Available online at www.sciencedirect.com
ScienceDirect
Tetrahedron: Asymmetry
Tetrahedron: *Asymmetry* 19 (2008) 157–159

Asymmetric reduction of ketones with a germinated plant

Kiyoko Matsuo,[a] Sei-ichiro Kawabe,[a,*] Yosuke Tokuda,[a] Takashi Eguchi,[a] Rio Yamanaka[b] and Kaoru Nakamura[c,*]

[a]*College of Life Science, Kurashiki University of Science and the Arts, 2640 Nishinoura, Tsurajima-cho, Kurashiki, Okayama 712-8505, Japan*
[b]*Faculty of Pharmaceutical Science, Himeji Dokkyo University, 7-2-1 Kami-Ohno, Himeji, Hyogo 670-8524, Japan*
[c]*Institute for Chemical Research, Kyoto University, Uji, Kyoto 611-0011, Japan*

Received 31 October 2007; accepted 6 December 2007

Abstract—A germinated radish sprout was used as a novel type of biocatalyst for the asymmetric reduction of ketones. The reactions proceeded with high enantioselectivities (>99% ee). The biocatalyst is easily obtainable from commercially available vegetable seeds and is very easy to use.

1. Introduction

Until now, many kinds of biocatalysts have been applied for useful biotransformations.[1] For asymmetric reductions, isolated enzymes, microbes such as yeasts and fungi, and plant cell cultures have been used.[2] Microbes and plant cell cultures are more useful than isolated enzymes due to the existence of many enzymes that catalyze various reactions. Furthermore, in the asymmetric reduction of substrates, whole living cell systems do not require cofactors and cofactor-regeneration systems since they already have these requirements. However, it is not easy to obtain these cell cultures for organic chemists who are not familiar with cell cultivation. On the other hand, another category of biocatalysts baker's yeast[2] and vegetables,[3] has been applied to organic synthesis because these biocatalysts are easily obtainable from markets and easily manipulated by organic chemists. However, an essential problem, the reproducibility of experiments with raw baker's yeast and vegetables, has remained since the conditions of these biocatalysts are easily changeable depending on the places they come from. Moreover, even from the same area, it is difficult to obtain the biocatalyst with the same condition constantly throughout a year. To solve these problems, we have developed a novel biocatalyst that does not depend on the place and/or time of isolation and still universally available. This is a sprout prepared from a vegetable seed. The merits of using vegetable seeds as pre-biocatalysts are as follows. (1) Vegetables seeds are obtainable all over the world. (2) Seeds are well preserved for a long time. (3) Seeds have an ability to germinate at any time of the year if suitable conditions for the germination are provided.

2. Results and discussion

We have investigated asymmetric reductions by germinated plants obtained from commercially available seeds. If conditions of germination and growth for plants can be optimized, uniform and reproducible germinated plants should be easily obtained. The radish sprout was selected as the biocatalyst since radish seed is one of the most obtainable seeds and the germination of radish is very easy. Indeed, the biocatalysts, germinated radish (*Raphanus sativus* L.), reduced ketones, α,α,α-trifluoroacetophenone and *o*-chloroacetophenone[4,5] to the corresponding alcohols with high enantioselectivities.

Radish seeds were obtained commercially and the surface of the seed was sterilized by rinsing with water and 1% sodium hypochlorite and soaked in water for a while. Then the seeds were placed on sterilized wet paper filters in the dark for 1–2 days at 20 °C to be germinated. A grain of germinated radish was cultivated in 30 mL of LS medium[6] with 3% sucrose for 10 days[7] to obtain the uniform radish sprout (Biocatalyst) (Fig. 1). The radish sprout (7 ± 1 g) was transferred to the flask containing 30 mL of LS

