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Microbiological transformations. Part 51: The first example of a dynamic kinetic resolution process applied to a microbiological Baeyer–Villiger oxidation

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Abstract—The dynamic resolution of racemic 2-benzyloxymethylcyclopentanone **1** upon microbiologically mediated Baeyer–Villiger oxidation allowed the corresponding (*R*)-lactone **2** to be prepared in 85% yield and 96% ee. © 2002 Elsevier Science Ltd. All rights reserved.

1. Introduction

The Baeyer–Villiger (BV) oxidation of ketones, described more than a century ago, is of great interest in organic chemistry. Nevertheless, in spite of numerous efforts due to the ever increasing demand for enantiopure drug manufacture, asymmetric Baeyer–Villiger oxidation using conventional chemistry still has not been satisfactorily achieved.[1] Conversely, the microbiological approach has been known for more than a decade to be an efficient method for achieving such reactions in a highly enantioselective manner.[2] Very often, nearly enantiopure lactones can thus be obtained, starting from either racemic or prochiral ketones. However, in the former case, one important drawback is the fact that, as for all conventional kinetic resolutions, the yield is limited to 50%. One way to circumvent this limitation is to set up a strategy based on a dynamic resolution process, allowing the product to be obtained in, theoretically, 100% yield and 100% ee.[3] The key point of such approaches is the in situ racemisation of the slow reacting substrate enantiomer. An impressive example of such an industrially used dynamic kinetic resolution process is the large scale production of D-aminoacids using a combination of two enzymes, i.e. an hydantoïnase and a racemase.[4]

In this paper, we wish to report the first example of a dynamic kinetic resolution applied to a microbiological Baeyer–Villiger oxidation.

2. Results and discussion

Most enzymatic (microbiological) Baeyer–Villiger oxidations have up to now been performed on structurally rather simple ketones. In order to expand the chemical potential of this (bio)oxidation, we have been interested in studying the possibility of achieving the oxidation of ketones bearing additional functionalities, as well as to set up a dynamic kinetic resolution during this process. To explore this strategy, 2-benzyloxymethylcyclopentanone **1** was chosen as the substrate. Its synthesis was achieved in four steps and 50% overall yield from commercially available ethyl 2-oxocyclopentane carboxylate (Scheme 1).[5,6]

2.1. Kinetic resolution

Ketone **1** was first submitted to a preparative scale Baeyer–Villiger (bio)oxidation following our standard procedure,[7] i.e. using a culture of (wild type) *Acinetobacter calcoaceticus* NCIMB 9871 at pH 7. Using this procedure, **1** was very smoothly oxidised within 24 h. This experiment was conducted on 0.5 g of **1** in a 1 L culture and, after usual work-up and flash chromatography, afforded 0.23 g of (*R*)-lactone **2** and 0.24 g of remaining ketone (*S*)-**1** (respectively, 43 and 48% isolated yield). The ees were 97% for lactone **2** ($[\alpha]_D^{20} = -8$

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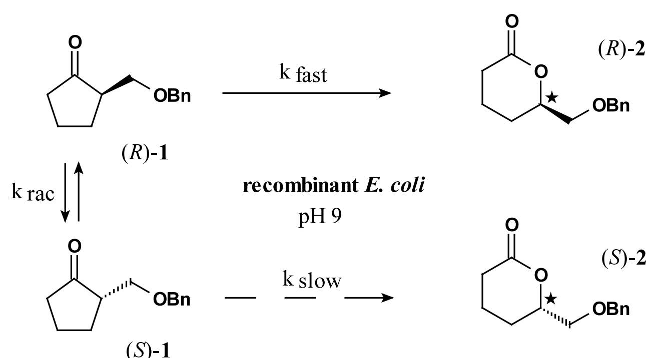


Scheme 1. Dynamic kinetic resolution of 2-benzyloxymethyl-cyclopentanone **2** using a recombinant *E. coli*.

