DOI: 10.1002/ejoc.200900084

Enzymatic Baeyer–Villiger Oxidation of Benzo-Fused Ketones: Formation of Regiocomplementary Lactones

Ana Rioz-Martínez,[a] Gonzalo de Gonzalo,[a] Daniel E. Torres Pazmiño,[b] Marco W. Fraaije,[b] and Vicente Gotor*[a]

Keywords: Bioorganic chemistry / Oxidoreductases / Oxidation / Lactones / Regioselectivity

Baeyer–Villiger monooxygenases (BVMOs) are enzymes that are known to catalyse the Baeyer–Villiger oxidation of ketones in aqueous media using O_2 as oxidant. Herein, we describe the oxidation of a set of diverse benzo-fused ketones by three different BVMOs in both aqueous and non-conventional reaction media. Most of the tested ketones, for example, 1-tetralone and 1- and 2-indanone, were converted by one of the employed biocatalysts. The catalytic efficiency could be improved by performing the oxidation reactions at a relatively high pH and by adding organic cosolvents. One striking observation is that absolute and complementary regioselectivities were obtained when oxidizing a range of 1-indanones using two different BVMOs. The conversion of 1-indanone by 4-hydroxyacetophenone monooxygenase (HAPMO) results in the formation of the expected lactone, 3,4-dihydrocoumarin. In contrast, by using a phenylacetone monooxygenase mutein (M-PAMO), conversion of 1-indanone leads to the formation of only the unexpected lactone, 1-isochromanone. This illustrates that by the appropriate choice of BVMO as biocatalyst, the effective and regioselective conversion of a wide range of benzo-fused ketones is feasible.

Introduction

Since its discovery more than 100 years ago, the Baeyer–Villiger reaction is one of the key reactions in organic synthesis.[1] In this reaction a carbon–carbon bond located adjacent to a carbonyl group is oxidatively cleaved, and the subsequent insertion of an oxygen atom into that position results in the conversion of (cyclic) ketones into esters and lactones. Baeyer–Villiger oxidations can be carried out by using peroxy acids or by employing hydrogen peroxide or oxygen as milder oxidants in the presence of Lewis acids or organometallic compounds.[2] The products of this reaction offer an attractive and simple entry to several classes of biologically active compounds.

In the last few years, the development of enzymatic methodologies using Baeyer–Villiger monooxygenases (BVMOs) has allowed the preparation of several compounds that are of high interest in organic synthesis.[3] These flavoproteins are oxidoreductases that catalyse the Baeyer–Villiger oxidation as well as other oxidative processes that employ atmospheric oxygen as the natural oxidant. When using BVMOs, mild reaction conditions are applied, and the environmental impact is minimized to a great extent when compared with those used in chemical approaches to this reaction. In addition, high regio- and/or enantioselectivities are obtained in processes that are difficult to perform when using other oxidizing reagents. The regioselectivity of the Baeyer–Villiger oxidation is established by steric, conformational and electronic effects leading to the migration of the higher substituted (the more nucleophilic) carbon centre. In some rare cases, however, the use of Baeyer–Villiger monooxygenases has led to the formation of unexpected lactones with high regioselectivities,[4] increasing the synthetic potential of this class of enzymes. It has been suggested that in these cases the chiral environment of BVMOs imposes restrictions that allow only a single conformation of the Criegee intermediate formed during the oxidation. Only the carbon centre located in a determined conformation will migrate, regardless of its nucleophilicity.

As biological catalysts, enzymes have evolved to perform their catalytic activity in aqueous medium. Nevertheless, it has also been demonstrated that some biocatalysts can act in the presence of organic solvents. In fact, organic solvents have been widely studied and applied in biocatalysis, with the main advantages as follows: (1) catalysis of processes unfavourable in aqueous medium, (2) the partial or total suppression of water-induced side-reactions and (3) the solubilization of hydrophobic substrates.[5] Recently, it has been shown that BVMOs can also be employed in non-conventional media (mixtures of aqueous buffer with miscible

[a] Departamento de Química Orgánica e Inorgánica, Instituto de Biotecnología de Asturias, Universidad de Oviedo, c/ Julián Clavería 8, 33006 Oviedo, Spain
Fax: +34-985-103448
E-mail: vgs@fq.uniovi.es
[b] Laboratory of Biochemistry, Groningen Biomolecular Sciences and Biotechnology Institute, University of Groningen, Nijenborgh 4, 9747 AG Groningen, The Netherlands
Supporting information for this article is available on the WWW under http://www.eurjoc.org or from the author.

or immiscible organic solvents).[6] For all the cosolvents tested, two main effects were observed: (1) a decrease in enzyme activity and stability and (2) an increase and in some cases a reversal in enantioselectivity. Furthermore, it has been shown that phenylacetone monooxygenase (PAMO) can be employed in the enantioselective Baeyer–Villiger oxidation of ketones on a preparative scale when working in biphasic aqueous buffer/organic cosolvent systems.[7]

Benzo-fused lactones have received considerable attention as useful intermediates in organic synthesis and are the key structural elements of a variety of biologically active compounds. In a recent study it was shown that a set of substituted 1-indanones could be oxidized with good to excellent yields by the wild-type bacterium *Pseudomonas* sp. NCIMB 9872. In these reactions it was shown that the kinetic behaviour of the reactions was influenced by the position and size of the substituents.[8] In this paper we report the BVMO-mediated oxidation of several benzo-fused ketones catalysed by three BVMOs employing non-conventional reaction media. The three biocatalysts are phenylacetone monooxygenase (PAMO) from *Thermobifida fusca*,[9] 4-hydroxyacetophenone monooxygenase (HAPMO) from *Pseudomonas fluorescens* ACB[10] and a recently created mutant of PAMO, the M446G PAMO mutein (M-PAMO).[11] All three BVMOs have previously been shown to be effective biocatalysts in the oxidation of racemic benzyl ketones and aromatic sulfides.

