P168 ENANTIODIVERGENT BAEYER-VILLIGER OXIDATION OF FUNCTIONALIZED PROCHIRAL CYCLOHEXANONE DERIVATIVES UTILIZING RECOMBINANT CELLS

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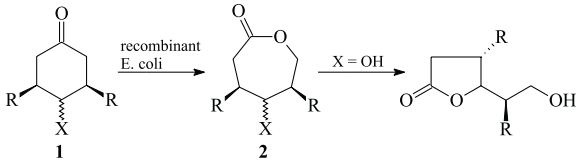
Keywords: Baeyer-Villiger oxidation, lactone, enantioselectivity, protein sequence analysis

Beside the enantioselective organo-metal catalyzed Baeyer-Villiger oxidation[1] the microbial Baeyer-Villiger oxidation[2] has become a powerful tool to synthesize asymmetric lactones as interesting intermediates in organic chemistry and frequently encountered precursors in enantioselective synthesis. The importance of enantioselective microbial reactions has grown in recent years due to an increased need for "green chemistry" approaches in industrial synthesis. Especially the field of chiral Baeyer-Villiger oxidations is one of the representative domains for biocatalysis.

In this study we present whole-cell mediated Baeyer-Villiger reactions on preparative scale using recombinant organisms as facile tools for organic chemists. Four expression systems for flavin dependent monooxygenases from *Acinetobacter* sp., *Comamonas* sp., and *Brevibacterium* sp. (I + II) were investigated for their substrate acceptance on prochiral 3,4,5 functionalized carbocyclic ketones **1**.



X = H, CH_3, $=CH_2$, *cis*-OH
trans-OH, *cis*-Cl, *trans*-Cl
R = H, CH_3

In this poster we present the results of the microbial Baeyer-Villiger oxidation of compounds **1**, which lead to lactones **2** in high optical purities and potential enantiodivergence. The influence of substrate polarity and sterical aspects of substituents R and X will be discussed in detail. The enantioselectivity of the enzymatic transformation, will be compared with protein sequence analysis of all four different expression systems.

A diastereoselective synthetic route to compounds **1** will be outlined together with potential applications of product lactones **2** in natural product and bioactive compound synthesis.

REFERENCES

1. Bolm C.: Peroxide Chemistry *2000*, 494.
2. Mihovilovic M. D., Müller B., Stanetty P.: Eur. J. Org. Chem. *2002*, 3711.

P169 MICROBIAL BAEYER-VILLIGER OXIDATION OF PROCHIRAL TETRAHYDROPYRANONS USING RECOMBINANT WHOLE-CELLS

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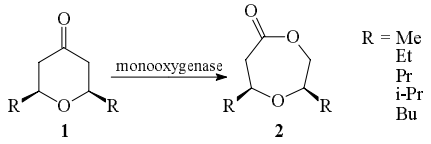
Keywords: Baeyer-Villigerase, heterocyclic ketone, stereoselectivity

Baeyer-Villigerases have been proven to be a versatile tool for the conversion of cyclic ketones to lactones. A key feature beside the regio- and chemoselectivity is the possibility of introducing chirality on a large number of non-natural substrates[1].

Based on our previous reports on recombinant whole cell mediated oxidations[2] of carbocyclic prochiral ketones[3], we are currently expanding our substrate profiling on heterocyclic substrates[4].



In this contribution we discuss our latest results on the conversion of heterocyclic ketones of type **1** to the corresponding lactones **2**. A detailed discussion of the synthetic approach towards the required tetrahydropyranone substrates will be presented. The stereoselectivity of the microbial transformation will be studied together with a survey of spatial requirements of the active site of the enzymes investigated based on substrate acceptance.

REFERENCES

1. Mihovilovic M. D., Müller B., Stanetty P.: Eur. J. Org. Chem. *2002*, 3711; Stewart J. D.: Curr. Org. Chem. *2*, 195 (1998); Willets A.: Trends Biotechnol. *15*, 55 (1997); Walsh C. T.; Chen Y.-C. J.: Angew. Chem. *100*, 342 (1988).
2. Chen G., Kayser M. M., Mihovilovic M. D., Mrstik M. E., Martinez C. A., Stewart J. D.: New J. Chem. *8*, 827 (1999).

3. Mihovilovic M. D., Müller B., Kayser M. M., Stanetty P.: Synlett *2002*, 700; Mihovilovic M. D., Müller B., Kayser M. M., Stanetty P.: Synlett *2002*, 703; Mihovilovic M. D., Chen G., Wang S., Kyte B., Rochon F., Kasyer M. M., Stewart J. D.: J. Org. Chem. *66*, 733 (2001).
4. Mihovilovic M. D., Müller B., Kayser M. M., Stewart J. D., Fröhlich J., Stanetty P., Spreitzer H.: J. Mol. Cat. B. Enzym. *11*, 349 (2001).

P170 MICROBIAL BAEYER-VILLIGER OXIDATION: SYNTHESIS OF OPTICALLY ACTIVE GEISSMAN-WAISS LACTONE

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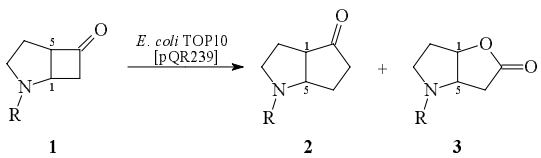
Keywords: whole cells biotransformation, asymmetric Baeyer-Villiger oxidation, Geissman-Waiss lactone

Pyrrolizidine alkaloids (PAs) are natural products containing an azabicyclic[3.3.0]heptane structural core. These compounds have a wide range of biological and pharmacological activity with therapeutic potential[1].

In the course of our studies on microbial Baeyer Villiger oxidation, we decided to investigate the biotransformation of the protected 2-azabicyclo[3.2.0]heptan-6-one **1**. It could be a direct route to obtain the Geissman-Waiss lactone (2-oxa-6-azabicyclo[3.3.0]octan-3-one) **3**, an important intermediate for preparing PAs necine base family[2] Baeyer-Villigerases (enzymes catalysing BV oxidation), in particular the cyclohexanone monooxygenase (CHMO), exhibit a broad substrate versatility[3], but only few examples of containing nitrogen compounds were described until now[4]. Following a methodology developed in our laboratory, we used a whole cell process involving recombinant *Escherichia coli*[5] as well as wild type strains.



1 **2** **3**

R = COOBn

Similarly to the well known bicyclo[3.2.0]hept-2-en-6-one[6] biotransformation, ketone **1** was oxidized to a mixture of the regioisomeric and optically active lactones **2** and **3**, the relative proportion of these lactones depends on the substrate and the microorganism employed.

REFERENCES

1. Michael J. P.: Nat. Prod. Rep. *18*, 520 (2001); Liddell J. R.: Nat. Prod. Rep. *18*, 441 (2001).
2. Wee A. G. H.: J. Org. Chem. *66*, 8513 (2001).
3. Milhovilovic M. D., Müller B., Stanetty P.: Eur. J. Org. Chem. *2002*, 3711.
4. Mihovilovic M. D., Müller B., Kayser M. K., Stewart J. D., Frölich J., Stanetty P., Spreitzer H.: J. Mol. Catal. B: Enzym. *11*, 349 (2001).
5. Kamerbeek N. M., Moonen M. J. H., Van der Ven J. G. M., Van Berkel W. J. H., Fraaije M. W., Hansse D. B.: Eur. J. Biochem. *268*, 2547 (2001); Doig S. D., Simpson H., Alphand V., Furstoss R., Woodley J. M.: Enzyme Microb. Technol. *32*, 347 (2003).
6. Alphand V., Furstoss R.: J. Org. Chem. *57*, 1306 (1992).

P171 MICROBIAL BAEYER-VILLIGER OXIDATION: A DYNAMIC KINETIC RESOLUTION USING A HETEROGENEOUS RACEMISATION CATALYST

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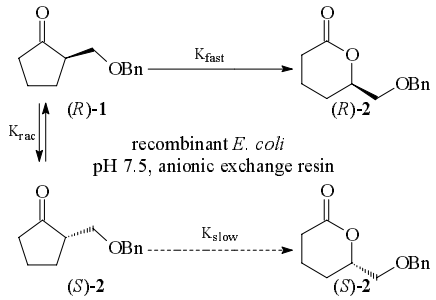
Keywords: dynamic resolution, whole cell biotransformation, asymmetric Baeyer-Villiger oxidation, heterogeneous catalysis, exchange resins

The conventional kinetic resolution is a powerful tool in asymmetric organic synthesis allowing to prepare enantiopure compounds starting from racemic substrates. However, the maximum theoretical yield of such process is intrinsically limited to 50 %. One way to circumvent this problem is the set up of a dynamic kinetic resolution process, which theoretically permits to reach a 100 % chemical yield as well as a 100 % ee starting from a racemate[1].

The Baeyer-Villiger (BV) oxidation of ketones is an important and interesting reaction because of its large number of applications. Asymmetric BV oxidation the ketones using organometallic reagents have only recently been described with moderate success[2]. On the contrary, the utilisation of enzymes (BV monooxygenases) offers efficient access to enantiomerically pure lactones and is considered an interesting "green chemistry" alternative to conventional catalysis[3].

We have recently described the first example of a dynamic kinetic resolution process applied to the microbiological Baeyer-Villiger (BV) oxidation of 2-benzyloxymethylcyclopentanone **(1)**. In this case, the racemisation process was achieved by a basic-catalysed method, at pH 9 (ref.[4]). However, only 0.3 $g.l^{-1}$ of ketone **1** could be transformed because of the extreme biotransformation conditions. In order to improve this result, we explored the possible racemisation of

1 using a procedure based on the application of heterogeneous catalysts. Using this approach, the (bio)oxidation of racemic ketone **1** by *E. coli* TOP10 [pQR239] (at higher concentration) afforded nearly enantiopure (*R*)-6-benzyloxymethyl-tetrahydropyran-2-one **(2)** in a yield of about 84 %.



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REFERENCES

1. Ebbers E. J., Ariaans G. J. A., Houbiers J. P. M., Bruggnik A., Zwanenburg B.: Tetrahedron *53*, 9417 (1997); Strauss U. T., Felber U., Faber K.: Tetrahedron: Asymmetry *10*, 107 (1999); Azerad R., Buisson D.: Curr. Opinion Biotechnol. *11*, 565 (2000).
2. Strukul G.: Angew. Chem. Int. Ed. *37*, 1199 (1998); Watanabe A., Uchida T., Ito K., Katsuki T.: Tetrahedron Lett. *43*, 4481 (2002).
3. Mihovilovic M. D., Müller B., Stanetty P.: Eur. J. Org. Chem. *22*, 3711 (2002); Alphand V., Carrea G., Wohlgemuth R., Furstoss R., Woodley J. M.: Trends Biotechnol., submitted.
4. Berezina N., Alphand V., Furstoss R.: Tetrahedron: Asymmetry *13*, 1953 (2002).

P172 MICROBIAL BAEYER-VILLIGER OXIDATION: A PROCESS CONCEPT COMBINING BIOTRANSFORMATION AND SOLID PHASE EXTRACTION IN A NOVEL WAY

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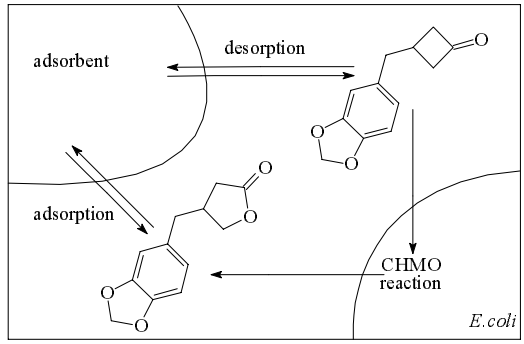
Keywords: asymmetric Baeyer-Villiger oxidation, adsorbent, whole cell biotransformation, preparative scale

During the last decades, enzymatic Baeyer-Villiger oxidation proved to be a successful tool in asymmetric synthesis which is well-known for its excellent enantioselectivities. A number of interesting synthetic applications has been published including the oxidation of bicyclic ketones and prochiral 3-substituted cyclobutanones[1].

The use of whole cells, avoiding the cofactor recycling necessity (the so-called “Baeyer-Villigerases” mostly belong to an NADPH-dependent flavoenzyme family), is the method of choice for preparative scale synthesis[2,3]. However, up to now, the use of this approach at a larger scale is hampered by the necessity to work at low substrate concentration, primarily due to inhibition phenomena or low solubilities of the organic substrate and product compounds.



Scheme 1. **Two-in-one concept of substrate feeding and product recovery**

The present work demonstrates on two substrates of the above mentioned families – namely bicyclo[3.2.0]hept-2-en-6--one and 3-piperonylcyclobutanone – how we have overcome this limitation by integrating solid phase extraction techniques into the process[4]. Ketone is loaded onto an adsorbent carrier material which is able to release it into the liquid phase in a way matching the needs of the cells during biotransformation. Likewise, the formed lactone is readsorbed onto the carrier to keep product concentration low. This “two-in-one” concept permits not only simple downstreaming processing but also intensified exploitation of biocatalyst productivity (Scheme 1).

We used whole cells of *E. coli* TOP10 [pQR239] into which had been cloned and overexpressed the cyclohexanone monooxygenase from *Acinetobacter calcoaceticus* NCIMB 9871 (ref.[5,6]). A wide range of adsorbent materials has been tested on the two substrates which differ in their adsorption/desorption behaviour. Biotransformations have been carried out in a laboratory scale reactor set up for this purpose and demonstrate the increase in productivity for biocatalytic Baeyer-Villiger oxidation on larger scale and for nearly enantiopure products.

REFERENCES

1. Mihovilovic M. D., Müller B., Stanetty P.: Eur. J. Org. Chem. *2002*, 3711.
2. Alphand V., Furstoss R., in: *Practical Approach in Chemistry: Asymmetric Oxidation Reactions* (Katsuki T., ed.), pp 214–227. Oxford University Press, Oxford 2001.
3. Alphand V., Carrea G., Furstoss R., Wohlgemuth R., Woodley J. M.: Trends Biotechnol., accepted.
4. Simpson H., Alphand V., Furstoss R.: J. Mol. Catal. B: Enzym. *16*, 101 (2001).
5. Doig S. D., O'Sullivan L. M., Patel S., Ward J. M., Woodley J. M.: Enzyme Microb. Technol. *28*, 265 (2001).
6. Doig S. D., Simpson H., Alphand V., Furstoss R., Woodley J. M.: Enzyme Microb. Technol. *32*, 347 (2003).

P173 MICROBIAL BAEYER-VILLIGER OXIDATION OF BICYCLO[4.3.0]- AND BICYCLO[3.3.0] KETONES USING RECOMBINANT WHOLE-CELLS

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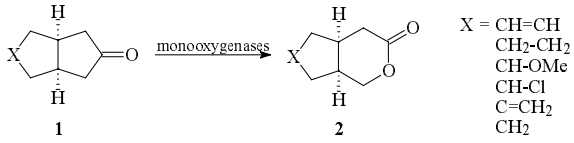
Keywords: biocatalysis, Baeyer-Villiger oxidation, monooxygenase, enatioselective synthesis, enantiodivergence

Over the last few years Baeyer-Villigerases have gathered increased attention for their ability to convert a large number of cyclic ketones in a regio- and chemoselective manner. But the most interesting feature for a synthetic chemist is the possibility to introduce chirality. The chiral lactones obtained, represent useful intermediates for the synthesis of natural products[1].

Based on our previous reports on recombinant whole cell mediated oxidations[2], we were interested in the prepartion of chiral lactones of type **2**.



1 **2**

In this contribution we discuss our results for the conversion of bicyclic ketones of type **1**. Both 5- and 6-membered fused carbocycles represent substrates for *Cyclohexanone monooxygenase* (EC 1.14.13.22)[3] and *Cyclopentanone monooxygenase* (EC 1.14.13.16)[4]. The stereoselectivity of the microbial transformation will be studied together with a survey of spatial requirements of the active site of the enzymes investigated based on substrate acceptance. This enables a novel approach to yohimbine-type alkaloids as potential α_2-adrenoceptor antagonists. An improved synthetic procedure to the corresponding ketones **1** will be presented together with conditions for the whole-cell biotransformation.