(*c* 1.1, $CHCl_3$)) but, surprisingly only 43% for the remaining ketone **1**. They were determined using chiral GC analysis (Lipodex®E column (Macherey–Nagel) for **2**, Chiralsil-Dex CB column (Chrompack) for **1**). The enantiomeric ratio *E*, calculated according to Sih's equations from both the conversion ratio and the ee of the formed lactone,[8] was greater than 100 (Fig. 1).

The (*R*)-absolute configuration of lactone **2** was assigned by comparison of retention times with an authentic sample obtained by chemical asymmetric synthesis. This was performed following the route described in Scheme 2. Thus, Sharpless asymmetric dihydroxylation[9] using ADmix-β of 5-hexenoic ethyl ester, followed by cyclisation of the obtained diol, afforded the known (*R*)-δ-hydroxymethyl-δ-valerolactone ($[\alpha]_D^{25}=-17$ (*c* 1, $CHCl_3$), lit.[10] $[\alpha]_D^{20}=-34.3$ (*c* 1.3, $CHCl_3$), i.e. about 50% op). This was further benzylated[6] to give (*R*)-**2** in 48% ee ($[\alpha]_D^{30}=-3.8$ (*c* 0.7, $CHCl_3$)).

2.2. Dynamic kinetic resolution

It has been well documented in the recent literature that, in order to obtain an efficient dynamic kinetic resolution of a given substrate, the racemisation rate of the less reactive substrate enantiomer must, at least, be of the same order of magnitude as the conversion rate of the most reactive enantiomer. Due to the structure of substrate **1** (α-alkylated cyclopentanone), we expected that a pH dependent spontaneous racemisation could occur via an keto-enol tautomerisation. Therefore, racemisation versus pH of optically active **1** (obtained by the kinetic resolution described above) was studied in order to determine the best experimental conditions for the dynamic kinetic resolution. Our results (Fig. 2) indicate that racemisation of optically active **1**, although slow, was not negligible at pH 7. This is in accordance with the unexpected low ee (43%) obtained in the above described wild type catalysed experiment, making it impossible to calculate the *E* value using the remaining ketone ee. As expected, racemisation was noticeably faster at pH 9 and was complete within 7 h. Since biooxidation of racemic **1** was shown in our preliminary experiments to necessitate a 10–15 h period, operating at pH 9 appeared to be adequate for achieving the desired dynamic resolution.

Thus, a preparative scale biotransformation was performed at this pH using a recombinant *Escherichia coli* strain recently constructed by Ward.[11] This microorganism, *E. coli* TOP10[pQR239], contains the cyclohexanone monooxygenase (CHMO) gene from the above used *A. calcoaceticus* wild type, induced by using L-(+)-arabinose instead of the more expensive conventional IPTG. We have previously checked that this strain was

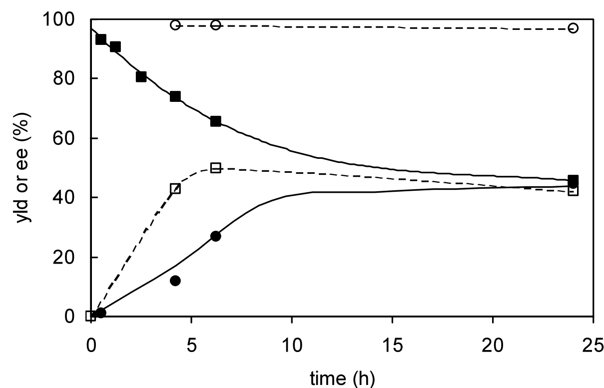
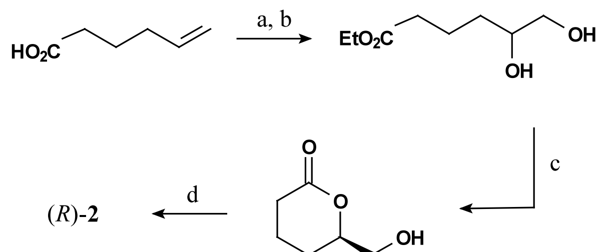


Figure 1. Biotransformation using wild type *A. calcoaceticus* NCIMB 9871 at pH 7: ■ residual ketone yield, □ residual ketone ee, ● lactone yield, ○ lactone ee.



