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Enantioselective kinetic resolution of 3-phenyl-2-ketones using Baeyer–Villiger monooxygenases

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Abstract—The enantioselective kinetic resolution of two 3-phenyl-2-ketones using four different Baeyer–Villiger monooxygenases (BVMO) expressed recombinantly in *Escherichia coli* was studied. The highest enantioselectivity ($E = 82$) was achieved for 3-phenyl-2-butanone using a BVMO originating from *Pseudomonas fluorescens*. A BVMO from *Pseudomonas putida* showed an opposite (*R*)-enantiopreference and $E = 12$.

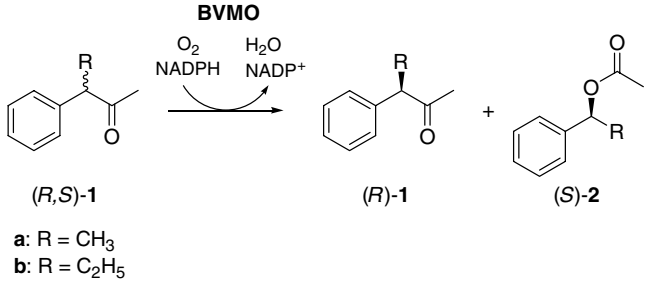
1. Introduction

Baeyer–Villiger monooxygenases (BVMOs) belong to the class of oxidoreductases and convert aliphatic, arylaliphatic and cyclic ketones into esters and lactones, respectively, using molecular oxygen.[1–5] Thus, they mimic the chemical Baeyer–Villiger oxidation,[6] which is usually peracid-catalyzed and proceeds by a two-step process as initially proposed by Criegee.[7] Since it is becoming more and more important to perform Baeyer–Villiger oxidations in an enantioselective manner,[8] BVMOs represent a valuable alternative to metal-based chiral catalysts.[9] Until now, stereoselective Baeyer–Villiger oxidations using enzymes were described for prochiral or racemic mono- and bicyclic ketones[10–15] as well as racemic arylaliphatic ketones.[3,16] Recently, we have shown that aliphatic acyclic ketones such as 4-hydroxy-2-ketones could be enantioselectively ($E \sim 50$) converted by a BVMO from *Pseudomonas fluorescens* DSM 50106, which was recombinantly expressed in *Escherichia coli*.[17,18]

Herein we report, the kinetic resolution of two 3-phenyl-2-ketones using four different BVMOs (Scheme 1).

2. Results and discussion

Kinetic resolutions of the two 3-phenyl-2-ketones **1a** and **1b** (Scheme 1), which were synthesized according to the literature,[19] were carried out using resting cells of recombinant *E. coli* expressing four different BVMOs: cyclohexanone monooxygenase from *Acinetobacter calcoaceticus* NCIMB 9871 (CHMO[20]), cyclopentanone monooxygenase from *Comamonas* sp. NCIMB 9872 (CPMO[21]), a BVMO from *P. fluorescens* DSM 50106 (PF-BVMO) and a BVMO from *Pseudomonas putida* KT2440 (PP-BVMO).



Scheme 1. Kinetic resolution of two 3-phenyl-2-ketones by Baeyer–Villiger monooxygenase-catalyzed oxidation.

After expression of the recombinant enzymes, cells were harvested by centrifugation, washed once with phosphate

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buffer and used directly afterwards for biocatalysis reactions on an analytical scale. Cofactor recycling was ensured by employing the whole cell *E. coli* and glucose was added to each reaction vial. Therefore, the addition of cofactor NADPH was not necessary. For efficient uptake of the substrates **1a** and **1b** by the cells, β-cyclodextrin had to be included.[22,23] Reaction progress was monitored by GC analyses using a chiral column.

3-Phenyl-2-butanone **1a** was converted by all four enzymes to the corresponding 1-phenylethyl acetate **2a**, while PF-BVMO and PP-BVMO did not oxidize 3-phenyl-2-pentanone **1b** to 1-phenylpropyl acetate **2b** (Tables 1 and 2). The enzymes exhibited different activities and enantioselectivities towards **1a**, for example, CPMO showed the highest activity but only low enantioselectivity ($E < 3$). On the other hand, PF-BVMO exhibited significantly less activity but at the same time was the most selective, yielding the highest *E*-value (analytical scale: $E = 43$, preparative scale: $E = 82$). This value substantially exceeds the selectivity ($E \sim 7$) estimated for a BVMO from *Thermobifida fusca*.[16] Interestingly, PP-BVMO preferentially converted the (*R*)-enantiomer of **1a** ($E = 12$), while the three other BVMOs were (*S*)-selective.

