ELSEVIER

Journal of Molecular Catalysis B: Enzymatic 21 (2003) 231–238

JOURNAL OF MOLECULAR CATALYSIS B: ENZYMATIC

www.elsevier.com/locate/molcatb

# Microbiological transformations 52. Biocatalysed Baeyer–Villiger oxidation of 1-indanone derivatives

María C. Gutiérrez, Véronique Alphand, Roland Furstoss*

*Faculté des Sciences de Luminy, Groupe Biocatalyse et Chimie Fine, UMR CNRS 6111, Université de la Méditerranée, Case 901, 163 Avenue de Luminy, 13288 Marseille Cedex 9, France*

Received 26 July 2002; received in revised form 26 July 2002; accepted 25 September 2002

**Abstract**

The microbiological Baeyer–Villiger oxidation of various substituted 1-indanones is described. Three bacterial strains have been explored: an *E. coli* TOP10 [pQR 239] constructed to overexpress the cyclohexanone monoxygenase (CHMO) of *Acinetobacter calcoaceticus* NCIMB 9871, an *E. coli* TOP10 [hapE] strain recently constructed to overexpress 4-hydroxyacetophenone monoxygenase (HAPMO) of *Pseudomonas fluorescens* ACB and the wild type *Pseudomonas* sp. NCIMB 9872 strain known to metabolise cyclopentanone. This last strain oxidised some of the proposed substrates, leading to the corresponding lactones with good to excellent yields depending on the aromatic ring substituents.
© 2002 Elsevier Science B.V. All rights reserved.

*Keywords:* Baeyer–Villiger oxidation; Whole cell biotransformation; Recombinant microorganisms; Overexpressed Baeyer–Villigerase; 1-Indanones

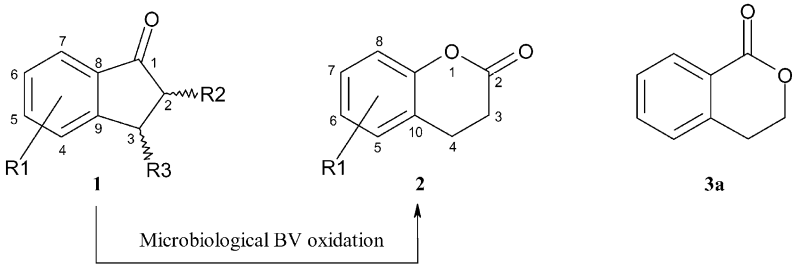
## 1. Introduction

The Baeyer–Villiger (BV) oxidation of ketones is an important and interesting reaction because of its large number of applications [1]. The typical chemical oxidants used to perform this reaction are essentially peracids, which are either commercially available—if sufficiently stable—or must be prepared in situ for more reactive (and therefore unstable) ones. In any case, these are rather delicate reagents for use at large (industrial) scale. Moreover only recently some transition metal based catalysts have been found which allow to achieve such reactions in an asymmetric manner, with generally only moderate success as far as enantioselectivity is concerned [2]. On the other hand, it has been demonstrated for more than a decade that BV oxidations can also be performed using enzymes, i.e. so-called Baeyer–Villigerases. In many cases these enzymes allow to reach very high enantioselectivity, and several examples demonstrating the possible preparative scale use of whole cell microorganisms, starting from either racemic or prochiral substrates, have been described [3]. Interestingly, this "green chemistry" approach provides a highly appealing alternative to the chemical methods, allowing enhanced safety and minimum environmental impact.

Most—if not all—biocatalysed BV oxidations which have been studied up to now, were performed using aliphatic ketones as substrates, in spite of the fact that several bacteria able to degrade aromatic ketones via a BV process have been isolated [4–7]. We describe in this paper our results aimed to explore the

* Corresponding author. Tel.: +33-491-829155; fax: +33-491-829145.
*E-mail address:* furstoss@luminy.univ-mrs.fr (R. Furstoss).