REFERENCES

1. Mihovilovic M. D., Müller B., Stanetty P.: Eur. J. Org. Chem. *2002*, 3711; Stewart, J. D.: Curr. Org. Chem. *2*, 195 (1998); Willets A.: Trends Biotechnol. *15*, 55 (1997); Walsh C. T., Chen Y.-C. J.: Angew. Chem. *100*, 342 (1988).
2. Mihovilovic M. D., Müller B., Kayser M. M., Stanetty P.: Synlett *2002*, 700; Mihovilovic M. D., Müller B., Kayser M. M., Stanetty P.: Synlett *2002*, 703; Mihovilovic M. D., Chen G., Wang S., Kyte B., Rochon F., Kasyer M. M., Stewart J. D.: J. Org. Chem. *66*, 733 (2001); Chen G., Mihovilovic M. D., Müller B., Kayser M. M., Stewart J. D., Fröhlich J., Stanetty P., Spreitzer H.: J. Mol. Catal. B: Enzym. *11*, 349 (2001); Kayser M. M., Mihovilovic M. D., Mrstik M. E., Martinez C. A., Stewart J. D.: New J. Chem. *8*, 827 (1999).
3. Donoghue N. A., Trudgill P. W.: Eur. J. Biochem. *60*, 1 (1975).
4. Griffin M., Trudgill P. W.: Biochem. J. *129*, 595 (1972).

P174 ENGINEERING A NADH-SPECIFIC BAEYER-VILLIGER MONOOXYGENASE

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Keywords: Baeyer-Villiger monooxygenase, coenzyme specificity, NAD(P)H, flavin, sulfoxidation

Baeyer-Villiger monooxygenases catalyse both nucleophilic and electrophilic oxygenation reactions. Both types of reactions can proceed with exquisite selectivity indicating that these enzymes can be of great value for the synthesis of interesting fine chemicals. So far, only a limited number of Baeyer-Villiger monooxygenases have been identified from bacteria and fungi. Recently, we have cloned the gene encoding 4-hydroxyacetophenone monooxygenase (HAPMO) from *Pseudomonas fluorescens* ACB into *E. coli*[1]. The enzyme efficiently catalyses Baeyer-Villiger oxygenation reactions of a variety of ketones and aldehydes (Fig. 1)[2]. Except for converting carbonyl compounds, HAPMO also efficiently catalyses enantioselective sulfoxidation reactions

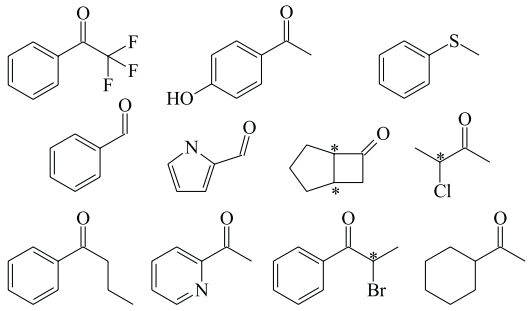

Fig. 1. **Several HAPMO substrates**

HAPMO is member of a recently recognised sequence--related flavin-dependent monooxygenase enzyme family[3]. As has been observed for all other family members, HAPMO is highly specific for NADPH. Since a switch in coenzyme specificity towards NADH would be beneficial for biocatalytic applications, we have started a study that aims at identifying the residues that modulate the coenzyme specificity. Alignment of the HAPMO sequence with sequences from characterised Baeyer-Villiger monooxygenases revealed several conserved arginine and lysine residues. It is known that arginines and lysines are often involved in binding the 2'-phosphate moiety of NADPH. Therefore, we probed the function of these conserved basic residues by site-directed and random-mutagenesis. By this, we have been able to engineer a HAPMO variant which displays a significantly increased activity with NADH. By a similar approach, we could also alter the coenzyme specificity of another sequence related Baeyer-Villiger monooxygenase; cyclohexanone monooxygenase. In conclusion, we have identified several residues that determine the coenzyme specificity of Baeyer-Villiger monooxygenases. However, to switch the coenzyme specificity of BVMOs from NADPH to NADH in terms of catalytic efficiency, more residues need to be mutated.

REFERENCES

1. Kamerbeek N. M., Moonen M. J. H., van der Ven J. G. M., van Berkel W. J. H., Fraaije M. W., Janssen D. B.: Eur. J. Biochem. *268*, 2547 (2001).
2. Kamerbeek N. M, Olsthoorn A. J. J., Fraaije M. W., Janssen D. B.: Appl. Environ. Microbiol. *69*, 419 (2003).
3. Fraaije M. W., Kamerbeek N. M., van Berkel W. J. H., Janssen D. B.: FEBS Lett. *518*, 43 (2002).

P175 DIRECTED EVOLUTION OF ENANTIOSELECTIVITY OF CYCLOHEXANONE – MONOOXYGENASE

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Keywords: cyclohexanone-monooxygenase, Baeyer-Villiger, directed evolution

Cyclohexanone – monooxygenase (CHMO) from *Acinetobacter* sp. *9871*, which is able to catalyse reactions of the Baeyer-Villiger-type and the oxidation of heteroatoms, has been overexpressed in *E. coli* as host organism. CHMO has been successfully applied in asymmetric synthesis and in kinetic resolutions[1]. Many substituted cyclic ketones have been converted into the corresponding lactones in high enantiomeric excess.

Directed evolution is a powerful tool to enhance enantioselectivity for those substrates in which the wild type enzyme gives only low *ee*'s (ref.[2]). 4-Hydroxycyclohexanone has been chosen as substrate for the directed evolution of enantioselectivity since it shows an *ee* of only 9.6 % with the CHMO – wild type.

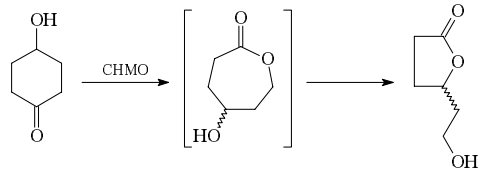

Fig. 1. **Baeyer-Villiger oxidation of 4-hydroxycyclohexanone**

GC screening[3] enables us to screen up to 500 mutants per day. In the first round of mutagenesis, using error prone PCR, mutants with enhanced and reversed enantioselectivity have been detected.

REFERENCES

1. Roberts S. M., Wan P. W. H.: J. Mol. Catal. B: Enzym. *4*, 111 (1998).
2. Reetz M. T.: Angew. Chem. Int. Ed. *40*, 284 (2001).
3. Reetz M. T., Kühling K. M., Wilensek S., Husmann H., Häusig U. W.: Cat. Today *67*, 389 (2001).

P176 BIOHYDROXYLATIONS OF KETONES AND ALDEHYDES WITH *Sphingomonas* SP. HXN-200 USING THE DOCKING/PROTECTING GROUP CONCEPT

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Keywords: biohydroxylation, *Sphingomonas* sp. HXN-200, docking/protecting group concept

Sphingomonas sp. HXN-200 has been found to be a very potent and easy to use hydroxylating microorganism[1–5]. In combination with the docking and protecting group concept, which was developed to ease the biohydroxylation of compounds containing nonactivated carbon atoms[6,7], the hydroxylation of a number of substance classes such as amines[1,3,4], amides[2,5], and carboxylic acids[8] could be achieved with this microorganism. In a few cases it was also possible to biohydroxylate ketones and aldehydes which were protected as *N*-benzoylated spirooxazolidines and oxazolidines. However, with most of the tested substrates, no or very little product formation was observed. For this reason, more appropriate docking/protecting groups are required for these substance classes. Indeed, the nature of the docking/protecting group has been found to have a significant influence[5] on hydroxylation regio- and stereoselectivity in other substrates, this adding impetus to our investigations.

Results will be disclosed in detail in this presentation.

REFERENCES

1. Li Z., Feiten H. J., van Beilen J. B., Duetz W., Witholt B.: Tetrahedron: Asymmetry *10*, 1323 (1999).
2. Chang D., Witholt B., Li Z.: Org. Lett. *2*, 3949 (2000).
3. Li Z., Feiten H. J., Chang D., Duetz W. A., van Beilen J. B., Witholt B.: J. Org. Chem. *66*, 8424 (2001).
4. Chang D., Feiten H. J., Engesser K.-H., van Beilen J. B., Witholt B., Li Z.: Org. Lett. *4*, 1859 (2002).
5. Chang D., Feiten H. J., Witholt B., Li Z.: Tetrahedron: Asymmetry *13*, 2141 (2002).
6. de Raadt A., Griengl H., Weber H.: Chem. Eur. J. *7*, 27 (2001).
7. Braunegg G., de Raadt A., Feichtenhofer S., Griengl H., Kopper I., Lehmann A., Weber H.: Angew. Chem. *38*, 2763 (1999).
8. de Raadt A., Engesser K., Griengl H., Li Z., Münzer D. F., van Beilen J. B., Weber H., Witholt B.: *Biocat 2002, Hamburg, Germany, 28–31 July 2002*.

P177 INVESTIGATIONS INTO THE BIOHYDROXYLATION OF CHIRAL ALCOHOLS EMPLOYING THE DOCKING/PROTECTING GROUP CONCEPT

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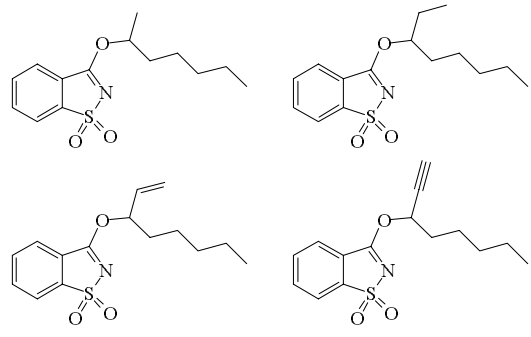
Keywords: biohydroxylation, docking/protecting group concept, alcohols

The development of the docking/protecting group concept has proved to be very useful for the biohydroxylation of nonactivated carbon atoms belonging to a variety of substance classes such as alcohols, aldehydes, ketones and carboxylic acids[1–3].

This work focuses on the biohydroxylation of open-chain alcohols protected as isosaccharine derivatives (Figure). On the basis of preliminary screening results with a range of bacteria and fungi, the most promising biohydroxylating microorganisms for each examined compound were selected, and the best biotransformations repeated on a preparative scale.



The results of these biotransformation experiments will be disclosed in more detail in this presentation.

REFERENCES

1. Braunegg G., de Raadt A., Feichtenhofer S., Griengl H., Kopper I., Lehmann A., Weber H.: Angew. Chem. *38*, 2763 (1999).
2. de Raadt A., Griengl H., Weber H.: Chem. Eur. J. *7*, 27 (2001).
3. de Raadt A., Fetz B., Griengl H., Klingler M. F., Kopper I., Krenn B., Münzer D. F., Ott R. G., Plachota P., Weber H., Braunegg G., Mosler W., Saf R.: Eur. J. Org. Chem. *2000*, 3835.

P178 EFFICIENT ENANTIOSELECTIVE BIO-OXIDATION OF *sec*-ALCOHOLS

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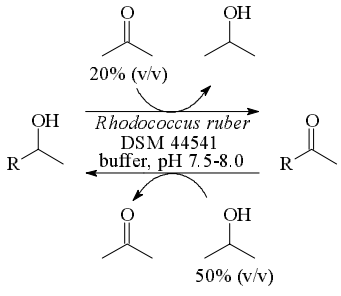
Keywords: alcohol dehydrogenases, *sec*-alcohol, Oppenauer--oxidation

In general, asymmetric biocatalytic hydrogen-transfer is based on alcohol dehydrogenases. By comparison of the biocatalytic approach with the conventional Oppenauer-oxidation and transition-metal catalysed hydrogen-transfer, the advantages of biocatalysis are the intrinsic asymmetry of enzymes[1], absence of side reactions[2] and essentially mild reactions conditions[3].

Whole resting cells of *Rhodococcus ruber* DSM 44541 are able to oxidise *sec*-alcohols at the expense of a sacrificial ketone (e. g. acetone) as hydrogen-acceptor. The process could be easily switched into the reverse mode to reduce ketones to *sec*-alcohols by replacing the auxiliary ketone with 2-propanol as hydrogen-donor.

The microorganism prefers to oxidise medium-chain alcohols and reduce ketones with the functional group in (ω-1)-position.



The substance tolerance is rather broad and encompasses not only simple *sec*-*n*-alkanols, but also a variety of substrates bearing alicyclic or aromatic functional groups. This poster focuses especially on diols in various positions of the substrate. Best results were obtained when one alcohol moiety is located in the (ω-1)-position.

This work was performed within the Spezialforschungsbereich Biokatalyse (project #F-115) and financial support by FWF (Vienna) is gratefully acknowledged.

REFERENCES

1. de Graauw C. F., Peters J. A., van Bekkum H., Huskens J.: Synthesis *1994*, 1007.
2. Stampfer W., Kosjek B., Moitzi C., Kroutil W., Faber K.: Angew. Chem. Int. Ed. Engl. *41*, 1014 (2002).
3. Sheldon R. A.: Pure Appl. Chem. *72*, 1233 (2000).

P179 BIOHYDROXYLATIONS OF BICYCLIC SATURATED γ-LACTONES WITH THE SUBSTITUTED CYCLOHEXANE SYSTEM

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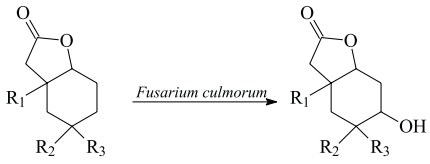
Keywords: lactones, biotransformation, hydroxylation, *Fusarium culmorum*

Many antifeedants with the lactone moiety isolated from the natural sources possess additional functional group, mainly hydroxy or acetoxy. Because of our interest in synthesis of structural analogues of natural antifeedants we have been studing methods for an efficient and regiospecific hydroxylation of lactones.One of the most used and attractive methods of introduction of such group in the unactivated position of molecule is a microbial oxidation of C-H bond to the alcohol catalysed by cytochrome P450 monooxygenases[1, 2].

On the basis of earlier studies with biohydroxylations of γ-lactones[2], we applied some fungi to biotransformate saturated γ-lactones with cyclohexane ring substituted with various number of methyl groups **(1 a, b)**.

The most effective transformation took place when different species *Fusarium culmorum* were used. The fungi were cultivated at 25 °C in Erlenmayer flasks, after 5 days the substrates dissolved in acetone were added to the grown cultures. The biotransformations were being continued for 14 days. The products were extracted with ethyl ether and analysed by GC and TLC. Then they were isolated and purified by column chromatography. Their structures were established on the basis of ^{1}H NMR and IR data.



1a,b **2a,b**

a: R_2,R_3 = CH_3; R_1 = H
b: R_1,R_2,R_3 = CH_3

The products of biotransformation of γ-lactone **1 a, b** were identified as the hydroxylactones **2 a, b** respectively, with the hydroxy group located at the C-3 in equatorial position.

REFERENCES

1. Kołek T.: Wiadomości chemiczne *56*, 449 (2002).
2. Atkinson J. K., Ingold K. U.: Biochemistry *32*, 9209 (1995).
3. Nobilec E., Anioł M., Wawrzeńczyk C.: Tetrahedron *34*, 10339 (1994).

P180 SOLVENTLESS YEAST MEDIATED REACTIONS

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Keywords: yeast, solventless, reduction, stereoselective

Yeast is capable of catalysing a wide range of reactions in a highly stereoselective manner[1]. The almost mandatory use of aqueous reaction media for this reaction causes problems associated with substrate solubility and product isolation. The use of an organic solvent for yeast mediated reactions greatly simplifies the isolation process and generally leads to higher isolated yields and enantioselectivity. For example, the yeast mediated reduction of β-keto esters in petroleum ether proceeds with isolated yields of up to 96 % and ee values of >99 % (ref.[2]).

We have now discovered that yeast reactions take place very efficiently if the substrate is simply added to moistened yeast. This is effectively a solventless reaction since only 0.8 ml water/g yeast is required, which is just sufficient to "wet" the yeast but not sufficient to form a distinct water layer. This reaction system is more reactive towards substrates than either a water or an organic solvent based system.

Using this system we have been able to reduce β-keto esters, β-keto amides, nitrostyrenes and enol ethers with high enantioselectivity and good isolated yield. Generally the isolated yields are better than those obtained using other yeast reaction systems.

The scope of this new reaction system along with the various factors (water content, temperature, substrate concentration, etc.) which influence the reaction outcome will be discussed.

REFERENCES

1. Servi S.: Synthesis *50*, 1 (1990).
2. Medson C., Smallridge A. J., Trewhella M. A.: Tetrahedron: Asymmetry *8*, 1049 (1997).

