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ARTICLE TYPE

Expanding the regioselective enzymatic repertoire: oxidative mono-cleavage of dialkenes catalyzed by *Trametes hirsuta*

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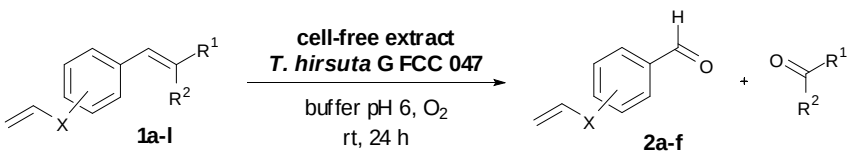
The first report of a biocatalytic regioselective oxidative mono-cleavage of dialkenes was successfully achieved employing a cell-free enzyme preparation from *Trametes hirsuta* at the expense of molecular oxygen. Selected reactions were performed on preparative scale affording high to excellent conversions and chemoselectivities.

Oxidative alkene cleavage is an important synthetic tool (i) to remove protecting groups; (ii) to tailor large molecules or (iii) to access carbonylic functional groups.[1] Among the different methodologies available to perform this reaction, the ones employing ozone[2] or metal-based oxidants[3] are the most frequently used. However, these methods present some disadvantages: for instance, (i) the use of special equipment and reaction conditions or (ii) over-oxidation of aldehyde products leading to by-products such as carboxylic acids. Furthermore, from an environmental point of view, it is desirable to find alternatives for metal catalysts and stoichiometric amounts of peroxides or salts. Consequently, novel greener methodologies have been developed to overcome some of these drawbacks.[4]

Although biocatalytic oxidative alkene cleavage is probably still in its infancy, enzyme preparations[5] and isolated enzymes[6] have proven to be valuable catalysts in order to oxidize terminal or internal alkenes leading to the corresponding carbonyl derivatives under mild conditions employing oxygen as oxidant in aqueous media. Enzyme-catalyzed processes offer, apart from the environmentally benign reaction conditions, a series of advantages such as high chemo-, regio-, and stereoselectivities, which has led to their increasing use at industrial scale.[7] Numerous regioselective reactions catalyzed by hydrolases such as lipases,[8] epoxide hydrolases,[9] or nitrilases,[10] and oxidoreductases like alcohol dehydrogenases,[11] have been reported to differentiate between identical functional groups thereby avoiding the time-consuming protection and deprotection steps and isolation of intermediates.

Chemical oxidative alkene cleavage of compounds bearing more than one (non-aromatic) C=C double bond generally leads to the cleavage of all the alkene groups. One remarkable exception was described by Neumann and co-workers using a ruthenium catalyst in the presence of hydrogen peroxide, showing that a terminal alkene group was regioselectively oxidized in the presence of other more sterically hindered internal (or cyclic) C=C double bonds in different aliphatic polyalkenes, displaying high to excellent selectivities.[12] The lack of more regioselective methods could be due to the fact that oxidative alkene cleavage is highly exothermic (*e.g.*, 47-64 kcal mol^{-1} for ozonolysis),[13] therefore hampering an easy control of the regioselectivity.

To the best of our knowledge, alkene cleaving biocatalysts have not yet been investigated for their ability to differentiate between two alkene groups within the same molecule.



1a, *para*, X = CH_2O, R^1, R^2 = H
1b, *meta*, X = CH_2O, R^1, R^2 = H
1c, *ortho*, X = CH_2O, R^1, R^2 = H
1d, *para*, X = CH_2, R^1, R^2 = H
1e, *meta*, X = CH_2, R^1, R^2 = H
1f, *ortho*, X = CH_2, R^1, R^2 = H
1g, *para*, X = CH_2O, R^1= Me, R^2 = H
1h, *meta*, X = CH_2O, R^1= Me, R^2 = H
1i, *ortho*, X = CH_2O, R^1= Me, R^2 = H
1j, *para*, X = CH_2O, R^1= H, R^2 = Me
1k, *meta*, X = CH_2O, R^1= H, R^2 = Me
1l, *ortho*, X = CH_2O, R^1= H, R^2 = Me

Scheme 1 Regioselective bio-oxidative mono-cleavage of dialkenes.

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An enzyme preparation from the fungus *Trametes hirsuta* G FCC 047 has been described to selectively cleave alkenes adjacent to a phenyl moiety affording the corresponding benzaldehyde derivatives.[5b,e] To test the regioselectivity of this biocatalyst, a series of dialkenes derivatives (**1a-l**) was prepared possessing one alkene moiety adjacent to a phenyl moiety and one not adjacent. Three main aspects that could influence the enzymatic alkene cleavage were considered for the choice of substrates: 1) the relative substitution on the phenyl ring; 2) the electronic character of the non-conjugated double bond and 3) the sterical hindrance (and stereochemistry) of the phenyl-conjugated alkene (Scheme 1).

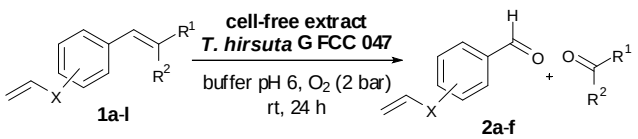
The substrates (**1a-l**) and the corresponding aldehyde products (**2a-f**) were synthesized in moderate to high yields using different methodologies, such as the Wittig olefination,[14] Suzuki-Miyaura[15] and Stille cross-couplings[16] (see Scheme S1 in ESI). The *Z*-configured alkenes **1j-l** were obtained as a *Z*:*E* mixture ranging from 85:15 to 80:20, while the *E*-configured alkenes could be prepared as single isomers.

