Available online at www.sciencedirect.com
SCIENCE DIRECT®
Tetrahedron Letters
Tetrahedron Letters 45 (2004) 4577–4579

Immobilized baker's yeast reduction in fluorous media

Arata Yajima, Kazumi Naka and Goro Yabuta*

Department of Fermentation Science, Faculty of Applied Bioscience, Tokyo University of Agriculture, Sakuragaoka 1-1-1, Setagaya-ku, Tokyo 156-8502, Japan

Received 26 January 2004; revised 17 March 2004; accepted 26 March 2004

Abstract—The first example of immobilized bakers' yeast mediated reduction of ketones in fluorous media is described. The use of fluorous media permits simple work-up and reuse of the solvent without any purification.

The development of environmentally benign chemical processes is widely recognized as an important goal. From this point of view, fluorous media such as perfluorocarbons (PFC) or perfluorinated solvents have been receiving increasing attention in organic synthesis due to their unique properties.[1] The immiscibility of fluorous solvents with both organic and aqueous solvents is the key property in the process.[2] For example, the use of the liquid–liquid biphase system with fluorous reagents or catalysts is the most accessible concept in organic synthesis.[1c] The common advantage of the use of fluorous media is the easiness of the separation or purification in the work-up of the reaction. In addition to the unique property, fluorous solvents exhibit low toxicity[3] and high solubility of gases such as oxygen, which can make it possible such mammals as mice and cats to breathe and survive in the liquid.[4,5]

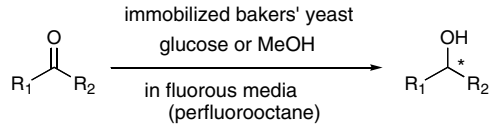
Baker's yeast (*Saccharomyces cerevisiae*) has been used in the various transformations as an environmentally benign reagent in organic synthesis. In most cases, aqueous solvent systems have been employed for the reaction media.[6] On the other hand, it has been reported that a variety of organic solvents can be employed in the immobilized baker's yeast (IBY)[7] or free bakers' yeast (FBY)[8] mediated reactions. The use of organic solvents is advantageous in terms of the solubility of the substrates, the avoidance of side reactions such as hydrolysis caused by water and the easiness of the isolation of the products. The disadvantages of employing such solvents are their potential toxicity to the bioreagent/catalyst and problems associated with their disposal. It is therefore necessary to develop alternative media, which possess unusual properties, for a more environmentally friendly chemical process.

For the purpose, ionic liquid is a possible candidate. In 2001, Howarth and co-workers reported the use of a moisture-stable ionic liquid, [bmim]PF_6, and water mixture as a reaction medium in IBY reduction of ketones.[9] According to them, the addition of water was necessary for the medium, because the yields and the enantiomeric excesses of the products greatly decreased without water, which might be caused by the inactivation of the bioreagent by [bmin]PF_6. To avoid the inactivation of the bioreagent, we speculated that fluorous media could be candidates for the reaction media. In 2002, Beckman and co-workers reported an enzymatic reaction with fluorinated nicatinamide adenine dinucleotide (NAD) as a coenzyme in fluorous media.[10] They have succeeded in combining the green benefits of fluorous media with that of green catalysts. Taking into consideration these results, the IBY mediated reaction in fluorous media would combine the advantages of IBY with that of fluorous properties. The employment of whole cells of the yeast could simplify the reaction, because there is no need to prepare an expensive coenzyme. This communication describes the first example of the whole cell biotransformation, the IBY-mediated reduction of ketones, in a fluorous medium Scheme 1.

Although the employment of FBY in organic solvents has been reported,[8] we speculated that the employment of IBY could simplify the work-up process. Immobilization of baker's yeast in calcium alginate beads was

Keywords: Fluorous media; Bakers' yeast; Green chemistry; Asymmetric reduction.
* Corresponding author. Tel.: +81-3-5477-2542; fax: +81-3-5477-2622; e-mail: yabta@nodai.ac.jp

0040-4039/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetlet.2004.03.172



Scheme 1. IBY mediated reduction of ketone.

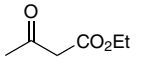
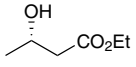
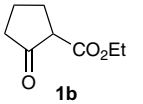
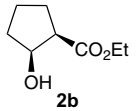
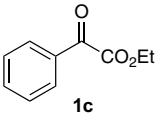
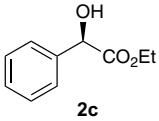
carried out according to Naoshima's procedure.[11] As a fluorous medium, perfluorooctane was selected, since its moderate boiling point and commercial availability (purchased from Fluorochem Ltd., bp 99–100 °C) are suitable for our experiment. The results of the reactions are shown in Table 1.

