Available online at www.sciencedirect.com
ScienceDirect
Tetrahedron: Asymmetry 17 (2006) 2043–2045
Tetrahedron: *Asymmetry*

Baker's yeast catalyzed asymmetric reduction in glycerol

Adi Wolfson,* Christina Dlugy, Dorith Tavor, Janine Blumenfeld and Yoram Shotland

Green Processes Centre, Chemical Engineering Department, Sami Shamoon College of Engineering,
Bialik/Basel Sts., Beer-Sheva 84100, Israel

Received 23 May 2006; accepted 17 July 2006

Abstract—Free and immobilized baker's yeast were successfully employed in the asymmetric hydrogenations of prochiral β-keto esters and ketones in glycerol. The activities with immobilized cells were always higher then with free cells while the enantioselectivity was very high (99%) with both catalysts. Using glycerol, a non-toxic, biodegradable and recyclable liquid, as an environmentally friendly solvent allowed easy separation of the product by simple extraction with diethyl ether.

1. Introduction

Chiral hydroxyl esters and alcohols are useful intermediates and auxiliaries in the production of fine chemicals.[1,2] One of the most attractive methods to synthesis pure alcohols enantiomer is via catalytic enantioselective reduction of the corresponding prochiral ketones (Fig. 1). Though chiral transition metal complexes were successfully employed for this purpose,[3,4] the use of biocatalytic reduction has some advantages since it proceeds at room temperature and does not require high pressures of hydrogen.[5] Several oxidoreductases are capable of efficiently reducing ketones with a combination of co-factors such as NADPH or NADH. However using whole cells, such as baker's yeast (*Saccharomyces cerevisiae*), for chiral reduction is more economically attractive due to its availability, low costs and ease of handling and disposal.

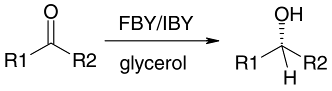


Figure 1. Baker's yeast catalyzed asymmetric reduction of prochiral ketones.

Water is the natural solvent of choice for biocatalysis.[6] However, performing the reduction of prochiral ketones with free baker's yeast (FBY) in water has several drawbacks: low solubility of the organic substrate, undesired side reactions such as hydrolysis, and difficult separation of the product. Thus the enantioselective reduction of various prochiral ketones with baker's yeast was also studied in different organic solvents.[7–9] Although employing organic reaction medium helps overcome most of the associated drawbacks of water, the main disadvantages of using such solvents are their toxicity, which damage the cells and has a severe environmental impact. Moreover, glucose, which is usually added to the reaction mixture as a hydrogen source and as an electron donor in the regeneration of the cofactor[9] has negligible solubility in organic solvents.

Immobilized baker's yeast (IBY) was also prepared to ease the cells separation and tested in the asymmetric reduction of β-keto esters in water[10] and organic solvents.[8] The preparation of yeast beads with alginate is the most frequently used immobilization technique due to the easy preparation method, low cost and relatively high affinity of alginate to water, and its ability to form a gel under mild conditions.[11,12] Besides improved separation of IBY from the reaction mixture, their preparation also keeps water around the yeast cells that is essential for their performance but may also stimulate unwanted side reactions.

Different organic solvents such as hexane, toluene, ethyl acetate,[7] light petroleum,[13] and liquefied petroleum gas[14] were tested with or without the addition of a small amount of water with both FBY and IBY. More recently IBY was also tested in ionic liquids[15] and fluorous media,[16] which offers recyclable and more environmentally friendly organic media and allows easy separation of the product.

---

* Corresponding author. Tel.: +972 8 6475731; fax: +972 8 6475636; e-mail: adiw@sce.ac.il

0957-4166/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetasy.2006.07.026

Nevertheless, their production is also associated with employing large amounts of hazardous and volatile organic solvents.

Glycerol is a non-toxic, biodegradable and recyclable liquid manufactured as a by-product of the transesterfication of a triglyceride in the production of natural fatty acid derivatives. These derivatives are utilized in many areas from pharmaceuticals and the food industry to alternative fuels, for example, biodiesel, and thus as the production of glycerol raises, its price decreases. Glycerol has also promising physical and chemical properties, which allows its use as an alternative environmentally friendly solvent. It has very high boiling point and negligible vapour pressure; it is stable up to its boiling temperature and compatible with most organic and inorganic compounds, as well as not requiring special handling or storage. The relatively high polarity of glycerol allows the dissolution of glucose and sucrose, yet it also dissolves organic compounds that are poorly miscible in water. Different hydrophobic solvents such as ethers and hydrocarbons, which are immiscible in glycerol allow removal of the products by simple extraction. Herein, we report on the asymmetric reduction of prochiral β-keto esters and ketones with both FBY and IBY in glycerol as a green medium (Fig. 1).[17]

