Biocatalytic Preparation of (*R*)-(−)-4-(Phenylthio)-2-butanol and (*R*)-(−)-4-(Phenylsulfonyl)-2-butanol by the Sequential Use of *Pichia farinosa* and *Rhodococcus rhodochrous*

Takeshi Sugai, Yoshikazu Ohtsuka, and Hiromichi Ohta*
Department of Chemistry, Keio University, 3-14-1 Hiyoshi, Yokohama 223

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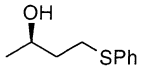
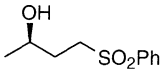
Preparation of (*R*)-4-(phenylthio)-2-butanol and (*R*)-4-(phenylsulfonyl)-2-butanol has been established based on the sequential use of two biocatalysts. A *Pichia farinosa* IAM 4682 mediated reduction of 4-(phenylthio)-2-butanone afforded (*R*)-4-(phenylthio)-2-butanol (91%*e.e.*) in 90% yield. Contaminating (*S*)-enantiomer in the resulting product was selectively oxidized by *Rhodococcus rhodochrous* IFO15564 to leave pure (*R*)-enantiomer in 87% yield. From this product, highly enantiomerically pure (*R*)-4-(phenylsulfonyl)-2-butanol was obtained by hydrogen peroxide oxidation.

Optically active secondary alcohols with a sulfur-containing functionality, such as sulfinyl and sulfonyl groups are important starting materials for synthesizing natural products, medicines, and other useful materials. Lipase catalized kinetic resolution of racemic 4-(phenylthio)-2-butanol **1a** has been reported, although the yields is essentially less than 50%.[1] Thus, preparation of such type of compounds by means of biocatalytic reduction[2] of corresponding ketones are recently gaining much attentions of many synthetic chemists, because of its efficiency. Among them, bakers' yeast-mediated synthesis of (*S*)-**1a** and (*S*)-4-(phenylsulfonyl)-2-butanol **2a** have been established.[3] So far, however, the low availability of the corresponding (*R*)-enantiomers from the corresponding ketones still remains unsolved. Here we report on the biocatalytic preparation of these (*R*)-enantiomers.

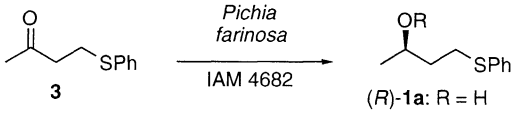


(*R*)-**1a**



(*R*)-**2a**

Our first attempt was the use of a yeast, *Pichia farinosa* IAM 4682.[4] The reduction of ketones using this yeast has been revealed to proceed in accordance with "*anti*-Prelog" selectivity. Thus, the reduction of **3** under an anaerobic condition smoothly proceeded to give (*R*)-**1a** in 90% yield.[5] However, the *e.e.* of **1a**, 91%,[6] was not satisfactory for being used as an optically active starting material.



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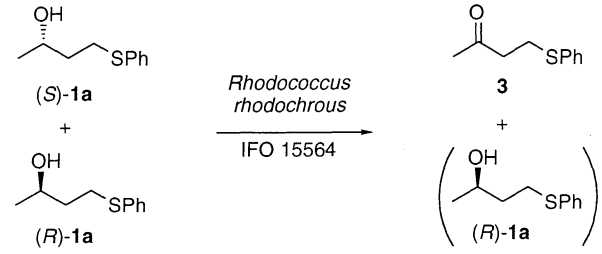
(*R*)-**1a**: R = H
90%Yd., 91%*e.e.*
1b: R = (*R*)-MTPA

At this point, we turned our attention to the enantioselective oxidation[7] of contaminating (*S*)-isomer (*ca.* 5%) back to the starting material. Toward this end, we investigated the possibility of enantioselective oxidation of (*S*)-**1a** mediated by *Rhodococcus rhodochrous* IFO15564, based on our own recent findings.[8]

The oxidation of (±)-**1a** by the resting cells of *R. rhodochrous* proceeded smoothly at pH 8.0 with bubbling of air.[9] The *e.e.* of the recovered substrate, (*R*)-**1a**, became as high as 75% after 26 h. The time course of incubation (Figure 1, solid lines) shows that the oxidation of (*S*)-**1a** is faster (3 : 1) than that of (*R*)-**1a** in the initial phase.