P181 ENANTIOSELECTIVE ENZYMIC REDUCTION OF A PROCHIRAL CYCLIC KETONE USING YEASTS

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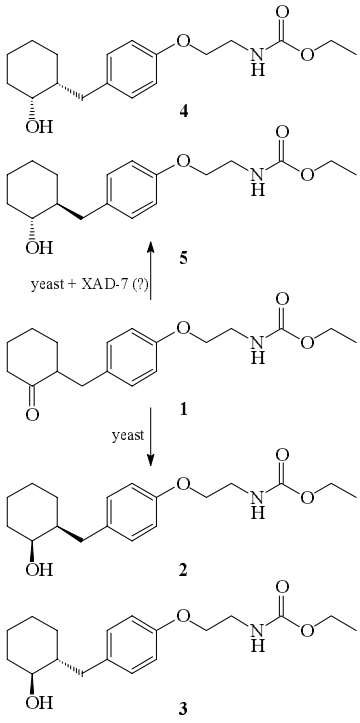
Keywords: *Geotrichum candidum*, *Saccharomyces cerevisiae*, ketone, bioreduction, polymer

Various synthetic tricks have been applied to stimulate microorganisms in producing opposite enantiomers of target structures under modified conditions[1]. One of the most often used ways is to introduce an auxiliary substituent, assisting in changing bulkiness of substituents at the stereogenic center, and, consequently, stereopreference during enzymic reduction. The auxiliary substituent can easily be removed or transformed once the new chiral center is contructed and the new product is obtained. This approach is usually applicable in the synthesis of chiral synthons or intermediates, however, can be hardly applied, when chirality is introduced into prochiral molecule in the final synthetic step. In such cases, change of environment (conditions or medium used) of the enzymic procedure has been studied. One of such approaches is to apply different immobilization factor to the microorganism, which may result in changing stereospecific ability of enzyme mediating the target process.

A hydrophobic polymer (Amberlite XAD-7)[2] has been used as auxiliary additive in the final step of the synthesis of enantiomerically pure stereoisomers **2** and **3** of ethyl *N*-{2-{4-[(2-hydroxycyclohexyl)methyl]phenoxy}ethyl}carbamate, an insect pest management agent, accessible from the parent ethyl *N*-{2-{4-[(2-oxocyclohexyl)methyl]phenoxy}ethyl}-carbamate **(1)**. In the former modification, absence of the polymer results in expected product bearing *(S)* absolute configuration at the C(2)-OH rising stereocenter. In the latter modification, addition of the polymer to the mixture is expected to immobilize yeast cell culture *in situ*, and to enable enzymic reaction to produce opposite stereoisomers **4** and **5** of the products in comparison with the former process. Se-

veral strains of the yeasts *Geotrichum candidum* and *Saccharomyces cerevisiae* have been subjected to this study.



This research was supported by the grants GA AVČR S4055104 and GAČR 203/02/0166.

REFERENCES

1. Roberts S. M.: J. Chem. Soc., Perkin Trans. 1 *2001*, 1475.
2. Nakamura K., Takenaka K., Fujii M., Ida Y.: Tetrahedron Lett. *43*, 3629 (2002).

P182 BAKER'S YEAST MEDIATED ASYMMETRIC REDUCTION OF CINNAMALDEHYDE DERIVATIVES

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Keywords: asymmetric reduction, cinnamaldehydes, baker's yeast

The enantioselective reduction of cinnamaldehyde derivatives is an attractive strategy to prepare various optically active multifunctional molecules that can be used as chiral building blocks for the synthesis of some HIV-protease inhibitors[1]. The asymmetric reduction of cinnamaldehydes **1a-c** mediated by baker's yeast *(Saccharomyces cerevisiae)* furnished alcohols **2a** and **2b** in excellent enantiomeric excesses and yields (Table I).

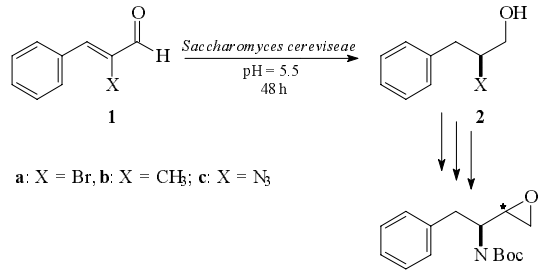

Table I
Reduction of cinnamaldeydes **1a–c** by baker's yeast[a]

Aldehyde	Alcohol	Yield (%)	$[\alpha]_D^{20}$	e. e.[c] (%)	Configuration
1a	**2a**	98	–22.5 (*c* 5, $CHCl_3$)[b]	>99	(*S*)
1b	**2b**	99	–11.0 (*c* 4.6, benzene)[b]	>99	(*S*)
1c	**2c**	98	–7.6 (*c* 0.75, $CHCl_3$)[b]	–	–

[a]T = 30 °C, 48 h, pH = 5.5, 4.7 mmol/25g (aldehyde/baker's yeast), 10.4 g of glucose, 250 ml H_2O (2.0 ml of EtOH); [b]Values of *c* (g /100 ml); [c]Determined by GC-MS analysis (capillary chiral column CHIRASIL-DEX)

REFERENCES

1. Tucker T. J., Lumma Jr. W. C., Payne L. S., Wai J. M., Solms S. J., Giuliani E. A., Darke P. L., Heimbacj J. C., Zugay A. J., Schlei, W. A., Quintero J. C., Emini E. A., Huff J. R., Anderson P. S.: J. Med. Chem. *35*, 2525 (1992); Evans B. E., Rittle K. E., Homnick C. F., Springer J. P., Hirshfield J., Veber D. F.: J. Org. Chem. *50*, 4615 (1991).

P183 ENANTIOSELECTIVE OXIDATION AND REDUCTION OF ACYCLIC COMPOUNDS BY A YEAST

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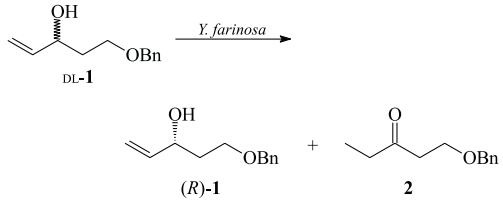
Keywords: yeast, oxidation, reduction, acyclic alcohols, acyclic ketones

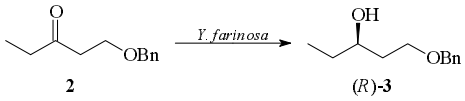
Optically active acyclic alcohols are very useful in synthesis of natural products. We have reported that *Yamadazyma*

farinosa IFO10896, a yeast, catalyzes the enantioselective oxidation of cyclic allyl alcohols to afford the optically active form[1]. Herein, we will report the enantioselective oxidation and reduction of acyclic compounds by the yeast.

First, the oxidation of an acyclic allyl alcohol DL-**1** was examined. As expected, the reaction proceeded with high enantioselectivity as well as that of cyclic compounds to give optically pure *(R)*-**1**. Interestingly, the microbial reduction of C = C double bond of the corresponding ketone also occurred in the reaction system to afford the saturated ketone **2**.



Second, we tried the reduction of the resulting **2** using *Y. farinosa*. While the reduction under aerobic conditions proceeded without enantioselectivity, asymmetric reduction of **2** occurred under anaerobic conditions to afford *(R)*-**3** with high ee.



REFERENCES

1. Matsumoto K., Kawabata Y., Hatanaka M.: *Abstracts of BIOTRANS 99, P214, 1999*.

P184 ASYMMETRIC REDUCTION OF KETONES BY PHOTOSYNTHETIC ORGANISMS

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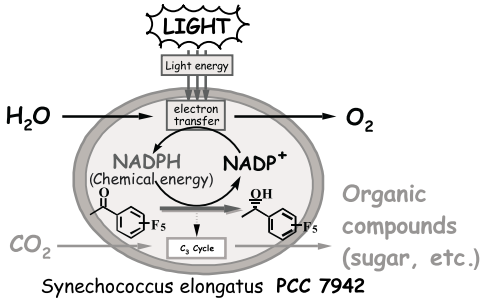
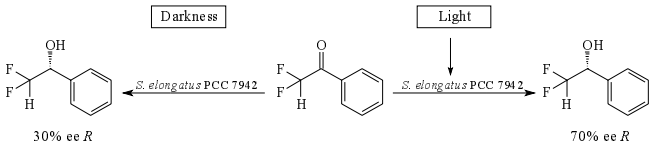
Keywords: photosynthetic organisms, asymmetric reduction, ketone, light-mediated, light-controlled

Biocatalytic reduction is a useful tool for obtaining optically active alcohols, and many scientists have studied reactions using isolated enzymes, microbes and plant cell cultures as biocatalysts. Reduction of substrates usually requires a large input of energy, and in microbial reductions, carbohydrates such as sugars have been used to recycle the coenzyme. These carbohydrates are generated through photosynthesis with sunlight energy. In other words, we have been indirectly using light energy for asymmetric reduction[1–3].

Now we propose the direct use of light energy for such reactions by using biocatalyst that fall into a new category, the photosynthetic organisms, because they can directly use light energy. Reduction of artificial ketones such as acetophenone derivatives by *Synechococcus elongatus* PCC 7942 proceeds smoothly by the aid of light. The efficiency of the reaction is very high since the coenzyme, NADPH is regenerated by using light energy.



In the reduction by *S. elongatus* PCC 7942, the stereochemical course of asymmetric reduction of ketones is largely regulated by light. Thus, we find that enantioselectivity in the reduction of α,α-difluoroacetophenone by *S. elongatus* PCC 7942 increases as a result of illumination by fluorescent light. Furthermore, DCMU, an inhibitor of photosynthesis affects to the stereoselectivity under illumination, and decreases the enantioselectivity.



REFERENCES

1. Nakamura K., Yamanaka R., Tohi K., Hamada H.: Tetrahedron Lett. *41*, 6799 (2000).
2. Nakamura K., Yamanaka R.: J. Chem. Soc., Chem. Commun. *2002*, 1782.
3. Nakamura K., Yamanaka R.: Tetrahedron: Asymmetry *13*, 2529 (2002).

P185 ASYMMETRIC REDUCTION BY *Geotrichum candidum* IN SUPERCRITICAL CARBON DIOXIDE USING SEMI-CONTINUOUS FLOW REACTOR

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Keywords: supercritical carbon dioxide, alcohol dehydrogenase, *Geotrichum candidum*, asymmetric reduction, chiral alcohols

Supercritical carbon dioxide (scCO2) has been used as a solvent for organic synthesis, extraction and chromatography due to it's environmentally benign nature, unique characteristics and high functionalities. The use of biocatalysts in scCO2 for organic synthesis has the additional attraction of combining a natural catalyst with a natural solvent. Moreover, if flow scCO2 reactors that discharge products and CO2 from a column packed with biocatalysts are used, the problem of product extraction, which obstructs the practical use of enzymes in an aqueous solvent for organic synthesis, can be solved because CO2 can be removed easily, whereas, with batch reactors, the separation of biocatalysts from the products is necessary after depressurization.

Although flow scCO2 systems using hydrolytic enzymes have been developed, no study has been conducted using dehydrogenases[1]. We used the resting cell of *Geotrichum candidum* as a catalyst for the reduction of ketones in a semi-continuous flow process using scCO2 for the first time. The reduction of cyclohexanone was successful, and the biocatalyst could be used repeatedly. It was also suitable for the asymmetric reduction of *o*-fluoroacetophenone, and resulted in excellent enantioselectivity (ee >99 %) and higher productivity than that of the corresponding batch process.

REFERENCES

1. Matsuda T., Harada T., Nakamura K.: Chem. Commun. *2000*, 1367.

P186 STUDY OF SUBSTRATE SPECIFICITY ON THE REDUCTION OF BICYCLIC DIKETONES WITH A YEAST STRAIN, *Torulaspora delbrueckii* IFO10921

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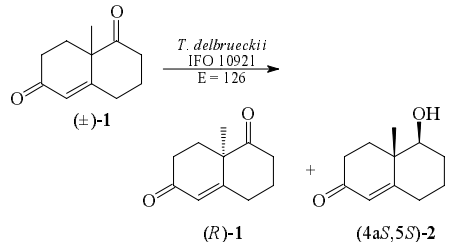
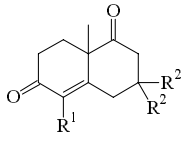
Keywords: kinetic resolution, yeast-mediated reduction, substrate specificity, whole cell biocatalyst

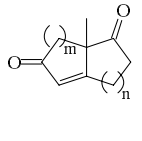
Both enantiomers of Wieland-Miescher ketone **1** in a highly enantiomerically enriched form became readily available by a newly-developed kinetic resolution with yeast--mediated reduction[1]. From a screening of yeast strains, *Torulaspora delbrueckii* IFO 10921 was selected. The collected stationary-phase cells of this strain, obtained by an incubation in a glucose medium, smoothly reduced only the isolated carbonyl group of the *(S)*-enantiomer, while the *(R)*-enantiomer remained intact (*E*: 126). Starting from both enantiomers (*ca.* 70 % ee) prepared by an established proline-mediated asymmetric Robinson annulation, the reduction with *T. delbrueckii* gave the *(R)*-enantiomer (98 % ee) and the corresponding alcohol **2** (94 % ee, 94 % de) in preparative scale in nearly quantitative yields.



Substrate specificity on the related compounds was further studied. Compared with a high level of recognition for Wieland-Miescher ketone **1**, the introduction of substituents on the octahydronaphthalene skeleton as well as the structural change into an octahydroindene skeleton retarded the enzymatic reduction and the enantiomeric ratio fell in 5–16. Further structural variation into a bicyclo[3.3.0] skeleton led to an exclusive 1,4-conjugate reduction of the α,β-unsaturated carbonyl group, and the above results suggested the participation of plural oxidoreductive enzymes in the whole cell.



3 (R^1 = Me, R^2 = H)
4 (R^1 = H, R^2 = Me)



5 (m = 2, n = 1)
6 (m = 1, n = 2)
7 (m = 1, n = 1)

An air-dried preparation of these cells was very effective both in terms of activitiy and enantioselectivity, with a high retention of a co-factor regeneration system. The *E* value (123) was nearly equal with that of the freshly harvested cells (126). Moreover, these air-dried cells showed a long-term stability. The activity was almost same, even after storage in a refrigerator (4 °C) for sixty days. In this way, by virtue of preservable whole cells of this yeast strain, the applicability of this yeast-mediated reduction in synthetic organic chemistry was greatly increased.

REFERENCES

1. Fuhshuku K., Funa N., Akeboshi T., Ohta H., Hosomi H., Ohba S., Sugai T.: J. Org. Chem. *65*, 129 (2000).

P187 APPLICATION OF *Torulaspora delbrueckii*--MEDIATED REDUCTION IN NATURAL PRODUCT SYNTHESIS

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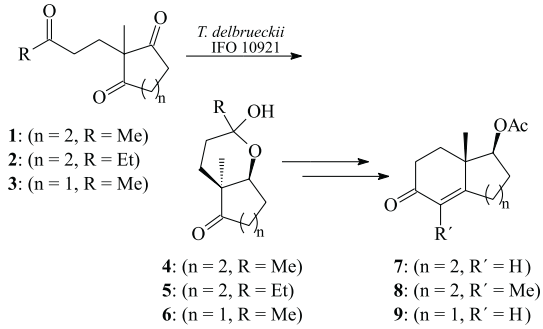
Keywords: asymmetric reduction, yeast-mediated reduction, substrate specificity, quaternary asymmetric center, radical--mediated β-fragmentation

Torulaspora delbrueckii IFO10921 catalyzes the enantiotopic group-selective reduction of triketone **1**, the prochiral precursor of Wieland-Miescher ketone, to give a stereomerically pure form of bicyclic hemiacetal **4**, which is the equivalent of the resulting hydroxyketone[1].

Through a study of substrate specificity among 2,2-disubstituted cycloalkane-1,3-diones **2** and **3**, there were found some good substrates to give the equivalents of the resulting hydroxyketone by yeast-mediated reduction. These products were isolated as cyclic hemiacetals **5** and **6**. The subsequent chemical transformation to **7**, **8** and **9** warranted the stereochemistry and the stereochemical purity of the yeast-mediated reduction products, with considerable values as the starting materials for natural product synthesis.