In our initial experiments, glucose was used as an energy source for the reactions (entries 1–4).[12] Since both the starting materials and glucose were insoluble in perfluorooctane, perfluorooctane acted as dispersion media in these reactions. All of the reactions proceeded smoothly, and the starting materials disappeared within 2 days (monitored by GC). After completion of the reaction, the IBY beads were separated by simple filtration. We chose methanol for the washing of beads and the extraction of the products because of its high immiscibility with perfluorooctane. In this step, perfluorooctane was recovered in 90–94%. Although the major cause of the loss of the solvent was the work-up operations, filtration and extraction, a small amount of the solvent was dissolved into methanol. After the work-up, perfluorooctane was detected in 0.1% in the combined extracts.[13] On the other hand the recovered perfluorooctane contained only 0.6% of methanol as an impurity. Since the solvent was pure enough to reuse in the next experiments without any purification (entries 1 and 4), the recovered perfluorooctane was used in the other experiments. Even if methanol remained in perfluorooctane, it can act as an energy source in the baker's yeast-mediated reactions.[7d] Thus we investigated the reactions using methanol as an energy source in fluorous media (entries 5–7). Since the reactions proceeded more slowly, the reaction times were prolonged for the completion of the reactions. However, this method is simpler in the work-up operations due to the lack of remaining glucose or their metabolites.

According to GC analyses, most of the starting materials disappeared in the reactions (Table 1). But the isolated yields of **2a** and **2b** were low (entries 1, 2, 4–6). Taking into consideration the relatively low boiling points of **2a** and **2b**, the products might be lost in the concentration of the solvents used in the extraction or the chromatography. In fact, **2c** with high boiling point was obtained in good yields (entries 3 and 7). The development of more efficient work-up processes is in progress in our laboratory. The absolute configurations of the obtained alcohols were determined by comparison with authentic samples in GC analyses.[14] The enantiomeric excesses of the obtained alcohols, determined by GC, were comparable to those of the alcohols obtained by yeast reduction in aqueous or organic media.[14] These results indicate that fluorous media is a possible candidate for the environmentally friendly solvent in the yeast-mediated reactions.

In conclusion, the first biotransformations employing whole cells of baker's yeast in fluorous media were investigated. The IBY-mediated reductions of various ketones either with glucose or methanol as energy sources proceeded in fluorous media without loss of

Table 1. The IBY mediated reductions of various ketones

Entry	Substrate	Product	Energy source	Time (h)	Conversion (%)	Isolated yield (%)	Enantiomeric excess (% ee)
1[a]	 **1a**	 **2a**	Glucose[c]	41	100	25	95
2[b]	 **1b**	 **2b**	Glucose[c]	29	100	28	99 (98% de)
3[b]	 **1c**	 **2c**	Glucose[c]	21	100	66	94
4[b]	**1a**	**2a**	Glucose[c]	41	100	26	95
5[b]	**1a**	**2a**	MeOH[d]	212	93	35	87
6[b]	**1b**	**2b**	MeOH[d]	118	100	19	99 (97% de)
7[b]	**1c**	**2c**	MeOH[d]	168	84	54	93

[a] Freshly distilled perfluorooctane was used.
[b] Recovered perfluorooctane was used.
[c] 1.0 g of glucose per 0.25 g of the starting material was used.
[d] 0.25 g of MeOH per 0.25 g of the starting material was used.

stereoselectivity. The used fluorous solvent was easily and sufficiently recovered by the filtration and methanol extraction, and was pure enough to reuse without any purification. The combination of the biotransformation with fluorous chemistry can act as an environmentally benign chemical process.

Acknowledgements

We wish to acknowledge Prof. Y. Naoshima (Okayama University of Science) for his helpful experimental advice in the preparation of IBY.