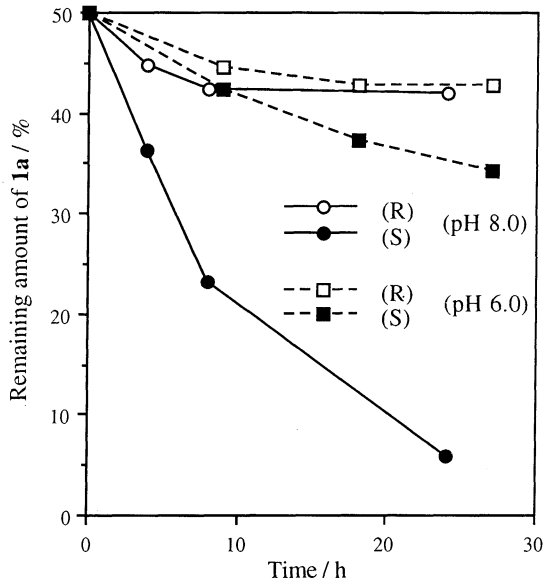
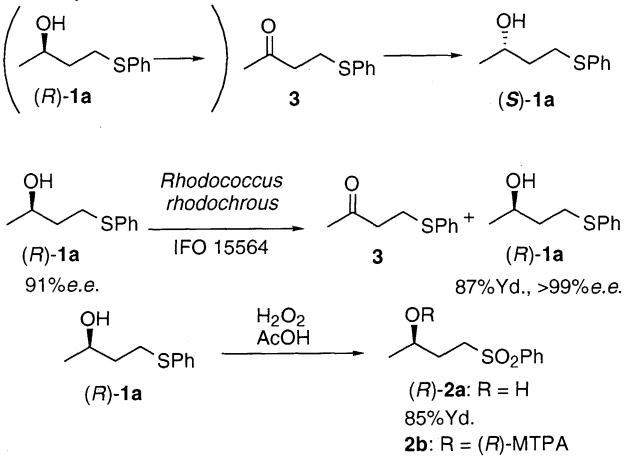


Figure 1.

At a lower pH (6.0), the *e.e.* of the recovered substrate (37%) was considerably lower than that obtained at pH 8.0. The detailed time-course study (Figure 1, dotted lines) indicated that the rate of oxidation of (*S*)-isomer at pH 6.0 was slower than that at pH 8.0, while the oxidation of (*R*)-isomer proceeded in a similar rate under either pH. This result suggests that a part of the corresponding ketone **3** was reduced to (*S*)-**1a**[10] under pH 6.0 in the reaction mixture accompanied with the oxidation of alcohols. An example of pH dependent inversion of oxidation-reduction of a related substrate, 1-(phenylthio)-2-propanol, mediated by *Rhodococcus equi* IFO3730,[11] has been observed.

The application of the above-mentioned *Rhodococcus*-mediated oxidation on an enantiomerically enriched (91% *e.e.*) (*R*)-**1a** worked well. The contaminating (*S*)-**1a** was effectively removed by this procedure to give (*R*)-**1a** of >99%*e.e.* in 87%

yield.[12] The total yield through the sequential use of these biocatalysts was 78%.



(*R*)-**1a** was converted to (*R*)-**2a** in 85% yield, by oxidizing its sulfide moiety with hydrogen peroxide in acetic acid.[13] In conclusion, (*R*)-**1a** and (*R*)-**2a** with high *e.e.* became available. The substrate specificity and selectivity of *Rhodococcus*-mediated oxidation of secondary alcohol is under investigation.

References and Notes

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4 a) T. Sugai and H. Ohta, *Agric. Biol. Chem.*, **54**, 1577 (1990); b) T. Sugai, D. Sakuma, N. Kobayashi, and H. Ohta, *Tetrahedron*, **47**, 7237 (1991); c) N. Mochizuki, H. Yamada, T. Sugai, and H. Ohta, *BioMed. Chem.*, **1**, 71 (1993).

5 Incubation condition of *Pichia farinosa* was according to lit.[4c] From 500 mg of **3**, 454 mg (90%) of (*R*)-(−)-**1a** was obtained. Analytical sample: $[\alpha]_D^{20}$ −25.9° (*c* 0.99, $CHCl_3$)].

6 (*R*)-(−)-**1a** was converted to the corresponding (*R*)-MTPA ester **1b**. ^1H NMR (270 MHz) δ = 1.26 (d, *J* = 6.3 Hz, 2.87H), 1.32 (d, *J* = 6.3 Hz, 0.13H), 2.47 (br.s, 2.87H), 3.54 (br. s, 0.13H).