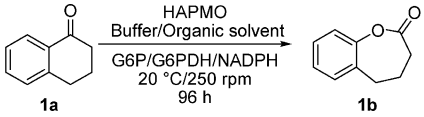
Results and Discussion

Our experiments were dedicated to the biocatalysed oxidation of different benzo-fused ketones (tetralones, indanones and benzocyclobutanone). All the reactions were carried out by using purified enzymes. An auxiliary enzymatic system (glucose-6-phosphate with glucose-6-phosphate dehydrogenase) was included in the reaction mixture to regenerate the NADPH coenzyme, which is required by BVMOs.[12]

BVMO-Catalysed Oxidation of Tetralones

We first analysed the enzymatic bio-oxidation of 1-tetralone (**1a**) by employing the three BVMOs. The reactions were performed in buffer (50 mM Tris-HCl, pH 9.0) at 30 °C for the two PAMO enzymes, whereas 20 °C was a more appropriate temperature for HAPMO.[9d] After 96 h, no reaction was observed with PAMO and M-PAMO, whereas only 5% of the expected regioisomer 4,5-dihydro-1-benzoxepin-2(3*H*)-one (**1b**) was obtained when employing HAPMO. As ketone **1a** is not very soluble in water, its oxidation catalysed by HAPMO in buffer containing 5% of different organic cosolvents was studied. Table 1 shows that only cosolvents with a high hydrophobic character ($\log P \geq 2.0$)[13] led to a significant increase in the formation of **1b**. Thus, the addition of 5% (v/v) of toluene or 2-octanol (Entries 8 and 9, respectively) allowed a 25% conversion to the lactone to be achieved after 96 h. The use of the same organic cosolvents was studied in the PAMO-catalysed oxidations; however, the formation of **1b** was not observed.

Table 1. Effect of organic cosolvents on the HAPMO-biocatalysed oxidation of 1-tetralone (**1a**).[a]



1a **1b**

Entry	Cosolvent	log *P*	Conv. [%][b]
1	none	–	5
2	5% MeOH	–0.76	≤3
3	5% 1,4-dioxane	–0.27	≤3
4	5% *i*PrOH	0.07	≤3
5	5% CH_2Cl_2	1.25	≤3
6	5% *t*BuOMe	1.35	7
7	5% iPr_2O	2.00	≤3
8	5% toluene	2.50	26
9	5% 2-octanol	2.72	25
10	5% hexane	3.50	15

[a] For the reaction conditions, see the Exp. Sect. [b] Determined by GC.

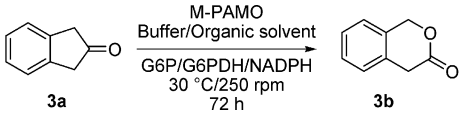
2-Tetralone (**2a**) was also subjected to BVMO-catalysed oxidation, but no reaction was observed with the three biocatalysts, even after modifying the reaction parameters (pH, temperature, and organic cosolvents).

Enzymatic Oxidation of 2-Indanone Catalysed by BVMOs

After analysing the oxidation reactions of tetralones, our efforts were devoted to the study of the enzymatic Baeyer–Villiger reactions of indanones. The oxidation reactions of 2-indanone (**3a**) carried out in buffer with HAPMO and PAMO did not show the formation of 3-isochromanone (**3b**). When modifying the reaction medium by adding different cosolvents, a slight improvement in the enzymatic activity was observed only in the HAPMO-biocatalysed oxidations. After 72 h, only 10% of **3b** was recovered in the media containing 5% hexane or CH_2Cl_2. No oxidation was observed by changing the reaction media when PAMO was used. Oxidation of **3a** catalysed by M-PAMO in 50 mM Tris-HCl buffer at pH 9.0 led to **3b** with 50% conversion after 72 h. Different water-miscible and -immiscible organic cosolvents (5% v/v) were tested, as shown in Table 2. In all cases, this resulted in a more effective oxidation of **3a**. The best results were obtained in both a hydrophilic (1,4-dioxane, Entry 3) and a hydrophobic solvent (DIPE, Entry 7), giving **3b** with conversions close to 90% after 72 h. As a further attempt at medium engineering and to optimize the reaction conditions, we decided to analyse the effect of pH on the oxidation of **3a** in buffer containing 5% 1,4-dioxane. As shown for PAMO and HAPMO in other oxidative reactions,[9b,9d] there was a significant increase in M-PAMO activity when working at relatively high pH values. No reaction was observed at pH 6.0, and low conversions were

measured below pH 9.0. At pH 9.0–10.5, the enzymatic activity remained high (conversion, c = 88–90% after 72 h, Entries 3, 14 and 15).

Table 2. M-PAMO-catalysed biooxidation of 2-indanone (**3a**) in non-conventional reaction media.[a]



3a **3b**

Entry	Cosolvent	log P	pH	Conv. [%][b]
1	none	–	9.0	50
2	5% MeOH	–0.76	9.0	83
3	5% 1,4-dioxane	–0.27	9.0	90
4	5% *i*PrOH	0.07	9.0	72
5	5% CH_2Cl_2	1.25	9.0	69
6	5% *t*BuOMe	1.35	9.0	69
7	5% iPr_2O	2.00	9.0	89
8	5% toluene	2.50	9.0	71
9	5% 2-octanol	2.72	9.0	55
10	5% hexane	3.50	9.0	77
11	5% 1,4-dioxane	–0.27	6.0	≤3
12	5% 1,4-dioxane	–0.27	7.0	11
13	5% 1,4-dioxane	–0.27	8.0	37
14	5% 1,4-dioxane	–0.27	10.0	90
15	5% 1,4-dioxane	–0.27	10.5	88

[a] For the reaction conditions, see the Exp. Sect. [b] Determined by GC.

