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Hydroxy Functionalization of Non-Activated C–H and C=C Bonds: New Perspectives for the Synthesis of Alcohols through Biocatalytic Processes**

*Harald Gröger**

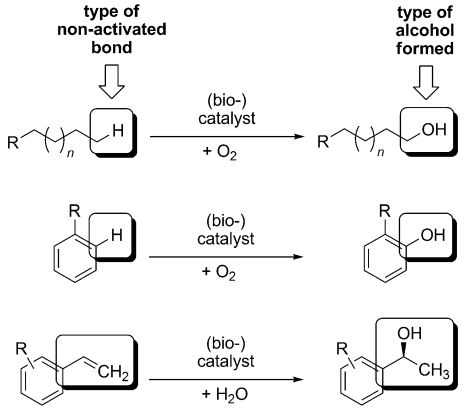
Dedicated to Professor Werner Hummel on the occasion of his 65th birthday

alcohols · biocatalysis · enzyme catalysis · hydration · hydroxylation

In organic chemistry, biocatalysis has become a valuable tool for academic and industrial synthesis and provides a complement to "classic chemical" and chemocatalytic processes.[1] In particular, enzyme-catalyzed processes are applied with tremendous success in the production of fine chemicals and active pharmaceutical ingredients.[2,3] A current focus in biocatalysis research is on the realization of chemical transformations, for which efficient and sustainable solutions do not exist. The demand for such new methodologies has been expressed in detail by representatives of the pharmaceutical industry.[4] One of the challenges is the selective hydroxy functionalization of non-activated C–H and C=C bonds. Examples of such "dream reactions" include the ω-hydroxylation of (substituted) alkanes and the regioselective hydroxylation of aromatic hydrocarbons with atmospheric oxygen as the oxidant in both cases as well as the asymmetric addition of water to non-activated alkenes (Scheme 1). Although in general enzymes appear to be predestined for this purpose, hydroxylations in particular are often hampered by low enzyme activities and stabilities, thus leading to biotransformations with low efficiency. In the past few months impressive solutions based on enzyme catalysis have been presented for the reactions mentioned above. In this Highlight this is exemplified by three pioneering studies,[5–8] which can all be considered to be breakthroughs also from the perspective of synthetically practical catalytic processes.

A current challenge in enzyme catalysis is broadening its application range in the production of specialty and bulk chemicals exceeding an annual output of 1000 tonnes, since in this field its use is still very limited.[2] One of the few examples is the production of acrylamide with a nitrile hydratase.[9] Currently, ω-aminolauric acid and its esters are interesting substitutes for laurin lactam,[10] which is required for the manufacture of polyamide-12 on a multi-10000 tonne scale annually. In a joint project with Evonik Industries AG the research group of Schmid and Bühler has now developed a new process for the synthesis of ω-aminolauric acid methyl ester based on sustainable feedstocks and the use of a monooxygenase.[5,6] The resulting catalytic selective hydroxylation of an alkyl chain as a reaction type, which up to now has been known to be synthetically difficult and in general connected with low efficiency, proceeds here with remarkably high process efficiency (Scheme 2). An *E. coli* whole-cell catalyst is used bearing a monooxygenase and an AlkL membrane protein (which is responsible for the transport of the ester through the hydrophobic membrane and into the cell) in recombinant form. Upon application of this catalyst dodecanoate **1** is first terminally hydroxylated and then transformed in part into the aldehyde **3**. In the process, a product concentration of 159 mM of alcohol **2** is obtained in the organic phase and additionally about 50 mM of the aldehyde **3** is formed by further oxidation.[5] Besides the monooxygenase, the AlkL protein also plays a key role: in its presence the whole-cell activity increases by 62-fold up to 87 $U\,g_{cdw}^{-1}$ (cdw = cell dry weight).

The subsequent conversion of aldehyde **3** with a transaminase and L-alanine as an amine donor then furnishes the desired ω-aminododecanoate, for which a whole-cell process



Scheme 1. Selected synthetic challenges in the field of hydroxy functionalization.

[*] Prof. Dr. H. Gröger
Faculty of Chemistry, Bielefeld University
Universitätsstrasse 25, 33615 Bielefeld (Germany)
E-mail: harald.groeger@uni-bielefeld.de

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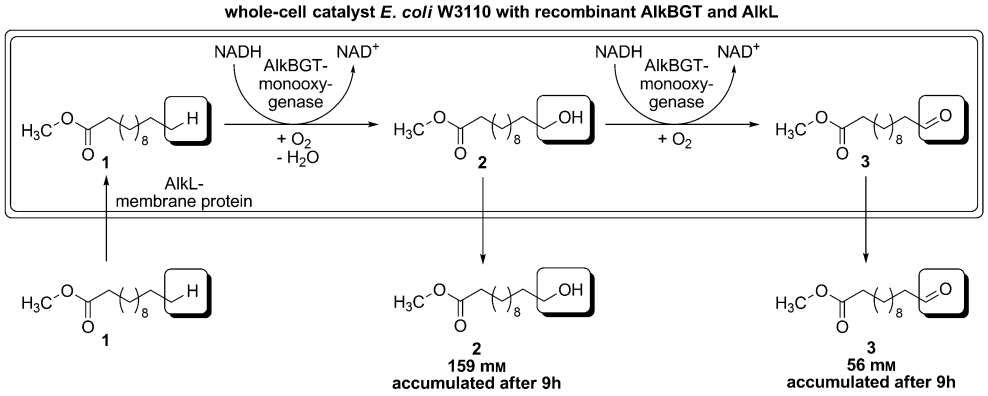
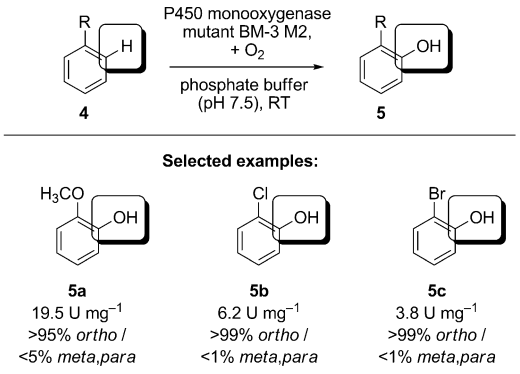