Scheme 2. *Reagents and conditions*: (a) EtOH, 3 Å molecular sieves, H_2SO_4 cat., reflux, 95%; (b) ADmix-β, ^{t}BuOH/H_2O, 0°C; (c) Amberlyst 15, 4 Å molecular sieves, MeCN, rt, 4 h, 60% (two steps), 50% op; (d) BnBr, NaH, anhydrous THF, reflux, 28 h, 85%, 48% ee.

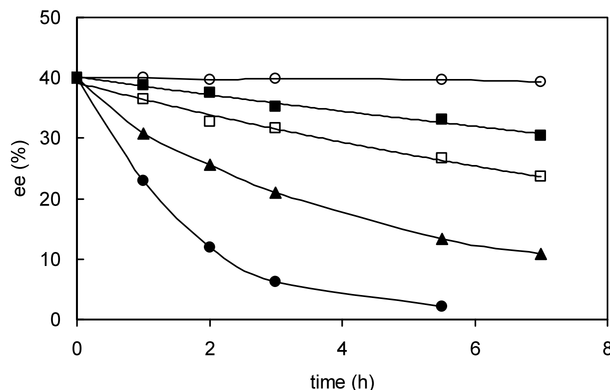


Figure 2. Time-course of ketone racemization at different pHs: ○ pH 2, ■ pH 6, □ pH 7, ▲ pH 8, ● pH 9.

able to perform biotransformations at rather high pH.[12] Thus, for analytical purposes, an experiment was conducted using 2.8 g (dry weight) of cells[13] suspended in 1 L of a pH 8.5 glycine/NaOH buffer (50 mM) containing 1% of glycerol. 300 mg (1.5 mM) of substrate **1** (solubilized in 10 mL ethanol) were added.[†] The pH was regulated at a value of 9 by automated addition of 0.1N NaOH. The reaction was followed by withdrawing aliquots which were analyzed for yield and ee. The results shown in Fig. 3 indicate that the substrate disappeared within a period of about 27 h, whereas the corresponding lactone was formed with an analytical yield of about 75% and an ee of about 98%. This high analytical yield clearly indicates that a dynamic kinetic resolution process did take place as expected. A similar bioconversion, conducted for preparative purposes and using 5.3 g (dry weight) of cells, was stopped after 24 h by addition of HCl until acidic pH. The medium was continuously extracted with dichloromethane containing Amberlite IR 120 resin. Purification by flash chromatography afforded a 85% isolated yield of nearly enantiopure (*R*)-6-benzyloxymethyl-tetrahydropyran-2-one **2** (275 mg, 96% ee, $[\alpha]_D^{25} = -8$ (c 1.1, $CHCl_3$)). This confirmed the above proposed dynamic kinetic resolution.

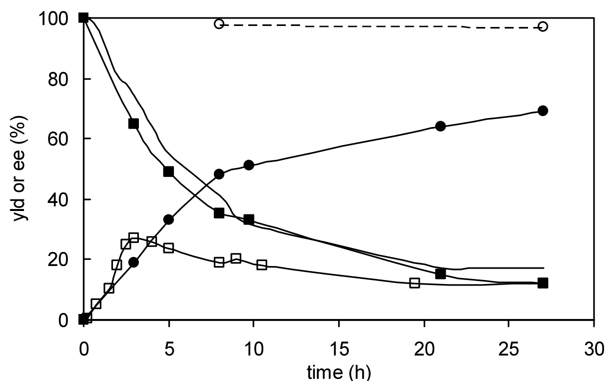


Figure 3. Biotransformation using *E. coli* TOP10(pQR239) at pH 9: ■ ketone yield, □ ketone ee, ● lactone yield, ○ lactone ee.

[†] The biotransformation was carried out in a 2 L Setric fermentor under the following conditions: 400 rpm, 0.27 vvm aeration at 30°C.

