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Valuable chemicals by the enzymatic modification of molecules of natural origin: Terpenoids, steroids, phenolics and related compounds

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ABSTRACT

A renewed interest for using natural organic molecules for the production of valuable chemicals is observed in current organic processes. Natural compounds provide the access to natural grade chemicals when submitted to physical treatments or biotechnological processes. Dealing with structurally complex molecules, they can provide complex core structures for hemisynthesis purposes, and in many instances they offer the advantage of providing sustainable processes when using renewable resources. These assets could be synergistic with the assets of biocatalytic processes, to end-up with efficient and sustainable processes in the organic synthesis of valuable products.

In this review, we have gathered a selection of examples on the use of enzymes for the modification of molecules of natural origin being either purified compounds (terpenoids, steroids, phenolics) or mixtures (essential oils, natural extracts) to access fine chemicals or organic polymers.

1. Introduction

Natural products have probably been, and remain, the main source of investigations and discoveries for organic chemists to date. The discipline of organic chemistry is considered to be born in 1828 when Friedrich Wöhler serendipitously achieved the synthesis of urea from cyanic acid and ammonia in an attempt to prepare ammonium cyanate, thereby breaking the dogma of the vital force theory of the influential Swedish Professor Jons J. Berzelius. Following this discovery, natural organic molecules such as glucose, quinine, camphor, indigo, have attracted the interest of renowned chemists for the study of their structure, synthesis and properties. Organic chemistry was born, and the 20th century has witnessed an exponential increase of activity in the field, providing the scientific community with problems to solve and questions to address in the structural elucidation of complex molecules, the description and understanding of their stereochemistry and of the stereoselectivity of their biosynthesis, their mode of interaction with their environment including living organisms, and the total synthesis of these complex molecules. As a reward, many conceptual and practical progresses were made, including biosynthetic pathways elucidation, analytical chemistry at the molecular level, the development of a plethora of efficient synthetic methodologies, including enantioselective catalysis, transition metals-catalysed coupling reactions, in addition to the access to bioactive natural products and relatives. In one and a half century, the status of organic compounds thus shifted from Nature-made to Man-made, and with the ever growing ability of organic chemists to create new molecules, their interests also shifted towards synthetic and/or artificial molecules, in other words molecules with no natural equivalents. This attraction for artificial molecules culminated in the nineties in particular in medicinal chemistry with the development of combinatorial chemistry. For several scientific and cultural reasons, a renewed interest for natural molecules has been observed lately.

This trend has also been observed in applied biocatalysis. While in its infancy, the focus of biochemists and chemists interested in using enzymes in organic synthesis was put on the natural metabolic substrate, a trend largely supported by Emil Fisher's dogma 1 enzyme = 1 substrate = 1 reaction. Many studies of the substrate scopes of enzymes followed, and showed that some enzymes could accommodate various structurally related substrates. The idea of a larger substrate scope was significantly improved with the use of enzyme in organic solvents and the pioneering work of Klibanov (Zaks and Klibanov, 1985), in particular for lipases with the interfacial activation phenomenon (Schmid and Verger, 1998). Synthetic substrates of artificial origin could thus be used in enzymatic reactions performed in biomimetic media or in non-conventional media as well (organic solvents, gas phase, RTILs, supercritical fluids, fluorinated solvents). Today, the novel interest for using natural organic molecules for the production of natural

Abbreviations: API, active pharmaceutical ingredient; CaLB, *Candida antartica* lipase B; CPDMO, cyclopentadecanone monooxygenase; CPO, chloroperoxidase; CrL, *Candida rugosa* lipase; DMF, dimethylformamide; LOX, lipoxygenase; PLE, pig liver esterase; RTIL, room temperature ionic liquid; SEM, scanning electron microscopy; XO, xanthine oxidase.

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grade chemicals, for the hemisynthesis of complex molecules, or for the sustainable use of renewable resources is also observed in biocatalysis in organic synthesis.

On another hand, even if the chemical toolbox seems infinite and virtually allows any kinds of transformations and the synthesis of any kinds of molecules, enzymatic steps have been increasingly implemented in synthetic strategies over the last two decades. Main reasons explaining this evolution are (1) the huge increase of the number, quality and diversity of purified enzymes available from recombinant organisms, (2) the unique ability of enzymes to operate selectively even in complex molecular environments providing chemo-, regio-, and stereoselective reactions (Faber, 2004), of critical importance in the late stages of the synthesis of complex molecules, (3) the need for more sustainable chemical processes, and in the case of active pharmaceutical ingredients (APIs), the need for metal-free products, even at the ppm scale (Galaffu et al., 2007), (4) the development of protocols for the use of enzymes in non-conventional media, useful in medium engineering (Carrea and Riva, 2000).

The progress of biosynthetic pathways elucidation and biotechnology have also contributed greatly to the popularity of enzyme-based strategy since it is possible to identify, isolate, clone, and finally over express the genes involved in the biosynthesis of a natural compound of interest, thereby opening the possibility to use the specific biocatalyst in vitro, and to use site-selective mutation if performance needs to be improved, for instance to change or enhanced the enantioselectivity, or to tailor the enzyme to better accommodate other substrates (Fig. 1).

In this review, we have gathered a selection of examples on the use of enzymes for the modification of molecules of natural origin either being purified compounds (terpenoids, steroids, phenolics) or mixtures (essential oils, natural extracts). Our purpose is not to provide a comprehensive encyclopaedia of all the transformations of that category reported to date, but more likely to expose the results published in the recent literature in a selection of compounds leading to valuable products. We will therefore mainly focus on terpenoids, steroids, natural flavours and odorants, phenolics, and natural extracts production and modification.