Biocatalysed Oxidation of 1-Indanone and Its Derivatives

The three Baeyer–Villiger monooxygenases were employed as biocatalysts in the enzymatic oxidation of 1-indanone (**4a**). The reaction performed in the presence of HAPMO led to 3,4-dihydrocoumarin (**4b**) with a 26% conversion after 72 h. When PAMO was employed, no oxidation products were formed in the same reaction time. Surprisingly, the oxidation catalysed by M-PAMO led to the formation of an unexpected compound (67% conversion) with the same molecular weight as **4b**. This compound corresponded to the product obtained from the oxidation of isochromane with $NaClO_2$ and is the unexpected Baeyer–Villiger product 1-isochromanone (**4c**). Thus, by the appropriate choice of the BVMO employed, two possible regioisomers from the Baeyer–Villiger oxidation of 1-indanone can be obtained.

As shown in Figure 1, the effect of different organic cosolvents (5% v/v) with HAPMO or M-PAMO as the biocatalyst was studied. The use of hydrophobic solvents in the HAPMO-catalysed oxidation reactions led to an increase in the enzymatic activity. Thus, the oxidation performed in 5% 2-octanol (log P = 2.70) or hexane (log P = 3.50) allowed the preparation of lactone **4b** with 39 and 44% conversions, respectively. On the other hand, the use of hydrophilic solvents led to a reduction of the enzymatic activity. M-PAMO showed the opposite behaviour in respect of the nature of the organic cosolvent. The use of hydrophilic solvents such as methanol, 1,4-dioxane or 2-propanol, as well as the ethers *t*BuOMe or iPr_2O, led to **4c** with conversions higher than 70% after 72 h (Table 3). The employment of hydrophobic cosolvents led to a deactivation of M-PAMO, giving the unexpected lactones in lower yields than those obtained for the oxidation performed in Tris-HCl buffer.

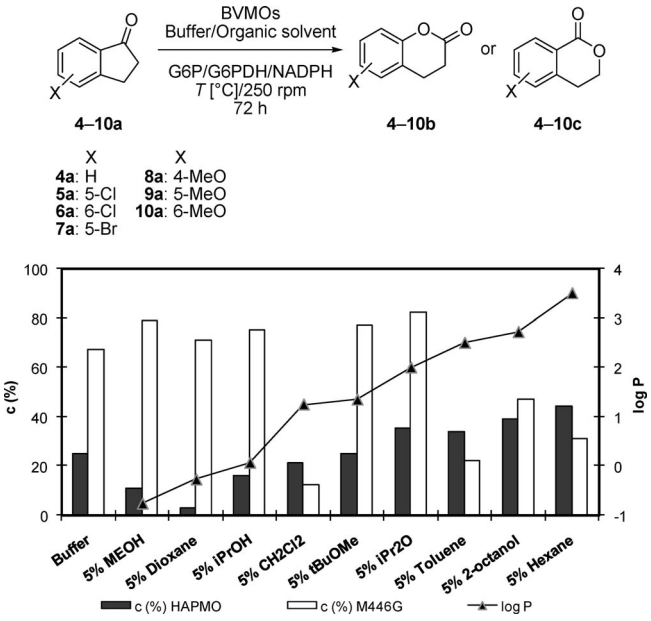


Figure 1. Effect of different organic solvents in aqueous/organic solvent (5% v/v) systems on the enzymatic oxidation reactions of **4a** catalysed by HAPMO to synthesize lactone **4b** or by M-PAMO to give the unexpected lactone **4c** (t = 72 h); log P (▲) values have been plotted to correlate enzyme activities with the hydrophobicity of the organic solvents.

Table 3. Effect of pH on the oxidation of 1-indanone (**4a**) catalysed by HAPMO or M-PAMO in different reaction media.[a]

Entry	BVMO	Cosolvent	pH	Conv. [%][b]	
				4b	**4c**
1	HAPMO	5% MeOH	7.0	≤3	≤3
2	HAPMO	5% hexane	8.0	39	≤3
3	HAPMO	5% hexane	9.0	44	≤3
4	HAPMO	5% hexane	10.0	47	≤3
5	HAPMO	5% hexane	10.5	21	≤3
6	M-PAMO	5% MeOH	7.0	≤3	≤3
7	M-PAMO	5% MeOH	8.0	≤3	17
8	M-PAMO	5% MeOH	9.0	≤3	79
9	M-PAMO	5% MeOH	10.0	≤3	72
10	M-PAMO	5% MeOH	10.5	≤3	72

[a] The reactions were performed at 30 °C for M-PAMO, whereas 20 °C was used for HAPMO. [b] Determined by GC.

The effect of pH on the enzymatic Baeyer–Villiger oxidations of **4a** catalysed by HAPMO in aqueous buffer with 5% hexane and by M-PAMO in Tris-HCl with 5% methanol were analysed in more detail. As described for previous oxidation reactions, low pH values led to no reaction or to low conversion after long reaction times. For both enzymes, no oxidation was observed at pH 7.0. An increase

in the pH led to higher activities. M-PAMO was even able to catalyse the formation of **4c** with high conversions at pH 10.5 (Entry 10, c = 72%), whereas HAPMO seemed to be deactivated at this pH: a moderate decrease in conversion was observed when the pH was increased from pH 10.0 (Entry 4, c = 47%) to pH 10.5 (Entry 5), giving only 21% of **4b** after 72 h.

