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Lipase-catalyzed transformations as key-steps in the large-scale preparation of vitamins

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Abstract

In this account, scope and limitations for the application of hydrolytic enzymes (lipases) to large-scale syntheses of economically important bulk products (vitamins) are discussed. In three case studies, biocatalytic alternatives were compared to "classical" chemical processes. While in the first example (pantolactone) the lipase-catalyzed kinetic resolution by esterification of the hydroxy-γ-lactone did not provide superior results for the overall-process, the synthetic strategies for the preparation of key-intermediates for fat-soluble vitamins could be improved significantly by using selected robust, immobilized lipases under optimized conditions. Regioselective mono-acetylation of a primary-secondary diol for a Vitamin A synthesis as well as mono-saponification of a hydroquinone-diacetate for a route to Vitamin E provided the monoacetylated key-intermediates in excellent yields and selectivities (generally >97%). Examples of laboratory procedures and continuous production on kilogram-scale are given. In consideration of environmental, technical, and economical aspects of modern industrial syntheses, these processes are, therefore, superior to classical (de)acetylation procedures.

Keywords: Acylation; Hydrolysis; Industrial application; Kinetic resolution; Regioselectivity

1. Introduction

Enzymes are generally considered to be advantageously used in organic synthesis in cases where stereochemical aspects are involved. This applies, in particular, to the field of high-value products like enantiopure pharmaceuticals and drugs of high structural complexity [1]. Our contribution [2] has the aim to show that lipase catalysis can be applied as a powerful method for the preparation of key-intermediates needed in the large-scale synthesis not only of specialities of complex stereochemistry but also of achiral or all-racemic low-cost bulk products. As typical examples, vitamins belong to an economically important group of products which are essential to human and animal nutrition and are, therefore, being produced world-wide in amounts of up to several hundreds of thousand tons per year, depending on their complexity of structures (for an overview, see [3]).

The driving force for process research and development at *Roche Vitamins* is to improve process economics, i.e. lowering running and/or investment costs, and to gain competitive advantage through superior technology. The consideration of ecologically benign processes is a prerequisite in this regard.

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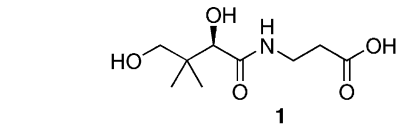
The development of cleaner methods of chemical production which minimize waste and avoid toxic reagents is based on the principles of atom economy and catalysis [4]. Atom efficiency [5] corresponds to the *E-factor* representing the kilogram amount of waste produced per kilogram of product which is a powerful tool for valuating production processes [4]. In view of the requirements of low-cost production in line with environmental responsibility, catalytic methods including enzyme-mediated transformations are preferred tools to meet such necessities [6–9].

Roche Vitamins is already one of the most extensive users of biotransformations for the large-scale production of vitamins and fine chemicals. Examples of processes are the production of Vitamin B_2 from glucose (several 1000 tons per year), in Vitamin C production the transformation of sorbitol from sorbose ("old" process; >10,000 tons per year), and ketogulonic acid from sorbitol ("new" process; several thousand tons per year), as well as the production of citric acid from sugars/starch on a scale of >100,000 tons per year. Due to the major impact of such key-steps on commercial success, serious efforts of our research programs are directed towards either whole-cell fermentation or individual enzyme catalysis. The following chapters describe three representative examples of recent activities using lipase-catalysis [10,11] in the field of vitamins and fine chemicals, including an assessment of scope and limitation of the methods described.

2. Results and discussion

2.1. Example 1: Pantothenates

Pantothenic acid (**1**) plays an important biological role as a component of coenzyme A [12]. Key-intermediate in the industrial synthesis of enantiopure **1** as well as panthenol (**2**) is (*R*)-pantolactone [(*R*)-**3**]. Routes to (*R*)-**3** involve enantioselective syntheses like oxynitrilase-catalyzed addition of HCN to β-substituted pivalaldehydes [13–15] and catalytic hydrogenation of prochiral ketopantolactone[3]. Resolution of racemic starting material by various methods and reagents is another general way to obtain the enantiopure product (see footnote 3 and [16]). The lipase-catalyzed



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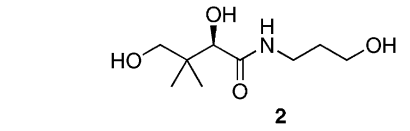

