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# Asymmetric Synthesis of (*S*)-Arylalkanols by Microbial Reduction

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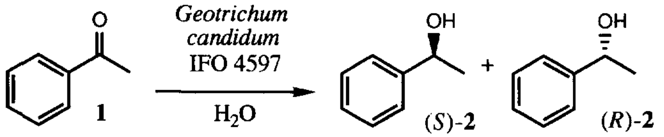
**Abstract:** Enantiomerically pure (*S*)-arylalkanols have been synthesized in excellent yields by the reduction of acetophenone derivatives under the catalysis of *Geotrichum candidum*.

Microbial transformations have been used widely for the syntheses of chiral compounds.[1] However, since there are many enzymes in a microbe to transform a substrate to products of different configurations, the selectivity of these reactions are usually low when no modification is applied to the reaction system. Various methods have been developed to improve the efficiency of microbial transformations; for example, modifications of substrates,[2] variations of substrate concentrations,[3] variations of solvents,[4] and the use of additives such as reductants or oxidants.[5,6] On the contrary, dependence of enantioselectivity in microbial transformations on the cultivation conditions of the microbe has not been investigated. We have investigated the effects of the medium concentrations for cultivation of *Geotrichum candidum* IFO 4597 on the selectivity of the asymmetric reduction of acetophenone derivatives catalyzed by the microbe. As the result, we have found that the reduction proceeds enantioselectively to give enantiomerically pure (*S*)-arylalkanols in excellent yields when they are reduced by the microbe cultivated on relatively dilute media (see experimental section for the standard conditions) under modified reaction conditions (shorter reaction time, larger amount of the microbe, and the use of an argon atmosphere).



When acetophenone (**1**) was reduced with the resting cells (0.5 g) cultivated under the standard conditions, (*R*)-1-phenylethanol ((*R*)-**2**) was obtained in 52% yield with 28% ee. On the other hand, it was found that ee of the product is greatly affected by the concentration of medium for the cultivation of the microbe. Figure 1 shows the relationship between the relative concentration of cultivation medium ($C/C_o$) and the yield of (*R*)-**2** after 24 h of the reduction by the resting cells. The yield of the (*R*)-enantiomer increases with an increase in the concentration of the cultivation medium.

Reaction conditions such as reaction time, amount of cells and atmosphere were also investigated using the resting cells cultivated under the standard conditions. Figure 2 shows the time course of the reduction of **1**. The reduction yields the (*S*)-**2** selectively before 12 h, but elongation of the reaction time leads to the decrease in ee, and the reduction affords the (*R*)-**2** after 40 h. At the initial stage, the selectivity of the reduction to the (*S*)-**2** is high, though not perfect, and the yield of the alcohol is kept low.

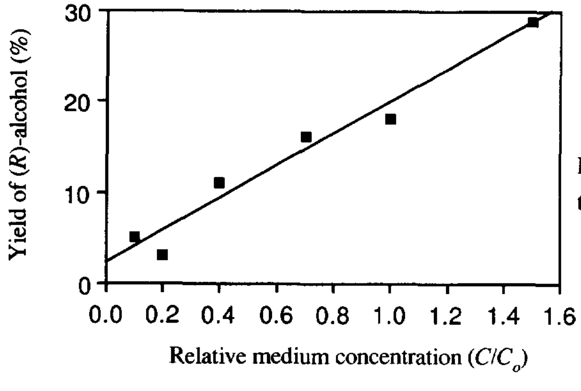


Figure 1. Effect of medium concentrations on the yield of (*R*)-**2** in the reduction of **1**.

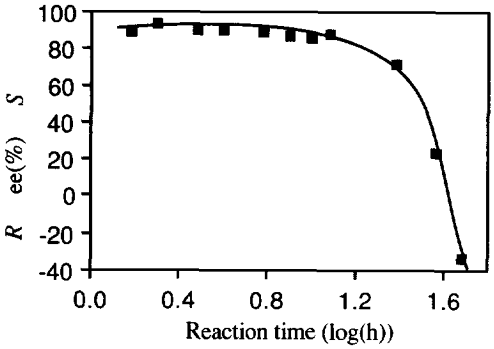


Figure 2. Effect of the reaction time on enantioselectivity in the reduction of **1**.

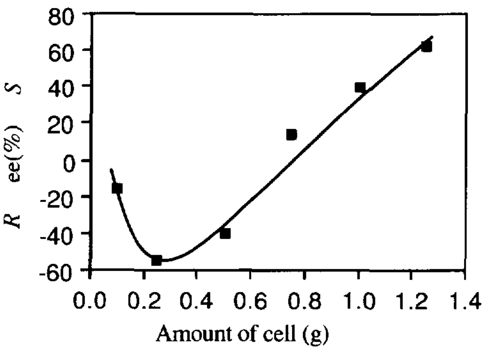


Figure 3. Effect of amount of cell on selectivity in reduction of **1**.

Figure 3 shows the effect of the amount of the resting cells (cultured under standard conditions) on enantioselectivity of the product after 24 h of reaction time. When more than 0.7 g wet wt. of the cells were used, the reduction becomes *S* selective, whereas the (*R*)-**2** was obtained when smaller amounts of the cells were used.