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9 Incubation condition of *Rhodococcus rhodochrous* is as follows. A sterilized medium (pH 7.2, 100 ml) containing glucose (15 g/l), KH_2PO_4 (0.4 g/l), K_2HPO_4 (1.2 g/l), $MgSO_4 \cdot 7H_2O$ (0.5 g/l), yeast extract (1 g/l), peptone (5 g/l) in a 500-ml Erlenmeyer flask with two internal projections was inoculated with a loopful of *R. rhodochrous* IFO15564, and the flask was shaken at 30 °C on a gyrorotary shaker for 2 days. The cells were harvested by centrifugation. The wet cells (12 g) were re-suspended in a phosphate buffer solution (pH 8.0, 0.01M, 100 ml) and the substrate [300 mg, 1.65 mmol, 0.3% (w/v)] was added. After adding antifoam (Nakarai Tesque Antifoam AF emulsion, 10%, 1 ml), the mixture was stirred at 30 °C with bubbling of air (70 ml / min). During the incubation, its pH was kept at 8.0 by a pH controller. The analytical sample (1.67 ml) was occasionally withdrawn. To this chalcone (11.1 mg, as a solution in ethyl acetate) was added as an internal standard and extracted with ethyl acetate. The progress of reaction was measured by analyzing the crude mixture by ^1H NMR (270 MHz) δ = 2.07 (s, H-1 of **3**), 2.69 (dd, *J* = 7.3, 7.3 Hz, H-4 of **3**), 3.91 (ddq, *J* = 6.3, 6.3, 6.3 Hz, H-2 of **1a**), 7.75 (d, *J* = 15.8 Hz, H-3 of chalcone).

10 Incubation of ketone **3** as the sole substrate at 30 °C for 21 h afforded (*S*)-**1a** (67% *e.e.*) in 33% yield.

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12 From 300 mg of (*R*)-(−)-**1a** (91%*e.e.*), 229 mg (87%) of (*R*)-(−)-**1a** was obtained. Analytical sample: $[\alpha]_D^{20}$ −27.5° (*c* 0.97, $CHCl_3$) [lit.[3c] (*S*)-isomer (96%*e.e.*) $[\alpha]_D^{23}$ +26.8° (*c* 1.34, $CHCl_3$)], $[\alpha]_D^{20}$ −35.7° (*c* 1.0, EtOH) [lit.[1b] (94%*e.e.*) $[\alpha]_D$ −33.0° (*c* 1.1, EtOH)] IR νmax 3380, 3060, 2980, 2940, 1585, 1482, 1440, 1380, 1279, 1229, 1130, 1090, 940, 900, 880, 850, 745, 700 cm^{-1}; ^1H NMR (270 MHz) δ = 1.28 (d, *J* = 5.9 Hz, 3H), 1.83 (ddd, *J* = 5.9, 7.3, 7.4 Hz, 2H), 3.07 (dt, *J* = 14.8, 7.4 Hz, 1H), 3.14 (dt, *J* = 14.8, 7.3 Hz, 1H), 4.04 (tq, *J* = 5.9, 5.9 Hz, 1H), 7.20-7.28 (m, 1H), 7.31-7.44 (m, 4H). Its IR and NMR spectra were in good accordance with those reported for (*S*)-isomer.[3c] Anal. Found: C, 65.59; H, 8.09%. Calcd for $C_{10}H_{14}OS$: C, 65.89; H, 7.74%. Its *e.e.* was confirmed by ^1H NMR analysis of corresponding MTPA ester **1b**.

13 From 100 mg of (*R*)-(−)-**1a** (>99%*e.e.*), 100 mg (85%) of (*R*)-(−)-**2a** was obtained. Analytical sample: $[\alpha]_D^{20}$ −21.8° (*c* 1.03, $CHCl_3$) [(*S*)-isomer (>95%*e.e.*)[3b] $[\alpha]_D^{23}$ +20.7° (*c* 1, $CHCl_3$)] IR νmax 3520, 3080, 2980, 2940, 1725, 1590, 1450, 1410, 1380, 1310, 1240, 1150, 1090, 1030, 940, 860, 800, 750, 700, 670, 600 cm^{-1}; ^1H NMR (270 MHz) δ = 1.20 (d, *J* = 6.1 Hz, 3H), 1.70-2.00 (m, 2H), 3.20 (ddd, *J* = 5.6, 9.9, 14.0 Hz, 1H), 3.30 (ddd, *J* = 5.9, 9.7, 14.0 Hz, 1H), 3.91 (ddq, *J* = 3.8, 12.2, 6.1 Hz, 1H), 7.52-7.70 (m, 3H), 7.88-7.94 (m, 2H). Its IR and NMR spectra were in good accordance with those reported for (*S*)-isomer.[3b] Anal. Found: C, 56.21; H, 6.82%. Calcd for $C_{10}H_{14}O_3S$: C, 56.05; H, 6.59%. Its *e.e.* was confirmed by ^1H NMR analysis of corresponding MTPA ester **2b**.