The concentration of the organic cosolvent in both enzymatic systems was also studied. As shown in Figure 2, the highest activity for HAPMO was found using 5% hexane. When this cosolvent concentration was increased to 30%, only a slight decrease in the conversion to **4b** was observed. Higher concentrations of hexane resulted in (partial) deactivation of the enzyme, although HAPMO was still able to oxidize **4a** in Tris-HCl buffer containing 70% hexane (c = 5% after 72 h). The enzymatic Baeyer–Villiger oxidation of **4a** catalysed by M-PAMO in the presence of methanol was found to be the highest at 5% (v/v), whereas higher cosolvent concentrations led to a progressive loss in enzymatic activity. The formation of lactone **4c** after 72 h was not observed using mixtures containing 50% methanol.

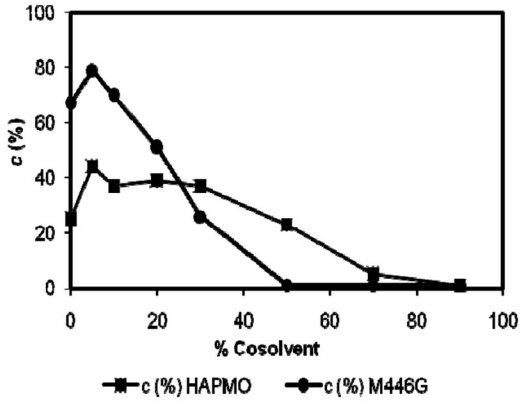


Figure 2. Effects of the hexane concentration on the HAPMO oxidation of 1-indanone (**4a**) and of the MeOH concentration on the M-PAMO oxidation.

To analyse the effect of different substituents on the aromatic moiety of 1-indanone, we studied the oxidation of various 1-indanone derivatives **5a**–**10a** catalysed by the three BVMOs. As described for 1-indanone, HAPMO and M-PAMO showed again a complete and opposite regioselectivity in the oxidation of the 1-indanone derivatives. Reactions performed with HAPMO led to the expected lactones **5b**–**10b**, whereas M-PAMO yielded the unexpected lactones **5c**–**10c**. In all the reactions tested, the use of 5% hexane with HAPMO and of 5% methanol with M-PAMO resulted in higher conversions. No reactions were observed for any of the substituted derivatives when wild-type PAMO was employed. Halogenated derivatives **5a**–**7a** were shown to be converted more efficiently by M-PAMO than by HAPMO, yielding high amounts of the unexpected lactones **5c**–**7c**. The oxidation of 5-chloro-1-indanone (**5a**) by HAPMO was less effective than that of the 6-chloro derivative (**6a**) when using only buffer, but in the presence of 5% hexane the opposite effect was observed: **5a** was converted more efficiently (c = 87%) than **6a** (c = 56%) after 72 h (Table 4). A similar behaviour was also observed for M-PAMO in the presence of 5% MeOH. HAPMO showed a lower activity towards the oxidation of 5-bromo-1-indanone (**7a**) than towards the chlorine derivative, whereas 6-bromoisochroman-1-one (**7c**) can be obtained with an excellent conversion (95%) in a process catalysed by M-PAMO. The oxidation of 1-indanones bearing a methoxy group in the aromatic ring occurred with lower activities than the halogenated substrates. Thus, 4-methoxy-1-indanone (**8a**) was not a substrate for the HAPMO-catalysed reaction, whereas the Baeyer–Villiger oxidations of **9a** and **10a** led to the expected lactones **9b** and **10b** with moderate conversions (32% after 72 h when employing 5% hexane). On the other hand, the best results of M-PAMO were achieved with ketone **8a** (58% conversion in the presence of 5% methanol). 6-Methoxyisochroman-1-one (**9c**) was obtained with a moderate conversion (c = 32% after 72 h), whereas 6-methoxy-1-indanone (**10a**) was not oxidized by this biocatalyst, which indicates that the position of the methoxy group has a strong effect on the enzymatic activity.

Table 4. Enzymatic oxidation of substituted 1-indanones catalysed by BVMOs in different reaction media.[a]

Entry	X	Cosolvent	BVMO	Conv. [%]	
				5–10b[b]	**5–10c**[b]
1	5-Cl	5% hexane	HAPMO	87	≤3
2	5-Cl	5% MeOH	M-PAMO	≤3	92
3	6-Cl	5% hexane	HAPMO	56	≤3
4	6-Cl	5% MeOH	M-PAMO	≤3	79
5	5-Br	5% hexane	HAPMO	52	≤3
6	5-Br	5% MeOH	M-PAMO	≤3	95
7	4-MeO	5% hexane	HAPMO	≤3	≤3
8	4-MeO	5% MeOH	M-PAMO	≤3	58
9	5-MeO	5% hexane	HAPMO	32	≤3
10	5-MeO	5% MeOH	M-PAMO	≤3	32
11	6-MeO	5% hexane	HAPMO	32	≤3
12	6-MeO	5% MeOH	M-PAMO	≤3	≤3

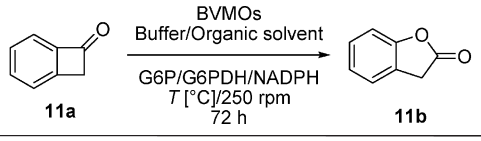
[a] The reactions were performed at 30 °C for M-PAMO, whereas 20 °C was used for HAPMO. [b] Determined by GC.