References and notes

1. Representative reviews of fluorous chemistry: (a) Cornils, B. *Angew. Chem. Int. Ed.* **1997**, *36*, 2057–2059; (b) Curran, D. P. *Angew. Chem. Int. Ed.* **1998**, *37*, 1174–1196; (c) Horváth, I. T. *Acc. Chem. Res.* **1988**, *31*, 641–650; (d) Barthel-Rosa, L. P.; Gladysz, J. A. *Coord. Chem. Rev.* **1999**, *192*, 587–605.
2. Scott, R. L. *J. Am. Chem. Soc.* **1948**, *70*, 4090–4093.
3. Lowe, K. C. *Blood Rev.* **1999**, *13*, 171–184.
4. Clark, L. C., Jr.; Gollan, F. *Science* **1966**, *152*, 1755–1756.
5. Riess, J. G.; Le Blanc, M. *Pure & Appl. Chem.* **1982**, *54*, 2383–2406.
6. Representative reviews of the yeast-mediated reactions: (a) Stefano, S. *Synthesis* **1990**, 1–25; (b) Rene, C.; Brigitte, I. G. *Chem. Rev.* **1991**, *91*, 49–97.
7. (a) Nakamura, K.; Inoue, K.; Ushio, K.; Oka, S.; Ohno, A. *J. Org. Chem.* **1988**, *53*, 2589–2593; (b) Naoshima, Y.; Nishiyama, T.; Munakata, Y. *Chem. Lett.* **1989**, 1517–1518; (c) Nakamura, K.; Miyai, T.; Inoue, K.; Kawasaki, S.; Oka, S.; Ohno, A. *Biocatalysis* **1990**, *3*, 17–24; (d) Naoshima, Y.; Maeda, J.; Munakata, Y.; Nishiyama, T.; Kamezawa, M.; Tachibana, H. *J. Chem. Soc. Chem. Commun.* **1990**, 964–965; (e) Naoshima, Y.; Maeda, J.; Munakata, Y. *J. Chem. Soc. Perkin. 1* **1992**, 659–660.
8. (a) Haag, T.; Arslan, T.; Seebach, D. *Chimia* **1989**, *43*, 351–353; (b) Fadnavis, N. W.; Prabhakar Reddy, N.; Bhalerao, U. T. *J. Org. Chem.* **1989**, *54*, 3218–3221; (c) Nakamura, K.; Kondo, S.; Kawai, Y.; Ohno, A. *Tetrahedron Lett.* **1991**, *32*, 7075–7078; (d) Jayasinghe, L. Y.; Smallridge, A. J.; Trewhella, M. A. *Tetrahedron Lett.* **1993**, *34*, 3949–3950; (e) Nakamura, K.; Kondo, S.; Kawai, Y.; Ohno, A. *Bull. Chem. Soc. Jpn.* **1993**, *66*, 2738–2743; (f) Jayasinghe, L. Y.; Kodituwakku, D.; Smallridge, A. J.; Trewhella, M. A. *Bull. Chem. Soc. Jpn.* **1994**, *67*, 2528–2531; (g) North, M. *Tetrahedron Lett.* **1996**, *37*, 1699–1702; (h) Bak, R. R.; McAnda, A. F.; Smallridge, A. J.; Trewhella, M. A. *Aust. J. Chem.* **1996**, *49*, 1257–1260; (i) Rotthaus, O.; Krüger, D.; Demuth, M.; Schaffner, K. *Tetrahedron* **1997**, *53*, 935–938; (j) Medson, C.; Smallridge, A. J.; Trewhella, M. A. *Tetrahedron Asymm.* **1997**, *8*, 1049–1054; (k) Johns, M. K.; Smallridge, A. J.; Trewhella, M. A. *Tetrahedron Lett.* **2001**, *42*, 4261–4262.
9. Howarth, J.; James, P.; Dai, J. *Tetrahedron Lett.* **2001**, *42*, 7517–7519.
10. Panza, J. L.; Russell, A. J.; Beckman, E. J. *Tetrahedron* **2002**, *58*, 4091–4104.
11. Naoshima, Y.; Munakata, Y.; Nishiyama, T.; Maeda, J.; Kamezawa, M.; Haramaki, T.; Tachibana, H. *World J. Microbiol. Biotech.* **1991**, *7*, 219–224.
12. Representative procedure for the IBY reduction in a fluorous medium: To a suspension of starting material (0.25 g) in 50 mL of perfluorooctane (90% purity, purchased from Fluorochem Ltd.) in a 500 mL bottle with a rubber seal, 50 g of IBY prepared from 2.5 g of dry baker's yeast (SIGMA, type II) and D-glucose (1.0 g) or distilled MeOH (0.25 g) were added. The bottle was sealed with an aluminum seal cap, and shaken at 100 rpm for 21–212 h at 30 °C. The reaction was monitored by GC analyses. After the completion of the reaction, the seal was removed and the reaction mixture was filtered with Celite. The Celite pad was washed with MeOH. The combined perfluorooctane was extracted with MeOH. The combined MeOH layers were concentrated in vacuo. The residue was chromatographed on silica gel (hexane/diethyl ether) to give the desired alcohol. The enantiomeric excess of the product was determined by the GC analysis [column: Chirasil Dex CB® (25 m × 0.25 mm, 0.25 μm thickness); for **2a**: carrier gas He 70 kPa, 77–81 °C (1 °C/min), $t_R(S) = 19.8$, $t_R(R) = 20.3$; for **2b**: carrier gas He 110 kPa, 100–130 °C (1 °C/min), t_R (1*R*,2*S*) = 15.6, t_R (1*S*,2*R*) = 15.9, t_R (1*S*,2*S*) = 22.2, t_R (1*R*,2*R*) = 22.6; for **2c**: carrier gas He 110 kPa, 100–137 °C (0.8 °C/min), $t_R(R) = 34.1$, $t_R(S) = 34.8$].
13. The method of the GC analyses of perfluorooctane (90% purity) and methanol; injection 60 °C, column: SUPELCOWAX™-10 (30 m × 0.25 mm, 0.25 μm thickness), carrier gas He 130 kPa, 31 °C (20 min), t_R (methanol) = 1.6, t_R (perfluorooctane) = 2.6 (minor) and 3.7 (major).
14. The authentic samples were obtained according to the known procedures; **2a**: Ref. 7d, **2b**: Ref. 15, and **2c**: Ref. 7d.
15. Nishimura, Y.; Mori, K. *Eur. J. Org. Chem.* **1998**, 233–236.