Scheme 2. Selective ω-hydroxylation of dodecanoate **1** using a whole-cell catalyst.

was also developed by Schmid and Bühler et al.[6] The preparative utility and industrial potential of the process concept to produce biotechnological ω-aminolauric acid from palm kernel oil for polyamide-12 synthesis are underlined by a recent press release from Evonik Industries AG,[11] according to which a pilot plant for this purpose started operation in Slovenska Lupka (Slovakia) in early 2013.

In analogy to aliphatic molecules the hydroxy functionalization of aromatic hydrocarbons also presents a challenge for the synthetic chemist. Up to now selective hydroxylations of substituted arenes have been considered to be difficult and are often accomplished only under harsh reaction conditions and with side-product formation and low selectivity. Here, again, enzyme catalysis provides an interesting means to solve this problem. Recently the group of Schwaneberg succeeded in developing a highly regioselective *ortho*-hydroxylation of substituted arenes **4** by protein engineering (Scheme 3).[7]

The P450-monooxygenase-catalyzed substitution of anisole (**4a**) proceeds with a high turnover frequency of 38.6 s^{-1}.[7] This corresponds to 19.5 U mg^{-1}, which represents the highest enzyme activity observed so far for aromatic hydroxylations with P450 monooxygenases. In addition, the product *ortho*-methoxyphenol (**6a**) is formed with an excellent *ortho*/*para* regioselectivity of >95:5 and the *meta*-substituted form does not occur. High activities and *ortho*/*para* selectivities are also obtained with other substrates, for

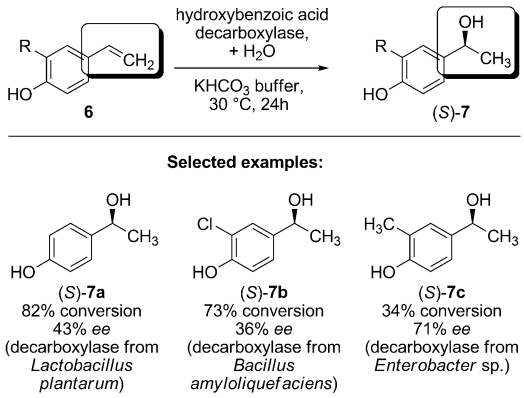


Scheme 3. Selective *ortho*-hydroxylation of arenes using P450 monooxygenases.

example halogenated arenes. The resulting substituted phenols are valuable intermediates for the synthesis of natural products and active pharmaceutical ingredients. A remaining challenge for this process, which proceeds with molecular oxygen in water at room temperature, consists in the increase of the product concentration, which is so far $\leq 0.67\ g\,L^{-1}$.

In connection with the selective enzymatic hydroxylation of arenes the recent contribution of Shoji and Watanabe et al. should also be mentioned at this stage: here with the P450 BM3 wild-type enzyme a high *ortho* selectivity was achieved when in addition perfluorinated carboxylic acids were used as so-called "decoy molecules".[12]

The catalytic enantioselective hydration of non-activated alkenes, for example styrene, represents an attractive synthetic approach for obtaining enantiomerically pure alcohols. In contrast to many other asymmetric reactions, however, to date no suitable chiral chemocatalyst is known for this type of reaction.[13] Recently, the enantioselective transformation of styrene to 1-phenylethanol was achieved by means of a chemoenzymatic one-pot synthesis.[14] However, this "formal addition of water" proceeds in two steps by means of a Wacker oxidation and subsequent enzymatic ketone reduction. Although direct biocatalytic hydrations of C=C bonds are known and applied on an industrial scale for the production of fumaric acid,[15] in this case the substrates are activated alkenes, for example enoates. Accordingly, the direct asymmetric catalytic addition of water to styrene and other non-activated alkenes remained a challenge, for which now a solution has been found by Faber et al. by means of enzyme catalysis (Scheme 4).[8] Interestingly hydroxybenzoic acid decarboxylases turned out to be suitable for this purpose and enabled the asymmetric addition of water to *p*-hydroxystyrene (**6a**) and substituted derivatives with conversions of up to 82% and enantioselectivities of up to 71% *ee*. For example, *p*-hydroxystyrene was transformed into the product (*S*)-**7a** with 82% conversion and 43% *ee*. In contrast, the carboxylation, which could be expected from this enzyme class, took place at only a low level (<5%) even in the presence of a high concentration of carbonate buffer of 3 M. Remarkable is the high proportion of enzymes suitable for this hydration of non-activated C=C bonds with six out of seven studied decarboxylases. In view of the broad applica-



Scheme 4. Asymmetric addition of water to substituted styrenes using decarboxylases.

tion of this method, future research tasks include increasing substrate scope (currently limited to *para*-hydroxy-substituted styrenes) and improving the enantioselectivity.

In conclusion, recent pioneering research has led to the development of synthetically practical catalytic processes for several hydroxy functionalizations of (substituted) alkanes and alkenes. Thereby the ω-hydroxylation of fatty acid esters and the regioselective *ortho*-hydroxylation of aromates are accomplished with atmospheric oxygen. A biocatalytic process was also established for the asymmetric addition of water to non-activated alkenes. These contributions offer promising perspectives for the increased application of such enzyme-catalyzed reactions in organic synthesis.

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