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Improvement of Enantioselectivity of Microbial Reduction by Using Organic Solvent Redox Coupler System

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Abstract: Enantioselectivity in reduction of aryl methyl ketones by *Geotrichum candidum* is improved in hexane in the presence of 2-hexanol when the microbe is entrapped with a water-adsorbent polymer.

Microbial reductions have been used widely for synthesizing chiral alcohols.[1] However, generally, a microbial reduction does not afford an alcohol of satisfactory optical purity, and a new method for controlling an enantioselectivity of microbial reduction is awaited. Recently we and others have developed stereochemical control of yeast reduction of keto esters.[2] Among them, the use of organic solvent is a powerful tool for controlling stereoselectivity of the reaction. Thus (*R*)-hydroxy esters are obtained in yeast reduction of α-keto esters in hexane, whereas the antipodes are obtained in water.[3-6] Diastereoselectivity of microbial reduction is also controlled by using hexane as the solvent.[7] However, these methods in stereochemical control have been restricted to keto esters as substrates and the reduction of ketones such as acetophenone has not been controlled enantioselectively. In the present paper, we would like to report that enantioselectivities in microbial reductions of aryl methyl ketones are enhanced by using a microbe immobilized on a water-adsorbent polymer (BL-100) in an organic solvent in the presence of redox coupler.

When acetophenone (**1a**) was reacted with *Geotrichum candidum* IFO 4597 in water, (*R*)-1-phenylethanol ((*R*)-**2a**) was obtained in 52% yield with 28% ee. The reduction in hexane, however, did not proceed smoothly and **2a** was obtained in only 1.2% yield. Since the microbe contains glycerol dehydrogenase and it has been found that several alcohols such as 2-propanol[8,9] and cyclopentanol[10] are oxidized with a coupled reduction of NAD^+ to NADH by glycerol dehydrogenase, it is plausible that addition of an alcohol to the system enhances the activity of microbe.[7] Alcohols such as 2-propanol and cyclopentanol have an advantage that they are soluble in organic solvents. Among alcohols tested for their abilities to reduce **1a**, 2-hexanol was found to be most effective. In fact, the reduction in hexane proceeded smoothly when 2-hexanol was added to the system and, surprisingly, it was found that (*S*)-**2a** was afforded with the immobilization/hexane/2-hexanol reduction system with perfect enantioselectivity (>99% ee). Thus, the use of hexane prohibits the reduction systems of both R- and S-enzyme, and 2-hexanol activates the S-enzyme system only to give the (*S*)-alcohol selectively.

Enantioselectivity and chemical yield in the reduction with 2-hexanol are both excellent. Other aryl methyl ketones, **1b-1h**, were also reduced smoothly by the aid of the same system to give the corresponding (*S*)-alcohols in excellent ees. When the microbe was immobilized with calcium alginate, a commonly used

immobilizing material, yield of **2a** was very low and ee was only 98%. Results are listed in Table 1.

In a typical experiment, the immobilized microbe was prepared by adding 0.5 g of BL-100[7,10,11] to a suspension of 0.5 g of *Geotrichum candidum* in 3 ml of water. A solution of **1a** (0.08 mmol), 2-hexanol (1.0 mmol) and dodecane (internal standard for GC) in 6 ml of hexane was added to the immobilized microbe and the mixture was shaken (130 stroke/min) at 30 °C for 24 h. After the reaction mixture was filtered, the biocatalyst was washed with ether and the filtrates were combined. Chemical yields and ees in products were measured using a chiral GC-column (G-TA, 0.25 mm × 30 m, 105 °C, He, 2 ml/min).

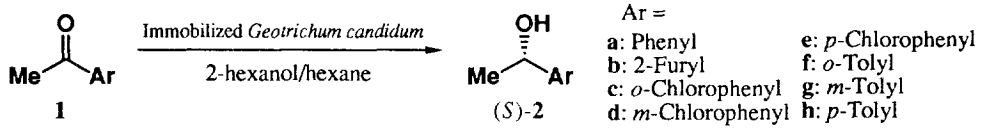

Table 1. Reduction of Aryl Methyl Ketone by Immobilized *Geotrichum candidum* in Hexane

Substrate	Additive	Yield,%	Ee,%	Config.
1a[a]	none	52	28	*R*
1a	2-propanol	29	>99	*S*
1a	cyclopentanol	58	>99	*S*
1a	2-hexanol[b]	73	>99	*S*
1a	2-hexanol	38[c]	98	*S*
1b	2-hexanol	81	>99	*S*
1c	2-hexanol	99	99	*S*
1d	2-hexanol	88	>99	*S*
1e	2-hexanol	41	92	*S*
1f	2-hexanol	59	>99	*S*
1g	2-hexanol	60	>99	*S*
1h	2-hexanol	40	99	*S*

[a] Reaction with unimmobilized microbe in water. [b] Racemic alcohol was used but (*S*)-2-hexanol was oxidized selectively. [c] The microbe was immobilized with calcium alginate.

Although mechanism for the improvement of enantioselectivity by an immobilized microbe in hexane is not clear at present, the novel stereochemical control of microbial reduction is believed to be a useful method to enhance enantioselectivity of microbial reduction. Further studies including isolation of enzymes are in progress in our laboratory.

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