Baeyer–Villiger Oxidation of Benzocyclobutanone

Finally, the three enzymes were tested in the enzymatic oxidation of benzocyclobutanone (**11a**). The Baeyer–Villiger reaction of this compound led to a five-membered lactone as the only regioisomer, 2-coumaranone (**11b**). When employing HAPMO at 20 °C in aqueous buffer, this lactone was obtained in high yield after 72 h (Entry 1, Table 5). The presence of 5% hexane increased the yield of **11b** slightly (c = 93%). Good results were also obtained when using PAMO. This biocatalyst showed low activity in the oxidation of indanones and tetralones, but was able to oxidize **11a** with a 51% conversion at 30 °C in a solution containing only buffer. By performing this reaction in the presence of 5% hexane, higher yields could be obtained (Entry 4). The use of 5% MeOH did not improve the results

obtained for this biocatalyst in buffer alone (c = 42% after 72 h, Entry 5). M-PAMO showed low activity towards **11a** (Entry 6), giving only a 14% yield of **11b** after 72 h. The yield of this lactone could be doubled by using 5% MeOH as cosolvent.

Table 5. Enzymatic oxidation of **11a** catalysed by BVMOs in non-conventional reaction media.[a]



Entry	Cosolvent	BVMO	T [°C]	Conv. [%][b]
1	none	HAPMO	20	87
2	5% hexane	HAPMO	20	93
3	none	PAMO	30	51
4	5% hexane	PAMO	30	81
5	5% MeOH	PAMO	30	42
6	none	M-PAMO	30	14
7	5% MeOH	M-PAMO	30	31

[a] For the reaction conditions, see the Exp. Sect. [b] Determined by GC.

Conclusions

The oxidation reactions of a set of benzo-fused ketones biocatalysed by three BVMOs have been performed in order to obtain benzo-fused lactones. Tetralones were poor substrates for the three enzymes, whereas the bio-oxidation of indanones produced the corresponding lactones in good yields. The appropriate choice of reaction medium, for example, by the addition of different organic cosolvents (5%, v/v) to Tris-HCl buffer, led to higher conversions. This demonstrates again that the addition of organic solvents can be beneficial for enzymatic reactions. For HAPMO, the best results were obtained when using hydrophobic organic cosolvents, whereas M-PAMO seemed to prefer hydrophilic solvents. Strikingly, these two enzymes also presented a divergent regiospecificity in the oxidation of 1-indanone and its derivatives. Employment of HAPMO as the biocatalyst resulted in the formation of the expected lactones, whereas the unexpected lactones were obtained with the PAMO mutant. Wild-type PAMO was unable to convert most of the 1-indanones. This illustrates that the M446G mutation creates an active site in PAMO that is dedicated to accepting 1-indanones (see below) or indoles.[11] Thus, by choosing carefully the reaction medium and the biocatalyst, both regioisomeric Baeyer–Villiger products of 1-indanone derivatives can be obtained in high yields. PAMO and HAPMO are also able to catalyse the synthesis of 2-coumaranone with excellent conversions, which illustrates the synthetic potential of BVMO using benzo-fused ketones as substrates.

Experimental Section

General Methods: Recombinant histidine-tagged phenylacetone monooxygenase (PAMO),[9a] its M446G mutant (M-PAMO)[11] and recombinant 4-hydroxyacetophenone monooxygenase (HAPMO)[10a] were overexpressed and purified as described previously. 1.0 unit of BVMO will oxidize 1.0 μmol of phenylacetone to benzyl acetate per minute at pH 9.0 and 25 °C in the presence of NADPH. Glucose 6-phosphate dehydrogenase from Leuconostoc mesenteroides was obtained from Fluka-Biochemika. Starting ketones **1**–**6a** and **8a** and lactone **11b** were supplied by Sigma-Aldrich-Fluka, ketone **7a** was a product of TCI Europe and compounds **9a** and **10a** were purchased from Acros Organics. All other reagents and solvents were of the highest quality grade available and were obtained from Sigma-Aldrich-Fluka and Acros Organics. Chemical reactions were monitored by analytical TLC, performed on Merck silica gel 60 F254 plates and visualized by UV irradiation. Flash chromatography was carried out with silica gel 60 (230–240 mesh, Merck). Melting points were measured in open capillary tubes with a Gallenkamp apparatus and are uncorrected. IR spectra were recorded with a Perkin-Elmer 1720-X IR Fourier-transform spectrometer using KBr pellets. ^{1}H and ^{13}C NMR and DEPT spectra were recorded with tetramethylsilane (TMS) as the internal standard with a Bruker AC-300 DPX spectrometer (^{1}H: 300.13 MHz; ^{13}C: 75.5 MHz). The chemical shifts (δ) are given in ppm. Mass spectra were recorded in EI$^+$ mode with a Hewlett-Packard 5973 mass spectrometer. GC analyses were performed with a Hewlett-Packard 6890 Series II chromatograph. For all the analyses, the injector temperature was 225 °C and the FID temperature was 250 °C. Lactones **1b**–**10b** were synthesized by Baeyer–Villiger oxidation of the corresponding starting ketones (100 mg) with *m*-CPBA in CH_2Cl_2 at 0 °C (yields from 30 to 85%). 1-Isochromanone (**4c**) was prepared according to the literature,[14] by oxidizing isochromane with $NaClO_2$ and *N*-hydroxyphthalimide in CH_3CN/H_2O (66% yield). Compounds **1b**,[15a] **2b**,[15b] **3b**,[15c] **4b**,[15d] **5b**,[8] **7b**,[15e] **9b**,[15f] **10b**,[15g] **4c**,[14] **6c**[15h] and **9c**[15i] exhibit physical and spectral properties in accord with those reported previously.

