Some Baeyer–Villiger Oxidations using a Monooxygenase Enzyme from *Pseudomonas putida* NCIMB 10007

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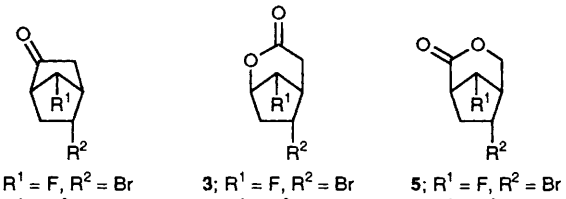
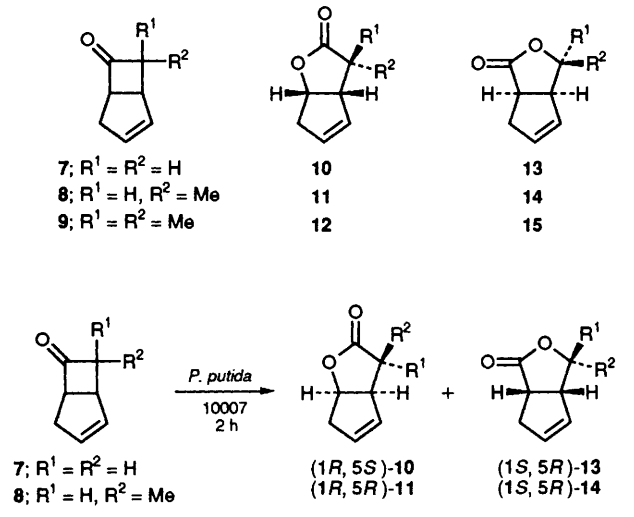
A monooxygenase from *Pseudomonas putida* NCIMB 10007 is shown to catalyse stereoselective Baeyer–Villiger-type oxidations on the bicyclic ketones **2**, **7**, **8** and **9**.

The Baeyer–Villiger reaction is an important transformation in synthetic organic chemistry. The same oxidation is promoted by a variety of microorganisms.[1] For example, the biological Baeyer–Villiger reaction has been used to convert simple 2-substituted cyclopentanones into optically active δ-lactones.[2] Similarly the dihalogenoketone **1** undergoes enantioselective oxidation using a microorganism (*Acinetobacter calcoaceticus* NCIMB 9871) to give the optically active lactone **3** and recovered optically active ketone.[3] None of the lactone **5**[4] was observed by NMR spectroscopy. The enantioselectivity of the process is due to one or both halogen atoms since incubation of the unsubstituted ketone **2** with *A. calcoaceticus* 9871 gave the lactone **4** [enantiomeric excess (e.e.) < 10%] contaminated with a small amount of racemic lactone **6**.

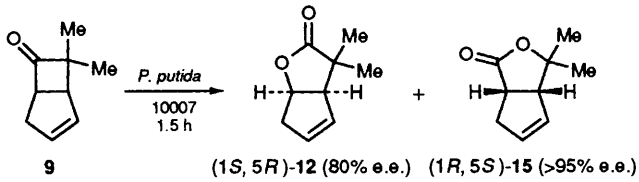


1; R^1 = F, R^2 = Br
2; R^1 = R^2 = H
3; R^1 = F, R^2 = Br
4; R^1 = R^2 = H
5; R^1 = F, R^2 = Br
6; R^1 = R^2 = H

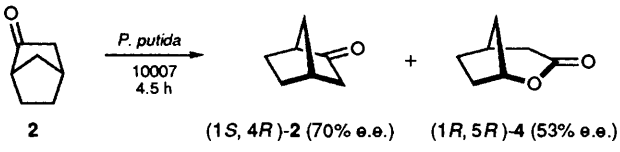
Oxidation of the ketone **7** with *A. calcoaceticus* furnished the (1*S*,5*R*)-lactone **10** (>95% e.e.) and the (1*R*,5*S*)-lactone **13** (> 95% e.e.).[5] Similarly, 7-*endo*-methylbicyclo[3.2.0]hept-2-en-6-one **8** produced the lactones (1*S*,5*S*)-**11** and (1*R*,5*S*)-**14**.[6] Oxidation of the dimethylbicycloheptenone **9** is much less enantioselective giving the lactone **15** in low optical purity (8–23% e.e). Very little of the lactone **12** is observed in the crude product from the latter transformation.



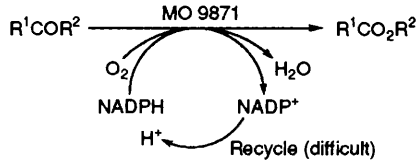
Scheme 1



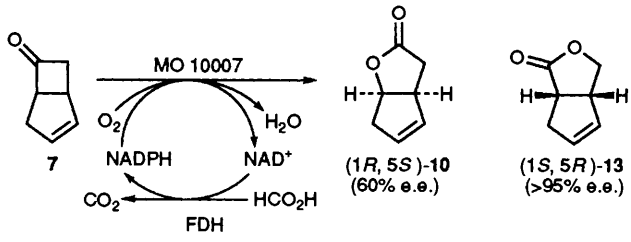
Scheme 2



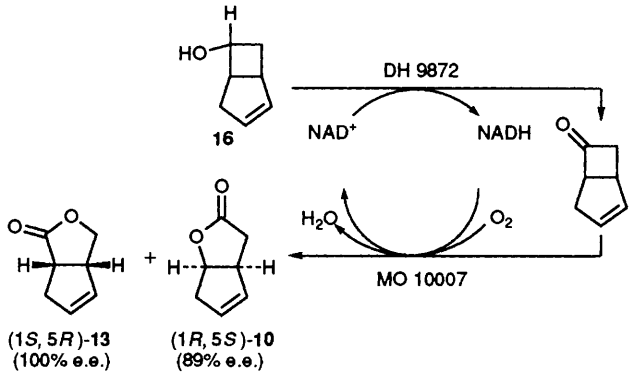
Scheme 3



Scheme 4



Scheme 5



Scheme 6

A report in the literature[7] led us to believe that the microorganism *Pseudomonas putida* NCIMB 10007 may provide a very useful catalyst for conducting Baeyer–Villiger oxidations. Our initial observations, reported hereunder, tend to support this view.