Table 1. Whole cell biocatalysis reactions (analytical scale) with different BVMOs expressed in recombinant *E. coli* for ketone **1a** at 30 °C (CHMO: cyclohexanone monooxygenase from *Acinetobacter calcoaceticus* NCIMB 9871; CPMO: cyclopentanone monooxygenase from *Comamonas* sp. NCIMB 9872; PF-BVMO: BVMO from *P. fluorescens* DSM 50106; PP-BVMO: BVMO from *P. putida* KT2440)

Enzyme	Time (h)	Conversion[a] (%)	Enantiomeric excess		E[a]
			% ee_S	% ee_P	
CHMO	8	41	2.6	8.3 (*S*)	<3
CPMO	2	93	90	6.0 (*S*)	<3
PF-BVMO	8	46	75	90 (*S*)	43
PP-BVMO	2	50	72	71 (*R*)	12

[a] Calculated according to Chen et al.[24]

Table 2. Whole cell biocatalysis reactions (analytical scale) with different BVMOs expressed in recombinant *E. coli* for ketone **1b** at 30 °C. For abbreviations, see Table 1

Enzyme	Time (h)	Conversion[a] (%)	Enantiomeric excess		E[b]
			% ee_S	% ee_P	
CHMO	4	36	n.d.[c]	73 (*S*)	4.5
CPMO	2	44	n.d.[c]	48 (*R*)	4.0
PF-BVMO		No conversion			
PP-BVMO		No conversion			

[a] Calculated by the following equation: (peak areas of product enantiomers)/(peak areas of substrate enantiomers + peak areas of product enantiomers).

[b] Calculated according to Chen et al.[24]

[c] n.d.—not determined due to unsatisfactory separation of enantiomers by GC analysis.

A similar pattern was found for **1b**, for which CHMO and CPMO again exhibited only low enantioselectivity, but the *E*-values were slightly higher compared to **1a** ($E \sim 4$; Table 2). Increasing the length of the alkyl side chain from methyl to ethyl changed the enantiopreference of CPMO from (*S*)- to (*R*)-selectivity, while CHMO remained (*S*)-selective.

Using resting cells of *E. coli* JM109 pGro7 pJOE4072.6, biocatalysis with **1a** was also performed in preparative scale (1 mmol substrate), this time using shake flasks and only 0.5 equiv of β-cyclodextrin. Previous experiments with different cyclodextrin concentrations revealed that conversions only marginally decreased when reducing the amount of cyclodextrin to 0.5 equiv (data not shown). After 6 h at 30 °C, GC analysis showed 46% conversion. The enantiomeric excess of substrate and product was 80% ee and 94% ee, respectively, corresponding to an $E = 82$. For better separation of the ketone and ester during work-up, **2a** was enzymatically hydrolyzed to the corresponding alcohol using *Candida antarctica* lipase A (CAL-A). CAL-A was identified to be non-selective and therefore could be used without affecting the enantiomeric excess of the ester. Thus, 1-phenylethanol **3a** could be isolated in 35% yield and 93% ee. GC analysis of the substrate fraction revealed that a small amount of ester **2a** was still present. Interestingly, using an esterase from *P. fluorescens* instead of CAL-A, the ee-value of **2a** could be increased up to 99% since this enzyme preferentially hydrolyzes the (*R*)-ester (data not shown).

3. Conclusions

Herein, we have shown that arylaliphatic ketones were accepted as substrates by different Baeyer–Villiger monooxygenases. Only the BVMO from *P. fluorescens* DSM 50106 gave satisfactory enantioselectivity in the kinetic resolution of 3-phenyl-2-butanone yielding the corresponding (*S*)-acetate with 93% ee. On a preparative-scale reaction the corresponding chiral alcohol 1-phenylethanol was obtained in 35% yield and 93% ee. Interestingly, one enzyme showed opposite enantiopreference towards **1a** and the increase of the alkyl chain from methyl to ethyl gave no conversion using the BVMOs originating from *Pseudomonas* sp. but inverted the enantiopreference of CPMO.

4. Experimental

4.1. General

All chemicals were purchased from Sigma–Aldrich (Taufkirchen, Germany), Fisher Scientific (Schwerte, Germany), VWR (Darmstadt, Germany), ABCR (Karlsruhe, Germany) and Roth GmbH (Karlsruhe, Germany) unless otherwise specified. ^1H and ^{13}C NMR-spectra were recorded on a 300 MHz or 600 MHz (Bruker) instrument.

4.2. Synthesis of the starting material

3-Phenyl-2-butanone **1a** and 3-phenyl-2-pentanone **1b** were synthesized according to the literature[19] by conversion of the corresponding carboxylic acids with methyllithium. Synthesis of 1-phenylpropyl acetate **2b** was carried out by acetylation of 1-phenylpropanol with acetylchloride.[25] Structural identity was confirmed by NMR spectroscopy.

