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Asymmetric synthesis of both enantiomers of secondary alcohols by reduction with a single microbe

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Abstract—Both enantiomers of secondary alcohols were prepared by reduction of the corresponding ketones with a single microbe. Thus, reduction of aromatic ketones with *Geotrichum candidum* IFO 5767 afforded the corresponding (*S*)-alcohols in an excellent ee when amberlite™ XAD-7, a hydrophobic polymer, was added to the reaction system and the same microbe afforded (*R*)-alcohols also in an excellent ee when the reaction was conducted under aerobic conditions.

Enzymatic reductions are now recognized as a valuable approach to the organic synthesis of optically active compounds.[1–3] Lots of optically active alcohols have been prepared by enzymatic reduction. However, enzymatic reactions have a disadvantage: it is difficult to obtain both enantiomers of an alcohol by using a single biocatalyst. Since the number of available enzymes is limited, it is usually impossible to find a suitable enzyme for an unnatural substrate. In this report, we would like to describe the enantioselective synthesis of both enantiomers of alcohols with a single biocatalyst.

Microbial whole cells have broader substrate specificities than isolated alcohol dehydrogenases from the same species. Furthermore, the use of microbial whole cells has economical advantages since isolated dehydrogenases require expensive coenzymes and a recycling system. However, the stereoselectivities of microbial reduction are usually low owing to the existence of plural dehydrogenases which exhibit different stereoselectivities in the microbe.[4–6] To increase the stereoselectivity of microbial reduction, several methods have been developed. For example, specific inhibitors such as methyl vinyl ketone,[6] allyl alcohol[7] and ethyl chloroacetate[6] change the stereochemical course of yeast reduction of β-keto esters. Since methyl vinyl ketone and allyl alcohol inhibit the *S*-enzymes (the enzymes that afford (*S*)-alcohol on reduction) and ethyl chloroacetate inhibits the *R*-enzyme, both enantiomers, (*R*)-hydroxy esters, and (*S*)-hydroxy esters could be prepared selectively using these inhibitors.

In fact, both enantiomers of β-hydroxy esters have been prepared with high enantioselectivities by bakers' yeast, a single microbe, with the reduction–inhibition method. However, this method can be applied only to β-hydroxy esters. The synthesis of the other chiral alcohols with a single microbe is still awaited. The inhibition method has another disadvantage in that the use of an inhibitor decreases the yield of the reduction of ketones, since the inhibitor decreases the reducing activities of the microbe. Therefore, the development of methods other than those which use an inhibitor is strongly desired. Here, we report the synthesis of both enantiomers of simple alcohols with a single microbe.

Synthesis of (*S*)-alcohol (Method A)

The stereoselectivities in the reduction of an aryl methyl ketone with the resting cell of *Geotrichum candidum* IFO 5767 were not satisfactory. For example, the reduction of acetophenone (**1a**) afforded (*R*)-1-phenylethanol ((*R*)-**2a**), with a 99% chemical yield in 64% ee. Modification of the reaction conditions is necessary to change the stereoselectivity to afford the (*S*)-alcohol. Previously, the synthesis of (*S*)-1-arylethanols from aryl methyl ketones with *G. candidum* IFO 4597, another species of *Geotrichum*, has been reported using the acetone powder method,[8] the organic solvent method,[9] and the hydrophobic polymer method.[10–12] In the former two procedures, addition of