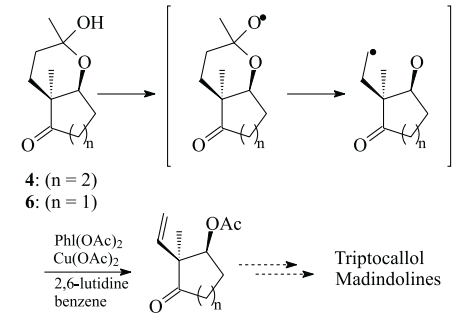


1: (n = 2, R = Me)
2: (n = 2, R = Et)
3: (n = 1, R = Me)

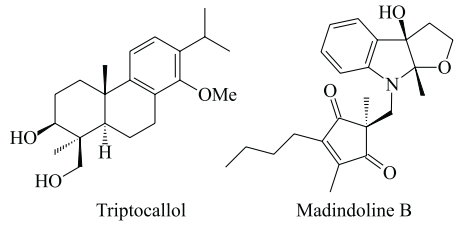
4: (n = 2, R = Me)
5: (n = 2, R = Et)
6: (n = 1, R = Me)

7: (n = 2, R′ = H)
8: (n = 2, R′ = Me)
9: (n = 1, R′ = H)

Also the above hemiacetals **4** and **6** served as the unique precursor of a sterically congested β-acetoxyketones containing a quaternary asymmetric center with three independent functional groups, by means of C-C bond cleavage through a β-fragmentation of alkoxy radicals. The resulted β-acetoxyketones **10** and **11** are expected for the multi-functional building blocks, and attempts to apply on the natural product syntheses, such as triptocallol and madindolines are now under way.



4: (n = 2)
6: (n = 1)



Triptocallol Madindoline B

REFERENCES

1. Fuhshuku K., Funa N., Akeboshi T., Ohta H., Hosomi H., Ohba S., Sugai T.: J. Org. Chem. *65*, 129 (2000).

P188 BIOREDUCTION OF ACETOPHENONES AND DERACEMIZATION OF ARYL ETHANOLS BY FUNGI

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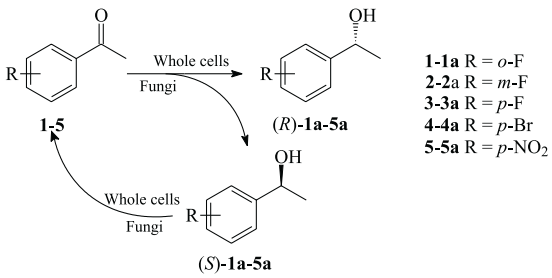
Keywords: bioreduction, deracemization, whole cells, chiral alcohol, biocatalysts

Biocatalysis is one of the most important methods for the preparation of optically active compounds with high stereoselectivity[1]. Among the biocatalysed reactions, we can mention the asymmetric reduction of ketones or kinetic resolution of racemic alcohols by whole cells or isolated enzymes, leading to chiral alcohols which are of great synthetic utility in view of the possible transformation of alcohols into other functionalities[1]. Recently, we have explored new microorganism strains, native of the Brazilian rain forests, for synthetic purposes, for instance in the oxidation of sulfides[2] and hydrolysis of epoxides[3]. As a part of this program, we initiated the search for microorganisms from tropical rain forests with oxidoreductase activity. The prochiral ketones **1-5** were reduced by whole cells cultures of *Rhizopus oryzae* CCT 4964, *Aspergillus terreus* CCT 3320 and *Aspergillus terreus* CCT 4083 (Scheme 1 and Table I)[4].



Scheme 1. **Mechanistic pathway proposed for the biotransformation of acetophenones and aryl ethanols by whole cells of fungi**

Different selectivities were observed for each of the mentioned microorganism. The deracemization of alcohols **1a-5a** was achieved with high enantiomeric excess when whole cells of *Aspergillus terreus* CCT 3320 and *Aspergillus terreus* CCT 4083 were used (Scheme 1 and Table II). The enantioselectivity was anti-Prelog in some cases. In conclusion, we developed an efficient method to reduce acetophenones to the corresponding alcohols using whole cells of new strains of fungi native of the rain forests, which proved to be excellent biocatalysts for this purpose. In addition, *A. terreus* CCT 3320 and *A. terreus* CCT 4083 promoted the efficient deracemization of aryl ethanols. These results demonstrate that the investigated microorganisms have a great bioenzymatic potential to perform reduction and deracemization reactions. Further studies with these microbial cells of native Brazilian fungi are in progress in our group.

Table I
Reduction of acetophenones by whole cells of fungi

#	*Aspergillus terreus* CCT 3320			*Aspergillus terreus* CCT 4083			*Rhizopus oryzae* CCT 4964		
ketone	t	c (%)	*e. e.* (%)*	t	c (%)	*e. e.* (%)*	t	c (%)	*e. e.* (%)*
1	3	69	65 *(S)*[a]	3	91	57 *(S)*[a]	1	91	98 *(S)*
	17	98	72 *(R)*[a]	17	94	66 *(R)*[a]	3	92	>99 *(S)*[b]
2	3	45	76 *(R)*	3	63	26 *(S)*	1	91	57 *(S)*
	6	91	>99 *(R)*[c]	8	75	83 *(S)*	3	92	62 *(S)*
3	3	35	94 *(S)*	3	52	70 *(S)*	1	23	76 *(R)*
	8	29	97 *(S)*[d]	8	31	22 *(S)*	3	84	66 *(R)*
4	2	55	81 *(R)*	1	96	91 *(R)*[a]	–	–	–
	5	57	70 *(R)*	11	98	65 *(S)*[a]	–	–	–
5	1	98	82 *(S)*	1	100	53 *(S)*	1	86	79 *(R)*
	6	100	96 *(S)*	9	99	51 *(S)*	3	63	87 *(R)*

t – time (days), c – conversion in alcohol, *e. e* – enantiomeric excess, [a]deracemization occured, [b]isolated yield *(S)*-(-)-**1a**: 90 %, [c]isolated yield *(R)*-(+)-**2a** 59 %, [d]isolated yield *(S)*-**3a** 18 %,. **e. e.* and conversion calculated by GC analysis (capillary column: chiral Chirasil-Dex CB β-cyclodextrin).

Table II
Deracemization of phenyl ethanols by whole cells of fungi

#	*Aspergillus terreus* CCT 3320				*Aspergillus terreus* CCT 4083			
alcohol	t	c (%)	c* (%)	*e. e.* (%)**	t	c (%)	c* (%)	*e. e.* (%)**
1a	3	4	8	21 *(S)*	3	–	4	14 *(S)*
	5	7	10	51 *(S)*	5	–	10	25 *(S)*
	8	2	6	61 *(S)*	8	–	21	52 *(S)*
2a	3	18	82	68 *(R)*	3	14	86	75 *(S)*
	5	12	88	>99 *(R)*	5	18	82	95 *(S)*
	7	12	88	>99 *(R)*[a]	7	16	84	97 *(S)*[b]
4a	4	32	68	70 *(R)*	3	12	88	6
	6	37	63	74 *(R)*	5	12	88	8
	9	43	57	74 *(R)*	17	21	79	>99 *(S)*
5a	4	98	02	–	5	18	82	84 *(R)*
	6	100	00	–	6	15	85	95 *(R)*
	9	100	00	–	7	14	86	>99 *(R)*

T – time (days), c – conversion in acetophenone, c* – conversion in alcohol, *e. e.*: enantiomeric excess, [a]isolated yield *(R)*-(+)-**2a**: 35 %, [b]isolated yield *(S)*-(-)-**2a** 59 %, ***e. e.* and conversion calculated by GC analysis (capillary column: chiral Chirasil-Dex CB β-cyclodextrin)

REFERENCES

1. Faber K.: *Biotransformations in Organic Chemistry, 4th edition*. Springer, New York 2000.
2. Porto A. L. M., Cassiola F., Dias S. L. P., Joekes I., Gushikem Y., Rodrigues, J. A. R. Moran P. J. S., Manfio G. P., Marsailoi A. J.: J. Mol. Catal B: Enzym. *1–8*, 810 (2002).
3. Cagnon J. R., Porto A. L. M., Manfio G. P., Eguchi S. Y., Marsaioli A. J.: Chemosphere *38*, 2237 (1999).
4. Comasseto J. V., Omori A. T., Andrade L. H., Porto A. L. M.: Tetrahedron: Asymmetry, in press.

P189 ENANTIOSELECTIVE REDUCTION OF 1-(1,3-BENZODIOXOL-5-YL)-2-HALO-1--ETHANONES BY *Rhodotorula glutinis* CCT 2182

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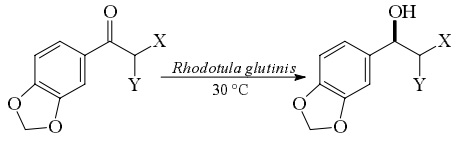
Keywords: asymmetric reduction, 1,2-aminoalcohols, *Rhodotorula glutinis*

The stereoselective synthesis of optically pure 1,2-aminoalcohols is an important issue since these compounds are versatile chiral building blocks for the synthesis of chemotherapeutic drugs, chiral auxiliaries and other chiral intermediates in organic synthesis[1,2].

Table I shows that *Rhodotorula glutinis* CCT 2182 was able to convert 1-(1,3-benzodioxol-5-yl)-2-halo-1-ethanones **1a-d** into the corresponding *(R)*-halohydrines **2a-d** in good yields and excellent enantiomeric excesses. However, the yeast was unable to reduce ketone **1e**. The *(R)*-halohydrines **2a-b** may be used as raw materials for the preparation of the pharmaceuticals *(R)*-(-)-epinefrine, *(R)*-(-)-norepinefrine and *(R)*-(-)-isoproterenol.



1 **2**

a: X = Cl, Y = H
b: X = Br, Y = H
c: X = Cl, Y = Cl
d: X = Br, Y = Br
e: X = H, Y = H

Table I
Asymmetric reduction of ketones **1a–e** by *Rhodotorula glutinis* CCT 2182[a]

Ketone	Alcohol	Yield (%)	$[\alpha]_D^{20b}$	e. e.[c] (%)	Configuration
1a	**2a**	98	–36.0	>99	*(R)*
1b	**2b**	57	–33.2	>99	*(R)*
1c	**2c**	88	–19.0	76	*(R)*
1d	**2b**	92	–11.7	72	*(R)*
1e	**2e**	–	–	–	–

[a]T = 30 °C, 24 h, 1 mmol/5 g (ketone/*Rhodotorula glutinis*), 100 ml H_2O (1.5 ml of EtOH); [b]*c* 1, $CHCl_3$; [c]determined by GC-MS analysis (capillary chiral column CHIRASIL-DEX)

REFERENCES

1. Cavallo S. A., Lapitajs G., Buchert P., Klein A., Colonna S., Manfredi A: J. Organomet. Chem. *330*, 357 (1987).
2. Ager D. J., Prakash I., Schaad D. R.: Chem. Rev. *96*, 835 (1996).

P190 HIGHLY EFFICIENT EXTRACTIVE BIOCATALYSIS WITH AMBERLITE XAD-7 IN THE ASYMMETRIC REDUCTION OF ENONES BY *Pichia kluyveri* AND *Rhodotorula glutinis*

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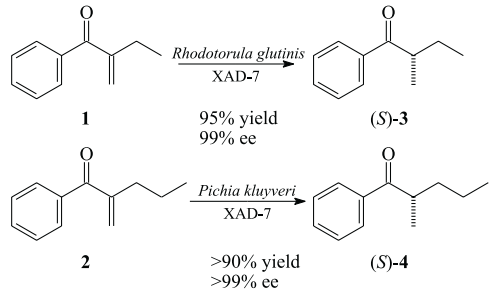
Keywords: extractive biocatalysis, asymmetric reduction, α-methyleneketones, non-conventional yeasts, Amberlite XAD-7

Chiral α-substituted ketones are versatile building blocks for the synthesis of natural products, especially in pheromone synthesis. Although it has been generally accepted that enzymatic transformations are suitable methods to obtain chiral compounds, little is known about stereoselective α-substituted ketone synthesis through α-methyleneketone reduction[1].

Herein, we describe the high regio- and enantioselective reduction of enones **1** and **2** to the corresponding saturated ketones **3** and **4**, by the yeasts *Pichia kluyveri* and *Rhodotorula glutinis* using Amberlite XAD-7 as adsorbing resin (extractive biocatalysis).



We found that the use of Amberlite XAD-7 as adsorbent of the enones played a crucial role in the reaction profile with two biocatalysts. In the absence of XAD-7 poor yields and enantiomeric excesses were achieved. On the other hand, growing amounts of XAD-7 sensibly improved the yields and ee's of the products. Best results with adsorbed enones **1** and **2** onto XAD-7 were achieved with *Rhodotorula glutinis* and *Pichia kluyveri* as biocatalysts, respectively.

In conclusion, the maintenance of low concentrations of the substrates in the aqueous phase by the use XAD-7 as adsorbing resin in the course of the reduction of enones **1** and **2** by suitable yeasts allowed us to obtain ketones **3** and **4** in high yields and enantiomeric excesses. Therefore, the extractive biocatalysis method proves again to be a powerful tool when inicial concentration and toxicity of the substrate are the limiting issues in the reaction[2].

REFERENCES

1. Filho E. P. S., Rodrigues J. A. R., Moran P. J. S.: Tetrahedron: Asymmetry *12*, 847 (2001).
2. Conceição G. J. A., Moran. J. S., Rodrigues J. A. R.: Tetrahedron: Asymmetry *14*, 43 (2003).

P191 ENZYMATIC DYNAMIC KINETIC RESOLUTION OF (±)-2-HYDROXY-1-INDANONE BY *Trichosporon cutaneum*: A SHORTCUT TO HOMOCHIRAL (1*S*,2*R*)-1,2-INDANDIOL

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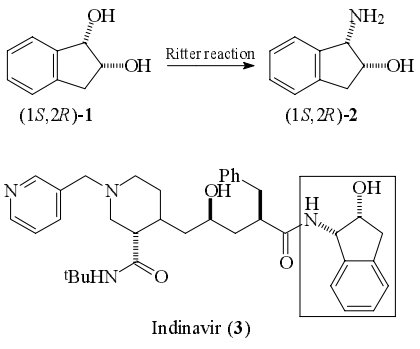
Keywords: enzymatic dynamic kinetic resolution, asymmetric reduction, (1*S*,2*R*)-1,2-indandiol, *Trichosporon cutaneum*

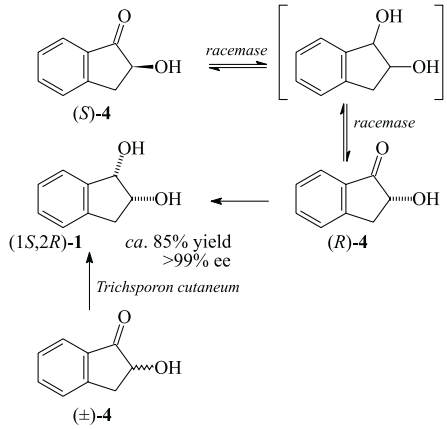
The (1*S*,2*R*)-1,2-indandiol **(1)** is a well stablished precursor to the (1*S*,2*R*)-1-amino-2-indanol **(2)**, a key raw material in the synthesis of the leading HIV-I protease inhibitor oligopeptide mimic Indinavir **(3)** (Scheme 1). Hitherto, only few biocatalytic roots to **1** have been delivered, *i. e.*: (i) microbial asymmetric dioxygenation of indene[1] and (ii) *Trichosporon cutaneum* MY 1506-mediated asymmetric reduction of 1,2-indanedione[2]. In turn, diol **1** can be promptly converted to **2** through a Ritter reaction[3].



Scheme 1

We wish to report a highly efficient enantio-convergent preparation of homochiral (1*S*,2*R*)-1,2-indandiol **(1)** through a enzymatic dynamic kinetic resolution of (±)-2-hydroxy-1--indanone **(4)** with stereoselective reduction mediated by resting cells of the non-conventional yeast *Trichosporon cutaneum* CCT 1903 (TC). Accordingly, when 1 g of racemic benzoin **4** was added to a slurry of resting cells of TC and glucose in distilled water, we were able to recover the (1*S*,2*R*)-1,2-indandiol **(1)** in *ca.* 85 % isolated yield plus >99 % e. e. after 3–4 days of incubation on an orbital shaker (150 rpm) at 28 °C (Scheme 2).

The intervention of a highly active racemase acting in the enzymatic dynamic kinetic resolution of the (±)-2-hydroxy--1-indanone **(4)** is evoked (Scheme 2). In conclusion, we have disclosed a valuable shortcut to homochiral (1*S*,2*R*)-1,2-indandiol in comparison with the previously reported method[2].



Scheme 2

REFERENCES

1. Chartrain M., Jackey B., Taylor C., Sandford V., Gbewonyo K., Lister L., DiMichele L., Hirsch C., Heimbuch B., Maxwell C., Pascoe D., Buckland B., Greasham R.: J. Ferm. Bioeng. *86*, 550 (1998).
2. Stahl S., Ikemoto N., King A., Greasham R., Chartrain M.: J. Biosc. Bioeng. *88*, 495 (1999).
3. Senanayake C. H., DiMichele L. M., Liu J., Fredenburgh L. E., Ryan K. M., Roberts F. E., Larsen L. D., Verhoeven T. R., Reider P. J.: Tetrahedron Lett. *36*, 7615 (1995).