1381-1177/02/$ – see front matter © 2002 Elsevier Science B.V. All rights reserved.
PII: S1381-1177(02)00227-8




| Compound | | a | b | c | d | e | f | g | h | i |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | $R_1$ | H | 5-F | 5-Cl | 5-Br | 5-OMe | 4-OMe | 6-OMe | H | H |
| | $R_2$ | H | H | H | H | H | H | H | Me | H |
| | $R_3$ | H | H | H | H | H | H | H | H | Me |
| 2 | $R_1$ | H | 6-F | 6-Cl | 6-Br | 6-OMe | - | - | - | - |
| | Yield(%)* | 91 | 93 | 93 | - | 94 | - | - | - | - |

Scheme 1. Structures of the substrates (**1a**–**1i**) used in the biotransformation studies and of their corresponding lactone derivatives (**2**). Possible regioisomeric lactone (isochromanone) (**3a**). (∗): Overall yield of lactone obtained by preparative scale experiments (after cyclisation of the hydroxyacid). (-): no reaction. Substrate essentially recovered.

possibility to carry out such BV oxidations on aromatic ketones, i.e. 1-indanone derivatives. The aim of this study was to extend our knowledge of the scope and limitations of these enzymes, to determine the regioselectivity and, eventually, the enantioselectivity of these reactions and to test the influence of the aromatic ring substituents on the kinetics of these oxidations. Our studies were carried out using compounds **1a**–**1i** as substrates (Scheme 1). These various substrates have been chosen because (a) ketones **1b**–**1e** are substituted at the same (C-5) position of the aromatic ring, thus allowing to test the influence of both the size and the electronic nature of the substituents, (b) ketones **1e**–**1g** were chosen because they bear the same substituent (methoxy group) at different positions of the aromatic ring, thus allowing to test the influence of this parameter, (c) ketones **1h** and **1i** are substituted at the aliphatic ring, allowing to explore the possibility of achieving their kinetic resolution using this biooxidation. We have tested three different whole cell biocatalysts, i.e. two recombinant bacteria and a wild type bacterium. The first one, i.e. *E. coli* TOP10 [pQR 239], has been constructed to overexpress the cyclohexanone monoxygenase (CHMO) of *Acinetobacter calcoaceticus* NCIMB 9871 [8], an enzyme very well known to catalyse BV oxidation of numerous monocyclic or polycyclic substrates [9]. The second one, i.e. *E. coli* TOP10 [hapE], has been recently constructed for overexpression of the 4-hydroxyacetophenone monoxygenase (HAPMO) of *Pseudomonas fluorescens* ACB [10]. The wild type—*Pseudomonas* sp. NCIMB 9872 strain—was selected because it is known to be equipped with a cyclopentanone monooxygenase (CPMO) [11].

## 2. Materials and methods

### 2.1. General procedures and materials

Gas chromatography analyses were performed with a Shimadzu GC-14A chromatograph equipped with an Optima 5 fused silica capillary column (diameter 0.5 μm, length 30 m) (Macherey–Nagel GmbH &

Co., Duren, Germany) with helium as the carrier gas. Melting points were determined on a Büchi apparatus and are uncorrected. $^{1}H$ and $^{13}C$ NMR spectra were recorded on a Bruker AC 250 spectrometer in $CDCl_3$ solutions. Chemical shifts ($\delta$) are quoted in ppm with $Me_4Si$ as reference. Growth of microorganisms was carried out in a 2 L fermentor (Setric).

Substrates were purchased from Aldrich Chem. Co. (**1a**, **1f**, **1h** and **1i**), Interchim (**1b**), Avocado (**1c**), Acros Organic (**1d** and **1g**), Fluka (**1e**), and used without further purification. 3,4-Dihydrocoumarin (**2a**) was purchased from ICN Biochemicals. Reagents: *m*-chloroperbenzoic acid and *para*-toluenesulfonic acid monohydrate from Fluka and trifluoromethanesulfonic acid from Lancaster. Bacterial growth media were purchased from commercial suppliers and used as provided. All solvents were distilled before use.

The two overexpressing strains *E. coli* TOP10 [pQR 239] and *E. coli* TOP10 [hapE] were generous gifts from, respectively, Prof. J. Ward (University College, London) and Prof. D. Janssen and Dr M.W. Fraaije (University of Groningen, Holland). The wild type strain *Pseudomonas* sp. NCIMB 9872 was obtained from the National Collection of Industrial and Marine Bacteria (UK). Stock cultures were grown on nutrient agar at 30 °C and stored at 4 °C.

### 2.2. Culture conditions

#### 2.2.1. Recombinant E. coli

*E. coli* TOP10 [pQR 239] contains a pBAD plasmid into which the CHMO gene from *A. calcoaceticus* NCIMB 9871 has been cloned. Similarly, the *E. coli* TOP10 [hapE] strain contains a pBAD plasmid into which the HAPMO gene from *P. fluorescens* ACB has been cloned. The expression of either the CHMO or the HAPMO gene was induced by L(+)-arabinose. The recombinant bacteria were cultivated essentially as described previously [8].

