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Microbiological Transformations 57. Facile and Efficient Resin-Based in Situ SFPR Preparative-Scale Synthesis of an Enantiopure "Unexpected" Lactone Regioisomer via a Baeyer–Villiger Oxidation Process

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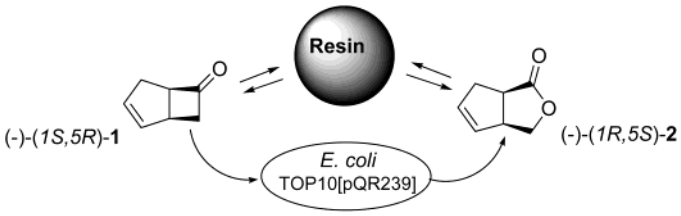
ABSTRACT



The microbiological Baeyer–Villiger oxidation of (–)-bicyclo[3.2.0]hept-2-en-6-one allowed exclusive formation of the "unexpected" lactone regioisomer in 84% yield, high chemical purity, and enantiopure form. Substrate (25 g) was transformed in a 1 L bubble column reactor, following a in situ substrate feeding/product removal methodology, which afforded high volumetric productivity (1.2 g L^{-1} h^{-1}). This illustrates the high "sustainable chemistry" advantages of such a process, simply conducted in aqueous medium, at room temperature and using atmospheric oxygen.

The Baeyer−Villiger (BV) oxidation of linear or cyclic ketones into their corresponding esters or lactones is one of the "fundamental" reactions of organic chemistry. Its regioselectivity is known to be essentially governed by electronic factors that, with only few exceptions, lead to migration of the more substituted carbon−carbon bond, affording the so-called "expected" lactone. Despite numerous efforts, the asymmetric version of this reaction still has been, up to now, largely unsuccessful using conventional (transition metal-based) chemistry.[1] The development of a (catalytic) reaction allowing (a) orientation of the reaction toward the opposite regioselectivity, i.e., to obtain exclusively the "unexpected" lactone, (b) achievement of this reaction in a highly asymmetric manner, and (c) set up of a process that would present reasonable potential for large-scale implementation remains a significant challenge. Over the last few decades, we (and others) have demonstrated that BV oxidation could be nicely achieved in its asymmetric version using a biocatalytic strategy (i.e., using whole cell or enzymatic biotransforma-

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(1) (a) Murahashi, S.-I.; Ono, S.; Imada, Y. *Angew. Chem., Int. Ed.* **2002**, *41*, 2366−2368. (b) Watanabe, A.; Uchida, T.; Ito, K.; Katsuki, T *Tetrahedron Lett.* **2002**, *43*, 4481−4485 and references therein.

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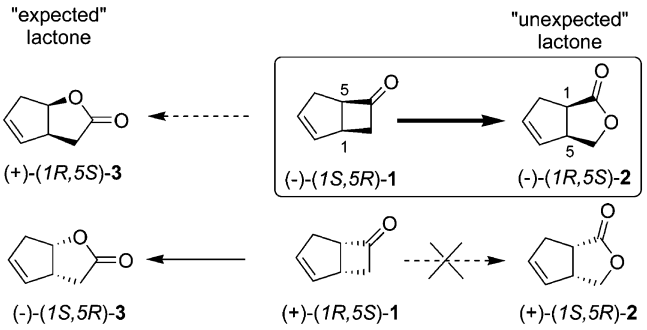
tion).[2] In many cases, the corresponding lactones were obtained in high, if not excellent, enantiomeric purity, thus opening potential new ways for improved synthetic routes aimed at the preparation of various biologically active compounds.[3]

Up to now, some severe practical bottlenecks have, however, hampered the implementation of this biocatalytic approach on a preparative (even industrial) scale. Among these, one can cite (a) occurrence of various side reactions (i.e., lactone hydrolysis and/or ketone reduction) when wild-type microbial strains[4] or overexpressing host strains containing other competitive enzymes were used,[5] (b) severe enzymatic inhibition by substrate and/or product (at concentrations generally as low as 1 to 2 g L^{-1}),[6] and (c) noticeable oxygen transfer limitation.[7]

We describe in this paper the biocatalyzed BV oxidation of enantiopure (−)-(1*S*,5*R*)-bicyclo[3.2.0]hept-2-en-6-one **1**, which overcame these drawbacks. Moreover, this process allowed the fast, facile, and exclusive obtention of the unexpected lactone regioisomer (1*R*,5*S*)-3-oxa-bicyclo[3.3.0]-octen-6-one-2 **2** in enantiomerically pure form (ee > 99%). The reaction was performed at a 25 g preparative scale using a 1 L bubble column reactor operated following an in situ substrate feeding/product removal (in situ SFPR) methodology.