* Corresponding authors. E-mail: nakamura@scl.kyoto-u.ac.jp

0957-4166/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetasy.2007.12.015

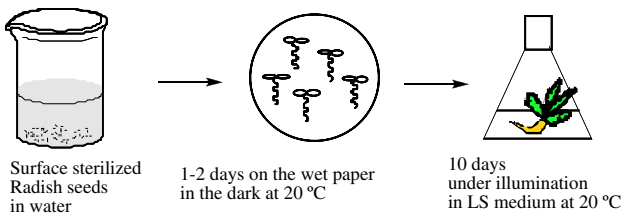
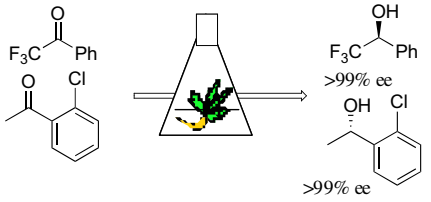


Figure 1. Preparation of the biocatalyst.

medium and 30 μL of α,α,α-trifluoroacetophenone solution (10% in DMSO) was added. The reaction was conducted under illumination (4000 Lux) for 1–4 days at 20 °C (Scheme 1).



Scheme 1. Reduction of ketones with germinated radish.

Then, the reaction mixture was extracted with ether and chemical yield and ee of trifluorophenylethanol were determined by GC analyses using an internal standard (naphthalene).[8,9] Thus, the corresponding (*S*)-alcohol was obtained in 30–40% yield with 90–100% ee. With the same method, *o*-chloroacetophenone was reduced and afforded the corresponding (*S*)-alcohol in >99% ee (Scheme 1). Interestingly, although (*S*)-alcohols were obtained in both reactions, the absolute configurations are different according to definition. The difference in stereoselectivities of the reduction is due to the existence of plural enzymes that participate in the reduction. The same phenomenon has been observed in the reduction with *Geotrichum candidum*.[9]

It is noteworthy that the addition of sucrose largely affected the growth of the plant and bioconversion of the ketone. Figure 2 shows the effect of sucrose on the fresh weight of radish (right vertical axis), chemical yields, and ee (left vertical axis); cultivation = 10 days and reaction = 1 day in the reduction of trifluoroacetophenone (data are the average of more than 3 experiments.). Sucrose was added at cultivation and also during reaction of the substrate.

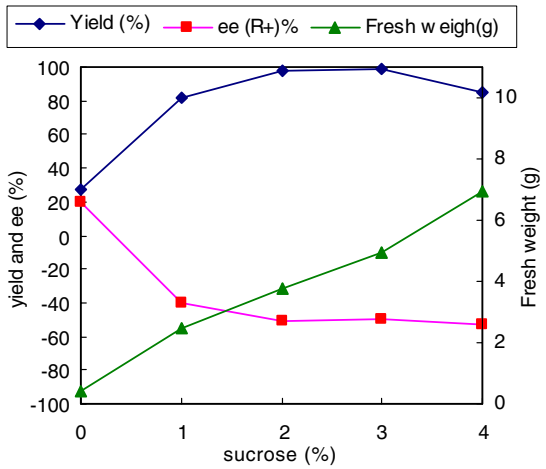


Figure 2. Effect of sucrose on yield, ee and fresh weight of radish on the reduction of trifluoroacetophenone with germinated radish.

The fresh weight of radish increased monotonically against the amount of sucrose (0.4 g without sucrose and 7 g with 4% sucrose added) and the yield of the reduction also increased from 40% (without addition of sucrose) to 100% (2 g of sucrose was added). The selectivity of the reduction changed from (*R*) to (*S*) by adding sucrose at cultivation time. The (*S*)-enzyme which gives the (*S*)-alcohol will be produced largely compared to the (*R*)-enzyme by the effect of sucrose. Then, the addition of sucrose exclusively enhances the reduction of the ketone to the (*S*)-alcohol and changes the stereoselectivity from (*R*) to (*S*). On the contrary, the addition of sucrose at the reaction time changed the stereoselectivity of the reduction slightly; the alcohol with 46% ee (*S*) was obtained from the reaction in the presence of sucrose and that of 40% ee (*S*) is produced from the reaction without adding sucrose (sucrose-cultivated radish was used in both the reactions; yields were 99% in both the cases). Thus, the change of stereoselectivity is thought to be the result of producing (*S*)-enzymes at cultivation time.

Sucrose affects the growth of the biocatalyst and also accumulates the reducing power. On the contrary, the addition of sucrose at the reaction time did not affect the chemical yield or the enantioselectivity of the reaction regardless of the pre-treatment of the biocatalyst.