#### 2.2.2. Pseudomonas sp. NCIMB 9872

A 2 L fermentor, filled with 1 L minimal mineral medium culture (4 g $Na_2HPO_4$, 2 g $KH_2PO_4$, 3 g $(NH_4)_2SO_4$, 0.1 g NaCl, 0.2 g $MgCl_2$, 0.01 g $CaCl_2$, 1 mL trace elements solution and 0.1 g yeast extract, pH 7) was supplemented with 1 g cyclopentanol as only carbon source. In order to get an improved cell (and enzymatic activity) production, 10 g sodium glutamate were also added in some experiments. The media was inoculated with 100 mL of an exponentially growing culture (10 h), which had been previously inoculated with 10 mL of 24 h pre-culture. Cells were grown for 15 h at 30 °C, with a vigourous aeration (35 L $h^{-1}$) and 500 rpm stirring. pH was controlled by the automatic addition of 1N $H_2SO_4$. The cell yield was 0.7 g DWL$^{-1}$ when cyclopentanol was used as the only carbon source, and 2.9 g DWL$^{-1}$ if sodium glutamate was also added.

### 2.3. Biotransformation conditions

#### 2.3.1. Analytical experiments

Harvested cells were resuspended in half the volume of their culture medium of phosphate buffer (50 mM, pH 7.1) and the suspension was used immediately for biotransformation. These were routinely performed at a 50 mL scale in 250 mL Erlenmeyer flasks in a reciprocal agitated water bath maintained at 30 °C. The substrate was pre-dissolved in the minimum volume of ethanol and was added to the cell suspension to obtain a final concentration of 0.5 g $L^{-1}$. Addition of glycerol (0.05%) was also necessary for biotransformations by recombinant *E. coli* cells. The biooxidations were monitored by GC (Optima 5), the sampling aliquots were extracted with an equal volume of ethyl acetate using an internal standard. The analytical studies were pursued by measuring the proportion of starting ketone remaining in the medium, since the formed lactone (3,4-dihydrocoumarins **2a** [12,13], **2b**, **2c** and **2e**) were hydrolysed in these experimental conditions to the undetectable hydroxyacid.

#### 2.3.2. Preparative scale experiments

Preparative scale biotransformation of compounds **1a**–**1c** and **1e** were achieved in a 2 L fermentor with *Pseudomonas* sp. cells. One litre of the bacterial culture was centrifuged and the cells were re-suspended in the same volume of a 50 mM (pH 7.1) phosphate buffer. The biotransformations were performed using experimental conditions identical to those used for the analytical experiments. Thus, 0.5 g of substrate, solubilized in 10 mL of EtOH, were added to the biocatalytic medium. The reactions were monitored by GC and stopped when the substrates had disappeared, then the media was acidified to pH 2 by addition of 1N $H_2SO_4$ and continually extracted using methylene

chloride (24 h). The extracts were dried over anhydrous sodium sulfate and the solvent removed under reduced pressure. The residues (i.e. the corresponding hydroxyacid) were dissolved into distilled toluene and cyclised back[1] to the corresponding lactones. In a typical experiment, 100 mg of hydroxyacid were dissolved into 10 mL of distilled toluene and refluxed for 1 h in the presence of *para*-toluenesulfonic acid (8 mg). The mixture reaction was diluted with toluene and washed with saturated $NaHCO_3$ solution. The organic phase was dried over anhydrous $Na_2SO_4$ and concentrated. The corresponding 3,4-dihydrocoumarins **2a**–**2c** and **2e** were isolated in good yields.

### 2.4. Inhibition studies of the biotransformation by *Pseudomonas sp.*

#### 2.4.1. Substrate inhibition

Ketone **1** was dissolved in the minimum amount of ethanol and added in the different flasks to a *Pseudomonas* sp. cell suspension at a final concentration of 0.5, 1, 2 and 3 g $L^{-1}$, respectively. The biotransformations were carried out and their results were analysed as described before. Total conversion of **1a** was only observed at the initial ketone concentrations of 0.5 and 1 g $L^{-1}$. Nearly 70% of the compound **1a** remained after 27 h of biotransformation at higher concentrations.