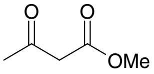
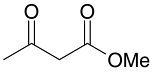
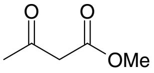
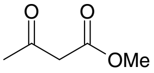
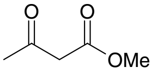
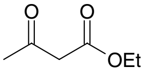
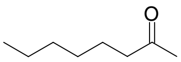
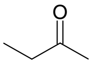
2. Results and discussion

We started the investigation with the asymmetric reduction of methyl acetoacetate in glycerol with both FBY and IBY (Table 1, entries 1–5).[17] As illustrated in Table 1, IBY always yielded higher activity than the corresponding reaction with FBY, while the enantioselectivity of the product, (*S*)-methyl hydroxybutyrate, was very high (>95%) with both catalysts. The higher performance with IBY might be attributed to the existence of water in the IBY beads that maintained their performance. For comparison, the reactions in water with both FBY and IBY under the same conditions were completed after 20 h. Indeed, the addition of 5 wt % of water to glycerol increased the activity without changing the enantioselectivity.

The effect of the addition of various hydrogen sources to the reaction mixture with regards to the performance of free and immobilized cells in glycerol was also tested. The addition of glucose (entry 2) or sucrose (entry 3) as an energy source equally increased the activity of the reaction when compared to the reaction without any additive (entry 1), keeping the high enantioselectivity. Conversely, the addition of ethanol as an energy source did not change either the activity or the enantioselectivity. Finally, representative prochiral carbonyl compounds were also reduced in glycerol under optimal conditions, using IBY and sucrose (Table 1, entries 6–8). The reaction with ethyl acetoacetate was more active than the reaction with methyl acetoacetate (entry 6). As expected, the activities of baker's yeast toward the reduction of aliphatic prochiral ketones, 2-octanone and 2-butanone (entries 7 and 8, respectively), were lower then those obtained with β-keto esters but the enantioselectivity remained very high.

Recovery of the product from the glycerol can be carried out by extraction with several glycerol immiscible solvents, such as diethyl ether, dichloromethane and ethyl acetate.

Table 1. Enantioselective reduction of prochiral ketone with baker's yeast in glycerol[a]

Entry	Substrate	Energy source (g)	FBY conversion (%)	ee (%)	IBY conversion (%)	ee (%)
1		No	61	>99 (*S*)	87	>99 (*S*)
2		Glucose	71	>99 (*S*)	96	>99 (*S*)
3		Sucrose	75 (17)[b]	>99 (*S*)	99 (95)[b]	>99 (*S*)
4		Ethanol	67	95 (*S*)	90	>99 (*S*)
5		No[c]	73	97 (*S*)	94	97 (*S*)
6		Sucrose	74[b]	>99 (*S*)	99[b]	>99 (*S*)
7		Sucrose	—	—	16	97 (*S*)
8[b]		Sucrose	—	—	22	>99 (*S*)

[a] 50 mL glycerol, 10 g FBY, 1 g substrate, 5 g additive 37 °C, 96 h.

[b] 48 h.

[c] Addition of 5 wt % water.

Table 2. Comparison of the performance of IBY in the asymmetric reduction of ethyl acetoacetate in recyclable reaction media

Medium	Ethyl acetoacetate (g)	Energy source (g)	IBY (g)	*t* (h)	Conversion (%)	Isolated yield (%)	ee (%)
Water	1	5	10	20	100	92	99
Glycerol	1	5	10	48	99	90	99
Ionic liquid[15]	1.3	7.9	10	72	70	—	95
Fluorous phase[16]	0.25	1	2.5	41	100	25	95

The product extractions were carried out in the presence of the yeast cells since part of the product existed in the cells. The highest extraction yields (>90%) were obtained with ethyl acetate, which is relatively less toxic than other glycerol immiscible solvents.[17] The product extractions were completed in the presence of the yeast cells, since part of the product existed in the cells.

The performance of IBY in the asymmetric reduction of ethyl acetoacetate in various green solvents was then compared (Table 2). The results in Table 2 illustrate that performing the reaction in glycerol is advantageous over ionic liquids or fluorous media as it is more active and enantioselective and it affords higher product isolation yield by simple extraction.

As previously mentioned, one of the advantages of using an organic solvent instead of water in yeast catalyzed enantioselective reductions is the ease of isolation of the products.[13,18] The asymmetric reduction of ethyl acetoacetate with FBY for example, was successfully performed in various organic solvents.[7,18] Employing light petroleum as the solvent resulted in 70% product isolation yield. The separation procedure involved yeast filtration and wash, solvent evaporation under reduced pressure and bulb to bulb distillation. Conversely, using glycerol as the reaction media allowed simple extraction of the product with ethyl acetate and after removal of the solvent at room temperature under reduced pressure, the isolation yield was 90%. Hence, not only is glycerol greener than light petroleum and other hydrocarbons but the separation procedure is simpler and the product isolation yield is also higher.