7-Chloro-3,4-dihydrochromen-2-one (6b): Chemical yield: 71.3 mg (65%). Colourless solid, m.p. 53–54 °C. IR (KBr): $\tilde{\nu}$ = 3086, 2918, 1744, 1487, 1450 cm^{-1}. ^{1}H NMR (300.13 MHz, $CDCl_3$, 25 °C): δ = 2.78 (t, $^3J_{H,H}$ = 6.8 Hz, 2 H), 2.98 (t, $^3J_{H,H}$ = 6.8 Hz, 2 H), 7.07–7.14 (m, 3 H) ppm. ^{13}C NMR (75.5 MHz, $CDCl_3$, 25 °C): δ = 23.2 (CH_2), 28.9 (CH_2), 117.3 (CH_{ar}), 121.1 (CH_{ar}), 124.5 (CH_{ar}), 128.8 (CH_{ar}), 133.4 (C_{ar}), 152.3 (C_{ar}), 167.6 (C=O) ppm. MS (EI): *m*/*z* (%) = 182 (100) [M]$^+$, 154 (94), 147 (21), 119 (56), 77 (56). HRMS (EI): calcd. for $C_9H_7ClO_2$ [M]$^+$ 182.01346; found 182.01217.

5-Methoxy-3,4-dihydrochromen-2-one (8b): Chemical yield: 23.1 mg (42%). Pale-yellow solid, m.p. 43–45 °C. IR (KBr): $\tilde{\nu}$ = 3096, 2965, 2360, 1770, 1612, 1469 cm^{-1}. ^{1}H NMR (300.13 MHz, $CDCl_3$, 25 °C): δ = 2.71 (t, $^3J_{H,H}$ = 7.0 Hz, 2 H), 2.96 (t, $^3J_{H,H}$ = 7.0 Hz, 2 H), 3.85 (s, 3 H), 6.67 (t, $^3J_{H,H}$ = 7.6 Hz, 2 H), 7.19 (t, $^3J_{H,H}$ = 8.2 Hz, 1 H) ppm. ^{13}C NMR (75.5 MHz, $CDCl_3$, 25 °C): δ = 17.4 (CH_2), 28.5 (CH_2), 55.7 (CH_3), 106.1 (CH_{ar}), 109.3 (CH_{ar}), 111.2 (C_{ar}), 128.2 (CH_{ar}), 152.5 (C_{ar}) 156.6 (C_{ar}), 168.5 (C=O) ppm. MS (EI): *m*/*z* (%) = 178 (100) [M]$^+$, 150 (48), 136 (97), 77 (42). HRMS (EI): calcd. for $C_{10}H_{10}O_3$ [M]$^+$ 178.06299; found 178.07031.

Synthesis of Benzocyclobutanone (11a): Ketone **11a** was synthesized according to the literature[16] by treating at reflux bromobenzene with $NaNH_2$ and 1,1-dimethoxyethylene in THF. The ketal obtained was hydrolysed without further purification using a mixture of THF/H_2O containing traces of HCl. The final product **11a** was obtained in 83% yield.

General Method for the Enzymatic Baeyer–Villiger Oxidation of Benzo-Fused Ketones: In a typical experiment, the starting ketones **1a**–**11a** (15–30 mm) were dissolved in a 50 mM Tris-HCl (pH from 6.0 to 10.5)/organic cosolvent system (0.5 mL) containing glucose-

6-phosphate (2.0 equiv.), glucose-6-phosphate dehydrogenase (10.0 units), NADPH (0.2 mm) and the corresponding Baeyer–Villiger monooxygenase (1.0 unit). The mixture was shaken at 250 r.p.m. and the selected temperature in a rotatory shaker for the times indicated. The reactions were then stopped, the mixtures worked up by extraction with ethyl acetate (2 × 0.5 mL), dried with Na_2SO_4 and analysed directly by chiral GC to determine the conversion. For all the reaction media tested, control experiments in the absence of enzyme resulted in no conversion.

General Procedure for the Enzymatic Synthesis of Lactones 5c, 7c and 8c on the Multimilligram Scale: Reactions were performed in duplicate. Ketones **5a**, **7a** and **8a** (50 mg) were dissolved in 50 mM Tris-HCl buffer (pH 9.0, 10 mL) containing glucose-6-phosphate (1.5 equiv.), glucose-6-phosphate dehydrogenase (20 units), NADPH (0.02 mm) and M446G phenylacetone monooxygenase (1.5 units). The mixtures were shaken at 30 °C and 250 r.p.m. for 72 h. Once finished, the reaction mixtures were extracted with EtOAc (3 × 25 mL), and the combined organic layers were dried with Na_2SO_4, filtered and concentrated under reduced pressure. The residues were purified by flash chromatography on silica gel with hexane/ethyl acetate (8:2) to afford the corresponding lactones **5c** and **7c** and with hexane/ethyl acetate (7:3) to obtain compound **8c**.

6-Chloroisochroman-1-one (5c): Yield: 39.4 mg (72%). Colourless solid, m.p. 58–59 °C. IR (KBr): $\tilde{\nu}$ = 3052, 2970, 1740, 1475 cm^{-1}. ^1H NMR (300.13 MHz, $CDCl_3$, 25 °C): δ = 3.08 (t, $^3J_{H,H}$ = 7.5 Hz, 2 H), 4.57 (t, $^3J_{H,H}$ = 7.5 Hz, 2 H), 7.40 (d, $^3J_{H,H}$ = 7.0 Hz, 1 H), 7.60 (s, 1 H), 7.94 (d, $^3J_{H,H}$ = 7.0 Hz, 1 H) ppm. ^{13}C NMR (75.5 MHz, $CDCl_3$, 25 °C): δ = 27.7 (CH_2), 67.2 (CH_2), 125.1 (CH_{ar}), 127.1 (CH_{ar}), 127.6 (C_{ar}), 130.2 (CH_{ar}), 133.5 (C_{ar}), 139.4 (C_{ar}), 164.9 (C=O) ppm. MS (EI): *m/z* (%) = 182 (90) $[M]^+$, 154 (80), 138 (12), 126 (70), 77 (60). HRMS (EI): calcd. for $C_9H_7ClO_2$ $[M]^+$ 182.01346; found 182.01504.