4.3. BVMO-production

Cyclohexanone monooxygenase (CHMO) from *Acinetobacter calcoaceticus* NCIMB 9871: *E. coli* BL21 (DE3) pKJE7 pMM4[26] was grown in 200 mL LB_{cm+amp} at 30 °C to an optical density of 0.5. Then, 0.5 mg/mL L-arabinose for induction of chaperone coexpression[27] and 0.1 mM IPTG for induction of CHMO expression were added. After further growth for 4 h at 30 °C, cells were harvested by centrifugation (10 min, 4400*g*, 4 °C) and washed once with sterile phosphate buffer (50 mM, pH 7.5).

Cyclopentanone monooxygenase (CPMO) from *Comamonas* sp. NCIMB 9872: expression of CPMO in *E. coli* DH5α pCMO206[28] was performed in 200 mL LB_{amp}. Cells were grown at 37 °C to an optical density of 0.5 where CPMO expression was induced by the addition of 0.1 mM IPTG. After further growth for 6 h at 25 °C, cells were harvested by centrifugation (10 min, 4400*g*, 4 °C) and washed once with sterile phosphate buffer (50 mM, pH 7.5).

BVMO from *P. fluorescens* DSM 50106 (PF-BVMO): *E. coli* JM109 pGro7 pJOE4072.6[18] was grown in 200 mL LB_{cm+amp} containing 0.5 mg/mL L-arabinose at 30 °C. At OD = 0.6, enzyme expression was induced by addition of L-rhamnose (0.2% (w/v) final concentration) and cells were further incubated at 30 °C for 4 h. Afterwards, cells were harvested by centrifugation (10 min, 4400*g*, 4 °C) and washed once with sterile phosphate buffer (50 mM, pH 7.5).

BVMO from *P. putida* KT2440 (PP-BVMO): expression of PP-BVMO in *E. coli* JM109 pGro7 pBVMO2440 was performed as described for PF-BVMO.

After expression of the recombinant BVMOs, the resting cells were used for biocatalysis reactions.

4.4. Biocatalysis reactions on an analytical scale

Recombinant *E. coli* cells carrying the BVMOs were resuspended in a sodium phosphate buffer (50 mM, pH 7.5) to a final OD of around 50. Aliquots (1 mL) of these cell suspensions were mixed in 2 mL Eppendorf (Hamburg, Germany) reaction vials with 15 μmol substrate, 15 μmol β-cyclodextrin and 10 μL of a sterile 1 M glucose solution. The vials were closed with air permeable caps (Lid_{Bac}, Eppendorf) and incubated in a thermoshaker (Eppendorf) at 1400 rpm. After certain time intervals, samples (300 μL) were taken, extracted twice with ethyl acetate and dried over anhydrous sodium sulfate. Excess solvent was removed under nitrogen and samples were analyzed via GC.

4.5. Biocatalysis reaction on a preparative scale

Recombinant *E. coli* cells containing the expressed PF-BVMO were resuspended in sodium phosphate buffer (50 mM, pH 7.5) to a final OD of around 20. To 100 mL of this suspension 1 mmol of substrate **1a**, 0.5 mmol β-cyclodextrin and 2 mL of a sterile 1 M glucose solution were added. The reaction mixture was incubated at 30 °C and 220 rpm. After four hours another 2 mL sterile glucose solution was added. The reaction was stopped after 6 h and extracted five times with 50 mL ethyl acetate. The combined organic phase was dried over anhydrous sodium sulfate and solvent was removed in vacuo.

For better separation of ketone **1a** and ester **2a**, the product mixture was hydrolyzed by *Candida antarctica* lipase A (CAL-A, Chirazyme L-5) in 60 mL of a 5:1 mixture of sodium phosphate buffer and hexane yielding unreacted **1a** and 1-phenylethanol **3a**. After 24 h, the reaction mixture was extracted four times with 30 mL of ethyl acetate. After drying over anhydrous sodium sulfate and evaporation of the organic solvent, the product was purified by column chromatography (hexane/ethyl acetate 5:1). Compound **3a** could be isolated in 35% yield (43 mg, 0.35 mmol).

4.6. Chiral GC analysis

GC analyses on a chiral stationary phase were carried out on a Shimadzu GC-14A gas chromatograph with a chiral β-cyclodextrin column (Hydrodex®-β-3P, Macherey-Nagel, Düren, Germany). Injection and detection temperature were set to 220 °C. Absolute configurations were determined by comparison of the retention times with those of optically active standards of (*R*)-**1a** and (*S*)-**2b** (see Table 3).

Table 3. GC analysis using a chiral column

Compound	T_{Column} (°C)	Retention times (min)
1a	100[a]	7.1/7.6[c]
2a	100[a]	6.7/9.7[d]
3a	100[a]	10.8/12.1[c]
1b	90[b]	35.6/36.1[c]
2b	90[b]	34.6/48.7[d]

[a] Column pressure: 125 kPa.
[b] Column pressure: 65 kPa.
[c] Elution order of enantiomers is (*R*) before (*S*).
[d] Elution order of enantiomers is (*S*) before (*R*).

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