Microbial Baeyer–Villiger Oxidation of Bicyclo[4.3.0]ketones by Two Recombinant *E. coli* Strains. A Novel Access to Indole Alkaloids

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Received 27 February 2002

Abstract: Recombinant *Escherichia coli* overexpressing *Pseudomonas sp.* NCIMB 9872 cyclopentanone monooxygenase (CPMO; E.C. 1.14.13.16) and *Acinetobacter* sp. NCIMB 9871 cyclohexanone monooxygenase (CHMO; E.C. 1.14.13.22) have been utilized in whole-cell biotransformations of prochiral bicyclo[4.3.0]ketones. The lactones produced in a biocatalytic Baeyer–Villiger oxidation represent key intermediates for the synthesis of several indole alkaloids. The two over-expression systems demonstrated a tendency for the formation of opposite enantiomers with CPMO giving (+)-lactones in good yields and excellent enantiomeric excess.

Key words: biocatalysis, recombinant whole-cell biotransformation, monooxygenase, Baeyer–Villiger oxidation, indole alkaloids, enantioselectivity

The utilization of enzymatic systems offers efficient access to enantiomerically pure lactones via Baeyer–Villiger oxidation[1] of the corresponding cyclic ketones[2–6] and is considered a 'green chemistry' alternative to organometallic catalysts.[7–14] Such chiral compounds are interesting precursors in enantioselective natural compound synthesis.

The widespread use of flavin-dependent monooxygenases as catalytic entities for these transformations throughout the synthetic community was hampered by the fact that cofactor recycling is essential for this class of enzymes, which makes the application of such systems more difficult. While the situation has been improved by the development of efficient and more robust recycling systems for NADPH required by these enzymes in recent years,[15,16] another way to circumvent this obstacle is to use whole-cells instead of isolated enzymes.

Advances in molecular biology mean that recombinant systems can be developed with significantly improved selectivity for the desired biotransformation and with higher efficiency compared to the native strains. Recently, we designed overexpression systems for the most extensively studied Baeyer–Villigerase to date, cyclohexanone monooxygenase (CHMO) from *Acinetobacter sp.* NCIMB 9871 (E.C. 1.14.13.22).[17] Both *Saccharomyces cerevisiae*[18] and *Escherichia coli*[19] were used as hosts and the new organisms demonstrated the same chemo-, regio-, and enantioselectivities as observed for the isolated enzyme.[5]

While more than 100 substrates for CHMO were identified in several studies,[4,5] CPMO from *Pseudomonas sp.* NCIMB 9872 (CPMO; E.C. 1.14.13.16)[20–22] received considerably less attention in biocatalytic applications,[23,24] probably because a substrate profile similar to CHMO was assumed. Just recently, an overexpression system has been constructed and utilized for the biotransformation of representative cyclohexanones.[25]

In this publication we are expanding our substrate-profiling studies of recombinant *E. coli* expression systems for Baeyer–Villiger monooxygenases[26,27] to bicycloketones **1** as precursors for chiral lactones **2** (Scheme 1).[28,29] Compounds **2** represent key intermediates in the total synthesis of several members of the indole alkaloid family. The use of prochiral substrate ketones in a biocatalytic asymmetrization step is highly advantageous, since it generates optically active products with a theoretical yield of 100%, while kinetic resolution of racemic samples leads only to a maximum of 50% enantiopure product.