To avoid side reactions, several groups have recently cloned and overexpressed some Baeyer−Villigerases,[8] cyclohexanone monooxygenase (CHMO) from *Acinetobacter calcoaceticus* NCIMB 9871 being the best known of these enzymes.[5,9] We have chosen to work with the recombinant strain *E. coli* TOP10(pQR239),[8] which offers the noticeable advantage of accepting L-(+)-arabinose for induction of CHMO overexpression, instead of the much more expensive isopropyl thio-*β*-D-galactoside (IPTG). However, here again, severe limitation due to substrate and product inhibition was observed, limiting the usable concentration of substrate (and product) and therefore the productivity of the process. To overcome this inhibition problem, we have recently developed a methodology that allowed operation at a much higher substrate concentration.[7,10] This was achieved by using a "two in one" resin-based in situ SFPR methodology, a particular

Scheme 1. Regiodivergent BV Oxidation of *rac*-Bicyclo[3.2.0]hept-2-en-6-one **1**



type of "extractive biocatalysis" pioneered by Vicenzi et al.[11] In contrast to the already known in situ product removal (ISPR) technique[12] in which an adsorbent resin is only used for extraction of the product (downstream processing), in situ SFPR uses the resin loaded with the substrate *prior* to the start of the bioprocess. The resin thus acts as an in situ-located reservoir, allowing on one hand slow release of the substrate, and concomitantly as a sink, allowing, on the other hand, continuous removal of the formed product. Therefore, both the substrate and product concentration can be maintained below the inhibitory level. As far as the third limitation, i.e., oxygen transfer limitation, was concerned, we used a specially designed bubble column reactor, equipped with an air sparger of sintered glass (located at the bottom of the vessel) to improve oxygenation of the medium.

We had described several years ago the surprising regiodivergent oxidation of *rac*-**1** using the wild-type strains *Acinetobacter* TD 63 and *Acinetobacter calcoaceticus* NCIB9871. Equimolar amounts of regioisomeric lactones **2** and **3** were obtained,[13] both with excellent enantiomeric purity (Scheme 1). Obviously, each one of the two enantiomers of **1** led, in a nonenantioselective but totally regioselective manner, to nearly exclusive formation of one single regioisomer, **3** being the expected one from the chemical point of view (migration of the more substituted carbon atom) and **2** being the unexpected regioisomer.[14] This BV oxidation of *rac*-**1** has been scaled up by Doig et al.[15] using a continuous substrate feeding strategy aimed at maintaining

(2) For recent reviews, see: (a) Alphand, V.; Carrea, G.; Wohlgemuth, R.; Furstoss, R.; Woodley, J. M. *Trends Biotechnol.* **2003**, *21*, 318−323. (b) Mihovilovic, M. D.; Müller, B.; Stanetty, P. *Eur. J. Org. Chem.* **2002**, 3711−3730.

(3) See for example: (a) Mazzini, C.; Lebreton, J.; Alphand, V.; Furstoss, R. *J. Org. Chem.* **1997**, *62*, 5215−5218. (b) Lebreton, J.; Alphand, V.; Furstoss, R. *Tetrahedron* **1997**, *53*, 145−160. (c) Adger, B.; Bes, M. T.; Grogan, G.; Mccague, R.; Pedragosa-Moreau, S.; Roberts, S. M.; Villa, R.; Wan, P. W. H.; Willetts, A. J. *Bioorg. Med. Chem.* **1997**, *5*, 253−261.

(4) Alphand, V.; Archelas, A.; Furstoss, R. *J. Org. Chem.* **1990**, *55*, 347−350.

(5) Chen, G.; Kayser, M. M.; Mihovilovic, M. D.; Mrstik, M. E.; Martinez, C. A.; Stewart, J. D. *New J. Chem.* **1999**, *23*, 827−832.

(6) Doig, S. D.; Simpson, H.; Alphand, V.; Furstoss, R.; Woodley, J. M. *Enzyme Microb. Technol.* **2003**, *32*, 347−355.