#### 2.4.2. Product inhibition

3,4-Dihydrocoumarin **2a** was added to the cell suspension at a final concentration of 0.5 and 1 g $L^{-1}$. Then, ketone **1a** was added to produce a final concentration of 1 g $L^{-1}$. Total consumption of substrate **1a** was detected in the presence of a 0.5 g $L^{-1}$ of additional lactone **2a**. At a lactone concentration of 1 g $L^{-1}$, 15% of ketone **1a** remained after more than 24 h.

### 2.5. Chemical Baeyer–Villiger oxidation of *1a*–*1e*

To a mixture of ketone (2 mmol) and trifluoromethanesulfonic acid (TfOH, 30 mg, 0.2 mmol) in 10 mL of anhydrous dichloromethane at 0 °C was added *m*-CPBA (863 mg, 4 mmol). The reaction was stirred at room temperature. After completion of the reaction, the mixture was diluted with $CH_2Cl_2$ and the excess of reagent was decomposed by addition of an aqueous $Na_2S_2O_3$ solution. The organic layer was washed with saturated $NaHCO_3$ solution. Conventional work up and purification by silica gel column chromatography afforded the desired lactones [14].

### 2.6. Characterisation of dihydrocoumarins *2a*–*2c* and *2e*

3,4-Dihydrocoumarin (**2a**) was compared with an authentic commercial sample.

6-Fluoro-chroman-2-one (**2b**): white crystals, mp 80 °C; $^1$H NMR (250 MHz, $CDCl_3$) $\delta$ 2.71 (2H, m, 2H-4), 2.93 (2H, t, $J$ = 7.2 Hz, 2H-3) and 6.99–6.82 (3H, m, H-5, H-7 and H-8), $^{13}$C NMR (250 MHz, $CDCl_3$) $\delta$ 23.8, 28.7, 114.4, 114.8, 115.1, 118.1, 157.0, 160.9, 168.1.

6-Chloro-chroman-2-one (**2c**): white crystals, mp 109 °C; $^1$H NMR (250 MHz, $CDCl_3$) $\delta$ 2.71 (2H, m, 2H-4), 2.92 (2H, t, $J$ = 7.2 Hz, 2H-3), 6.92 (1H, $J_{8,7}$ = 8.4 Hz, H-8) and 7.20–7.12 (2H, m, H-5 and H-7), $^{13}$C NMR (250 MHz, $CDCl_3$) $\delta$ 25.9, 31.1, 120.6, 126.5, 130.2, 130.6, 131.7, 152.8, 170.1.

6-Methoxy-chroman-2-one (**2e**): yellow crystals, mp 47 °C; $^1$H NMR (250 MHz, $CDCl_3$) $\delta$ 2.69 (2H, m, 2H-4), 2.90 (2H, t, $J$ = 7.2 Hz, 2H-3), 3.71 (3H, s, group methoxy), 6.64 (1H, d, $J_{5,7}$ = 2.8 Hz, H-5), 6.70 (1H, dd, $J_{7,8}$ = 8.8, $J_{7,5}$ = 2.8 Hz, H-7) and 6.90 (1H, $J_{8,7}$ = 8.8 Hz, H-8), $^{13}$C NMR (250 MHz, $CDCl_3$) $\delta$ 23.9, 29.1, 55.6, 113.0, 113.2, 117.6, 123.5, 145.8, 156.0, 168.9.

## 3. Results and discussion

Each one of the three strains mentioned above was cultured following the standard procedure. Before using these cells, activity tests were performed against bicyclo[3.2.0]hepten-2-one [15,16] which was proven to be a substrate for each one of these enzymes. The biotransformations were carried out using the resting cell technique at the appropriate optimal pH phosphate buffer. The results for the biooxidation of **1a**–**1i** (at a standard substrate concentration of 0.5 g $L^{-1}$) were obtained from analytical assays by the measure of residual ketone. The corresponding lactone concentration was not measurable because

[1] Dichloromethane is not an appropriate solvent for cyclisation because partial lactone dimerisation takes place in these conditions.

these 3,4-dihydrocoumarins were hydrolysed to their hydroxyacids [12,13], which were not detectable in our analysis conditions. Chemical oxidation of **1a**–**1c** and **1e** using *m*-chloroperbenzoic acid in the presence of catalytic amounts of triflic acid was performed in order to prepare the corresponding lactones **2a**–**2c** and **2e** as authentic samples.

