assay for the detection of the aldehydes. It is based on the rapid reaction of aldehydes with 2-aminobenzamidoxime (ABAO), forming quinazolines **5** that exhibit UV absorption and fluorescence properties. Using this assay, biotransformations with several pooled substrates are analyzed by detecting the photometric/fluorometric responses. So far, substrates such as isoeugenol (**1a**), hydroxyanethole (**1b**), 4-vinylguaiacol (**1c**), and resveratrol (**1m**) were found to be successfully converted to the respective aldehydes in a whole-cell system reaction. Among the tested enzymes, especially the MapADO from *Moesziomyces aphidis,* showed promising properties and converted 10 mM isoeugenol to vanillin in a whole cell approach within 1 h. Furthermore, the crystal structure of MapADO could be obtained, and several rational mutants were generated. So far, the screening revealed that the *para*-hydroxy group is essential for substrate acceptance.

(1) Kazimírová, V.; Rebroš, M. Production of Aldehydes by Biocatalysis. *Int. J. Mol. Sci.* **2021,** *22* (9), 4949.

(2) Fisher, T. J.; Dussault, P. H. Alkene ozonolysis. *Tetrahedron* **2017,** *73* (30), 4233.

(3) Ressmann, A. K.; Schwendenwein, D.; Leonhartsberger, S.; Mihovilovic, M. D.; Bornscheuer, U. T.; Winkler, M.; Rudroff, F. Substrate-Independent High-Throughput Assay for the Quantification of Aldehydes. *Adv. Synth. Catal.* **2019,** *361* (11), 2538.