Since the product, (*S*)-**2**, is known to be oxidized back to **1** by the same microbe,[6] and it is reported that anaerobic conditions increase the ee for a microbial reduction in an interface-bioreactor,[7] we conducted the reaction under an argon atmosphere. As the result, the use of an argon atmosphere in the reduction of **1** improved both yield and ee of the reaction as listed in Table 1. As expected, the oxidation of **2** was inhibited under an argon atmosphere.

Table 1. Reduction of **1** and Oxidation of **2** under Air or an Argon Atmosphere.

| Reaction | Reaction Atmosphere | Yield / % | ee / % (Config.) |
|---|---|---|---|
| Reduction of **1** | Air | 52 | 28(*R*) |
| Reduction of **1** | Argon | 95 | 85(*S*) |
| Oxidation of **2** | Air | 38 | |
| Oxidation of **2** | Argon | 11 | |

As shown in Figures 1-3 and Table 1, the enantioselectivity in the reduction of **1** is affected by several factors: medium concentration for the cultivation of the microbe, reaction time, the amount of cells used for the reaction, and reaction atmosphere. Under selected reaction conditions to optimize each factor, the yield of and ee in the product from the reduction of **1** were improved greatly to 98% and 95%(*S*), respectively; the conditions employed were the use of 2 g of wet wt. of resting cells cultured in a diluted medium and the conduction of reaction under an argon atmosphere for 3 h (Conditions C). A dramatic improvement in the enantioselectivity of the reduction from 28%(*R*) under the Conditions A to 95%(*S*) under the Conditions C is noteworthy. Table 2 shows the results from the reduction of **1** and its derivatives. Optically active (*S*)-alcohols were obtained in excellent ee by employing the Conditions C.

We believe that the reduction is valuable in organic syntheses because of its excellent selectivity and quantitative chemical yield. Detailed mechanism for the improvement of the selectivity of the reduction of acetophenone derivatives by altering the cultivation and reaction conditions is under investigation in our laboratory.

Table 2. Reduction of Acetophenone Derivatives.[a]

| Substrate | Microbe Cultured in Standard Medium | | Microbe Cultured in Diluted Medium | | | |
|---|---|---|---|---|---|---|
| | Under Air (Conditions A) | | Under Air (Conditions B) | | Under Argon (Conditions C) | |
| | Yield | ee(config.) | Yield | ee(config.) | Yield | ee(config.) |
| Acetophenone | 52 | 28(*R*) | 87 | 95(*S*) | 98 | 95(*S*) |
| Propiophenone | 14 | 19(*S*) | 98 | 98(*S*) | 99 | 98(*S*) |
| Butyrophenone | 7 | >99(*S*) | 27 | >99(*S*) | 15 | >99(*S*) |
| 1-Phenyl-2-propanone | 47 | 88(*S*) | 89 | >99(*S*) | 97 | >99(*S*) |
| Benzylacetone | 31 | 60(*S*) | 81 | 85(*S*) | 93 | 88(*S*) |
| *m*-Chloroacetophenone | 22 | 90(*S*) | 23 | >99(*S*) | 99 | >99(*S*) |
| *p*-Chloroacetophenone | 49 | 46(*R*) | 98 | 72(*S*) | 97 | 67(*S*) |

[a] The conditions are described in the experimental section.

## Experimental

Organic reagents were purchased from Nacalai Tesque, Inc. or Wako Pure Chemical Industries, Ltd. unless otherwise indicated. $^1$H NMR spectra were recorded at 200 MHz on a Varian VXR-200 Fourier transform NMR spectrometer. Gas chromatographic analyses were performed using a Shimadzu GC-14A gas chromatograph with a Shimadzu CR-6A Chromatopac equipped with a chiral GC-column (Chiraldex G-TA, 40 m, He 2 mL/ min or CP-Cyclodextrin-B-236-M-19 (CP), 25 m, He 2 mL/ min).

**Cultivation of Microbe under the Standard Conditions.** *Geotrichum candidum* IFO4597 was grown in a complex medium consisting of 3% (wt/vol) glycerol, 1% (wt/vol) yeast extract, and 0.5% (wt/vol) polypeptone. Medium adjusted to pH 6.2 with 0.1 M potassium phosphate buffer was placed in a Sakaguchi flask, sterilized (121 °C, 20 min), and inoculated with the preincubated culture. The cultivation was carried out for 24 h at 30 °C with shaking. The cells were obtained by filtration.

**Cultivation of Microbe on the Diluted Medium (relative medium concentration: 0.25).** *Geotrichum candidum* IFO4597 was grown in a complex medium consisting of 0.75% (wt/vol) glycerol, 0.25% (wt/vol) yeast extract, and 0.125% (wt/vol) polypeptone. The medium adjusted to pH 6.2 with 0.025 M potassium phosphate buffer was placed in a Sakaguchi flask, sterilized (121 °C, 20 min), and inoculated with the preincubated culture. The cultivation was carried out for 24 h at 30 °C with shaking. The cells were obtained by filtration.

