Conversion of Substrates and Recovery of Metals from Solutions

14 Industrial Biotransformations with Fungi

T. Zelinski and B. Hauer

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I. Introduction

A great challenge for industrial chemistry during the next few decades will be the development of selective and sustainable processes. One contribution to this task is made by biocatalysts, because biochemical conversions are performed under selective and mild conditions. The foundation for the discovery of new biocatalysts is the enormous diversity of organisms.

About 70,000 fungi are described in the literature and the number of species is estimated to about 1,500,000 (Bull et al. 1992). In nature, they are involved in the breakdown of complex compounds, biopolymers and man-made xenobiotica. For this task they express numerous robust exoenzymes and they are rather tolerant against toxic substrates and products. They catalyze a great variety of reactions and their synthetic potential is therefore used in industrial biotransformations.

If the target reaction is known, the first step will be the set-up of a screen to isolate microorganisms capable of catalyzing this reaction. This screen has to be fast, efficient and give you access to a broad variety of microorganisms. The chosen strain should meet the following criteria: high selectivity with no side products, lack of degradation of the product, tolerance of substrate concentrations $>5\,g\,l^{-1}$, amenable to strain improvement and scale-up. These standards are quite often met by fungi.

However, the performance of natural isolates is limited and for a technically feasible biotransformation the strain and the process has to be improved. The target of these efforts is high spacetime yield, high product concentration and low medium costs.

Natural isolates frequently grow as mycelium, form no spores, or form spores with numerous nuclei. The first step in a strain improvement program is to overcome these features. Filamentous fungi are difficult to grow in a fermenter. They float on the top or they build up large balls. These structures are difficult to supply with oxygen. This morphology can be overcome by selecting mutants which have a more yeast-like appearance. For example, *Inonotus hispidus* is capable of selectively hydroxylating phenyacetic acid to 4-hydroxy-phenylacetic acid, but the spacetime yield is much too low for an industrial process. However, this fungus is inaccessible to strain improvement programs. Spores, which are the basis of making mutants, are not formed (B. Hauer, unpubl. data). Such examples stress the necessity of having molecular biology tools available in order to have full access to the biocatalytic reservoirs of fungi.

The second approach to improving biotransformation is process development. This is based on the physiology of the fungi, which has to be studied in great detail. The parameters which need to be known are growth rate, carbon source, oxygen supply, pH, temperature, substrate and product concentrations, osmolality and cell morphology.

BASF AG, Fine Chemicals & Biocabalysis Research GVF – A 030, 67056 Ludwigshafen, Germany

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The potential of fungi for the synthesis of chemical compounds is by no means exhausted. To push this technology to its outer limits, we need fast methods for screening and developing microorganisms, as well as unbiased collaborations between chemists, biologists and engineers.

II. Biotransformations at an Industrial Scale

A. Oxidations

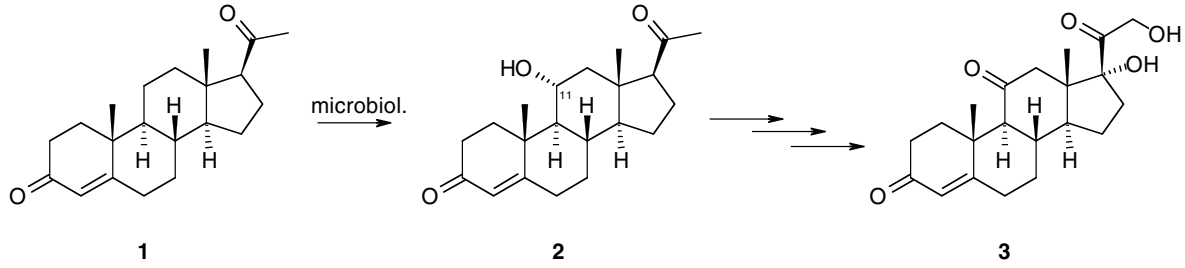
1. Hydroxylation of Saturated Positions

Numerous fungi were found to be suitable for 11-hydroxylation and for attacking steroids in various positions. The results have been summarized in comprehensive books and reviews (Kieslich 1980; Iizuka and Naito 1981; Mahato and Mukerjee 1989; Sedlaczek 1989; Mahato and Garai 1997).