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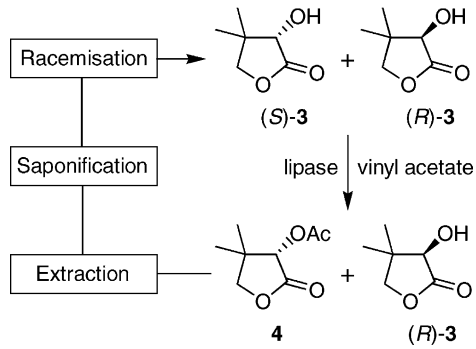


Scheme 1.

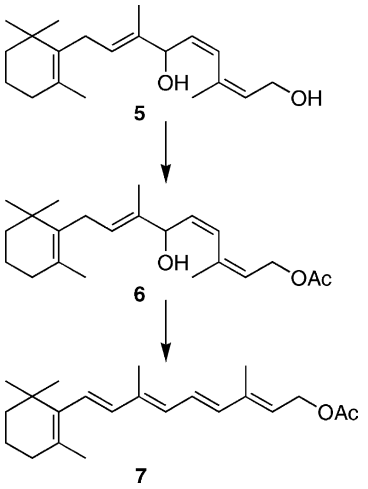
kinetic resolution of *rac*-**3** using vinyl acetate has been investigated as depicted in Scheme 1.

Taking into account not only the (high) enantiomeric excess of (*R*)-**3** obtained when using lipases, but also the additional process operations needed for recycling of the unwanted acetate **4** (extraction, followed by saponification and racemisation), we came to the conclusion that the enzymatic kinetic resolution is, in this case, not superior to known "classical" resolution procedures utilizing enantiopure amines.

2.2. Example 2: Vitamin A

Retinol (Vitamin A) is an essential component in animal tissue which plays a number of important roles in the organism, including cell differentiation and growth and involvement in the visual process [17]. In a *Roche* Vitamin A synthesis, intermediate **5** is partially acetylated and transformed to Vitamin A ester **7** [(all-*E*)-retinyl acetate] as indicated in Scheme 2. Conventional procedures, however, generally deliver mixtures of mono- and diacetylated products. Since

[3] See citations in [12] and [13].



Scheme 2.

it is known that yields of the subsequent dehydration/isomerization reaction to **7** are higher when pure monoester **6** is used as a starting material, a selective preparation of **6** is highly desirable.

While "classical" chemistry under a variety of conditions did not work very selectively, the lipase-catalyzed mono-acetylation proved to be the method of choice [18,19]. In a screening of commercially available hydrolases, the immobilized *Chirazyme L-2* (on C-2 carrier) proved to be the best with regard to conversion rate and selectivity. Moreover, the advantage of a heterogeneous catalyst could be exploited for carrying out the transformation in a continuous fashion. Vinyl acetate displayed highest activity as an acyl donor compared to other acetates (Fig. 1).

For the successful transfer of the continuous lab procedure to miniplant scale, however, several requirements had to be fulfilled. In particular, long-term stability of the enzyme dropped considerably within a few days of continuous operation at 50 °C. The inactivation caused by impurities could be prevented efficiently by using a pre-column containing a complexing agent (EDTA tetrasodium salt) and addition of 100 ppm each of an organic base (e.g. triethylamine) and an antioxidant (e.g. hydroquinone) to the substrate solution, when carrying out the experiment at 22–24 °C over hundred days. When the pre-column was removed after this period, a considerable decrease of conversion was detected (Fig. 2). Under the conditions

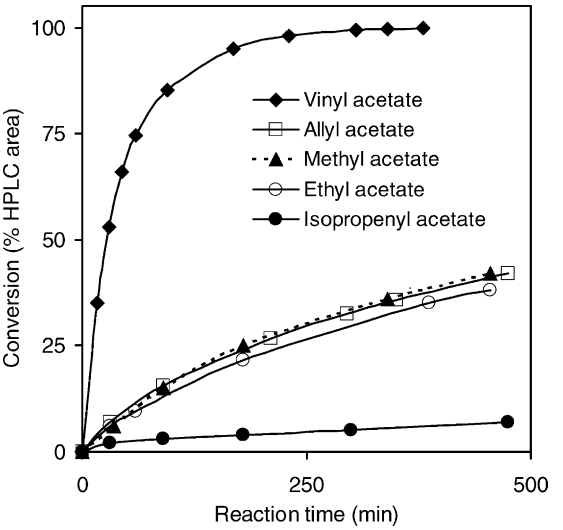


Fig. 1. Comparison of acetylating agents in the lipase(*L2-C2*)-catalyzed mono-acylation of Vitamin A precursor **5** to **6**; conditions: 10% (w/v) **5**, 50 °C, batch, lipase PLC (taken from [18]).