6-Bromoisochroman-1-one (7c): Yield: 34.9 mg (65%). Pale-yellow solid, m.p. 63–65 °C IR (KBr): $\tilde{\nu}$ = 3052, 2980, 1736, 1470 cm^{-1}. ^1H NMR (300.13 MHz, $CDCl_3$, 25 °C): δ = 3.12 (t, $^3J_{H,H}$ = 6.8 Hz, 2 H), 4.60 (t, $^3J_{HH}$ = 6.8 Hz, 2 H), 7.50 (d, $^3J_{H,H}$ = 7.2 Hz, 1 H), 7.55 (s, 1 H), 7.91 (d, $^3J_{H,H}$ = 7.2 Hz, 1 H) ppm. ^{13}C NMR (75.5 MHz, $CDCl_3$, 25 °C): δ = 26.5 (CH_2), 65.6 (CH_2), 127.3 (C_{ar}), 128.8 (CH_{ar}), 132.0 (CH_{ar}), 132.4 (C_{ar}), 132.6 (CH_{ar}), 144.1 (C_{ar}), 165.2 (C=O) ppm. MS (EI): *m/z* (%) = 226 (100) $[M]^+$, 188 (70), 182 (10), 154 (60), 77 (80). HRMS (EI): calcd. for $C_9H_7BrO_2$ $[M]^+$ 225.96294; found 225.95931.

5-Methoxyisochroman-1-one (8c): Yield: 15.4 mg (28%). Pale-yellow solid, m.p. 44–46 °C. IR (KBr): $\tilde{\nu}$ = 3040, 2975, 1747, 1482 cm^{-1}. ^1H NMR (300.13 MHz, $CDCl_3$, 25 °C): δ = 3.13 (t, $^3J_{H,H}$ = 7.0 Hz, 2 H), 3.80 (s, 3 H), 4.61 (t, $^3J_{H,H}$ = 7.0 Hz, 2 H), 7.03–7.09 (m, 2 H), 7.67 (d, $^3J_{H,H}$ = 7.8 Hz, 1 H) ppm. ^{13}C NMR (75.5 MHz, $CDCl_3$, 25 °C): δ = 28.5 (CH_2), 55.7 (CH_3), 65.9 (CH_2), 106.2 (CH_{ar}), 109.4 (CH_{ar}), 123.2 (C_{ar}), 128.2 (CH_{ar}), 140.8 (C_{ar}), 152.6 (C_{ar}), 168.3 (C=O) ppm. MS (EI): *m/z* (%) = 178 (100) $[M]^+$, 150 (90), 134 (45), 106 (12), 77 (40). HRMS (EI): calcd. for $C_{10}H_{10}O_3$ $[M]^+$ 178.06299; found 178.06547.

Supporting Information (see footnote on the first page of this article): Complete results of the enzymatic oxidation of 1-indanone and its derivatives in all the reaction media, as well as the GC data and ^1H and ^{13}C NMR spectra of lactones **5c**, **6b**, **7c**, **8b** and **8c**.

Acknowledgments

A. R.-M. (FPU Program) thanks the Spanish Ministerio de Ciencia e Innovación (MICINN) for her predoctoral fellowship which is financed by the European Social Fund. G. d. G. (Juan de la Cierva Program) thanks MICINN for personal funding. This work was supported by the MICINN (Project CTQ2007-61126). M. W. F. and D. E. T. P. received support from the EU-FP7 "Oxygreen" project.

[1] A. Baeyer, V. Villiger, *Ber. Dtsch. Chem. Ges.* **1899**, *32*, 3625–3633.

[2] a) S. Xu, Z. Wang, X. Zhang, X. Zhang, K. Ding, *Angew. Chem. Int. Ed.* **2008**, *47*, 2840–2843; b) G. Peris, S. J. Miller, *Org. Lett.* **2008**, *10*, 3049–3052; c) G.-J. Ten Brink, I. W. C. E. Arends, R. A. Sheldon, *Chem. Rev.* **2004**, *104*, 4105–4123; d) S.-I. Murahashi, S. Ono, Y. Imada, *Angew. Chem. Int. Ed.* **2002**, *41*, 2366–2368; e) M. Renz, B. Meunier, *Eur. J. Org. Chem.* **1999**, *4*, 737–750.

[3] For some recent bibliographies, see: a) M. M. Kayser, *Tetrahedron* **2009**, *65*, 947–974; b) D. E. Torres Pazmiño, M. W. Fraaije in *Future Directions in Biocatalysis* (Ed.: T. Matsuda), Elsevier, Dordrecht, **2007**, pp. 107–127, and references cited therein; c) M. W. Fraaije, D. B. Janssen in *Modern Biooxidation, Enzymes, Reactions and Application* (Eds.: R. D. Schmid, V. D. Urlacher), Wiley-VCH, Weinheim, **2007**, pp. 77–94; d) M. D. Mihovilovic, *Curr. Org. Chem.* **2006**, *10*, 1265–1287; e) N. M. Kamerbeerk, D. B. Janssen, J. H. van Berkel, M. W. Fraaije, *Adv. Synth. Catal.* **2003**, *345*, 667–678; f) V. Alphand, G. Carrea, R. Wohlgemuth, R. Furstoss, J. M. Woodley, *Trends Biotechnol.* **2003**, *21*, 318–323; g) M. D. Mihovilovic, B. Müller, P. Stanetty, *Eur. J. Org. Chem.* **2002**, 3711–3730.