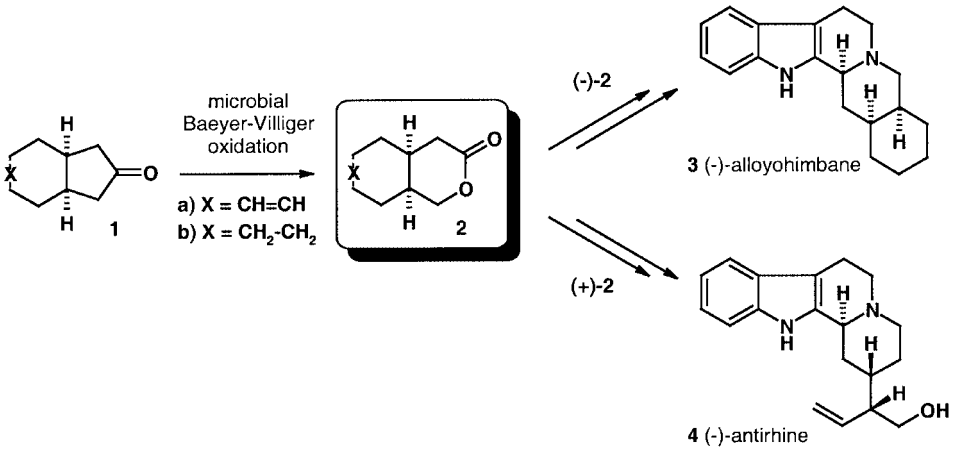
Lactone (–)-**2a** has been used by Riva and coworkers,[30] in a total synthesis of (–)-alloyohimbane **3**, a member of the yohimbine–respirine alkaloid group. This class of compounds has interesting pharmacological properties, acting as α_2-adrenoceptor antagonists, serotonin and dopamine receptor antagonists, as well as an aphrodisiac.[31] Representatives of this class have been detected in three plant families: *Apocynaceae*, *Rubiaceae* and *Loganiaceae*. Recently, new bisindoles of the yohimbine–gambarine type have been found also in different *Uncaria* species.[32] The antipodal (+)-**2a** was utilized by Danieli in the total synthesis of (–)-antirhine **4**,[33] another indole alkaloid originally isolated from *Antirhea putaminosa*.[34]

Since both enantiomeric lactones **2** have been fully characterized they are interesting model compounds for us to establish the absolute configuration of the fermentation products. In addition, the compounds served as key intermediates in natural compound syntheses, demonstrating applications of our expression systems.

Our biocatalytic approach to lactones **2** via microbial Baeyer–Villiger oxidation required rapid and efficient access to ketone **1**. We adapted[35] and optimized protocols[36] for the tosylation of diol **5** and conversion to the corresponding dinitrile, followed by hydrolysis to diacid **6**

Synlett 2002, No. 5, 03 05 2002. Article Identifier:
1437-2096,E;2002,0,05,0700,0702,ftx,en;D04402ST.pdf.

ISSN 0936-5214



Scheme 1 Access to lactones **2** via microbial Baeyer–Villiger oxidation as key intermediates for some indole alkaloids.

(Scheme 2). One-pot cyclization in acetic anhydride/pyridine and decarboxylation of compound **8** represents a shortcut to ketone **1a** compared to the usual Dieckmann condensation route.[37,38] Catalytic hydrogenation of olefin **1a** afforded compound **1b**.

Biocatalytic Baeyer–Villiger oxidations were performed with two recombinant *E. coli* strains: In both the CHMO-system *BL21*(*DE3*)(*pMM4*) and the CPMO construct *DA5a*(*pCMP201*) the production of the corresponding monooxygenase is induced by addition of isopropyl-β-D-thiogalactopyranoside (IPTG).

Results of the biotransformations of substrates **1** with whole-cells expressing CHMO and CPMO are summarized in the Table. All ketones were converted to the corresponding lactones with high chemoselectivity. The double bond in substrate **1a** was not oxidized under fermentation conditions. Conversion was improved by addition of β-cyclodextrin, a known effect by cyclic sugars of this type.[39]

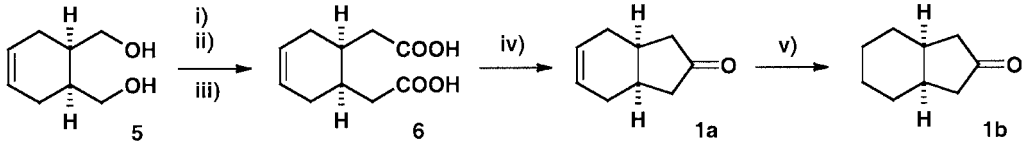
Fermentations of substrates **1** with the CHMO expression system proceeded rather slowly, with substantial amounts of starting material remaining after 24–36 hours when biocatalytic activity of this system came to a halt. We attribute this to the size and steric arrangement of the compounds reaching the spatial limitations of the active site. Biotransformations of **1a** and **1b** gave the corresponding lactones in almost racemic mixtures (reference material of *rac*-**2b** was prepared by chemical oxidation with mCPBA) with a slight preference for the (–)-4a*S*,8a*S* configuration. Absolute configuration was established by comparing the sign of the specific rotation to literature data.[30,33,40]