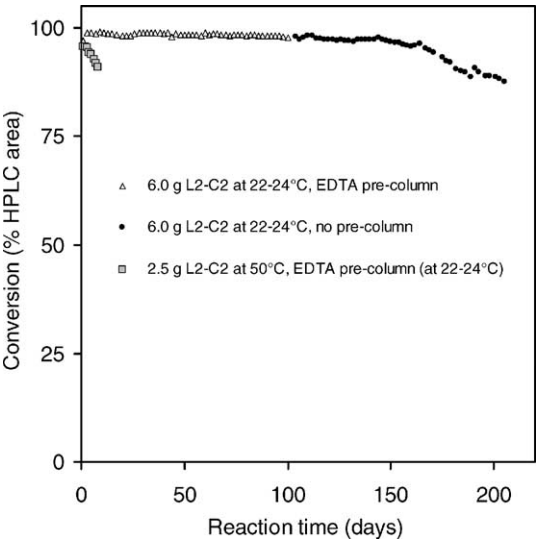
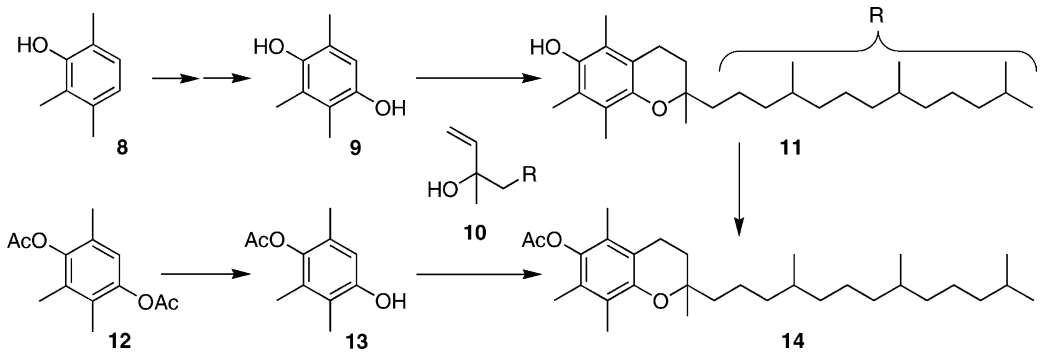


Fig. 2. Continuous lipase(*L2-C2*)-catalyzed mono-acylation of Vitamin A precursor **5** to **6**; conditions: 10% (w/v) **5** in vinyl acetate, 100 ppm triethylamine, 100 ppm hydroquinone, fixed-bed reactor, flow rate 1 ml/min, detection RP-HPLC; in the experiment with 6.0 g *L2-C2*, the pre-column was removed after 100 days (taken from [18]).



Scheme 3.

specified, high conversion rate (>99%) and selectivity (>97% for the primary hydroxy group) could be obtained.

For miniplant scale-up, the substrate concentration could be increased to 30% (w/w) by using acetone (vinyl acetate/acetone 30:70 v/v) as a co-solvent thus preventing crystallisation problems. At a flow rate of 10 g substrate solution per minute and 100% conversion, 1.6 kg of monoacetate **6** were prepared per day. For work-up, acetaldehyde produced was separated by distillation, and excess vinyl acetate was recycled after rectification.

