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Oxidation with *Bacillus stearothermophilus* in Heptane

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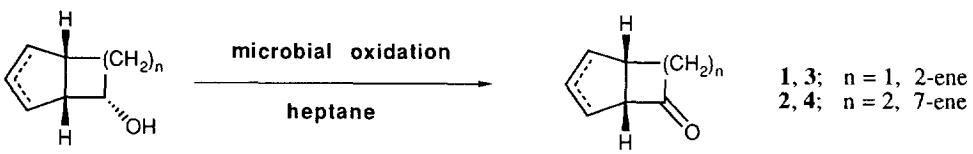
Abstract The first example of oxidation with bacteria in heptane is reported. The kinetic resolution of endo-bicyclic octen- and heptenols is obtained. The methodology is simpler and the volume of the reactions is smaller than in water.

Lipase-catalyzed kinetic resolution of secondary alcohols *via* hydrolysis, esterification and transesterification was widely used to have homochiral compounds.[1] Recently in our laboratory we achieved the kinetic resolution of heteroaryl ethanols *via* oxidation both with Baker's yeast [2] and *Bacillus stearothermophilus*.[3] This microorganism was also utilized with excellent results in the kinetic resolution of bicyclic octen- and heptenols.[4] Moreover, while hydrolytic enzymes such as lipases are known to retain their activity in organic solvents,[5] dehydrogenases and oxido-reductases are different because they require the assistance of stoichiometric amount of co-factor which in the microbe has to be reproduced in order to elaborate the substrates catalytically. However, the organic solvents often cause a serious damage on the hydrophobic cell membranes of a microbe resulting in a leakage of the contents of the cell. In order to avoid this problem, the organic solvents were used with immobilized microorganisms[6] because the immobilization enhances its stability against denaturation by organic solvents,[7] but only a few reports have concerned the reduction with microorganisms, i.e. Baker's yeast, in organic solvents[8] and only one example of oxidation with a yeast-like microbe, i.e. *Geotrichum candidum*, has been very recently reported by Nakamura.[9]

In this work we describe the oxidation with *Bacillus stearothermophilus*, of bicyclic racemic alcohols **1-2** in heptane (Scheme).



1-2 **3-4**

1, 3; n = 1, 2-ene
2, 4; n = 2, 7-ene

A *Bacillus stearothermophilus* ATCC 2027 culture (30 ml),[10] grown for 48 h at 39 °C, is centrifugated (3000 *g*) and water was removed by decantation. Heptane (10 ml) and the selected substrate (40 mg) were added to the cells and the suspension was vigorously stirred at 39 °C. In the same way the reactions were carried out in water adding the substrate (40 mg) directly to the grown culture and in only one case (i.e. alcohol **1**) the microorganism was immobilized.[11] The results are summarized in the Table.

Table. Oxidation of the bicyclic alcohols **1-2** with *Bacillus stearothermophilus*

alcohol	conditions	time (h)	ketone (%)[a]	ee (abs. conf.)[a]
1	water	6	**3** (41)	99 (1*S*,5*R*)
1	heptane	6	**3** (49)	100 (1*S*,5*R*)
1	immobilized[b]	3	**3** (41)	100 (1*S*,5*R*)
2	water	4	**4** (37)	100 (1*S*,5*S*)
2	heptane	4	**4** (48)	98 (1*S*,5*S*)

[a] The data are obtained by GLC on chiral column and the configurations are assigned on the basis of our recent work (ref. 4). [b] After immobilization the reaction is carried out in heptane.