3. Conclusion

A dynamic kinetic resolution process was successfully applied, for the first time, to a Baeyer–Villiger microbiological oxidation using a recombinant *E. coli* strain overexpressing the well known cyclohexanone monooxygenase enzyme. Nearly enantiopure (*R*)-6-benzyloxymethyl-tetrahydropyran-2-one **2** (96% ee) was thus obtained with a 85% overall yield from racemic 2-benzyloxymethylcyclopentanone **1**. The application of this dynamic kinetic resolution process to other substrates is in progress in our laboratory. We also are currently studying the possibility of increasing the productivity of this process, i.e. to increase the substrate concentration as we have previously described in the case of bicyclo[3.2.0]hept-2-en-6-one.[13]

Acknowledgements

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References

1. (a) For reviews about chemical asymmetric Baeyer–Villiger oxidation, see: Strukul, G. *Angew. Chem., Int. Ed.* **1998**, *37*, 1199–1209; (b) Watanabe, A.; Uchida, T.; Ito, K.; Katsuki, T. *Tetrahedron Lett.* **2002**, *43*, 4481–4485 and references cited therein.
2. For reviews about biocatalysed asymmetric Baeyer–Villiger oxidation see for instance: (a) Willetts, A. *Trends Biotechnol.* **1997**, *15*, 515–62; (b) Roberts, S. M.; Wan, P. W. H. *J. Mol. Catal. B: Enz.* **1998**, *4*, 111–136; (c) Alphand, V.; Furstoss, R. Baeyer–Villiger oxidations. In *Enzyme Catalysis in Organic Synthesis: a Comprehensive Handbook*, Drauz, K.; Waldmann, H., Eds.; VCH: Weinheim, 1995; Vol. 2, pp. 745–772.
3. For reviews about dynamic resolution, see: (a) Strauss, U. T.; Felfer, U.; Faber, K. *Tetrahedron: Asymmetry* **1999**, *10*, 107–117; (b) Kitamura, K.; Tokunaga, M.; Noyori, R. *Tetrahedron* **1993**, *49*, 1853–1860; (c) Azerad, R.; Buisson, D. *Curr. Opin. Biotechnol.* **2000**, *11*, 565–571.
4. Schulze, B.; Wubbolts, M. G. *Curr. Opin. Biotechnol.* **1999**, *10–6*, 609–6615.
5. Taylor, R. J. K.; Wiggins, K.; Robinson, D. H. *Synthesis* **1990**, 589–590.
6. Borjesson, L.; Csoregh, I.; Welch, C. J. *J. Org. Chem.* **1995**, *60*, 2989–2999.
7. Alphand, V.; Furstoss, R.; Pedragosa-Moreau, S.; Roberts, S. M.; Willetts, A. J. *J. Chem. Soc., Perkin Trans. 1* **1996**, 1867–1872.
8. Chen, C. S.; Fujimoto, Y.; Girdaukas, G.; Sih, C. J. *J. Am. Chem. Soc.* **1982**, *104*, 7294–7299.
9. Sharpless, K. B.; Amberg, W.; Bennani, Y. L.; Crispino, G. A.; Hartung, J.; Jeong, K. S.; Kwong, H. L.; Morikawa, K.; Wang, Z. M.; Xu, D.; Zhang, X. L. *J. Org. Chem.* **1992**, *57*, 2768–2771.
10. Liu, Z. Y.; Ji, J. X.; Li, B. G. *J. Chem. Soc., Perkin Trans. 1* **2000**, 3519–3521.
11. (a) Doig, S. D.; O'Sullivan, L. M.; Patel, S.; Ward, J. M.; Woodley, J. M. *Enzyme Microb. Technol.* **2001**, *28*, 265–274; (b) Zambianchi, F.; Pasta, P.; Carrea, G.; Colonna, S.; Gaggero, N.; Woodley, J. M. *Biotechnol. Bioeng.* **2002**, *78*, 489–496.
12. Doig, S. D.; Simpson, H. D.; Alphand, V.; Furstoss, R.; Woodley, J. M. *Enzyme Microb. Technol.* in press.
13. Simpson, H. D.; Alphand, V.; Furstoss, R. *J. Mol. Cat. B: Enz.* **2001**, *16*, 101–108.