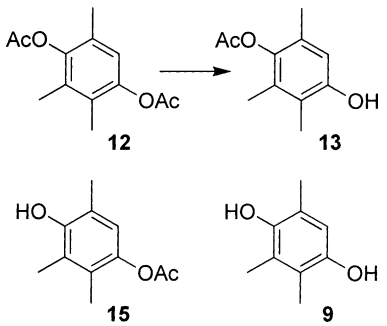
2.3. Example 3: Vitamin E

(all-*rac*)-α-Tocopherol (**11**) is the economically most valuable product from the group of Vitamin E compounds, due to its biological and antioxidant activities. All technically relevant syntheses use trimethylhydroquinone (**9**) and (all-*rac*)-isophytol (**10**) as starting materials for an acid-catalyzed condensation reaction (Scheme 3). The Vitamin E ester **14** [(all-*rac*)-α-tocopheryl acetate], which is the major application form for feed and food industry, is obtained by subsequent acetylation [20,21].

The alternative route to acetate **14** via trimethylhydroquinone-1-monoacetate (**13**) is an attractive one, since diacetate **12** is accessible from cheap α-isophorone, thus avoiding use of the difficult-to-handle trimethylhydroquinone (**9**) prepared from expensive 2,3,6-trimethylphenol (**8**). The selective synthesis of monoacetate **13** is, however, difficult to achieve by standard chemical methods. An elegant solution of this synthetic problem was, again, found by application of hydrolytic enzymes [22].

Screening of a set of commercially available lipases and esterases in small batch experiments with free enzymes revealed that *Pseudomonas* sp. lipase (PSL), *Thermomyces lanoginosus* lipase (TLL), and *Pseudomonas fluorescens* lipase (PFL) were most active and selective. We were particularly pleased to find that TLL was among the best catalysts, since it is recombinantly produced in *Aspergillus oryzae* on large scale [Novo Nordisk (now Novozymes)/Roche Diagnostics] as detergent additive for laundry, and is, therefore, a very cheap reagent.

Furthermore, immobilization on the hydrophobic polypropylene carrier *Accurel MP1001* (Membrana GmbH, Obernburg) [23] increased the hydrolytic activity tenfold without affecting the high regioselectivity. In addition, it should be mentioned that enzymes from *Thermomyces lanoginosus* used in laundry industry exhibit a considerable thermal stability which



Scheme 4.

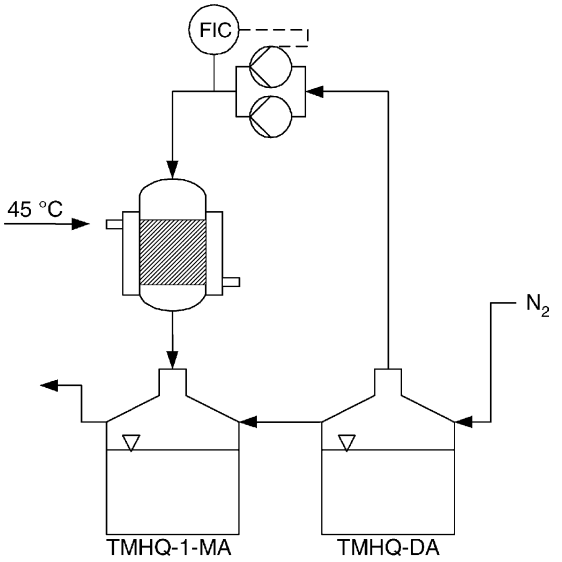


Fig. 3. Experimental set-up for the continuous lipase-catalyzed mono-deacetylation of trimethylhydroquinonediacetate (**12**) to the 1-monoacetate **13** in a fixed-bed reactor.

enables higher conversion rates by increasing the temperature. Even at 55 °C no traces of regioisomer **15** and no total hydrolysis to trimethylhydroquinone (**9**) could be detected. Water-saturated *tert*-butyl methyl ether showed to be the most suitable solvent for the transformation ***12*** → ***13*** (Scheme 4). The experimental set-up for a continuous mono-deacetylation is sketched in Fig. 3.

3. Conclusion

For three industrially important processes, biocatalytic alternatives to "classical" transformations have been investigated and discussed. While in one example (pantolactone) the lipase-catalyzed kinetic resolution did not provide superior results for the overall-process, the synthetic strategies in the field of Vitamins A and E could be improved significantly by using selected robust, immobilized lipases under optimized conditions (e.g. solvent systems, substrate/catalyst ratio, temperature). Monoacetylated key-intermediates have been obtained in excellent yields and selectivities (generally >97%). The processes described are, therefore, superior to classical (de)acetylation procedures.

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