Racemic 6-*endo*-bicyclo[3.2.0]hept-2-en-6-ol (**1**) was oxidized and kinetically resolved in heptane to give (1S,5R)-bicyclo-heptenone **3** in 49% (ee 100%). Parallel experiment in water and with immobilized cells in heptane gave lower yields (41%) but equally high enantiomeric excess (99-100%). Similar results were obtained in the oxidation of the racemic 2-*endo*-bicyclo[3.3.0]oct-7-en-2-ol (**2**) but the reaction rate was faster. In fact after only 4 h in heptane the (1S,5S)-bicyclo-octenone **4** was obtained in 48% yield and with 98% of ee. In water the yield was lower (37%). Various experiments have been made to test the organic solvent resistance of this microorganism. We have verified that the microbe after 24 h in heptane is still living.[12] Other solvents such as toluene, diethylene glycol dimethyl ether, t-butyl methyl ether kill the cells (no reproduction after 30 min). The oxidation of the alcohol **1** was carried out also on preparative scale starting from 1 l of culture medium. The cells obtained were immobilized, the alcohol **1** (1g) was added in heptane (150 ml) and after 18 h the ketone **3** (48% yield, 94% ee) was achieved. This is also an alternative method to the chemical oxidation of this compound: after 80 h the conversion to ketone is almost quantitative.

In conclusion, the oxidations with *Bacillus stearothermophilus* in heptane are the first example of reactions with bacteria carried out in an organic solvent and are as efficient as in water. The advantges of this methodology are that the procedure is simpler since the filtration of the cells and concentration of the solvents afford the crude reaction mixture and, moreover, that under the same conditions the volume of the reaction is threefold lower. On the other hand the oxidation with the immobilized cells is very promising for their use on preparative scale.

References

1. Davies, H. G.; Green, R. H.; Kelly, D. R.; Roberts, S. M. *Biotransformations in Preparative Organic Chemistry*; Academic Press: London 1989; Chapter 2.
2. Fantin, G.; Fogagnolo, M.; Medici, A.; Pedrini, P.; Poli, S.; Sinigaglia, M. *Tetrahedron Lett.* **1993**, *34*, 883.
3. Fantin, G.; Fogagnolo, M.; Medici, A.; Pedrini, P.; Poli, S.; Gardini, F. *Tetrahedron: Asymm.* **1993**, *4*, 1607.
4. Fantin, G.; Fogagnolo, M.; Medici, A.; Pedrini, P.; Rosini, G *Tetrahedron: Asymm.* , **1994**, *5*, 1635.
5. Klibanov, A. M. *Acc. Chem. Res.* **1990**, *23*, 114.
6. Nakamura, K.; Inoue, K.; Ushio, K.; Oka, S.; Ohno, A. *J. Org. Chem.* **1988**, *53*, 2589. Naoshima, Y.; Maeda, J.; Munakata, Y.; Nishiyama, T.; Kamezawa, M.; Tachibana, H. *J. Chem. Soc., Chem. Commun.* **1990**, 964. Nakamura, K. Takano, S. Ohno, A. *Tetrahedron Lett.* **1993**, *34*, 6087.
7. Klibanov, A. M. *Anal. Biochem.* **1979**, *93*, 1.
8. Nakamura, K.; Kondo, S.; Kawai, Y.; Ohno, A. *Tetrahedron Lett.* **1991**, *32*, 7075. Nakamura, K.; Kondo, S.; Kawai, Y.; Ohno, A. *Bull. Chem. Soc. Jpn.* **1993**, *66*, 2738. Jayasinghe, L. Y.; Smallridge, A. J.; Trewhella, M. A. *Tetrahedron Lett.* **1993**, *34*, 3949.
9. Nakamura, K.; Inoue, Y.; Ohno, A. *Tetrahedron Lett.* **1994**, *35*, 4375.
10. A synthetic culture medium containing for 1 l of water bactotryptone (20g), yeast extract (10 g), saccharose (40 g), K_2SO_4 (2.6 g), and $Na_2HPO_4.2H_2O$ (6.4 g), adjusted to pH 7.1 with KOH 6N, is inoculated with a spore suspension.
11. Bulow, L.; Birnbaum, S.; Mosbach, K. *"Methods in Enzymology, Immobilized Enzymes and Cells"*, Mosbach, K.; Ed.; Academic Press: San Diego (USA), 1988, vol. 137, p. 632.
12. The microorganism reproduces on Bacto m Plate count agar .

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