The other substrate, *o*-chloroacetophenone was also used to check the stereochemical conversion by the addition of sucrose. However, the reduction of the substrate without adding sucrose at cultivation time did not proceed regardless of adding sucrose at the reaction time.

The moderate enantioselectivity of the product was improved up to 100% ee by using long reaction times (3 days) although the chemical yield of the alcohol decreased slightly. This phenomenon is explainable with the idea of enantioselective decomposition of (*R*)-alcohol by radish. Indeed, the reaction of the racemic alcohol with radish afforded (*S*)-alcohol in 80% ee after 3 days.

3. Conclusion

To conclude, we present herein a novel method for asymmetric reduction. The proposed system should be universally applicable not only for asymmetric reduction, but also for other biocatalytic reactions. Other plant seeds may be applicable with the present method.

References

1. *Enzyme Catalysis in Organic Synthesis*; Drauz, K., Waldmann, H., Eds.; Wiley-VCH, Verlag GmbH: Weinheim, 2002.

2. Nakamura, K.; Yamanaka, R.; Matsuda, T.; Harada, T. *Tetrahedron: Asymmetry* **2003**, *14*, 2659–2681; Nakamura, K.; Matsuda, T. Enzyme-catalyzed Reduction Reactions, 2nd ed. In *Enzyme Catalysis in Organic Synthesis*; Drauz, K., Waldmann, H., Eds.; Wiley-VCH, Verlag GmbH: Weinheim, 2002; Vol. III, pp 991–1047.
3. Utsukihara, T.; Watanabe, S.; Tomiyama, A.; Chai, W.; Horiuchi, C. A. *J. Mol. Catal. B: Enzym.* **2006**, *41*, 103–109, and references cited therein.
4. Nakamura, K.; Matsuda, T.; Harada, T. *Chirality* **2002**, *14*, 703–708.
5. Nakamura, K.; Matsuda, T. *J. Org. Chem.* **1998**, *63*, 8957–8964.
6. LS medium: Linsmaier, E. M.; Skoog, F. *Physiol. Planta.* **1965**, *18*, 100–127.
7. Preparation of radish sprout: Radish seeds were obtained commercially from Takii Co. Ltd, Kyoto, Japan. Surface sterilized seeds were produced by 5 min rinsing in water, 10 min exposure to 1% sodium hypochlorite, and exhaustive rinsing with sterilized water. The seeds were stored on sterilized wet paper filters for 1–2 days in the dark at 20–25 °C to provide the germinated radish. A portion of LS liquid medium[6] with 0–4% sucrose (30 mL) was placed in an 100-ml Erlenmeyer flask, which was covered with silicone caps and sterilized (120 °C, 20 min). A germinated radish was added to this Erlenmeyer flask, and was shaken for 10 days at 20–25 °C at 120 rpm, 4000 Lux.
8. Reaction: Sterilized LS medium (30 mL) with the same sucrose concentration, as used for germination, was freshly prepared in a 100-ml Erlenmeyer flask. 10% (w/v) trifluoroacetophenone in DMSO (30 μL) and a radish obtained as above were added to the flask, and shaken for 24 h at 20–25 °C at 120 rpm, 4000 Lux. After the reaction, the resulting mixture was extracted with ether (5 mL × 2) and eluted by Extrelut (Merck). The chemical yield and ee of trifluorophenylethanol were determined by GC analyses using an internal standard (naphthalene).[9] Gas chromatograph: column; CP-cyclodextrin-B2,3,6-M-19; 25 m, trifluoroacetophenone; temperature 120 °C; retention time ketone, 2.94 min; (*S*)-alcohol, 15.64 min; (*R*)-alcohol, 16.36 min, *o*-chloroacetophenone; temperature 130 °C, ketone, 7.67 min; (*S*)-alcohol, 15.04 min; (*R*)-alcohol, 17.77 min.
9. Matsuda, T.; Harada, T.; Nakajima, N.; Itoh, T.; Nakamura, K. *J. Org. Chem.* **2000**, *65*, 157–163.