(7) Hilker, I.; Alphand, V.; Wohlgemuth, R.; Furstoss, R. *Adv. Synth. Catal.* **2004**, *346*, 203−214.

(8) (a) Kamerbeek, N. M.; Janssen, D. B.; van Berkel, W. J. H.; Fraaije, M. W. *Adv. Synth. Catal.* **2003**, 667−678. (b) Kyte, B. G.; Rouviere, P.; Cheng, Q.; Stewart, J. D. *J. Org. Chem.* **2004**, *69*, 12−17.

(9) Doig, S. D.; O'Sullivan, L. M.; Patel, S.; Ward, J. M.; Woodley, J. M. *Enzyme Microb. Technol.* **2001**, *28*, 265−274.

(10) Simpson, H. D.; Alphand, V.; Furstoss, R. *J. Mol. Catal. B* **2001**, *16*, 101−108.

(11) (a) Vicenzi, J. T.; Zmijewski, M. J.; Reinhard, M. R.; Landen, B. E.; Muth, W. L.; Marler, P. G. *Enzyme Microb. Technol.* **1997**, *20*, 494−499. (b) D'Arrigo, P.; Lattanzio, M.; Fantoni, G. P.; Servi, S. *Tetrahedron: Asymmetry* **1998**, *9*, 4021−4026. (c) Nakamura, K.; Tanekada, K.; Fuji, M.; Ida, Y. *Tetrahedron Lett.* **2002**, *43*, 3629−3631. (d) Conceiçao, G. J. A.; Moran, P. J. S.; Rodrigues, J. A. R. *Tetrahedron: Asymmetry* **2003**, *14*, 43−45.

(12) Lye, G. J.; Woodley, J. M. *Trends Biotechnol.* **1999**, *17*, 395−402.

(13) Compound **3**, well-known as "Corey's lactone", is an important precursor for prostaglandine synthesis. Lactone **2** is also a valuable chiral synthon. (For example, see ref 3b or: Hudlicky, T.; Reddy, D. B.; Govindan, S. V.; Kulp, T.; Still, B.; Sheth, J. P. *J. Org. Chem.* **1983**, *48*, 3422−3428).

(14) Such peculiar behaviour was also observed, albeit with generally lower selectivity, using transition metal-catalyzed oxidation. Therefore, **1** became a rather popular model substrate for BV oxidation.

(15) Doig, S. D.; Avenell, P. J.; Bird, P. A.; Gallati, P.; Lander, K. S.; Lye, G. J.; Wohlgemuth, R.; Woodley, J. M. *Biotechnol. Prog.* **2002**, *18*, 1039−1046.

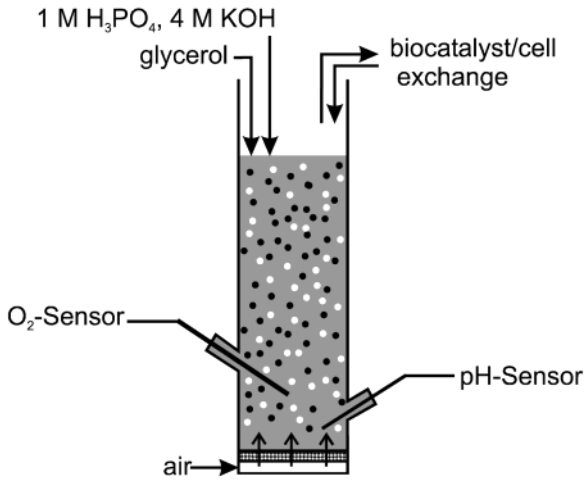


Figure 1. Scheme of the "bubble column" setup.