Biotransformations with the CPMO-expression system proceeded generally to completion after 48 hours incubation at room temperature. Both yields and enantioselectivities were superior to the CHMO strain. Two stereogenic centers were established in the course of the biotransformation with preference for the (+)-4a*R*,8a*R* configuration.

Table Whole-cell Biotransformations of Bicyclo[4.3.0]ketones

Substrate	Strain	Product	Yield[a]	ee[b]	$[\alpha]_D^{20}$	Abs. config.
1a	CHMO	**2a**	33% (85%)	~ 5%	–0.7 *c* 0.36, CH_2Cl_2	(–)-4a*S*,8a*S*
1a	CPMO	**2a**	76%	> 99%	+24.5 *c* 1.0, $CHCl_3$	(+)-4a*R*,8a*R*
1b	CHMO	**2b**	21% (65%)	~ 3%	–1.2 *c* 0.46, EtOH	(–)-4a*S*,8a*S*
1b	CPMO	**2b**	83%	99%	+39.1 *c* 1.0, $CHCl_3$	(+)-4a*R*,8a*R*

[a] Isolated yield after chromatographic purification; yield in brackets is based on consumed starting material.
[b] Ee determined by chiral phase gas chromatography; racemic reference material prepared by mCPBA oxidation of ketone **1b**.



Scheme 2 (i) TsCl, pyridine, 83%; (ii) NaCN, EtOH, reflux, 88%; (iii) 6 N KOH, then H_3PO_4, 92%; (iv) Ac_2O, pyridine, then 0.5 N HCl, 61%; (v) Pd/C, H_2 81 psi, THF, 77%.

Hence, CPMO promotes formation of the opposite enantiomer than CHMO for this class of substrates.

In conclusion, we have demonstrated that a recombinant expression system for CPMO can produce chiral lactones **2a** and **2b** chemoselectively via microbial Baeyer–Villiger oxidation with high optical purities and in good yields. Our biotransformation provides a simple and 'green' route to (+)-**2a**, a key intermediate for the synthesis of some indole alkaloids, and represents the first formal total synthesis of (–)-antirhine **4** and (+)-alloyohimbane **3** using a recombinant strain expressing CPMO. With respect to both yield and optical purity the CPMO-system is superior to a recombinant CHMO producing strain for this class of bicyclo-ketones.

Fresh LB-amp medium (1% Bacto-Peptone, 0.5% Bacto-Yeast Extract, 1% NaCl supplemented by 200 ppm ampicillin) was inoculated with a 1/100 aliquot of an overnight preculture of *DH5a*(*pCMP201*) in a baffled Erlenmeyer flask. The culture was incubated at 120 rpm at 37 °C on an orbital shaker for 2 h, then IPTG was added to a final concentration of 0.025 mM. The substrate **1** (3–5 mM) was added neat along with β-cyclodextrin (1 equiv). The culture was incubated at r.t. for 48 h. The biomass was removed by centrifugation (3500 rpm, 10 min), the aq layer was passed through a bed of Celite®, and the product was isolated by repeated extraction with EtOAc. The combined organic layers were dried over sodium sulfate and concentrated. Lactones **2**[41] were purified by flash column chromatography.

Acknowledgement

This project was funded by the Oesterreichische Nationalbank (grant no. JF-7619). Support by Baxter Immuno Austria and Novartis Donation and Sponsoring is gratefully acknowledged. We thank Dr. Erwin Rosenberg (Vienna University of Technology) for his assistance in the determination of enantiomeric purity.

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