P192 ASYMMETRIC HYDROGENATION OF NITROALKENES USING *Clostridium sporogenes*

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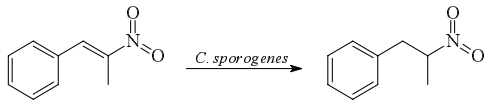
Keywords: asymmetric hydrogenation, nitroalkenes, *Clostridium sporogenes*, anaerobic bacteria

Asymmetric hydrogenation of nitroalkenes would be extremely valuable for the synthesis of chiral building blocks. In general, these substrates cannot be reduced enantioselectively by metal catalysts, whilst Baker's yeast and aerobic microorganisms have a limited substrate range and reduce the carbon-carbon double bond with very low efficiency. We have developed several anaerobic bacteria as novel biocatalysts which reduce nitroalkenes with good enantioselectivity and efficiency, and we are now studying the optimisation of the reactions.

Initially, we optimized the growth conditions for production of *C. sporogenes* to obtain maximal biocatalytic activity in harvested cells. Although glucose supported better growth than phenylalanine, the rate of cinnamate reduction was approximately 2-fold higher after growth on phenylalanine than on glucose. Addition of phenylalanine or cinnamate to cultures growing on glucose did not improve growth or increase the rate of cinnamate reduction using harvested cells. When *C. sporogenes* was grown in chemostat culture, the rate of cinnamate reduction increased with pH and with decreasing dilution rate. The reaction conditions for C = C reduction using harvested cells were also optimised. The efficiency of electron donors for reducing cinnamate was in the order hydrogen > phenylalanine > glucose.



The optimised biocatalyst production and reaction systems were used for the reduction of 2-nitro-1-phenyl-1-propene, using an anaerobic two liquid phase reaction system to protect the microorganism from substrate toxicity. The enantiopurity of the nitroalkane product depended on the growth substrate used to produce the biocatalyst and on the substrate concentration in the reaction. We are currently investigating the reasons for the varying enantioselectivity, with the aim of developing methods to control enantiopurity of the nitroalkane product.

P193 MULTI BIOREACTION SCREENING, A TOOL TO DISCOVER NEW ENZYMATIC ACTIVITIES

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Keywords: multi bioreaction screening, *Serratia rubidea*, *Saccharomyces cereviseae*

Biocatalysis has been pushed towards high throughput screening (HTS)[1, 2] in order to monitor enzymatic activity of libraries of natural or modified microorganisms. Among the most popular HTS methodologies are the high performance liquid chromatography and gas chromatography associated to mass spectrometry. Reagent based assays are also good alternatives which require the modification of the substrate by inserting a fluorogenic or chromogenic moieties. In principle all are efficient and are based on one reaction and several microorganisms or several enzymes (namely in 96 or 398 microtiter plates). Hoping to contribute with this worldwide trend we visualized a high throughput screening of reactions namely several substrates possessing different functional groups to be simultaneously by several microorganisms. This would increase the speed of the HTS *n* times, where *n* is the number of added substrates.

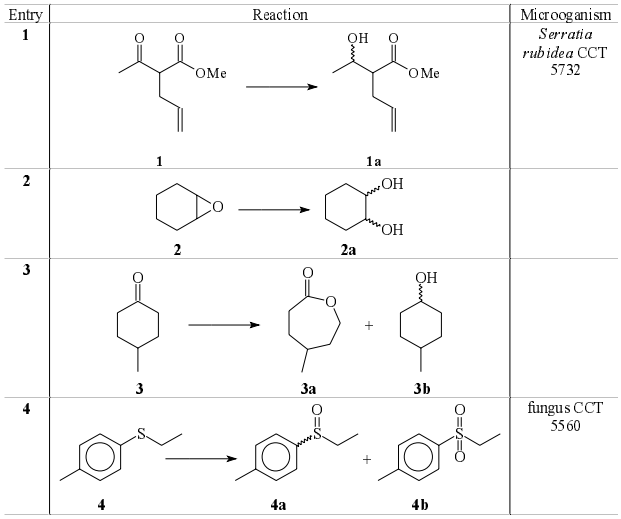
To test the effectiveness of the methodology we have selected 2 Brazilian microorganisms (*Serratia rubidea* CCT 5732 and the fungus CCT 5560) under study in our laboratory and one strain of *Saccharomyces cereviseae* DSM 0195. We selected substrates **1-4** with the objective of simultaneously monitoring ketone oxidation and reduction, sulfoxidation and epoxide hydrolysis. These reactions had been previously studied detecting microorganisms that could better perform them in terms of reaction time, yield and enantiomeric excesses. In biotransformations the substrate concentration has to be below the minimum inhibitory concentration (MIC) but taking into consideration that each reagent will act individually we could add 4 times the usual amount of xenobiotics to the cell suspensions and none of the individual substrates would be above the MIC. Notwithstanding this rationale we added only 50 mg of xenobiotic (mixture of compounds **1-4**) per 2 g (wet weight) of MO cells.

The reactions were monitored by GC/MS and indeed the *Serratia rubidea* CCT 5732 known to have oxidoredutase activity preferentially transformed substrate **1** into *syn* and *anti* **1a** in 2 hours leaving the remaining substrates with little or no modification. The same behavious was observed when treating the reaction individually (Table I). The reaction monitoring was continued for 20 hr when 1a was degraded and phenyltioderivatives was observed.

Fungus CCT 5560 had been previously selected as a good microorganism for sulfoxidation yielding chiral sulfoxides in

good yield and high enantiomeric excess. During this multibioreaction screening this same fungus was also selected for sulfoxidation just like in the individual screening.

Table I
Multibioreaction screening



Finally we are conscious that this methodology requires optimization but the central idea is sound and proved to be correct allowing the screen of a chemical mixture and reaching the same conclusion of individual substrate screening but n times faster were n is the number of added substrates.

The authors are indebted to FAPESP, CNPq and CAPES for grants and scholarship.

REFERENCES

1. Reetz M. T.: Angew. Chem. Int. Edit. *41*, 1335 (2002); Reetz M. T.: Tetrahedron *58*, 6595 (2002).
2. Reymond J.: Chimia *55*, 1049 (2001); Reymond J.: Curr. Opin. Biotechnol. *12*, 535 (2001).

P194 ENANTIOSELECTIVE OXIDATION OF SULFIDES AND SULFINIC ACID ESTER WITH THE AID OF BRAZILIAN MICROORGANISMS

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Keywords: organosulfur compounds oxidation, chiral sulfinates, chiral sulfoxides

The asymmetric oxidation of sulfide is one of the most convenient route to chiral sulfoxides which depends on chiral environments (electrodes[1], clays[2], helices[3], cyclodextrins[4]) and this is either limited to specific substrates or gives products in only moderate to low enantiomeric excesses. A new horizon was envisaged with Kagan-Modena's method using the modified Sharpless reagent[5,6] and giving access to useful chiral sulfoxides. The classic Andersen's chiral sulfoxide synthesis relies on the nucleophilic substitution on diastereomerically pure *(S,S)* menthyl *p*-toluenesulfinate[7] but high enantiomeric excesses are mainly obtained with *p*-tolyl derivatives. Focusing on the enzymatic oxidation of organo sulfur derivatives by Brazilian microorganisms as an alternative way to obtain chiral sulfoxides we have selected two major approaches: one by direct oxidation of sulfide derivatives and the second by an enzymatic Andersens's synthesis, that is, kinetic resolution of sulfinates viewing the production of chiral sulfoxides. In a previous program we obtained chiral sulfoxides in high enantiomeric excesses from organosulfur compounds using fungi whole cells either resting or immobilized. The conversions were not satisfactory due to the subsequent transformation of one of the sulfoxides into sulfone. At the time the selected microorganisms produced sulfoxides with *(S)* configuration from a wide range of benzyl alkyl and phenyl alkyl sulfide[8,9]. To access the oxidation of sulfides with little or no production of sulfone derivatives we have screened novel microorganisms using substrate **1**. The results are summarized in Table I.

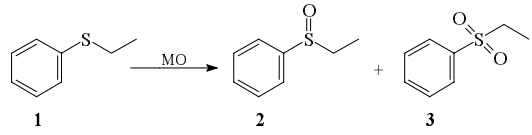

Table I
Biotransformation of phenyl ethyl sulfide **1** with Brazilian microorganisms

Entry	Microorganism	C (%)	2 (%)	3 (%)	ee (%)	Conf.
1	*Geotrichum candidum* CCT 5551	100	32	68	65	*S*
2	*Aspergillus niger* CCT 1435	100	34	66	76	*S*
3	Rhizopus oryzae CCT 4964	100	100	–	62	*S*
4	Fungi CCT 5551 (*Trigona sp*. bee)	97	97	–	68	*R*
5	*Nodulisporium sp.* CCT 5552	100	32	68	45	*R*
6	Fungus CCT 5553	75	71	4	>99	*R*
7	Fungus CCT 5560	83	83	–	85	*R*
8	Fungus CCT 5630	100	67	33	3	*S*
9	Fungus CCT 5632	80	80	–	>0	*R*
10	Fungus CCT 5635	100	96	4	53	*R*
11	Fungus CCT 5661	98	74	24	96	*S*
12	*Aspergillus ochraceus*	96	69	27	38	*S*

Thus fungus CCT 5553 isolated from a *Trigona* sp bee[10] was selected as the best candidate to transform sulfide **1** into sulfoxide **2** in >99 ee and 75 % conversion. Reaction scale up allowed a gram scale production of **2** which will be used in asymmetric synthesis. For the second approach we focused on the resolution of racemic sulfinates and sulfinates we first tested the stability of **4** and **5** in aqueous medium but only **5** did not hydrolyzed spontaneously. Therefore substrate **5** was

used to screen the enzymatic activity present in several microorganisms (Table II). As it can be appreciate by scrutinizing Table II, hydrolysis and oxidation are two competitive reactions. In all cases the hydrolysis was not enantioselective while sulfinate **5** of high enantiomeric purity was obtained by oxidative kinetic resolution. Notwithstanding this apparent sucesss the conversions obtained so far are not acceptable. Finally our results further improved the access to **2** in gram-scale with the alternative of choosing **2-***(R)* or the **2-***(S)* configuration. The biocatalytic method of obtaining chiral sulfoxides using the chiral sulfinate was also investigated by selecting microorganisms that resolved sulfinate **5**.

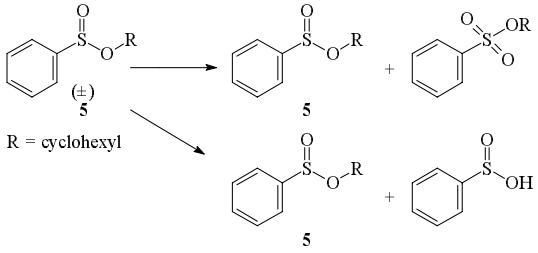

Table II
Kinetic resolution of sulfinates 5 with Brazilian microorganisms

Entry	Microorganism	5 ee (%)	Reaction
1	*Geotrichum candidum* CCT 5551	95	oxidation
2	*Aspergillus niger* CCT 1435	–	hydrolysis
3	*Rhizopus oryzae* CCT 4964	25	oxidation
4	*Aspergillus terreus* CCT 3320	95	oxidation
5	*Nodulisporium sp.* CCT 5552	–	hydrolysis
6	Fungus CCT 5553	44	oxidation
7	Fungus CCT 5560	–	hydrolysis
8	*Aspergillus ochraceus*	99	oxidation

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REFERENCES

1. Komori T., Nonako T.: J. Am. Chem. Soc. *106*, 2656 (1984).
2. Yamagishi A. J.: Chem Soc., Chem. Commun. *1986*, 290.
3. Vögtle F., Struck J., Puff H., Woller P., Reuter H.: J. Chem. Soc., Chem. Commun. *1986*, 1248.
4. Czarnik A. W.: J. Org. Chem. *49*, 924 (1984).
5. Pichen P., Kagan H. B.: Tetrahedron Lett. *25*, 1953 (1984).
6. Di Fúria F., Modena G., Seraglia R.: Synthesis *1984*, 325.
7. Andersen K. K.: Tetrahedron Lett. *3*, 93 (1962).
8. Porto A. L. M.: *DSc. Thesis*, Instituto de Química – UNICAMP (2002).
9. Porto A. L. M., Cassiola F., Dias S. L. P., Joekes I., Gushikem Y., Rodrigues J. A. R., Moran P. J. S., Manfio G. P., Marsaioli A. J.: J. Mol. Catal. B: Enzym. *19*, 327 (2002).
10. Bicalho B., Gonçalves R. A. C., Zibordi A. P. M., Manfio G. P., Marsaioli A. J.: Z. Naturforsch., in press.

P195 ASYMMETRIC SYNTHESIS OF ARYLSELENOALCOHOLS BY MEANS OF THE REDUCTION OF ORGANOSELENO ACETOPHENONES BY FUNGI

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Keywords: bioreduction, organoselenium, ketone, chiral, alcohol

In recent years chiral organoselenium compounds have been prepared and some of them were applied in asymmetric synthesis[1]. This concern constitutes a new trend in this field of organoelemental chemistry. Thus, the search for efficient methods for preparing organoselenium compounds with high enantiomeric purities is an important goal in this area of chemistry. In this context, biocatalysis has attracted much attention for being an important method in the preparation of optically active compounds with high stereoselectivity under environmentally friendly conditions[2]. In this communication we report the bioreduction of organoseleno acetophenones **3a-f** with whole cells of fungi (Fig. 1).

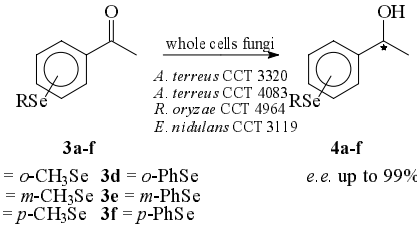

Fig. 1. **Asymmetric bioreduction of seleno ketones 3a–f with whole cells fungi**

We screened for microorganisms which are able to perform the reduction of the carbonyl group. The biotransformation was performed by re-suspending the wet cells of the appropriate fungi (4 g) in a phosphate buffer solution (50 ml, pH 7.0, 0.1 M) in a 125-ml Erlenmeyer flask. To these cells suspensions, solutions of ketones **3a-f** (20 mg) in ethanol (0.5 ml) were added and the mixtures were incubated in a rotary shaker (170 rpm) at 32 °C for times indicated in Table I. The progress of the biotransformation was monitored by GLC. The results are summarized in the Table I. As can be observed, two of the six seleno ketones **3**, namely *ortho*-methylseleno acetophenone **3a** and *ortho*-phenylseleno acetophenone **3d**, were unreactive toward the four investigated fungi under the bioreduction conditions employed. The other selenoacetophenones were reduced with good conver-

sion rates and ee. The results obtained in the bioreduction of seleno ketones **3c**, **3e** and **3f** showed that the fungus *Rhizopus oryzae* CCT 4964 gave the best values of conversion and enantiomeric excess for preparation of organoseleno-α--methylbenzyl alcohol **4**. To our knowledge this is the first reported biotransformatiom of a selenium compound for synthetic purposes.