Dialkenes **1** were tested on a microscale for the oxidative reactions with a cell-free extract from the fungus *T. hirsuta*. A pressure equipment[5b] was employed to adjust 2 bar of oxygen pressure and the reaction vessels (riplate® sw 10 mL deep-well plate) allowed screening of a total of 48 samples at the same time. Since most of these derivatives were volatile, cross-peak contamination from other substrates and products was observed in the GC chromatograms. Among alternative vessels tested, the best option was the riplate covered with parafilm and holes punched through for better oxygen exchange. To improve solubility of the styrene compounds in the aqueous buffer used, 10% v v^{-1} of DMSO was added, and the reactions ran for 24 h at room temperature under oxygen pressure. Employing these conditions, *para*-, *meta*- and *ortho*-allyloxy styrene substrates **1a-c** were successfully transformed with excellent conversions. Even more important, the non-conjugated C=C bond was not cleaved; thus only the alkene adjacent to the phenyl ring was transformed leading exclusively to the formation of benzaldehyde derivatives **2a-c**. Performing these biocatalytic reactions on a preparative scale (100-350 mg substrate) in a Parr hydrogenation apparatus (using molecular oxygen instead of hydrogen), the scalability was successfully demonstrated: again excellent conversions and good to excellent chemoselectivities were achieved (entries 1-3, Table 1). To the best of our knowledge, this reaction is the first example of a regioselective alkene mono-cleavage differentiating between two terminal alkenes. Interestingly, conversions into the corresponding aldehyde products were excellent in the case of *para*- and *ortho*-substituted compounds, being lower for the *meta*-derivative due to the formation of by-products detected by GC.

The allyl styrene substrates **1d-f** were found to be poorer substrates for *T. hirsuta*, as lower conversions were observed, especially for the dialkene **1f** (entries 4-6, Table 1). This effect can be ascribed to the oxygen atom in the allyloxy moiety (substrates **1a-c**), since activating groups have been shown to enhance the reaction outcome.[5b] Nevertheless, the transformation with **1d** was performed on a preparative scale and **1d** was cleaved with excellent chemoselectivity affording the aldehyde **2d** (entry 4, Table 1).

In a subsequent step, *para*-, *meta*-, and *ortho*-allyloxy β-methylstyrene substrates with *E*- or *Z*-configuration (**1g-l**) were tested to investigate the effect of the additional β-methyl group and the influence of the *E*/*Z*-stereochemistry of the cleaved double bond. It turned out that *E*-configured derivatives **1g-i** led to very similar results (entries 7-9, Table 1) as observed for dialkenes **1a-c** on preparative scale, resulting in benzaldehyde products **2a-c** with high to excellent conversions (62-98%). Due to the fact that only phenyl-conjugated double bonds can be oxidized by this fungus,[5b] the more hindered internal alkene was preferentially transformed over a terminal one (less hindered), complementing the previous methodology described by Kogan et al.[12] *Z*-Configured isomers **1j-l** showed low conversions into the aldehydes, the highest one obtained with the *meta*-derivative **1k** (entries 11, Table 1). These results indicated that the biocatalyst prefers transforming the *E*- over the *Z*-isomer. This preference was confirmed when employing *meta*-substituted substrate **1k** with an *E*:Z-ratio of 15:85, which changed during the transformation to an *E*:Z-ratio of 4:96, clearly indicating that the *E*-isomer was preferentially consumed. The cleavage of the non-conjugated double bond was never observed, as neither monoaldehyde regioisomer nor dialdehyde products were detected by GC/MS.

Table 1 Biocatalytic oxidative alkene cleavage of dialkene substrates with *Trametes hirsuta* G FCC 047 (t= 24 h)[a]



Entry	Substrate	Product	*c* (%)[b]	Aldehyde (%)[b]
1	**1a**[c]	**2a**	96	96
2	**1b**[c]	**2b**	98	61
3	**1c**[c]	**2c**	98	96
4	**1d**[c]	**2d**	28	28
5	**1e**[d]	**2e**	45	33
6	**1f**[d]	**2f**	33	6
7	**1g**[c]	**2a**	97	92
8	**1h**[c]	**2b**	>99	62
9	**1i**[c]	**2c**	>99	98
10	**1j**[d,e]	**2a**	<10	n.d.[f]
11	**1k**[d,e]	**2b**	47[g]	28
12	**1l**[d,h]	**2c**	<10	n.d.[f]

[a] For experimental details, see ESI. [b] Determined by GC and GC/MS. [c] Preparative scale. [d] Microscale. [e] *E*:*Z* ratio 15:85. [f] n.d. = not determined. [g] *E*:*Z* ratio 4:96. [h] *E*:*Z* ratio 20:80.

For *meta*-allyloxy substituted dialkenes, a main side-product was observed with a mass spectrum matching to the corresponding epoxide. This fact explains the lower conversions

into aldehydes observed in comparison with the results obtained for *ortho*- or *para*-substituted dialkenes.

In summary, a series of dialkene substrates was designed and synthesized to study the regioselectivity of the *Trametes hirsuta* catalyzed oxidative alkene cleavage in buffer solely employing oxygen as an innocuous oxidant. After optimization of the biocatalytic reaction parameters, the reaction afforded exclusively the benzaldehyde derivatives with moderate to excellent conversions and chemoselectivities. Thus, from the two C=C double bond present, only the one adjacent to the aromatic group was transformed. This biocatalytic methodology allowed the unprecedented differentiation between two terminal alkenes and the preferential oxidative cleavage of an internal double bond over a terminal one. Selected reactions were successfully performed on a preparative scale with >100 mg of substrate leading to excellent results.

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† Electronic Supplementary Information (ESI) available: [experimental details on the synthesis of all compounds, enzymatic reactions, analytics and spectral data are available]. See DOI: 10.1039/b000000x/

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Graphical Abstract