### 3.1. Analytical scale studies

#### 3.1.1. Biotransformation of *1a–1i* using E. coli TOP10 [pQR 239]

The CHMO enzyme has been the better-studied and has become over years the prototype for the enzymatic BV oxidation. This enzyme has been shown to be of high potential as a synthetic reagent, due to its generally high enantioselectivity and, also, to the surprising "abnormal" regioselectivity sometimes provided [15–18]. Biotransformation of 1-indanone (**1a**) only led to trace amounts of 3,4-dihydrocoumarin (**2a**) and no regioisomeric lactone **3a** was detected. In all the other cases (**1b**–**1i**) no biotransformation was observed at all (after 24–48 h), the starting ketone being recovered. Blank experiments indicate that the partial loss of substrate observed during the reaction was not due to biooxidation, but rather to substrate instability in the medium.

#### 3.1.2. Biotransformation of *1a–1i* using E. coli TOP10 [hapE]

The structural similarity between the natural substrate of the HAPMO enzyme [10] (4-hydroxyacetophenone) and our substrates led us to select this other BV enzyme as a potential biocatalyst. As in the previous case, biotransformations of 1-indanones **1a**–**1i** were carried out using this active strain. However, very surprisingly—owing to the aromatic nature of our substrates—the initial ketones were again almost quantitatively recovered after 24 h biotransformation. Clearly these indanones were not substrates for these whole cells overexpressing the HAPMO enzyme.

#### 3.1.3. Biotransformation using the wild type strain Pseudomonas sp. NCIMB 9872

It has been shown previously that this *Pseudomonas* sp. NCIMB 9872 wild type strain expresses a cyclopentanone monooxygenase (CPMO) which transforms cyclopentanone to its corresponding lactone [11]. Therefore, this strain appeared to be another potential biocatalyst for oxidation of our substrates. The bacteria were grown in a mineral minimum medium using cyclopentanol as the sole carbon source and as an enzyme inducer. Addition of monosodium glutamate to the mineral medium produced an increase of cell production, as observed previously by Barclay et al. in the case of *A. calcoaceticus* [19]. This allowed to obtain a four-fold increase of cell density per litre of culture medium. The apparent enzymatic activity, measured using resting cells suspended in the same buffer solution as the one used for biotransformations (50 mM phosphate, pH 7.1) was thus increased by a factor of 3.4.

Interestingly, biotransformation using these cells led to the total oxidation of compounds **1a**–**1c** and **1e** to their corresponding 3,4-dihydrocoumarins **2a**–**2c** and **2e** whereas only trace amounts of product could be detected from **2d**. No regioisomeric 1-chromanone derivatives were observed. On the other hand, substrates **1f**–**1i** were not oxidised and were mostly recovered (Table 1). These results interestingly indicate that the determining factor for the biotransformation of these 1-indanones derivatives is the position of the substituent, rather than its electrophilic character.

### 3.2. Evaluation of the molecular factors influencing the kinetics of the biotransformations

In order to compare the rate of the biocatalysed BV oxidation of our substrates with their chemical

Table 1
Percentage of ketone remaining after biotransformation with resting cells of *Pseudomonas* sp. NCIMB 9872

| Substrate | **1a** | **1b** | **1c** | **1d** | **1e** | **1f** | **1g** | **1h** | **1i** |
|---|---|---|---|---|---|---|---|---|---|
| *Pseudomonas* sp. NCIMB 9872 (CPMO) (23 h) | 0 | 0 | 0 | 69 | 0 | 93 | 90 | 70 | 76 |
| Blank (22 h) | 75 | 76 | 67 | 62 | 79 | 90 | 96 | 75 | 73 |

Yields were measured by GC analysis after extraction (internal standard).

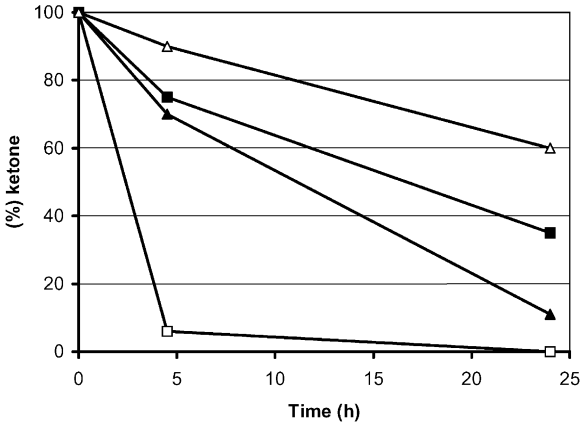



Fig. 1. Kinetic of the chemical BV oxidation with *meta*-chloroperbenzoic acid for compounds: (▲) **1a**, (△) **1b**, (■) **1c** and (□) **1e**.