Table I
Bioreduction of seleno ketones

Entry	Substrate	Whole cells fungi	Time (day)	Conversion (%)[a]	Product **4** ee (%)[b]
1	**3a**	*Rhizopus oryzae* CCT 4964	7	n. c.	–
2	**3b**	*Rhizopus oryzae* CCT 4964	2	99 (55)[c]	94
3	**3c**	*Rhizopus oryzae* CCT 4964	2	91 (50)[c]	96
4	**3d**	*Rhizopus oryzae* CCT 4964	7	n. c.	–
5	**3e**	*Rhizopus oryzae* CCT 4964	7	90 (27)[c]	87
6	**3f**	*Rhizopus oryzae* CCT 4964	7	85 (25)[c]	71
7	**3a**	*Aspergillus terreus* CCT 4083	7	n. c.	–
8	**3b**	*Aspergillus terreus* CCT 4083	2	76	90
9	**3c**	*Aspergillus terreus* CCT 4083	2	86	55
10	**3d**	*Aspergillus terreus* CCT 4083	7	n. c.	–
11	**3e**	*Aspergillus terreus* CCT 4083	9	21	47
12	**3f**	*Aspergillus terreus* CCT 4083	9	12	45
13	**3a**	*Aspergillus terreus* CCT 3320	7	n. c.	–
14	**3b**	*Aspergillus terreus* CCT 3320	2	75	86
15	**3c**	*Aspergillus terreus* CCT 3320	10	75	95
16	**3d**	*Aspergillus terreus* CCT 3320	7	n. c.	–
17	**3e**	*Aspergillus terreus* CCT 3320	7	41	99
18	**3f**	*Aspergillus terreus* CCT 3320	7	n. c.	–
19	**3a**	*Emericella nidulans* CCT 3119	7	n.c.	–
20	**3b**	*Emericella nidulans* CCT 3119	3	9	67
21	**3c**	*Emericella nidulans* CCT 3119	5	99	99
22	**3d**	*Emericella nidulans* CCT 3119	7	n. c.	–
23	**3e**	*Emericella nidulans* CCT 3119	10	n. c.	–
24	**3f**	*Emericella nidulans* CCT 3119	7	n. c.	–

[a]Conversion determined by GC, isolated yield in parentheses, n. c. = = no conversion, [b]determined by GC analysis using a chiral column (Chirasil-Dex CB β-cyclodextrin 25 m × 0.25 mm) **(4a-d)** , by chiral HPLC analysis after reaction with 3,5-dinitrobenzoyl chloride (column Astec-Cyclobond I 2000 SN: hexane/isopropanol = 99/1) **(4e, 4f)**, [c]100 mg of substrate was used

REFERENCES

1. Wirth T.: Angew. Chem. Int. Edit. *39*, 3740 (2000).
2. Faber K.: *Biotransformations in Organic Chemistry, 4th edition*. Springer, New York 2000.

P196 ENANTIOSELECTIVE SULFIDE OXIDATION CATALYSED BY RECOMBINANT *Escherichia coli* WHOLE CELLS

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Keywords: whole-cells, cyclohexanone monooxygenase, hydrophobic resin

A recombiant strain of *Escherichia coli* (TOP 10 pQR239)[1] expressing cyclohexanone monooxygenase (CHMO) from *Acinetobacter calcoaceticus* sp. NCIMB 9871 has been used as whole cell biocatalyst for the oxidation of 1,3-dithiane[2,3] to the corresponding enantiopure *(R)*-sulfoxide (ee > 98 %) (Fig. 1).

CHMO suffers from substrate and product inhibition and, furthermore, 1,3-dithiane shows low solubility in the biotransformation medium. These limitations have been overcome by using a polymeric hydrophobic resin[4]. After a series of substrate/product binding tests on a variety of resins with different hydrophobic properties, we choose SP207 (Sepabeads – Resindion). This acrylic resin was able to adsorb 1,3-dithiane, thus acting as a reservoir for high substrate concentrations. Furthermore, the product was also adsorbed onto the resin, thus allowing to remove it from the reaction mixture as it formed.

This approach has made it possible to increase 1,3-dithiane concentration in the reaction medium from 1 g.l^{-1} to 5 g.l^{-1} with complete substrate conversion.

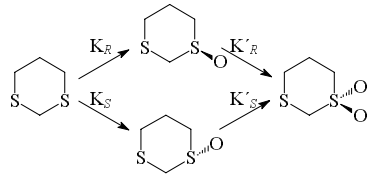

Fig 1. **CHMO catalysed oxidation of 1,3-dithiane to *(R)*-sulfoxide**; sulfone started forming only after complete substrate consumption.

REFERENCES

1. Doig S. D., O'Sullivan L. M., Patel S., Ward J. M., Woodley J. M.: Enzyme Microb. Technol. *28*, 265 (2001).
2. Colonna S., Gaggero N., Bertinotti A., Carrea G., Pasta P., Bernardi A.: Chem. Commun. *1995*, 1123.
3. Alphand V., Gaggero N., Colonna S., Pasta P., Furstoss R.: Tetrahedron *53*, 9695 (1997).
4. Lye G. J., Woodley J. M.: Tibtech *17*, 395 (1999).

P197 APPLICATION OF BACTERIAL ALDEHYDE OXIDATION SYSTEM FOR SIMPLE PREPARATION OF USEFUL ACIDS

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Keywords: application, bacterial aldehyde oxidation, useful acids

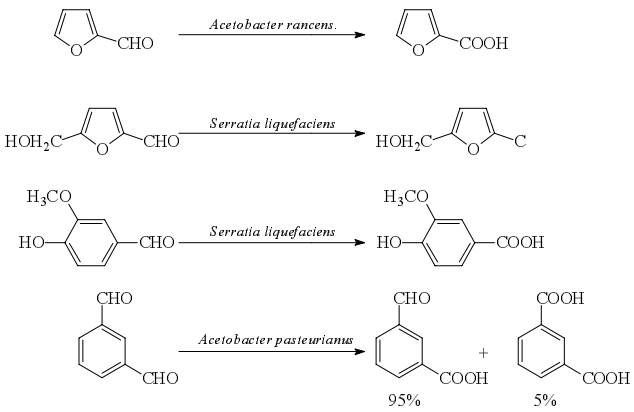
For the accomplishment of "green chemistry", the environmental friendly bioprocess should be introduced into chemical industry. The bacterial aldehyde oxidation system has been applied for the synthesis of various useful acids.

We surveyed the powerful aldehyde-oxidizing bacteria through the conventional enrichment culture technique. In addition to *Acetobacter* and *Gluconobacter* strains, we found that *Pseudomonas fluorescens* and *Serratia liquefaciens* exhibit the powerful aldehyde oxidation activity. We apply these bacterial aldehyde oxidation system for the rapid and convenient preparation of various useful acids from the corresponding aldehyde.

1) Syntheses of 2-furancarboxylic acid and 5-hydroxymethylfuran-2-carboxylic acid: Furfural and 5-hydroxymethyl-2-furaldehyde are abundant natural renewable resources obtained easily through the heat-distillation of pentosan and hexosan treated with the diluted sulfuric acid, respectively. 2-Furancarboxylic acid is used for the synthesis of various pharmaceuticals, perfumes, agricultural chemicals and resins. Although several synthetic methods of 2-furancarboxylic acid from furfural have been reported, there have been some problems to be resolved, particularly, for its production on a large scale. We focused on the bacterial aldehyde oxidizing capability. *Acetobacter rancens* and *Gluconobacter cerinus* oxidized furfural to produce 2-furancarboxylic acid. The accumulation of 2-furancarboxylic acid was 110 $g.l^{-1}$ with 83 % molar conversion yield for 30 h with the resting cells of *Acetobacter rancens*. *Serratia liquefaciens* converted 5-hydroxymethyl-2-furaldehyde into 5-hydroxymethylfuran-2-carboxylic acid. The accumulation of 5-hydroxymethylfuran-2-carboxylic acid reached 32.6 $g.l^{-1}$ with 88 % molar conversion yield for 26 h. The hydroxymethyl group of 5-hydroxymethyl-2-furaldehyde was inert and the oxidation was very specific to aldehyde. Compared to the oxidation of 5-hydroxymethyl-2-furaldehyde by chloroperoxidase[1], *Serratia liquefaciens* oxidation activity was very specific to aldehyde.

2) Synthesis of vanilinic acid: We also established the efficient conversion of vanillin into vanillic acid. Among tested aldehyde-oxidizing bacteria, *Serratia liquefaciens* showed highest productivity of vanillic acid. The accumulation of vanillic acid was 70.6 $g.l^{-1}$ with 89 % molar conversion yield for 12 h.

3) Selective oxidation of dialdehyde (*o*, *m*, and *p*-phthaladehyde): The selective oxidation of dialdehydes such as *o*, *m*, and *p*-phthaladehyde was also examined using aldehyde-oxidizing bacteria. When the oxidation of *m*-phthaladehyde as a substrate was examined using *Acetobacter pasteurianus* resting cells, *m*-phthalaldehydic acid was preferentially formed with 95 % conversion, and then formation of *m*-phthalic acid was only 5 %. The accumulation of *m*-phthalaldehydic acid reached 36.8 $g.l^{-1}$ with 88 % molar conversion yield for 40 h. On the other hand, the selectivity of oxidation of *p*-phthaladehyde was poor and the oxidation of *o*-phthaladehyde almost did not proceed.



REFERENCES

1. van Deurzen M. P. J., van Rantwijk F., Sheldon R. A.: J. Carbohydr. Chem. *16*, 299 (1997).

P198 MICROBIAL DERACEMIZATION OF α-SUBSTITUTED CARBOXYLIC ACIDS

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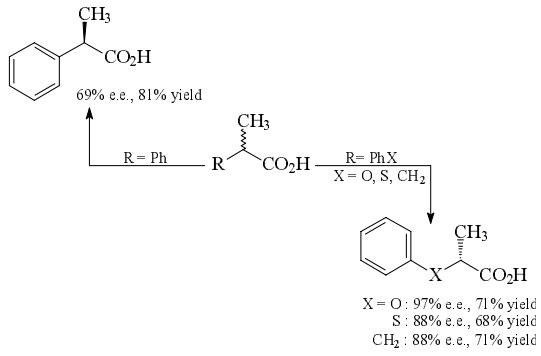
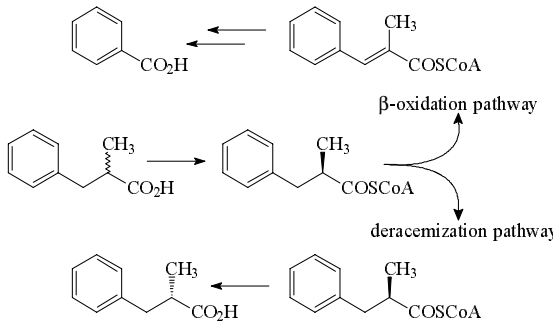
Keywords: deracemization, chiral inversion, suppression of metabolism, reaction mechanism, α-substituted carboxylic acids

Biocatalytic methods such as kinetic resolution for racemates provide you with an easy access to the optically active compounds. Unfortunately, however, these techniques are not ultimate methods obtaining the optically active products. These have disadvantages. In case of kinetic resolution, the maximum yield of the desired enantiomer is limited to 50 %, although this technique is already well-established to prepare the optically active products from their racemates. Recently, we have reported a new approach obtaining the optically active *(R)*-2-aryl- and *(R)*-2-aryloxypropanoic acid starting from their racemates by the aid of the growing cells of *Nocardia diaphanozonaria* JCM3208 (Scheme 1)[1]. This method is known as deracemization reaction and is capable of overcoming the drawback of the kinetic resolution process. Theoretically, deracemization reaction could give the desired enantiomer in 100% yield. It means that the synthesis of racemates is almost equal to the synthesis of optically active compounds and this concept is entirely different from the commonly accepted one in the asymmetric synthesis.



Scheme 1

In this poster, we would like to present the new design of reaction conditions to suppress the metabolic reactions for two types of compounds, 2-phenylthiopropanoic acid and 2-methyl-3-phenylpropanoic acid, and to proceed preferentially the deracemization reaction (Scheme 1). In addition, the investigation of the reaction mechanism using the cell free extracts as well as the whole cells indicate that this deracemization process is competitive reaction against the β-oxidation pathway of fatty acid metabolism (Scheme 2). Also, it was found that new types of enzymes take part in this system, although the reaction proceeds by way of the same mechanism as that in rat liver[2].



Scheme 2

REFERENCES

1. Kato D., Mitsuda S, Ohta H.: Org. Lett. *4*, 371 (2002).
2. Shieh W.-R., Chen C.-S.: J. Biol. Chem. *268*, 3487 (1993).

P199 MICROBIAL DERACEMISATION OF β-HYDROXY ESTERS – AN IMPORTANT STRATEGY TOWARDS VARIOUS CHIRAL INTERMEDIATES

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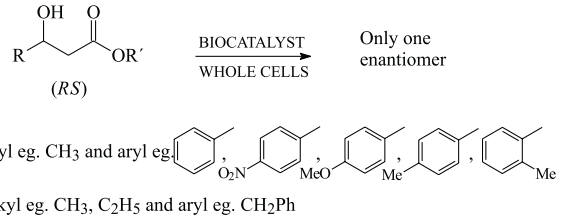
Keywords: deracemisation, β-hydroxy esters, microbial whole cells

Chiral β-hydroxy esters are important building blocks in organic synthesis especially in the preparation of natural and pharmaceutical products[1]. Several chemical[2] and biocatalytic[3] methods are known for preparing optically pure β-hydroxy esters. Among the biocatalysts, lipases, generally known for esterification and transesterifications[4] are extensively employed for the resolution[5] of secondary alcohols. The disadvanages of the reported methods are mainly low yields (maximum 50 %) and separation of the products after resolution or the use of expensive cofactors for isolated enzyme mediated asymmetric reduction. Deracemisation[6] is an attractive alternative as it permits the conversion of racemic

β-hydroxy esters to optically pure hydroxy esters in high enantiomeric excess as well as high yield. It is a novel approach for the synthesis of optically pure β-hydroxy esters . The starting materials for this reaction are racemic β-hydroxy esters[7]. This study will highlight the biocatalytic synthesis of optically pure β-hydroxy esters by deracemisation using microbial whole cells. The substrate specificity of the biocatalyst will be presented using a variety of aryl and alkyl β-hydroxy esters. A possible mechanism of the microbial deracemisation reaction will also be presented.



REFERENCES

1. Zhou B., Gopalan A. S., Van Middlesworth F., Shieh W. R., Sih C. J.: J. Am. Chem. Soc. *105*, 5925 (1983); Corey E. J., Reichard G. A.: Tetrahedron Lett. *30*, 5207 (1989).
2. Wang Z., Zhao C., Pierce M. E, Fortunak J. M.: Tetrahedron: Asymmetry *10*, 225 (1999).
3. Seebach D., Zuger M. F., Giovannini F., Sonnleitner B., Fiechter A.: Angew. Chem. Int. Ed. Engl. *23*, 151 (1984); Rodriguez S., Schroeder K. T., Kayser M. M., Stewart J. D.: J. Org. Chem. *65*, 2586 (2000).
4. Priya K., Venugopal T., Chadha A.: Ind. J. Biochem. Biophysics. *39*, 259 (2002).
5. Hoff B. H., Anthonsen T.: Tetrahedron: Asymmetry *10*, 1401 (1999); Chadha A., Goergens U., Schneider M. P.: Tetrahedron: Asymmetry *4*, 1449 (1993); Chadha A., Manohar M.: Tetrahedron: Asymmetry *6*, 651 (1995).
6. Strauss U. T., Felfer U., Faber K.: Tetrahedron: Asymmetry *10*, 107 (1999); Allan G. R., Carnell A. J.: J. Org. Chem. *66*, 6495 (2001); Chadha A., Baskar B.: Tetrahedron: Asymmetry *13*, 1461 (2002).
7. Padhi S. K., Chadha A.: Synlett, accepted.

P200 MICROBIAL MODELS OF ANIMAL DRUG METABOLISM: MICROBIAL PREPARATION AND IDENTIFICATION OF HYDROXYLATED METABOLITES OF IRBESARTAN

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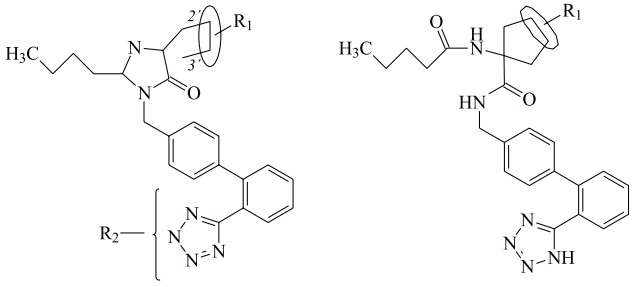
Keywords: drug metabolism, irbesartan, bacteria, *N*-glycosylation, hydroxylation

Irbesartan **(1)**, a recently developed and highly selective orally active non-peptidic angiotensin II receptor antagonist, is metabolized in animals and humans to give at least eight urinary metabolites[1–3]. Two of the minor metabolites correspond to monohydroxylated derivatives resulting from the oxidation of the spirocyclopentane ring. The stereochemical features of these metabolites have not been fully elucidated: indeed, symmetrical positions (2' and 3') on the cyclopentane ring are enantiotopic, due to a symmetry plane involving the substituted spiroheterocyclic ring. Hydroxylation on each of these positions, on either face, should generate enantiomeric pairs of *cis*- or *trans*-hydroxylated derivatives.