[4] For some examples, see: a) M. D. Mihovilovic, P. Kapitan, P. Kapitánová, *ChemSusChem* **2008**, *1*, 143–148; b) P. Černuchova, M. D. Mihovilovic, *Org. Biomol. Chem.* **2007**, *5*, 1715–1719; c) R. Snajdrova, G. Grogan, M. D. Mihovilovic, *Bioorg. Med. Chem. Lett.* **2006**, *16*, 4813–4817; d) F. Petit, R. Furstoss, *Tetrahedron: Asymmetry* **1993**, *4*, 1341–1352; e) A. J. Carnell, S. M. Roberts, V. Sik, A. J. Willets, *J. Chem. Soc. Perkin Trans. 1* **1991**, 2385–2390.

[5] a) G. Carrea, S. Riva in *Asymmetric Organic Synthesis with Enzymes* (Eds.: V. Gotor, I. Alfonso, E. García-Urdiales), Wiley-VCH, Weinheim, **2008**, pp 3–20, and references cited therein; b) A. M. Klibanov, *Nature* **2001**, *409*, 241–246.

[6] a) G. de Gonzalo, G. Ottolina, F. Zambianchi, M. W. Fraaije, G. Carrea, *J. Mol. Catal. B* **2006**, *39*, 91–97; b) C. Rodríguez, G. de Gonzalo, D. E. Torres Pazmiño, M. W. Fraaije, V. Gotor, *Tetrahedron: Asymmetry* **2008**, *19*, 197–203.

[7] F. Schulz, F. Leca, F. Hollman, M. T. Reetz, *Bels. J. Org. Chem.* **2006**, *1*, 10.

[8] M. C. Gutiérrez, V. Alphand, R. Furstoss, *J. Mol. Catal. B* **2003**, *21*, 231–238.

[9] a) M. W. Fraaije, J. Wu, D. P. H. M. Heuts, E. W. van Hellemond, J. H. Lutje Spelberg, D. B. Janssen, *Appl. Microbiol. Biotechnol.* **2005**, *66*, 393–400; b) G. de Gonzalo, D. E. Torres Pazmiño, G. Ottolina, M. W. Fraaije, G. Carrea, *Tetrahedron: Asymmetry* **2005**, *16*, 3077–3083; c) M. Bocola, F. Schulz, F. Leca, A. Vogel, M. W. Fraaije, M. T. Reetz, *Adv. Synth. Catal.* **2005**, *347*, 979–986; d) C. Rodríguez, G. de Gonzalo, M. W. Fraaije, V. Gotor, *Tetrahedron: Asymmetry* **2007**, *18*, 1338–1344.

[10] a) 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.* **2001**, *268*, 2547–2557; b) N. M. Kamerbeek, A. J. J. Osthorrn, M. W. Fraaije, D. B. Janssen, *Appl. Environ. Microbiol.* **2003**, *69*, 419–426; c) M. D. Mihovilovic, P. Kapitan, J. Rydz, F. Rudroff, F. H. Ogink, M. W. Fraaije, *J. Mol. Catal. B* **2005**, *32*, 135–140.

[11] D. E. Torres Pazmiño, R. Snajdrova, D. V. Rial, M. D. Mihovilovic, M. W. Fraaije, *Adv. Synth. Catal.* **2007**, *349*, 1361–1368.

[12] C.-H. Wong, G. M. Whitesides, *J. Am. Chem. Soc.* **1981**, *103*, 4890–4899.

[13] Logarithm of the partition coefficient of a given compound in a standard *n*-octanol/water two-phase system.
[14] S. M. Silvestre, J. A. R. Salvador, *Tetrahedron* **2007**, *63*, 2439–2445.
[15] a) P. Gorka, S. J. Miller, *Org. Lett.* **2008**, *10*, 3049–3052; b) E. Metay, E. Leonel, C. Sulpice-Gaillet, J. Nedelec, *Synthesis* **2005**, *10*, 1682–1688; c) E. W. Lindsell, W. Edward, D. D. Palmer, P. N. Preston, G. M. Rosair, R. V. Jones, A. J. Whitton, *Organometallics* **2005**, *24*, 1119–1133; d) V. Kumar, A. Sharma, A. K. Sinha, *Helv. Chim. Acta* **2006**, *89*, 483–495; e) S. G. Davies, D. Pyatt, C. Thompson, *J. Organomet. Chem.* **1990**, *387*, 381–390; f) R. C. Cambie, L. H. Mitchell, P. S. Rutledge, *Aust. J. Chem.* **1998**, *51*, 1167–1174; g) D. J. Collins, L. M. Downes, A. G. Jhingran, S. B. Rutschmann, G. J. Sharp, *Aust. J. Chem.* **1989**, *42*, 1235–1248; h) G. Ordasso, G. Winters, *Farmaco, Ed. Sci.* **1978**, *33*, 148–155; i) P. Mohr, M. Decker, C. Enzensperger, J. Lehmann, *J. Med. Chem.* **2006**, *49*, 2110–2116.
[16] R. V. Stevens, G. S. Bissacchi, *J. Org. Chem.* **1982**, *47*, 2393–2396.

Received: January 26, 2009
Published Online: April 9, 2009