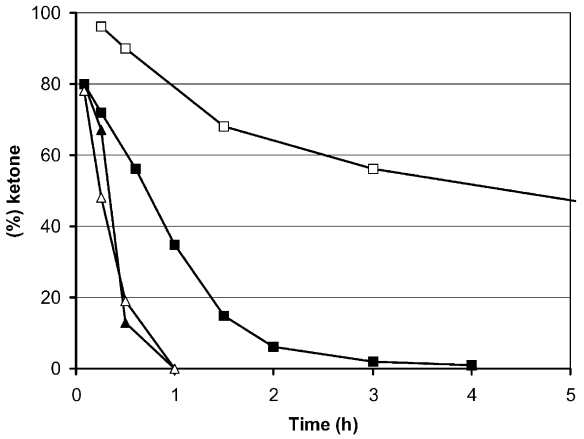



Fig. 2. Kinetic of the microbiological BV oxidation by *Pseudomonas* sp. NCIMB 9872 for compounds (▲) **1a**, (△) **1b**, (■) **1c** and (□) **1e**.

oxidation rate, we investigated their oxidation using *m*-chloroperbenzoic acid. Surprisingly, the results were quite different. Thus, for the chemical oxidation ketone **1e** showed the highest rate. The reaction slowed down for **1a** and **1c** and became even slower for ketone **1b** (Fig. 1). These results are very comparable to the ones previously described for the chemical BV oxidation of various *para*-substituted acetophenone derivatives [20]. Obviously, the determining factor in these chemical oxidations is the electronic nature of the substituents, which essentially influences the rate of the second step of the reaction, i.e. the migration step.

On the contrary, in the case of the biocatalysed BV oxidation, the best enzymatic activities were observed for compounds **1a** and **1b** bearing an hydrogen or a fluorine atom at C-5, respectively (Fig. 2). Only 1 h was necessary to cleanly and quantitatively oxidise these ketones to their corresponding lactones **2a** and **2b**. The rate of oxidation of **1c**, bearing a chlorine at C-5, was slower and about 4 h were needed for total conversion. Finally, more than twenty h were necessary to perform total oxidation of C-5 methoxy substituted **1e**, whereas the biotransformation of the *para*-bromide substituted ketone **1d** was very slow and only led to trace amounts of the corresponding lactone after 23 h.

Therefore, it appears that the influence of the electronic nature of the substituents was not the predominant factor. Obviously, the position and the intrinsic size of the substituents are most significantly involved in the rate determining step, the oxidation being faster for small substituents. Interestingly, a similar observation was made for biotransformation of substituted phenyl cyclobutanones [21] by *A. calcoaceticus* NCIMB 9871, where the stereoselectivity (enantiotopos-selection) of the reaction appeared to be essentially governed by the size of the substituent rather then by their electronic properties. This can of course be explained on the base of the spatial arrangement of the active site involved. However, other factors like for instance cell wall permeation or active transport processes, etc. ... could also be involved, and no real conclusion can be drawn without having the purified enzyme in hand.

### 3.3. Preparative scale biotransformation

Preparative scale biotransformations of **1a**–**1c** and **1e** were carried out on 0.5 g of substrate in a 1 L cell culture. The corresponding lactones, i.e. 3,4-dihydrocoumarins **2a**–**2c** and **2e** were recovered by continuous extraction (after acid catalysed cyclisation of their corresponding hydroxyacids) in overall yields of 90, 93, 93 and 94%, respectively. The thus obtained lactones were identical to the ones prepared by chemical oxidation of the corresponding ketone, thus confirming the fact that only the “normal” regioselectivity was obtained in each case.