1: R_1 = H; R_2 = H
2: R_1 = OH; R_2 = H
3: R_1 = H; R_2 = 1-glycosyl

4: R_1 = OH

We describe some results about the biotransformation of irbesartan by various microbial species in order to prepare some of its metabolites in sufficient amounts to complete the determination of their structural and stereochemical characteristics. Depending on the strain used, each isomer **(2)** could be obtained in good yield together with the corresponding hydrolysed (open form) metabolite **(4)**. In addition, a *N*(tetrazole)-glycosylated metabolite **(3)**, analogous to the *N*-glucuronyl animal metabolite, was produced by some strains and fully characterized.

REFERENCES

1. Chando T. J., Everett D. W., Kahle A., Starrett A. M., Vachharajani N., Schyu W. C., Kripalani K. J., Barbhaiya R. H.: Drug Metab. Dispos. *26*, 408 (1998).
2. Bourrié M., Meunier V., Berger Y., Fabre G.: Drug Metab. Dispos. *27*, 288 (1999).
3. Davi H., Tronquet C., Miscoria G., Perrier L., Dupont P., Caix J., Simiand J., Berger Y.: Drug Metab. Dispos. *28*, 79 (2000).

P201 A HIGH THROUGHPUT SCREENING METHOD FOR THE STUDY OF MICROBIAL METABOLISM OF XENOBIOTICS AND THE GENERATION OF MOLECULAR DIVERSITY

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Keywords: biocombinatorial, microorganisms, microplate, metabolism, molecular diversity

A fast 96-well microplate system has been developed to explore the biotransformation activities of various microorganisms (fungi, yeasts, bacteria) upon synthetic or natural compounds of interest in the pharmaceutical, agrochemical or cosmetic fields. This miniaturized method, associated with sensitive and efficient microanalyses of the products formed (LC, GC-MS, LC-MS…), allows to select the best strains 1) to study the formation of microbial metabolites[1]; 2) to generate molecular diversity, in a biocombinatorial approach[2].

Several examples of this technique will be described, and compared with the corresponding results obtained in classical incubation conditions.

REFERENCES

1. Azerad R., in: *Adv. Biochem. Engin./Biotechnol. (Biotransformations)* (Faber K., Scheper T., ed.), Vol. 63, p. 169. Springer-Verlag, Berlin, Heidelberg 1999.
2. Rich J. O., Michels P. C., Khmelnitsky Y. L.: Curr. Opin. Chem. Biol. *6*, 161 (2002).

P202 PREDICTIVE BIOTRANSFORMATION OF POTENTIAL TOXIC CHEMICALS

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Keywords: predictive biotransformation, toxic chemicals, functional groups, enzymes

Over 18 million chemical substances are known, with more than 65,000 currently used in commerce. For past several decades, considerable progress has been made towards understanding environmental fates of these naturally occurring and synthetic compounds. The important role of microorganisms in these transformations and mineralization has been clearly established. Biodegradability is one of the most important characteristics of an organic compound to assess its life and fate in the environment[1]. For mineralization of a chemical to occur, several enzymes must act sequentially to transform or breakdown chemicals to simpler molecules that enter intermediary metabolism. With expert knowledge of enzyme substrate specificity, sequential steps in the metabolism of naturally occurring compounds, and catabolic pathways, one can identify structural features of synthetic chemicals that are likely to be substrates for enzyme(s)[2].

The ability to predict biodegradability of chemicals in the environment is of increasing importance due to concerns about the persistence and toxicity of the parent compounds and/or their metabolites in addition to high cost of empirically assessing their fate. The predictive information of how a particular chemical compound may be degraded has enormous implications for industry as well as for regulatory agencies[3]. Though, the biodegradability of chemicals in the environment is largely predicted on their ability to serve as substrates for microbial enzymes, enzymatic mechanisms have been determined for only a small percentage of these chemicals.

Focusing on the above aspect, the present study was carried out to predict plausible biotransformation routes for potential toxic chemicals using chemical heuristics and information resources (published and unpublished scientific literature). The chemical substances were chosen based on the extent of utilization by chemical industries and the listing in hazardous substances registers. The predictions were based within a framework of certain rules: (i) prediction of one possible chemical route, (ii) prediction of one possible biochemical route – a sequence of plausible enzymatic reactions, (iii) prediction of all possible biochemical routes to intermediary metabolism, and (iv) evaluation of biochemical reactions for chemical feasibility.

The first step is to predict the possible transformation of the most reactive site (in the test chemical substance) based

on chemical heuristics. The second step is to examine the scientific/experimental literature to verify the occurrence of the above transformation biochemically. Deriving on the similarity of this transformation to the known enzymatic reactions, an enzyme and its characteristics are assigned to the step catalyzing the biotransformation. Similar process is followed further on the test molecule and a predictive pathway is proposed until the final product formed enters the intermediary metabolism[4].

Conversion of an aromatic isocyanate to an aromatic amine is an example of such biotransformation route, which has been contributed under the present study and accepted as a rule (BTrule) in Pathway Prediction System, developed at University of Minnesota Biocatalysis and Biodegradation Database (UMBBD)[5]. As of now, predictive biodegradation pathways have been studied for allyl amine (107-11-9), allyl alcohol (107-18-6), benzyl sulfide (538-74-9), glycolonitrile (107-16-4), methylene diphenyl diisocyanate (101-68-8) and toluene diisocyanate (584-84-9) which would be presented.

REFERENCES

1. Kameya T., Murayama T., Kitano M., Urano K.: Sci. Total Environ. *170*, 31 (1995).
2. Punch B.: URL = http://garage.cse.msu.edu/~punch/ /projects/biodeg.html
3. Wackett L. P., Ellis L. B. M., Speedie S. M., Hershberger C. D., Knackmass H.-J., Spormann A. M., Walsh C. T., Forney L. J., Punch W. F., Kazic T., Kanehisa M., Berndt D. J: ASM News *65*, 87 (1999).
4. Kanehisa M., Goto S., Kawashima S., Nakaya,A.: Nucleic Acid Res. *30*, 42 (2002).
5. Hou B. K., Ellis L. B. M.: URL=http://umbbd.ahc.umn.edu/ /predict.html.

P203 INDUCIBLE AND CONSTITUTIVE CYTOCHROMES P-450 INVOLVED IN OXIDATION OF TERFENADINE BY *Streptomyces platensis*

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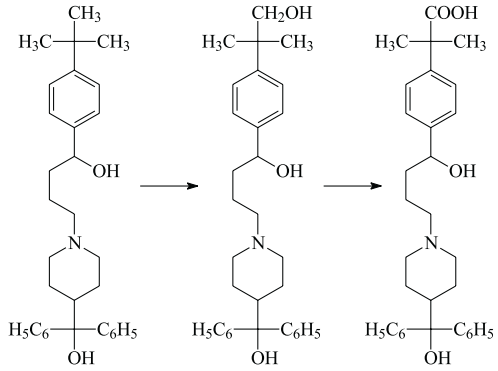
Keywords: *Streptomyces*, hydroxylation, oxidation, cytochromes, inducible

In the course of the preparation of the fexofenadine **3**, which is the active metabolite of terfenadine **1**, an antihistaminic drug, it has been found that *S. platensis* NRRL 2364 was able to oxidise the *t*-butyl group of **1** to give two products[1]. The hydroxylation of one methyl group gave the alcohol derivative **2** (so-called triol) and a subsequent oxidation could be observed and furnished the fexofenadine **3** in variable amounts.



Terfenadine **1** Triol **2** Fexofenadine **3**

We report here our investigations concerning the enzymes involved in these transformations. We have showed that two cytochromes P-450 catalysed the oxidation of terfenadine **1**. One was present only when the microorganism grew in soybean-peptone-containing culture medium, which was able to transform terfenadine **1** in fexofenadine **3** in short time incubation. The activity of this enzyme was higher after 48 hours of cultivation and decreased rapidly.

The second enzyme, more stable, was present in *S. platensis* grown in culture medium without soybean peptone and was able to transform terfenadine **1** and triol **2**. However, the oxidation of triol took place only without terfenadine in incubation medium. When the incubation of triol **2** was performed under oxygen-18, one atom of oxygen-18 was incorporated into fexofenadine. These observations suggest that the monooxygenase, which catalysed the hydroxylation of terfenadine was implicated in the oxidation of triol in fexofenadine. In order to optimise the formation of triol **2** we also studied the conditions of incubation (pH, temperature, concentration).

REFERENCES

1. Azerad R., Biton J., Lacroix I.: International Patent WO 99/47693 (1999).

P204 BIOTRANSFORMATION OF TERFENADINE, EBASTINE AND ANALOGUES BY SOME MICROORGANISMS

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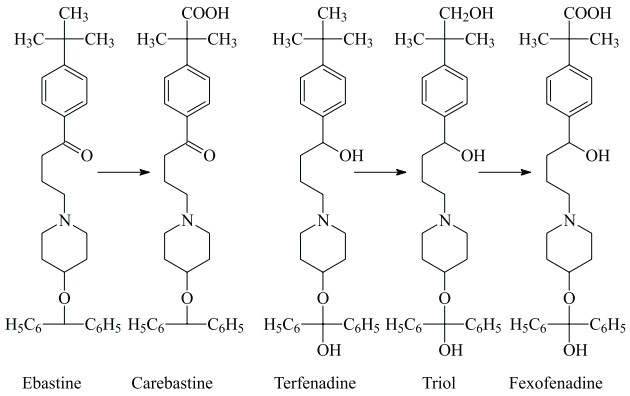
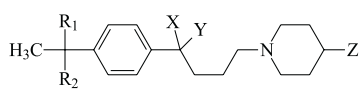
Keywords: microorganism, hydroxylation, oxidation, cytochrome P450

Terfenadine and ebastine, two antihistaminic drugs, are oxidised by hepatic cytochromes P-450 to fexofenadine and carebastine respectively, which are responsible for the pharmacological effects. Described chemical synthesis of fexofenadine and carebastine are laborious and products are obtained in low yields compared to synthesis of terfenadine and ebastine. Thus, the microbial oxidation of the *tert*-butyl groups of terfenadine and ebastine has been developed as an alternative method.



Cunninghamella echinulata is efficient to oxidise ebastine to carebastine[1] but transforms terfenadine into triol[2], the hydroxylated intermediate. The formation of fexofenadine has been reported[3] by oxidation with the bacteria *Streptomyces platensis* and the fungi *Absidia corymbifera*.

We present here our study concerning the biotransformation of several analogues of formula **1**. The aim of this work was to investigate the structural requirements necessary for the oxidative enzymatic activity of these microorganisms.



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REFERENCES

1. Schwartz H., Liebig-Weber A., Hochstätter H. Böttcher H.: Appl. Microbiol. Biotechnol. *44*, 731 (1996); Schwartz H., Böttcher H.: US Patent Nr. 5, 204, 249 (1993).
2. Schmitz G., Franke D., Stevens S., Takors R., Weuster-Botz D., Wandrey C.: J. Mol. Catal. B: Enzym. *10*, 313 (2000).
3. Azerad R., Biton J., Lacroix I.: Int. Patent WO 99/47693 (1999).

P205 IS THE OXIDATIVE CLEAVAGE OF HETEROCYCLIC NAPHTHOQUINONES IN *Streptomyces* CATALYZED BY A MEMBER OF THE HYDROQUINONE-EPOXIDASE FAMILY?

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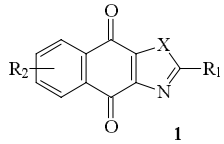
Keywords: dioxygenase, hydroquinone-epoxidase, *Streptomyces*, naphthoquinone, heteroaromatic

We have recently demonstrated that several *Streptomyces* strains are able to oxidatively cleave a large number of heterocyclic naphthoquinones (**1**, X = S, O, N) into isomeric carboxylic bis-(hetero)aromatic ketones (**2** or **3**) by a formal H_2O_2 addition, which is the result of a dioxygenase-type mechanism, as shown by the simultaneous incorporation of both oxygen atoms from an O_2 molecule[1]. The mechanism of this reaction will be compared to the hydroquinone-epoxydase reaction catalyzed by several *Streptomyces* strains with polyketide antibiotic precursors as substrates (antibiotic LL-C10037α (ref.[2]), tetracenomycin C, elloramycin[3], etc…) and the formation of a non-epoxide cleaved product will be discussed.



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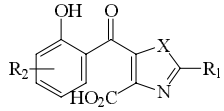

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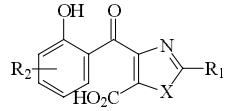

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REFERENCES

1. Le Texier L., Roy S., Fosse C., Neuwels M., Azerad R.: Tetrahedron Lett. *42*, 4135 (2001).
2. Gould S. J., Kirchmeier M. J., LaFever R. E.: J. Am. Chem. Soc. *118*, 7663 (1996).
3. Rafanan E. R., Hutchinson C. R., Shen B.: Org. Lett. *2*, 3225 (2000).

P206 BIPHASIC BIOCONVERSION OF NAPHTHALENES INTO DIHYDRODIOLS: SOLVENT EFFECTS AND SUBSTRATE DIFFERENCES

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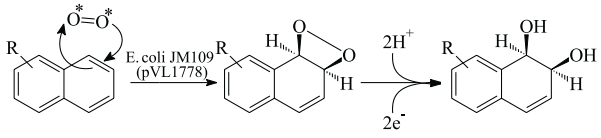
Keywords: dioxygenase, bioconversion, whole cell, naphthalene dihydrodiols, solvent effects

Since many years, we have been interested in whole cell bioconversions of aromatic hydrocarbons into derivatives that can be of interest as synthetic intermediates. We have developed recombinant bacteria containing oxygenases from *Pseudomonas* strains. In particular, we isolated the naphthalene dioxygenase of *P. fluorescens* N3 responsible of the conversion of substituted naphthalenes into the corresponding dihydrodiols with unique absolute configuration.



In order to increase the bioconversion yield and the system performance we studied the possibility of using a culture where the substrate is dissolved in an appropriate hydrophobic solvent. Preliminary results concerned the effects on the naphthalene transformation. Now, to further our understanding, we extend our experiments to some substituted naphthalenes. In this perspective, several flask transformations and some reactor experiments have been performed. The results show that the solvent role is highly affected by the substrate used and that some other important bioconversion variables influence the transformation. It appears clear that substrate bioavailability is definitely an essential condition, which is influenced by the solvent selection, the solvent-water phase ratio, the presence of surfactants, and, not last, the transfer mechanism. On the other hand, the absolute yield also depends on the possibility of maintaining the enzyme activity for long enough time. Our results will be presented and discussed together with future directions and developments.

In this respect, we began a series of reactor experiments using a multivariate approach where the role of some variables has been explicitly investigated.

P207 BIOTRANSFORMATION OF PYRIDINES WITH *Pseudomonas* SP STRAIN NCIB 9816-4

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Keywords: dioxygenases, *cis*-dihydroxylation, pyridines, naphthalene *cis*-dihydrodiol dehydrogenase

Bacterial degradation of aromatic compounds starts with stereospecific dioxygenation catalyzed by dioxygenases with formation of *cis*-dihydro-dihydroxy compounds. In wild strains of microorganisms these diols undergo dehydrogenation by *cis*-diol-dehydrogenases (DDH) to corresponding catechols. In mutant strains deprived of DDH, intermediate *cis*-dihydro-diols can be obtained as sole products. These *cis*--diols are valuable synthons in preparation of useful synthetic intermediates[1].

More than ten years ago Nikolai S. Zefirov (Moscow State University) suggested that application of such dioxygenase-catalyzed reaction to pyridines could lead to creation of enzymatic approach to synthesis of the naturally-occurring polyhydroxylated piperidine alkaloids[2]. By this time it was already known that bacterial catabolism of pyridines does not involve dioxygenation as a first step[3]. That is why microorganisms containing aromatic dioxygenases were chosen to explore such reaction on pyridines. However already first steps in this direction showed that the more electron poor pyridine ring is an unsuitable substrate for dioxygenation. For example, it was shown that *Pseudomonas* sp. NCIB 9816-4 containing naphthalene dioxygenase (NDO) transformed 4-pyridinecarboxaldehyde to isonicotinic acid and 4-hydroxymethylpyridine without any oxidation of heteroaromatic ring[4].

However when 1-methyl-2-pyridone (where electron-releasing substituents are present) was chosen as a substrate for NDO, dioxygenation led to corresponding dihydro-dihydroxypyridones (L. M., unpublished results), the structure of which and absolute stereochemistry were determined as *cis*-(5*S*,6*S*)-1-methyl-5,6-dihydroxy-5,6-dihydro-2-pyridone

(1) and *cis*-(3*S*,4*S*)-1-methyl-3,4-dihydroxy-3,4-dihydro-2-pyridone **(2)**[5, 6].