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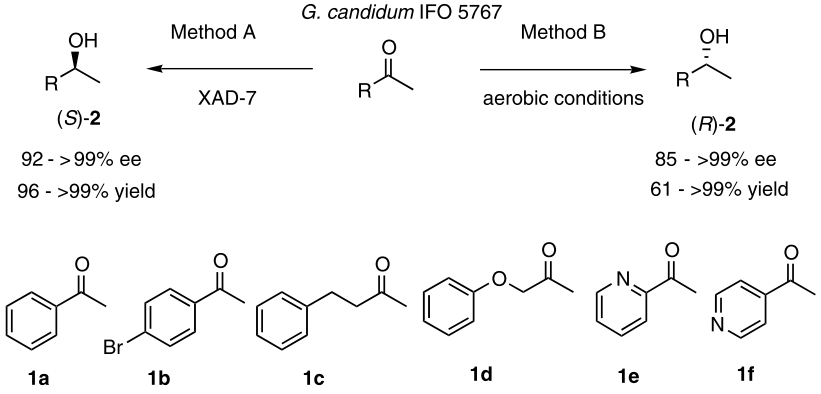
secondary alcohols such as 2-propanol and cyclopentanol increases the chemical yield and changes the selectivity of the reduction toward affording the (*S*)-alcohols. The last procedure did not need an additive to change the stereochemical course of the reduction.

To obtain (*S*)-alcohol preferentially, we chose the hydrophobic polymer method,[10] which was effective for reduction using *G. candidum* IFO 4597. We assumed that reduction with *G. candidum* IFO 5767 could also be controlled by the addition of the polymer. Thus, *G. candidum* IFO 5767 was cultivated in a glycerol medium for 24 h, and the cells were obtained by filtration. The cells (15 g wet wt.) were added to a mixture of a ketone (300 mg) and XAD-7 (18 g) in water (90 ml), and the mixture was shaken at 130 rpm for 1 day at 30°C under a nitrogen atmosphere. The usual work up afforded the corresponding (*S*)-alcohol as expected. For example, the reduction of acetophenone (**1a**) gave (*S*)-phenylethanol ((*S*)-**2a**) in >99% ee with a 98% chemical yield. The effectiveness of the hydrophobic polymer XAD-7 is noteworthy when the result is compared with that without the polymer. The hydrophobic polymer change the stereochemical course of the reduction from 64% ee (*R*) to >99% ee (*S*) toward affording the (*S*)-alcohol. Results are listed in Table 1.

Synthesis of (*R*)-alcohol (Method B)

Previously, (*R*)-1-arylethanols were obtained with *G. candidum* IFO 5767 from the corresponding racemic alcohols using the stereoinversion method.[13,14] Since stereoinversion is thought to be conducted with plural enzymes—one enzyme oxidizes the (*S*)-alcohol to the ketone and the other reduces the ketone to the (*R*)-alcohol—enhancement of oxidation should increase the (*R*)-enantioselectivity of the product in reduction. *G. candidum* IFO 4597 is known to oxidize only the (*S*)-alcohol, not the (*R*)-alcohol.[9] We assumed that *G. candidum* IFO 5767 also has similar enzymes. Although the reduction afforded both enantiomers of the alcohol (the reduction of **1a**: ee = 64%), the yield of the (*R*)-alcohol is thought to have increased when the reaction was conducted under moderately aerobic conditions. The cells (20 g wet wt.) and a ketone (200 mg) were added into a Sakaguchi flask containing water (100 ml), and shaken at 130 rpm for 1 day at 30°C. The usual work up afforded the corresponding *(R)*-alcohol. For example, (*R*)-**2a** was obtained with a 99% chemical yield in over 99% ee by the reduction of **1a**. The other ketones, **1b**–**1f** were also reduced using the same method and afforded the (*R*)-alcohols in excellent ee except for the reduction of **1c**. The reduction of ketones

Table 1. Asymmetric reduction of ketones with *G. candidum* IFO5767[a]



Substrate	Method A				Method B			
	Yield (%)	Isolated (%)	Ee (%)	Config.	Yield (%)	Isolated (%)	Ee (%)	Config.
1a	98	74	>99	*S*	99	73	>99	*R*
1b	>99	92	92	*S*	>99	99	98	*R*
1c	96	90	>99	*S*	61	56	85	*R*
1d	>99	88	99	*S*	>99	82	95	*R*
1e	99	77	98	*S*	>99	89	99	*R*
1f	>99	79	99	*S*	98	60	>99	*R*

[a] The chemical yield, the ee and the absolute configurations were determined by GLC analysis.