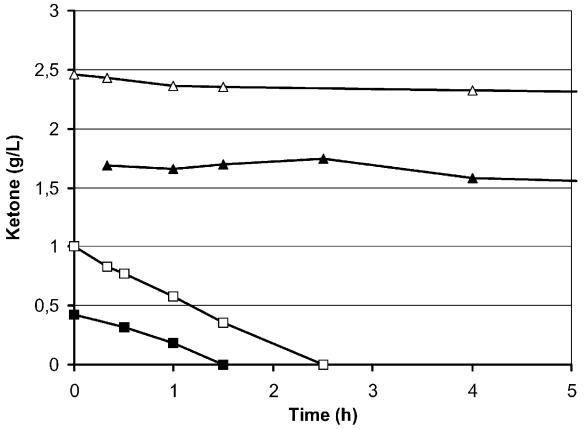



Fig. 3. Kinetic study of the microbiological BV oxidation by *Pseudomonas* sp. NCIMB 9872 at different concentrations of substrate **1a**: (■) 0.5 g $L^{-1}$, (□) 1 g $L^{-1}$, (▲) 2 g $L^{-1}$ and (△) 3 g $L^{-1}$.

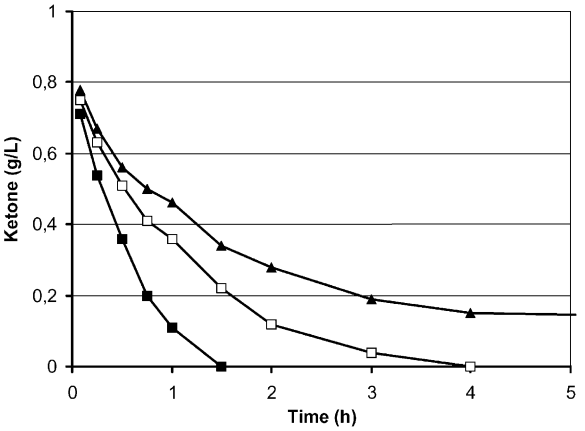



Fig. 4. Influence of the initial addition of lactone **2a** on the microbiological BV oxidation of **1a** by *Pseudomonas* sp. NCIMB 9872 (1 g $L^{-1}$ ketone concentration): (■) without initial lactone addition, (□) with 0.5 g $L^{-1}$ of **2a**, (▲) with 1 g $L^{-1}$ of **2a**.

### *3.4. Improving the experimental conditions for preparative scale biotransformation*

In view of further preparative scale experiments, we explored the possibility of achieving the biotransformation of **1a** at higher ketone concentration (i.e. at 1–3 g $L^{-1}$). Our analytical studies showed total transformation of this ketone at a 1 g $L^{-1}$ concentration. However, at 2 g $L^{-1}$ concentration, severe substrate inhibition occurred (Fig. 3). A parallel study also showed a moderate inhibition by the lactone (or hydroxyacid) product. Thus, when a 1 g $L^{-1}$ substrate **1a** concentration experiment was conducted after initial addition of lactone **2a** (at 0.5 or, respectively, 1 g $L^{-1}$ concentration), the biotransformation still occurred in the presence of 0.5 g $L^{-1}$ lactone. However, at 1 g $L^{-1}$ lactone concentration, the biooxidation stopped before completion, leaving about 15% residual ketone (Fig. 4). Therefore, controlled substrate addition as well as product removal will be necessary in order to practically improve the process. We have recently shown that, using increased cell concentration, as well as improved aeration and an ISPR technique (in situ product recovery on a specific adsorbent resin), allowed one to reach a substrate concentration of up to 20 g $L^{-1}$ for the microbiological BV oxidation of bicyclo[3.2.0]hepten-2-one [22]. We, therefore, plan to explore the possibility to set up such a process for this particular type of substrates.

## 4. Conclusion

In conclusion, we have shown that the BV oxidation of some aromatic ketones, i.e. the 1-indanone derivatives **1a**–**1c** and **1e** can be successfully carried out using whole cells of the wild type bacteria *Pseudomonas* sp. NCIMB 9872. On the contrary, the two *E. coli* recombinant strains overexpressing either the well known CHMO or the recently discovered HAPMO Baeyer–Villigerase, were unable to perform this oxidation. The oxygen atom was inserted between the aromatic ring and the ketone side chain, exhibiting a regioselectivity similar to the chemical oxidation. The corresponding 3,4-dihydrocoumarins **2a**–**2c** and **2e** were thus formed in excellent yields (>90%) and could be performed at preparative scale. Unfortunately, none of the three strains tested was able to oxidise substrates **1h** and **1i** substituted at the cyclopentane ring, thus precluding the possibility of a kinetic resolution and the preparation of the enantiomerically enriched corresponding lactones. Owing to our results, it appears that the kinetic behaviour of the enzymatic reaction is mainly influenced by the position and the size of the substituents, rather than by their electronic nature as is the case of chemical oxidation.