Finally, the enantioselective reductions of simple ketones in a yeast fermenting aqueous system usually results in moderate enantioselectivities.[19] This is probably due to the fact that the ketones are unnatural substrates for alcohol dehydrogenase from baker's yeast. Reduction of 2-hexanone in an ionic liquid yielded higher activity and similar enantioselectivity when compared with an aqueous system (% ee = 80).[15] Performing the reduction of 2-octanone and 2-butanone in glycerol resulted in much higher enantioselectivities (% ee >97). The separation of 2-butanol from an aqueous system is problematic since it forms azeotropic mixture with water. Employing glycerol as the solvent allowed the simple distillation of the alcohol with high isolation yield due to the low vapour pressure of glycerol.

3. Conclusions

In conclusion, glycerol, which is a renewable and biodegradable green solvent was employed for the first time as a reaction medium in the asymmetric reduction of prochiral carbonyl compounds with baker's yeast. Both activity and enantioselectivity were high and competitive with the reactions in water. IBY showed higher activity than FBY with the same enantioselectivity. However, it should be taken into account that although the immobilization procedure is simple it requires some effort and time. High products extraction yields were obtained.

References

1. Tsachen, D. M.; Fuentes, L. M.; Lynch, J. E.; Laswell, W. L.; Volante, R. P.; Shinkai, I. *Tetrahedron Lett.* **1988**, *29*, 2779.
2. Mori, K. *Tetrahedron* **1989**, *45*, 3233.
3. Noyori, R. *Asymmetric Catalysis in Organic Synthesis*; John Wiley: New York, 1994.
4. Wolfson, A.; Vankelecom, I. F. J.; Geresh, S.; Jacobs, P. A. *J. Mol. Catal. A* **2003**, *198*, 39.
5. Nakamura, K.; Yamanaka, R.; Matsuda, T.; Harada, T. *Tetrahedron: Asymmetry* **2003**, *14*, 2659.
6. Stefano, S. *Synthesis* **1990**, 1.
7. Rotthaus, O.; Krüger, D.; Demuth, M.; Schaffner, K. *Tetrahedron* **1997**, *53*, 935.
8. Qun, J.; Shanjing, Y.; Lehe, M. *Enzyme Microb. Technol.* **2002**, *30*, 721.
9. Leon, R.; Fernandes, P.; Pinheiro, H. M.; Cabral, J. M. S. *Enzyme Microb. Technol.* **1998**, *23*, 483.
10. Buque, E. M.; Chin-Joe, I.; Straathof, A. J. J.; Jongejan, J. A.; Heijnen, J. J. *Enzyme Microb Technol.* **2002**, *31*, 656.
11. Poncelet, D.; Lencki, R.; Beaulieu, C.; Halle, J. P.; Neufeld, R. J.; Fournier, A. *Appl. Microbiol. Biotechnol.* **1992**, *38*, 39.
12. Hulst, A. C.; Tramper, J.; Van't Riet, K.; Westerbeek, J. M. M. *Biotechnol. Bioeng.* **1985**, *27*, 870.
13. Medson, C.; Smallridge, A. J.; Trewhella, M. A. *Tetrahedron: Asymmetry* **1997**, *7*, 1049.
14. Johns, M. K.; Smallridge, A. J.; Trewhella, M. A. *Tetrahedron Lett.* **2001**, *42*, 4261.
15. Howarth, J.; James, P.; Dai, J. *Tetrahedron Lett.* **2001**, *42*, 7517.
16. Yajima, A.; Naka, K.; Yabuta, G. *Tetrahedron Lett.* **2004**, *45*, 4577.
17. The representative procedure for methyl acetoacetate reduction with FBY and IBY in glycerol: 10 g of FBY (SIGMA, type II) or 50 g of IBY (prepared from 10 g of FBY as described in 10) was added to a mixture of 50 mL of glycerol (99% purity, purchased from Frutarom Ltd) in a 250 mL bottle and shaken for 30 min. Then 5 g of energy source was added and the bottle was shaken for 10 min before 1 g of methyl acetoacetate was added. The bottle was shaken at 300 rpm for 96 h at 37 °C. At the end of the reaction, the products were extracted with ethyl acetate (3 × 50 mL). The conversion and the enantiomeric excess of the product were determined by GC analysis with Astec, Chiraldex G-TA® (30 m × 0.25 mm, 0.25 μm thickness).
18. Jayasinghe, L. Y.; Kodituwakku, D.; Smallridge, A. J.; Trewhella, M. A. *Bull. Chem. Soc. Jpn.* **1994**, *67*, 2528.
19. MacLeod, R.; Prosser, H.; Fikentscher, L.; Lanyi, J.; Mosher, H. S. *Biochemistry* **1964**, *3*, 838.