Thus, biotransformation of bicycloheptenone **7** with (+)-camphor-grown *Pseudomonas putida* NCIMB 10007 for 2 h gave the lactones (1*R*,5*S*)-**10** (50% e.e.) and (1*S*,5*R*)-**13** (>95% e.e.) (86% yield) in a transformation that is complementary to the one observed for *A. calcoaceticus*.[8] Similarly, the racemic ketone **8** gave the (1*R*,5*R*)-lactone **11** (80% e.e) and the (1*S*,5*R*)-lactone **14** (>95% e.e.) (63% yield) in roughly equal proportions (Scheme 1). These enantiomeric excesses (and other enantiomeric ratios reported later) were measured by GC using Lipodex D as the stationary phase.

Transformation of the dimethylbicycloheptenone **9** was also highly stereocontrolled affording the lactone (1*S*,5*R*)-**12** and (1*R*,5*S*)-**15** in 73% yield as shown in Scheme 2.

Using whole-cell preparations of (+)-camphor-grown *P. putida* 10007 with norbornanone **2**, it was apparent that the biocatalyst was able to biotransform the two enantiomers at different rates. Thus, stopping the biotransformation after 4.5 h (*ca.* 60% biconversion) resulted in a mixture of lactone **4** (53% e.e) and recovered optically active ketone (70% e.e.) (Scheme 3), whereas lactone **4** (13% e.e.) was the only recovered material (50% yield) when the reaction was allowed to go to completion (8 h).

The whole-cell biotransformations catalysed by *A. calcoaceticus* NCIMB 9871 and *P. putida* NCIMB 10007 suffer from a common problem namely that the yields of isolated product(s) are rarely near-quantitative through over-metabolism and/or side reactions due to other active enzymes in the microorganisms.

The monooxygenase(s) (MO 9871) responsible for the Baeyer–Villiger reactions in *A. calcoaceticus* has (have) been isolated and used to convert selected ketones into the corresponding lactones.[9] However, the enzyme utilizes NADPH as cofactor and $NADP^+$ recycling is notoriously difficult (Scheme 4).

In contrast, the readily available[7] monooxygenase from *P. putida* NCIMB 10007 is NADH-linked and this cofactor is much more readily recycled, for example using formate dehydrogenase (FDH) from *Candida boidinii*. Using this coupled enzyme system the ketone **7** was transformed into (1*R*,5*S*)-lactone **10** and (1*S*,5*R*)-lactone **13** with very good selectivity (Scheme 5). The two lactones were isolated in practically quantitative yield. In this system the cofactor is used in a less-than-stoichiometric amount and *ca.* 10 mol% of catalyst is sufficient to drive the biotransformation at a viable rate. The overall biotransformation is very simple [eqn. (1)].

$$\text{Ketone} + O_2 + HCO_2H \Rightarrow \text{Lactone(s)} + CO_2 + H_2O \quad (1)$$

Finally, enzyme cofactor recycling can be accomplished using MO 10007 and a NADH dependent dehydrogenase from *Pseudomonas* sp. NCIMB 9872 (DH 9872).[10] In this case the 6-*endo*-alcohol **16** forms the substrate and the lactones **10** and **13** are formed in high yield and in good to excellent optical purity (Scheme 6).

In summary, the (+)-camphor-grown microorganism *P. putida* NCIMB 10007 performs highly stereoselective oxida-

tions on two different classes of bicyclic ketone. The enzyme responsible for these oxidations is readily isolated and can be used in conjunction with dehydrogenases to provide highly effective and efficient bioconversions.

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References

1 H. G. Davies, R. H. Green, D. R. Kelly and S. M. Roberts, *Biotransformations in Preparative Organic Chemistry: the Use of Isolated Enzymes and Whole Cell Systems in Synthesis*; Academic Press, 1989.
2 V. Alphand, A. Archelas and R. Furstoss, *Biocatalysis*, 1990, **3**, 73; *J. Org. Chem.*, 1990, **55**, 347.
3 M. S. Levitt, R. F. Newton, S. M. Roberts and A. J. Willetts, *J. Chem. Soc., Chem. Commun.*, 1990, 619.
4 C. A. Fletcher, H. Hilpert, P. L. Myers, S. M. Roberts and R. Storer, *J. Chem. Soc., Chem. Commun.*, 1989, 1707.
5 V. Alphand, A. Archelas and R. Furstoss, *Tetrahedron Lett.*, 1989, **30**, 3663.
6 A. J. Carnell, S. M. Roberts, V. Sik and A. J. Willetts, *J. Chem. Soc., Perkin Trans. 1*, 1991, 2385.
7 D. Taylor and P. W. Trudgill, *J. Bacteriol.*, 1986, **165**, 489.
8 G. Grogan, S. M. Roberts and A. J. Willetts, *Biotechnol. Lett.*, 1992, **14**, 1125.
9 A. J. Willetts, C. J. Knowles, M. S. Levitt, S. M. Roberts, H. Sandey and N. F. Shipston, *J. Chem. Soc., Perkin Trans. 1*, 1991, 1608.
10 F. Rebolledo, S. M. Roberts and A. J. Willetts, *Biotechnol. Lett.*, 1991, **13**, 245.