**Reduction of Acetophenone Derivatives under Standard Conditions (Conditions A).** A substrate (0.08 mmol, 10 μL) was added to a mixture of 3 mL of water and 0.5 g wet wt. of resting cells cultivated on a standard medium. The mixture was shaken at 130 rpm for 3 h at 30 °C, put on Extrelut, and eluted with ether. The chemical yield and ee were determined from GC analysis.

**Reduction of Acetophenone Derivatives under Air by the Cell Cultured on Diluted Medium (Conditions B).** A substrate (0.08 mmol, 10 μL) was added to a mixture of 3 mL of water and 2 g wet wt. of resting cells cultivated on a diluted medium. The mixture was shaken at 130 rpm for 3 h at 30 °C, put on

Extrelut, and eluted with ether. The chemical yield and ee were determined by GC analysis.

**Reduction of Acetophenone Derivatives under an Argon Atmosphere by the Cell Cultured on Diluted Medium (Conditions C).** A substrate (0.08 mmol, 10 μL) was added to a mixture of 3 mL of water and 2 g wet wt. of resting cells cultivated on a diluted medium. The mixture was shaken at 130 rpm for 3 h at 30 °C in an argon atmosphere, put on Extrelut, and eluted with ether. The chemical yield and ee were determined by GC analysis.

**Preparation of (*S*)-*m*-Chloro-1-Phenylethanol.** Cells (18 g) cultivated on a diluted medium were added to the mixture of a substrate (0.68 mmol, 105 mg) and water degassed and filled with argon (30 mL). The mixture was stirred at 30 °C in an argon atmosphere for 5 h. The reaction was quenched by adding 1N HCl aqueous solution (10 mL) and Extrelut (40 g) at 0 °C. The product was extracted with ether (800 mL) from the Extrelut, and the ether solution was concentrated to 50 mL, then washed with water, aqueous sodium hydrogen carbonate, and water successively. The yield and ee were determined from GC analysis of the ether solution to be 92% and >99%, respectively. The ether was evaporated under reduced pressure. The resulting residue was subjected to silica gel column chromatography (eluent, hexane : ethyl acetate = 5 : 1 to 2 : 1). The product was purified by bulb-to-bulb distillation: yield 83%; GC conditions CP 130 °C; $[\alpha]_D^{24}$= -42.4 (*c* 0.484, $CHCl_3$) (*lit.*[8] $[\alpha]_D^{20}$ = +36.7 (*c* 1.00, $CHCl_3$, 84.6% ee(*R*))); $^1H$ NMR ($CDCl_3$) δ 1.48 (d, 3H, $CH_3$, *J* = 6.4 Hz), 1.81 (br s, 1H, OH), 4.88 (q, 1H, CH, *J* = 6.4 Hz), 7.22-7.27 (m, 4H, Ph), 7.36-7.37 (m, 1H, Ph); IR (neat) 787, 810, 1012, 1078, 1111, 1202, 1298, 1335, 1372, 1431, 1478, 1574, 1599, 2928, 2976, 3356 $cm^{-1}$; Anal. Calcd for $C_9H_{12}O$: C, 61.35; H, 5.79. Found: C, 61.21; H, 5.78 .

**Preparation of Various (*S*)-Arylalkanols.** The corresponding ketones were reduced in the same manner as described above for (*S*)-*m*-chloro-1-phenylethanol. Chemical yields and ee as well as GC-analysis conditions are summarized in Table 3 for each product.

Table 3. Products from the Asymmetric Reductions.

| Product | Time/h | Yield(Isolated)/% | ee/% | GC conditions, $[\alpha]_D$ |
|---|---|---|---|---|
| (*S*)-1-Phenylethanol | 3 | 78(46) | 98 | G-TA 115 °C, $[\alpha]_D^{24}$ = -49.7 (*c* 0.423, $CH_2Cl_2$)<br>*lit.*[9] $[\alpha]_D$ = -49.5 (*c* 0.0489, $CH_2Cl_2$, 97% ee(*S*))) |
| (*S*)-1-Phenyl-1-propanol | 5 | 98(76) | 99 | CP 110 °C, $[\alpha]_D^{20}$ = -49.3 (*c* 0.425, $CHCl_3$)<br>*lit.*[9] $[\alpha]_D$ = -46.7 (*c* 0.0409, $CHCl_3$, 95% ee(*S*)) |
| (*S*)-1-Phenyl-2-propanol | 3 | 98(78) | >99 | G-TA 110 °C, $[\alpha]_D^{24}$ = +38.8 (*c* 0.366, $CHCl_3$)<br>*lit.*[10] $[\alpha]_D^{15}$ =+39.7 (*c* 0.515, $CHCl_3$, >99.9% ee(*S*)) |
| (*S*)-4-Phenyl-2-butanol | 3 | 98(91) | >98 | CP 110 °C, $[\alpha]_D^{24}$ = +21.7 (*c* 0.502, $C_6H_6$)<br>*lit.*[10] $[\alpha]_D^{22}$ = +22 (*c* 0.380, $C_6H_6$, >99.9% ee(*S*)) |

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