The hydroxylation of progesterone (**1**) to 11α-hydroxy-progesterone (**2**) was the key step in the synthesis of the antiarthritic compound cortisone (**3**). The selective introduction of a hydroxy group at position 11 was achieved with the fungus *Rhizopus arrhizus* or *Rhizopus nigricans* (Peterson and Murray 1952). The substrate **1** could be synthesized in five steps from diosgenine (from the root of *Dioscorea composita*) or from stigmasterol (from soy beans) resulting in a 13-step route to cortisone. Previously, this product could only be prepared starting at deoxycholic acid via 26 chemical steps. In this reaction sequence, the displacement of the 12α-hydroxy group of deoxycholic acid for formation of the 11 keto function required nine steps. The fungi first used were later replaced in industry by *Aspergillus ochraceus* for the 11α-hydroxylation, and in part by *Curvularia lunata* for the 11β-hydroxylation (Weaver et al. 1960).

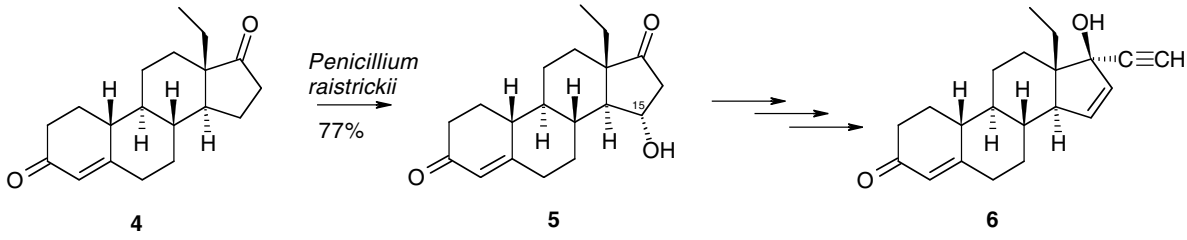
Apart from the 11-hydroxylations which are implemented in industry for the production of antiphlogistic corticosteroids only the introduction of a hydroxy group in the 15α-position makes use of a fungus at an industrial scale. Hydroxylation at the saturated carbon atom 15 in 18-methyl-4-estrene-3,17-dione (**4**) at a 77% yield was obtained with *Penicillium raistrickii* (ATCC 10490) at concentrations of 4 g l^{-1}. The product **5** is chemically dehydrated to a 15,16-double bond and ethinylated to gestodene (**6**), which increases the activity of the anticonceptivum D-norgestrel and is marketed in combination (Hofmeister et al. 1986).

The hydroxylation of an aryl methyl group has a privileged status in oxidative transformations of saturated carbon atoms. In contrast to aliphatic hydrocarbons, carbon atoms in benzylic positions are activated by the aromatic π-electron system.