Method A: **1a**: $[\alpha]_D$ −57.0° (*c* = 1.00, $CHCl_3$), (lit.[14] $[\alpha]_D$ +53.5° (*c* = 1.13, $CHCl_3$), 99% ee (*R*)), **1b**: $[\alpha]_D$ −35.3° (*c* = 1.00, $CHCl_3$), (lit.[14] $[\alpha]_D$ +39.6° (*c* = 1.24, $CHCl_3$), >99% ee (*R*)), **1c**: $[\alpha]_D$ +21.0° (*c* = 1.02, C_6H_6), (lit.[14] $[\alpha]_D$ −21.1 (*c* = 1.00, C_6H_6), 99% ee (*R*)), **1d**: $[\alpha]_D$ −2.8° (*c* = 1.50, EtOH) and +28.9° (*c* = 1.10, $CHCl_3$), (lit.[14] $[\alpha]_D$ +2.8° (*c* = 0.58, EtOH), 97% ee (*R*)), **1e**: $[\alpha]_D$ −24.5° (*c* = 0.50, $CHCl_3$), (lit.[14] $[\alpha]_D$ +24.5° (*c* = 0.31, $CHCl_3$)), 98% ee (*R*)), **1f**: $[\alpha]_D$ −44.9° (*c* = 0.99, MeOH), (lit.[14] $[\alpha]_D$ +44.9° (*c* = 0.94, MeOH), 100% ee (*R*)). **Method B**: **1a**: $[\alpha]_D$ +56.8° (*c* = 1.01, $CHCl_3$), **1b**: $[\alpha]_D$ +37.6° (*c* = 1.24, $CHCl_3$), **1c**: $[\alpha]_D$ −18.3° (*c* = 0.99, C_6H_6), **1d**: $[\alpha]_D$ −27.8° (*c* = 0.96, $CHCl_3$), **1e**: $[\alpha]_D$ +24.7° (*c* = 0.31, $CHCl_3$), **1f**: $[\alpha]_D$ +44.9° (*c* = 0.98, MeOH).

by *G. candidum* IFO 5767 under aerobic conditions gives (*R*)-alcohols with high yields and high ee (85–>99%), as shown in Table 1.

Thus, reduction of **1a**, **1d**, **1e** and **1f** by *G. candidum* IFO 5767 gives the (*S*)- and (*R*)-enantiomers with excellent ee for both conditions. In a previous study of stereoinversion by *G. candidum* IFO 5767,[14] it was determined that the oxidation from ketone to the corresponding (*S*)-alcohol is reversible and the reduction from ketone to the (*R*)-alcohol is irreversible. Moreover, aerobic conditions could activate the oxidation from the (*S*)-alcohol to the corresponding ketone, and anaerobic conditions could inhibit the oxidation.[15] Thus, the reduction in aerobic conditions afford the (*R*)-alcohol preferentially.

On the contrary, the addition of XAD-7 afforded the (*S*)-alcohol preferentially. A hydrophobic polymer absorbs a hydrophobic substrate and decreases the substrate concentration in the water phase. Since an enzyme with the smallest K_m value reacts preferentially at low substrate concentration, the *S*-enzyme (which is thought to have a smaller K_m value to the ketone than that of the *R*-enzyme) may predominantly contribute to the reduction. Then the stereochemical course of the reduction shifts to afford the (*S*)-alcohol preferentially.

In this study, we demonstrated that asymmetric reduction of ketones by *G. candidum* IFO 5767 managed to afford both enantiomers with high ee and high yield under different conditions. Our system of stereochemical control, which uses very simple methods only to change the air conditions and to include the addition of XAD-7, is highly suitable for the practical synthesis of optically active secondary alcohols.

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