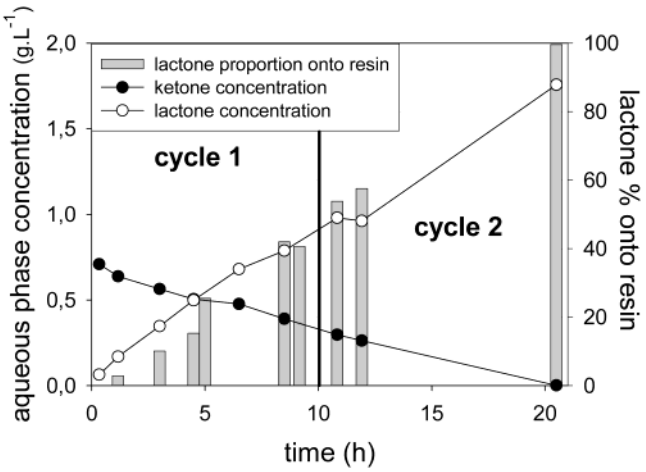


Figure 2. Kinetic profile of the BV oxidation of 25 g of (−)-(1*S*,5*R*)-**1** in the 1 L bubble column, over two cycles: exchange of biocatalyst after 10 h. (Shaded bars) proportion of re-adsorbed lactone **2** extracted from a resin sample, (●) ketone concentration, and (○) lactone concentration of the cell broth.

the substrate concentration below the inhibitory level. Starting from an initial cell culture volume of 300 L, 174 g of combined regioisomeric lactones **2** and **3** were thus produced from 182 g of *rac*-**1**, (at a ca. 3.8 g/L final product concentration). However, a delicate chromatographic separation was necessary to obtain these two lactones (i.e., the expected and the unexpected one) in pure form. Another strategy leading to one single product, i.e., to the valuable unexpected lactone **2**, which cannot be prepared via chemical BV oxidation of **1**, was therefore an interesting alternative to be explored.[16]

The recent availability of nearly enantiopure (−)-(1*S*,5*R*)-**1**, obtained by resolution of *rac*-**1** recently achieved by one of us,[17] offered such a new alternative. We therefore performed the biocatalyzed BV oxidation of (−)-(1*S*,5*R*)-**1** following the above-described in situ SFPR methodology, using a repeated batch process operated in the bubble column reactor shown in Figure 1, and the above cited (recombinant) strain *E. coli* TOP10(pQR239) overexpressing the *A. calcoaceticus* CHMO.

Prior to introduction into the reactor, 25 g of (−)-(1*S*,5*R*)-**1** (ee = 94%) was adsorbed on 75 g of Optipore L-493 resin. The adsorbent was then introduced into the reactor containing 1 L of a fresh culture of a recombinant strain *E. coli* TOP10-(pQR239) (concentrated twice prior to use). The oxygen flow was established, and the reactor was operated over a few hours. The kinetic profile of the reaction is shown in Figure 2. Biooxidation occurred rapidly over the first 10 h, leading to about 50% conversion. A preliminary experiment having shown that the reaction rate decreased considerably after this time (likely due to exhaustion of cells), we then proceeded to a second cycle run. Thus, the airflow was stopped and the resin decanted, and the exhausted culture broth was replaced with a fresh 1 L cell culture (kept in the refrigerator at 4 °C for 1−5 days). This was operated for an additional 11 h period.

The resin was then filtered off, and both aqueous media (run 1 and 2) were pooled. Continuous extraction (with CH_2Cl_2 for the liquid medium and AcOEt for the resin) was performed. The composition of the organic phases, analyzed by GC, is reported in Table 1. Careful concentration, followed by simple bulb-to-bulb distillation of these pooled phases, afforded 26 g of unexpected lactone **2** in a high chemical purity (93%) (which corresponds to a 84% yield of pure **2**). This corresponds to a space−time yield of about 1.2 g L^{-1} h^{-1} (9.7 mmol L^{-1} h^{-1}), which is significantly higher than that obtained up to now for any biocatalyzed BV oxidation.[15,18] Additional impurities were the expected lactone **3** (4%) and enantiopure *endo*-bicyclo[3.2.0]hepten-2-ol-6 (3%). No epoxides, which could have been generated

Table 1. Composition of Aqueous Phases Determined by GC Analysis

	(−)-3-oxa lactone **2** weight (yield)[a]	(−)-2-oxa lactone **3** weight (yield)[a]	*endo* alcohol weight (yield)[a]
aqueous phase	3.2 g (11%)	0.3 g (1%)	0.3 g (1%)
adsorbent resin	21.5 g (75%)	1.3 g (4%)	1.2 g (5%)
total	24.7 g (86%)	1.6 g (5%)	1.5 g (6%)

[a] Weights and yields were calculated from GC analysis using an internal standard.

(16) We also have previously observed the exclusive formation of enantiopure (−)-(1*R*,5*S*) **2**, using whole cells of the fungus *Cunninghamella echinulata* (see ref 3b). However, the BVMO of this wild-type strain has not been further studied or overexpressed (see ref 3b).