It was shown that besides NDO this reaction can be also catalyzed by toluene and biphenyl dioxygenases[6]. However, all these reactions were carried out by mutant strains of microorganisms – of which there are not many. Moreover those dioxygenases cannot accept the more polar, than 1-methyl-2--pyridone, pyridines as substrates.

Now we have found that *cis*-dihydro-dihydroxypyridones **1** and **2** can also be obtained successfully with *Pseudomonas* sp. NCIB 9816-4 (a wild-type strain containing both NDO and naphthalene *cis*-dihydrodiol dehydrogenase (NDD).

What is even more important, we have not found any traces of products of aromatization: dihydroxypyridones or phenols. This clearly indicates that dihydro-diols **1** and **2** are not substrates for NDD.

This finding allows us to broaden the circle of microorganisms, which can be applied for dioxygenation of various pyridine derivatives.

REFERENCES

1. Hudlicky T., Gonzalez D., Gibson D. T.: Aldrichim. Acta *32*, 35 (1999).
2. Stütz A. E.: *Iminosugars as Glycosidase Inhibitors: Nojirimycin and Beyond*. Wiley-VCH, Weinheim 1999.
3. Zefirov N. S., Agapova S. R., Terentiev P. B., Bulakhova I. M., Vasyukova N. I., Modyanova L. V.: FEMS Microbiol. Lett. *118*, 71 (1994).
4. Modyanova L. V., Piskunkova N. F.: *Biotrans '97 Abstracts*. *233* (1997).
5. Modyanova L., Azerad R.: Tetrahedron Lett. *41*, 3865 (2000).
6. Boyd D. R., Sharma N. D., Modyanova L. V., Carroll J. G., Malone J. F., Allen C. C. R., Hamilton J. T. G., Gibson D. T., Parales R. E., Dalton H.: Can. J. Chem. *80*, 589 (2002).

P208 CARBAZOLE HYDROXYLATION BY *Aspergillus flavus* VKM Ac-1024

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Keywords: biotransformation, carbazole, hydroxycarbazoles

Carbazole (Cz) is the tricyclic aromatic *N*-heterocyclic compound well known as a highly persistent environmental pollutant. On the other hand, its hydroxylated derivatives represent valuable pharmaceutical precursors[1].

The strain of *Aspergillus flavus* VKM Ac-1024 was selected among 300 species as showed high hydroxylase activity towards *N*-heterocyclic compounds. No 4- or 5-hydroxy indoles were formed from indole, while 1-benzoyl indole was converted by introducing hydroxyl function at the position 4 followed by cleavage of benzoyl substituent to form 4-hydroxy indole as a major product[2].

In the present study biocatalytic potential of this organism in respect of Cz and its *N*-substituted derivatives was estimated. The structure of metabolites was determined by H1-NMR, MS and GLC analyses.

3-Hydroxy carbazole was revealed as a major bioconversion product, while 1-hydroxy and 2-hydroxy carbazoles were formed in minorities at Cz conversion. The hydroxylation position shifted to preferable accumulation of 2-hydroxy carbazole and the formation of double hydroxylated 2,6- and 2,7-dihydroxy carbazoles in the presence of 1-benzoyl indole. The effect was never described so far. The conversion of *N*-substituted carbazole derivatives was found to depend on the type of substituent. No conversion of *N*-benzoyl carbazole was observed, while *N*-acetyl carbazole was transformed to carbazole and its 2- and 3-hydroxy derivatives.

REFERENCES

1. Shanmugasundaram K., Prasad K. J. P.: Heterocycles. *51*, 2163 (1999).
2. Sukhodolskaya G. V., Nikolayeva V. M., Donova M. V., Gulevskaya S. A., Baskunov B. P., Koshcheyenko K. A., Turchin K. F.: Appl. Microbiol. Biotechnol. *53*, 695 (2000).

P209 ONE-POT EXTRACTION-SOLVOLYSIS OF TRIACYLGLYCERIDES CATALYZED BY *Rhizopus oryzae* RESTING-CELLS IN SOLVENT AND SOLVENT-FREE MEDIA

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Keywords: resting-cells, *Rhizopus oryzae*, hydrolysis, esterification, one-pot extraction

The synthesis of heterocyclic hydrocarbons with one or several hydroxy groups in a ring is one of the most challenging tasks in modern organic chemistry. Biocatalysis allows obtaining of ring hydroxylated *N*-heterocycles not accessible by conventional chemical synthesis.

Acylglycerides are usually extracted from plant or animal material using physico-chemical or physical methods. Crude extracts are then purified and finally modified[1]. Enzymatic transformations are a potentially attractive method for the modification of the physical and chemical properties of edible

fats and oils[2]. The mild conditions associated with these transformations should permit one-pot extraction and transformation of acylglycerides to the desired compounds. Thus, Klass and Warwel have recently proposed using a commercial immobilized enzyme, deposited at the bottom of a soxhlet--apparatus, to transesterify triacylglycerides extracted from the oilseed-reservoir[3]. For the present study, an endophytic fungus isolated from a plant was selected. The ability of its resting-cells to carry out the one-pot extraction and transformation of acylglycerides in solvent and solvent-free systems was then studied.

Milled oilseeds or coffee grounds and fungal resting-cells were shaken at 50 °C. An appropriate amount of water and/or alcohol was added at the due time. An organic solvent was added either at starting or near the end of the process. The resulting organic solution was recovered by filtration and evaporated. Aliquots of the residue were dissolved and analyzed.

The one-pot extraction-solvolysis produced a 56–99 % of crude extract containing 33–95 % fatty acid esters and 3–75 % free fatty acids. The amount of water present in each material, the solvent, the alcohol, and the hardness of the solid material used have influence on the final results of the described process.

REFERENCES

1. Noureddini H., Medikonduru V.: J. Amer. Oil Chem. Soc. *74*, 419 (1997).
2. Ujang Z., Vaidya A. M.: Appl. Microbiol. Biotechnol. *50*, 318 (1998).
3. Klaas M. R. G., Warwel S.: Eur. J. Lipid Sci. Tech. *103*, 810 (2001).

P210 NOVEL HYDROLASES FROM THERMOFILIC FUNGI FOR STEREOSELECTIVE BIOTRANSFORMATIONS

VIKTÓRIA BÓDAI[a, b], CSABA PAIZS[c], MONICA TOŞA[c], CORNELIA MAJDIK[c], SAROLTA PILBÁK[c], LAJOS NOVÁK[a], FLORIN-DAN IRIMIE[c], GYÖRGY SZAKÁCS[b], and LÁSZLÓ POPPE[a]

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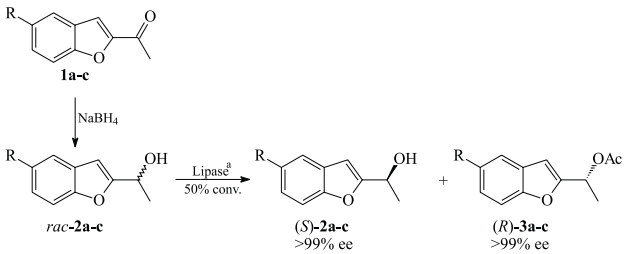
Keywords: hydrolases, thermophilic filamentous fungi, enzyme catalysis, kinetic resolution

By a novel method involving an enzymatic alcoholysis as key step benzofurane derivatives were prepared[1]. From the benzofurane-2-yl ketones **(1a-c)** obtained by this method, asymmetric baker's yeast reductions were performed, resulting the corresponding alcohols (*(S)*-**2a-d)** of moderate enantiomeric purities[2].

As an alternative to this bioreduction, the racemic alcohols **(2a-c)** were acylated in a highly enantiomer selective way (Scheme). For this transformations, a series of novel hydrolases from thermophilic fungi were produced and tested as biocatalysts[3].



R = H, Br, NO_2

[a] cca 80 different lipases were tested as biocatalysts in acylation

The lipase-catalysed acylation results were extended to further heterocyclic compounds. Using the appropriate lipases, some of these highly enantiopure compounds were also produced in a continuously operating bioreactor – extractor cascade.

REFERENCES

1. Paizs Cs., Toşa M., Majdik C., Bódai V., Novák L., Irimie F.-D., Poppe L.: J. Chem. Soc., Perkin Trans. 1, *2002*, 2400.
2. Paizs Cs., Toşa M., Majdik C., Moldovan P., Novák L., Kolonits P., Marcovic, A., Irimie F.-D., Poppe L.: Tetrahedron: Asymmetry, accepted.
3. Bódai V., Peredi R., Bálin J., Egri G., Novák L., Szakács Gy., Poppe L.: Adv. Synth. Catal., submitted.

P211 NOVEL HYDROLASES FROM THERMOPHILIC FILAMENTOUS FUNGI FOR ENANTIOMER AND ENANTIOTOPIC SELECTIVE BIOTRANSFORMATIONS

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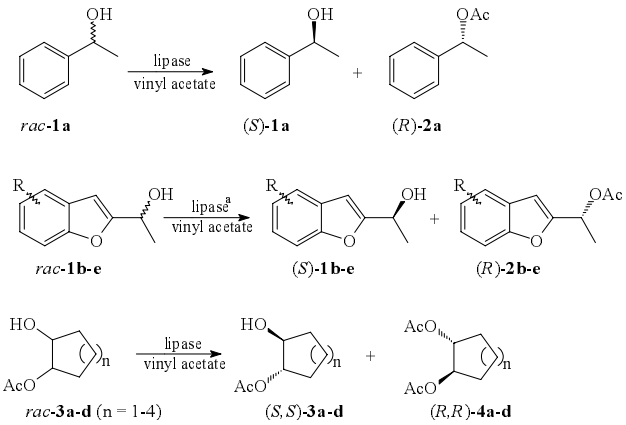
Keywords: hydrolases, thermophilic filamentous fungi, enzyme catalysis, kinetic resolution, asymmetric catalysis

A series of thermophilic filamentous fungi were cultivated under different conditions and were assayed for lipase/carboxylesterase activities[1]. The enzyme preparations were tested as biocatalysts in organic solvents. Kinetic resolution of racemic aryl/hetaryl methyl carbinols (*rac*-**1**)[1] or racemic *trans*-cycloalkane-1,2-diol monoacetates (*rac*-**3**)[2] (Fig. 1) and desymmetrisation of 2-acyloxypropan-1,3-diols **(5)**[1] (Fig. 2) by acetylation with vinyl acetate were chosen for testing the biocatalytic abilities of these preparations.



a – cca 70 different lipases were tested as biocatalyst in acylation

Fig. 1. **Kinetic resolution of racemic alcohols**

First, acetylation of racemic 1-phenylethanol (*rac*-**1a**) was performed[1]. Then the lipase-catalysed acylation was extended to further heterocyclic compounds, such as 1-(benzofuran-2--yl)ethanols (*rac*-**1b-e**). Using the appropriate lipases, some of these highly enantiopure compounds were also produced in a continuously operating bioreactor – extractor cascade. Next, kinetic resolution of a series of racemic *trans*-cycloalkane-1,2-diol monoacetates (*rac*-**2a-d**) was performed.

Finally, asymmetric acylation of 2-acyloxypropan-1,3--diols **(5a, b)** have been chosen for testing the enantiotopic selectivities of the novel biocatalysts.

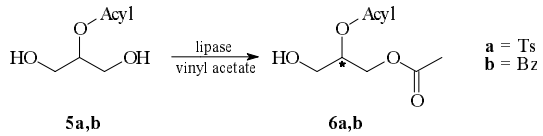


Fig. 2. **Asymmetric acetylation of prochiral 1,3-diols**

In summary, the tested biocatalysts proved to be superior over the commercially available enzymes with respect to the degree of enantiomer selectivity, and they exhibited a wider range of enantiotopic selectivity than the most common commercial enzymes.

REFERENCES

1. Bódai V., Peredi R., Bálint J., Egri G., Novák L., Szakács Gy., Poppe L.: Adv. Synth. Catal., submitted.
2. Bódai V., Orovecz O., Szakács Gy., Novák L., Poppe L.: Org. Biomol. Chem., submitted.

P212 CLONING AND EXPRESSION IN *E. coli* OF THE GENE ENCODING *Streptomyces* PMF PLD, A PHOSPHOLIPASE D WITH HIGH TRANSPHOSPHATIDYLATION ACTIVITY

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Phospholipases D (PLDs) hydrolyze phospholipids to yield phosphatidic acid and the corresponding alcohol and catalyze a transesterification (transphosphatidylation) reaction when alcohol is present as a nucleophilic donor. Bacterial forms of PLDs have shown to be suitable as biocatalysts for the synthesis of phospholipid derivatives of industrial interest.

Recently, PLD from *Streptomyces* PMF, an enzyme with a high transphosphatidylation activity, was purified and its crystallographic structure was solved at 1.4 Å.

A 315 bp fragment of the *pld* gene of *S.* PMF was amplified by PCR using chromosomal DNA as template and a pair of heterologous primers based on *S. antibioticus pld* gene sequence. The complete *pld* gene was isolated by colony hybridization and sequenced. DNA sequence analysis revealed a significant similarity with known *pld* gene sequences and showed the presence of highly conserved sequence motifs, namely the HKD motifs, shared by other members of the PLD superfamily. In order to promote the secretion of the protein into the medium, the mature PLD gene was fused in a pET derivative with the PelB signal sequence, and expression was performed in *E. coli* BL21(DE3)pLysE cells after induction with IPTG. Recombinant PLD activity detected in the culture supernatant was purified to homogeneity and structural and functional analyses confirmed the identity of the recombinant PLD with the wild-type protein.

P213 INVERSION OF THE ENANTIOSELECTIVITY OF ARYLMALONATE DECARBOXYLASE BY POINT MUTATION

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We have demonstrated that arylmalonate decarboxylase (AMDase; EC. 4.1.1.76) catalyzes asymmetric decarboxylation of arylmalonates to give optically pure *(R)*-α-arylpropionates. This enzyme has four cysteine residues and one of which, Cys188, is estimated to be located in the active site. It is estimated that it delivers a proton from the *si*-face of the intermediate enolate to give *(R)*-product (Fig. 1)[1].

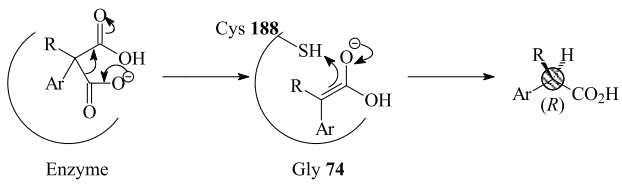

Fig. 1. **Active site of AMDase**

From the result of the data base searches, it is revealed that AMDase has some homology with Glu racemase. Glu racemase has a pair of Cys, 73 and 188, in the active site, which are estimated to provide a proton from both side. On the other hand AMDase has only one Cys at 188, and this is the reason why this enzyme gives optically pure products (Fig. 2). The estimated 3D-structure of AMDase suggested that Ser71 is in the opposite side of Cys188 (Fig. 3).

Glu racemase	-NDPI ~	ALVIA**C**NTAT ~	LVG**C**THFPL ~
Asp racemase	MFFSI ~	FIIMP**C**NTAH ~	VILG**C**TELSL ~
Hyd* racemase	MKVIN ~	AFVIA**C**WGDP ~	ILLG**C**AGMAE ~
Mal* isoracemase	---MK ~	VMAYA**C**LVAI ~	LSA-**C**VQMPS ~
AMDase	-MQAS ~	VVSLM**G**TSLS ~	ILLS**C**GGLLT ~
		74	**188**

Hyd* – hydantoin, Mal* – maleate

Fig. 2. **Homology of AMDase with some enzymes**

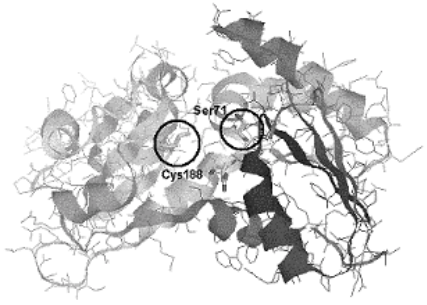

Fig. 3. **Estimated 3D-structure of AMDase**

Then we expected that introduction of one Cys around 71 to 76 and replacement of Cys188 with less acidic Ser might invert the enantioselectivity of the enzyme. Thus we prepared 6 mutants in which Cys188 was changed with Ser, and either one of the amino acid from 71 to 76 was changed with Cys. As the result, two double mutants, S71C/C188S and G74C/ /C188S exhibited decarboxylation activity and gave the opposite enantiomer of the products from wild type enzyme.

REFERENCES

1. Ohta H.: Bull. Chem. Soc. Jpn. *70*, 2895 (1997).