Although—due to its low productivity—this biocatalytic process can obviously not presently compete with the classical peracid (stoechiometric) approach,

it still is interesting since (a) it opens up a possible "green chemistry" strategy for the preparation of these targets and (b) it allows to extend our knowledge of the scope and limitations of these interesting Baeyer–Villigerases enzymes. Work is in progress in our laboratory to further explore ways to overcome the present substrate and product inhibition limitation.

## Acknowledgements

We would like to express our thanks to Prof. J. Ward (University College, London) and Prof. D. Janssen and Dr. M.W. Fraaije for having provided us with, respectively, the *E. coli* TOP10 [pQR 239] construct overexpressing the cyclohexanone monoxygenase (CHMO) of *A. calcoaceticus* NCIMB 9871, and the *E. coli* TOP10 [hapE] strain recently constructed for overexpression of the 4-hydroxyacetophenone monoxygenase (HAPMO) of *P. fluorescens* ACB. One of us (M.C.G.) would like to express her best acknowledgements to the Foundation Ramón Areces for having financially supported this work via a postdoctoral stipend.

## References

[1] G.C. Krow, Org. React. 43 (1993) 251.
[2] A. Watanabe, T. Uchida, K. Ito, T. Katsuki, Tetrahedron Lett. 43 (2002) 4481.
[3] M.D. Mihovilovic, B. Müller, P. Stanetty, Eur. J. Org. Chem. (2002) 3711.
[4] F.K. Higson, D.D. Focht, Appl. Environ. Microbiol. 56 (12) (1990) 3678.
[5] J. Havel, W. Reineke, Appl. Environ. Microbiol. 59 (8) (1993) 2706.
[6] A. Tanner, D.J. Hopper, J. Bacteriol. 182 (23) (2000) 6565.
[7] M.J.H. Moonen, I.M.C.M. Rietjens, W.J.H. van Berkel, J. Ind. Microbiol. Biotechnol. 26 (2001) 36.
[8] S.D. Doig, L.M. O'Sullivan, S. Patel, J.M. Ward, J.M. Woodley, Enzyme Microb. Technol. 28 (2001) 265.
[9] V. Alphand, R. Furstoss, Asymmetric Baeyer–Villiger oxidation using biocatalysis, in: T. Katsuki (Ed.), Practical Approach in Chemistry: Asymmetric Oxidation Reactions, Oxford University Press, Oxford, 2001, pp. 214–227.
[10] N.M. Kamerbeek, M.J.H. Moonen, J.G.M. van der Ven, W.J.H. van Berkel, M.W. Fraaije, D.B. Janssen, Eur. J. Biochem. 268 (2001) 2547.
[11] M. Griffin, P.W. Trudgill, Biochem. J. 129 (1972) 595.
[12] M. Caswell, G.L. Schmir, J. Am. Chem. Soc. 102 (1980) 4815.
[13] D.F. DeTar, J. Am. Chem. Soc. 104 (1982) 7205.
[14] H. Kotsuki, K. Arimura, T. Araki, T. Shinohara, Synletters 4 (1999) 462.
[15] V. Alphand, A. Archelas, R. Furstoss, Tetrahedron Lett. 30 (28) (1989) 3663.
[16] V. Alphand, R. Furstoss, J. Org. Chem. 57 (1992) 1306.
[17] V. Alphand, R. Furstoss, Tetrahedron: Asym. 3 (1992) 379.
[18] F. Zambianchi, P. Pasta, G. Ottolina, G. Carrea, S. Colonna, N. Gaggero, J.M. Ward, Tetrahedron: Asym. 11 (2000) 3653.
[19] S.S. Barclay, J.M. Woodley, M.D. Lilly, P. L Spargo, A.J. Pettman, Biotechnol. Lett. 23 (2001) 385.
[20] M.F. Hawthorne, W.D. Emmons, J. Am. Chem. Soc. 80 (1958) 6393.
[21] V. Alphand, C. Mazzini, J. Lebreton, R. Furstoss, J. Mol. Catal. B: Enzyme 5 (1998) 219.
[22] H.D. Simpson, V. Alphand, R. Furstoss, J. Mol. Catal. B: Enzyme 16 (2001) 107.