(17) This resolution process, performed by the Fluka company will be published elsewhere.

(18) Walton, A. Z.; Stewart, J. D. *Biotechnol. Prog.* **2002**, *18*, 262−268.

by oxidation of the double bond of **1** or **2**, could be detected, indicating that this oxidation was also highly chemoselective.

The enantiomeric purity of **2** was excellent (ee > 99%), the 3% of (+)-(1*R*,5*S*)-**1** enantiomer present in the starting substrate leading exclusively to formation of the expected regioisomeric lactone **3**. The fact that about 4% of this product was obtained in 40% ee indicates that only about 1% of **3** was formed from (−)-**1**. The absolute configuration of **2** could be directly deduced from that of the starting ketone (−)-(1*S*,5*R*)-**1** as being (1*R*,5*S*). For the sake of comparison, (−)-**1** was chemically oxidized with *meta*-chloroperbenzoic acid and with peracetic acid, affording a mixture of both regioisomeric lactones (−)-(1*R*,5*S*)-**2** and (+)-(1*R*,5*S*)-**3**, respectively, in 12:88 and 4:96 proportion.

Interestingly, no noticeable inhibition phenomenon was observed during this biotransformation thanks to the fact that substrate and product were adsorbed onto the resin, which allowed the concentration of dissolved material to stay quite below the inhibitory level (about 0.5 g L^{-1} for the substrate, 3−4 g L^{-1} for the product). Moreover, this low concentration also prevented noticeable formation of the expected lactone **3** from (−)-**1**, which could have been triggered at higher concentrations as described in experiments conducted using purified CHMO.[19] It is to be mentioned that, recently, a 50 mL feed batch reactor using this purified enzyme, immobilized on Eupergit C, has been run at a 10 g/L (92 mM) concentration. However, the difficult purification and the low stability of this enzyme obviously hampers any scale-up of this methodology.[20]

This work describes a highly efficient, exclusive, and logistically simple biocatalytic process allowing for the BV oxidation of (−)-(1*S*,5*R*)-bicyclo[3.2.0]hept-2-en-6-one **1** into the unexpected lactone regioisomer, which cannot be performed via chemical oxidation.[21] Due to the exquisite chemo- and regioselectivity of this reaction, this was thus obtained with an ee as high as 99% and a 84% preparative yield. As much as 25 g of (−)-**1** could thus be transformed over 21 h using a 1 L vessel, corresponding to a volumetric productivity of about 1.2 g L^{-1} h^{-1} (9.7 mmol L^{-1} h^{-1}).

As a more general feature, it is noteworthy that the use of such a whole cells approach, combined with an in situ SFPR technology, is by far the most efficient way to perform an asymmetric BV oxidation at a preparative scale. It is logistically easy to perform due to its practical simplicity. Thus, the entire process can be run in the same vessel without added practical complexity like membranes, additional pumps, and associated controls. Also, the (relatively important) mass of resin remains within the reactor upon withdrawing of the exhausted microbial culture, thus allowing multiple reuse with no need of operators to handle the resin. Moreover, the product formed, which is adsorbed onto the resin, could be simply recovered by continuous extraction using small volumes of ethyl acetate. Bottom-up aeration of the reactor additionally provides low shear agitation at any practical stage. This methodology clearly opens the way to further preparative-scale "green chemistry" (industrial) implementation, en route to the synthesis of natural and/or biologically active compounds. We are presently exploring other potential applications of this approach.

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Supporting Information Available: Experimental procedures and GC analyses of compounds **2** and **3** (chiral and racemic). This material is available free of charge via the Internet at http://pubs.acs.org.

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(19) Zambianchi, F.; Pasta, P.; Ottolina, G.; Carrea, G.; Colonna, S.; Gaggero, N.; Ward, J. M. *Tetrahedron: Asymmetry* **2000**, *11*, 3653−3657.

(20) Zambianchi, F.; Pasta, P.; Carrea, G.; Colonna, S.; Gaggero, N.; Woodley, J. M. *Biotechnol. Bioeng.* **2002**, *78*, 489−496.

(21) Some authors have very recently described an analytical-scale study on various enantioenriched 3-cyclopentanones and hexanones using overexpressed CHMO and CPMO. See: Wang, S.; Kayser, M. M.; Jurkauskas, V. *J. Org. Chem.* **2003**, *68*, 6222−6228.