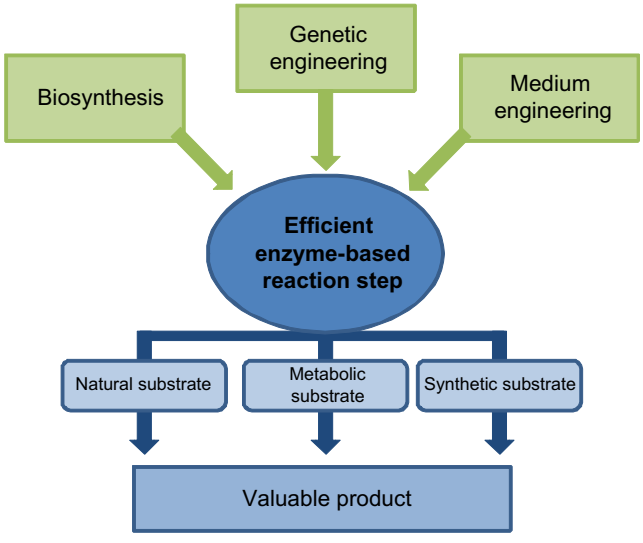

Fig. 1. Rationale of the development of an enzymatic reaction step in organic synthesis. The desired biocatalyst could be identified from biosynthetic studies, isolated and cloned. It could be an existing biocatalyst genetically modified to fit the requirements of the target reaction, and its activity could be tailored by medium engineering. It is used for the transformation of a substrate into a valuable product, the substrate being the natural metabolic substrate of the enzyme, or another substrate, natural or synthetic, structurally related to the metabolic substrate, or not (enzyme promiscuity).

The topic of the enzymatic processing of bioactive glycosides from natural sources has been reviewed in 2010 (Rauter et al., 2010) and will not be covered in the present review. Large-scale synthesis of commodity compounds such as surfactants or processes for the synthesis of biofuels will not covered by this review neither. The general topic of the biocatalytic modification of natural products was reviewed in 2001 (Riva, 2001), and we will not consider older references, excepted for clarity or comparison purposes. In vitro studies of enzymatic reactions involving natural substrates in the elucidation of biosynthetic routes will be excluded from the scope of this review.

2. Terpenoids and related compounds

Terpenoids are an amazing class of natural products found both in the marine and terrestrial realms, with a wide molecular diversity ranging from simple linear non-functionalised to complex polycyclic chiral molecules (Sell, 2003). The wide spectrum of their biological activities has also contributed to their popularity amongst chemists and biologists with compounds with functions of odorants, pheromones, vitamins, and hormones, or properties such as anti-oxidant, anti-inflammatory, anti-bacterial, anti-fungal, anti-tumoral, for example. The high efficiency of terpene cyclases, the enzymes responsible for a significant part of this diversity, has been a source of inspiration for organic chemists for a long while (Wendt et al., 2000) for their regio- and stereoselectivity, and biomimetic catalytic cyclisation reactions of simple polyenes have been proposed only recently (Godeau et al., 2011).

A wide array of terpenoids bearing an alcohol functional group has been used in many instances as substrates in transesterification reactions catalysed by lipases. Lipases are belonging to the class of hydrolases and are known to accommodate a great deal of substrates, particularly when an alcohol is used as nucleophile instead of water, typically in an organic solvent, resulting in a transesterification instead of an hydrolysis (Bornscheuer and Kazlauskas, 2004). They have been the most studied enzymes for applications in organic synthesis to date, and one of their most useful assets is undoubtedly their use in kinetic resolution of racemates, achieved through the enantioselective acylation reactions of alcohols (Ghanem and Aboul-Enein, 2004). In specific applications like fragrance chemistry, the production of enantiomerically enriched material is sometimes required, enantiomers having sometimes different olfactory properties (Abate et al., 2004b). For example, the resolution of racemic lavandulol has been reported by using CaLB and acetic acid as acyl donor to yield at ca. 55% conversion (*S*)-lavandulol in 42% yield and 52% *ee* and (*R*)-lavandulyl acetate in 51% yield and 48% *ee* (Cross et al., 2004). Many other examples of enzymatic resolution for the access to chiral synthetic fragrance materials have been reported with lipases (Abate et al., 2004a).

A parallel protocol has been described to evaluate the substrate scope of hydrolases and notably lipases with various terpenyl alcohols and acyl donors, in a combinatorial fashion (Antoniotti et al., 2008). The reactions were performed in toluene in the presence of 1% (w/w) hydrolases such as CaLB, CrL, PLE, etc. and 1–3 equiv. of vinyl esters as acyl donors. The success of the reaction was greatly influenced by the class of the nucleophilic alcohol used, primary alcohols reacting much better. Tertiary alcohols react indeed much slowly, and efficient enzymatic reactions are preferably based on enzymes isolated from mutant or rationally designed, in particular for resolution purpose (Barsch et al., 2008).

The prenyl side chain is a common feature of terpenes and is sometimes referred to as a hemiterpene (C5) derivative. Prenyl (dimethylallyl) transferases isolated from plants, bacteria or fungi catalyse the transfer of a prenyl moiety from a donor like dimethylallyldiphosphate DMAPP to an acceptor like tryptophan, in-