1 **2** **3**

Scheme 1



4 **5** **6**

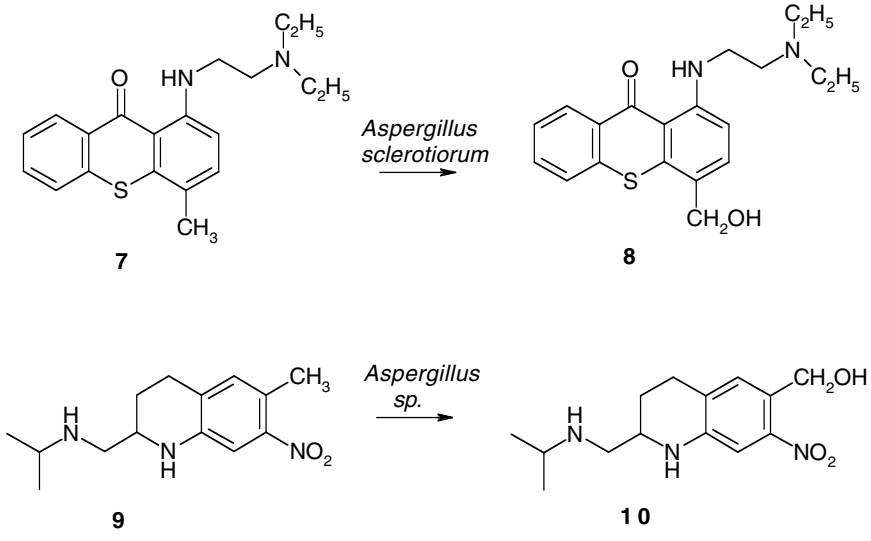
Scheme 2

The hydroxylation of an aryl methyl group was demonstrated by the transformation of the schistosomicidal compound lucathone (**7**) into the more potent hycanthone (**8**) by *Aspergillus sclerotiorum* (Rosi et al. 1967). The reaction is used in industry for the production of oxamniquin (**10**) starting from the corresponding methyl compound (**9**) (Richards 1979).

The ω-oxidation of n-alkanes by fungi was first studied in *Torulopsis* strains, especially, the biterminal oxidation (Tulloch and Spencer 1966). Improvement of the yield could be achieved by using mutations which inhibit the undesired β-oxidative degradation of the carboxy acids or by enhancing the alkane hydroxylation activity. A *Torulopsis magnoliae* mutant yielded 27.3 g l^{-1} sebacic acid (**12**) from n-decane (**11**) in 83 h (Kaneyuki et al. 1980), whereas a genetically engineered *Candida maltosa* strain is claimed to produce **12** with a yield of 28.8 g l^{-1} in 69 h (Fallon and Picataggio 1998). The oxidation of n-tridecane (**13**) to brassylic acid (**14**) by a classical mutant of *Candida tropicalis* grown on dodecane is already being used in industry, with a yield of 140 g l^{-1} in 120 h (Koichi and Namio 1982). A genetically engineered mutant blocked in the β-oxidation pathway has been described with a yield of 102 g l^{-1} of **14** in 114 h (Picataggio et al. 1989). Brassylic acid is used as the starting material for the synthesis of polymers and moschus compounds. Previously, it could only be prepared at low purity by ozonization of rape seed.

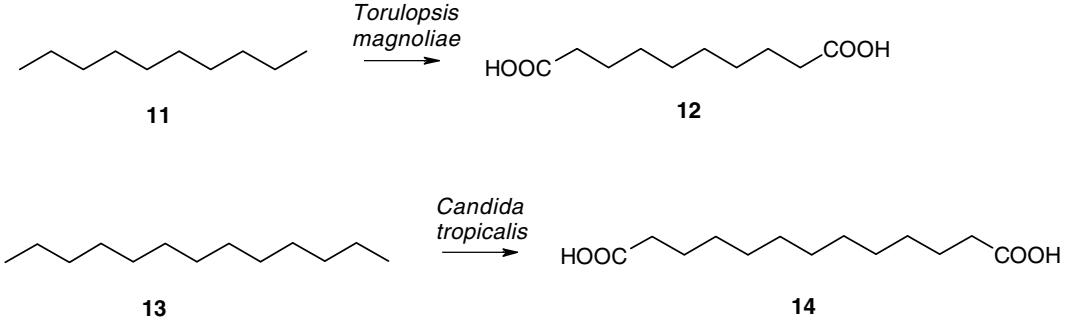
2. Hydroxylation of Arenes

With *Beauveria bassiana* Lu 700 as a biocatalyst, an ecologically beneficial process has been developed for the production of (*R*)-2-(4-hydroxyphenoxy)propionic acid (**16**). The substrate, (*R*)-2-phenoxypropionic acid (**15**), is also transformed



7 **8** **9** **10**

Scheme 3



11 **12** **13** **14**

Scheme 4