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Asymmetric Reduction of Ketones by the Acetone Powder of *Geotrichum candidum*

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Abstract: Reduction of ketones with a reductant, 2-alkanol, by the acetone powder of *Geotrichum candidum* affords the corresponding (*S*)-alcohols of excellent ee in high yield.

Microbial reductions have been used widely for synthesizing chiral alcohols.[1-3] However, generally, a microbial reduction does not afford an alcohol of satisfactory optical purity since a microbe possesses several enzymes to reduce ketones into different configurational alcohols. Several methods have been developed to remove this drawback. For example, stereochemical control of microbial reduction to obtain an optically pure alcohol of the desired configuration has been achieved by the use of organic solvents,[4-6] additives,[5,7] or inhibitor of unnecessary enzyme(s).[8,9] On the other hand, if an isolated enzyme is used for a reaction, selectivity is usually high,[10-12] although isolated enzymes are not always available or affordable. An alternative way to access an enzymatic system is the use of crude enzymes such as acetone powder,[13] microbial dried cell dehydrated by using acetone, the preparation of which is facile in contrast to isolation of enzymes. In this report, asymmetric reduction of ketones by the acetone powder of *Geotrichum candidum* is described.

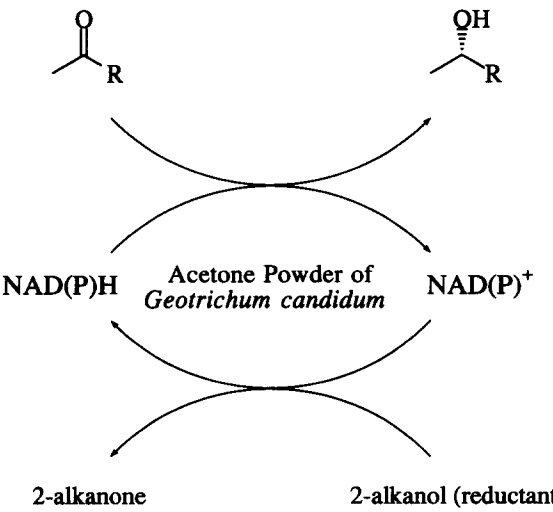
We found that the acetone powder from *G. candidum* IFO4597(APG4) catalyzes reduction of ketones giving outstanding results in the presence of a reductant. For example, when methyl 3-oxobutanoate was reduced by APG4 in the presence of 2-hexanol and a small amount of a coenzyme, NAD^+, methyl (*S*)-3-hydroxybutanoate of >99% ee was obtained in >99% yield, while the reduction with a resting cell system afforded the corresponding (*S*)-alcohol of 39% ee. Comparison of the reduction of methyl 3-oxobutanoate by APG4 with that by the resting cell is shown in Table 1. The optical purity of the product is markedly improved by the use of the acetone powder.

The reaction scheme is shown below. As the substrate, a ketone, is reduced to an alcohol, $NAD(P)^+$ is formed, which in turn is reduced to NAD(P)H by the coupled oxidation of 2-alkanol. Racemic 2-alkanol is used for the reaction, but (*S*)-2-alkanol is oxidized selectively to reduce the substrate to (*S*)-alcohol.

Table 1. Comparison of the Acetone Powder System with the Resting Cell System on Reduction of Methyl 3-Oxobutanoate

Catalysts	Yield[c] / %	Ee [c] / % (Config.)
Resting Cell[a]	97	39(*S*)
Acetone Powder[b]	>99	>99(*S*)

[a] [Substrate]=30 mM, [Water]=3 mL, [Cell]=0.5 g. [b] The reaction conditions are described in the text. [c] Yield and ee were measured using a chiral GC-column (G-TA, 0.25 mm x 30 m, 80 °C, He: 2 mL/min).



Besides the high selectivity of the reduction, there are many advantages in the use of APG4: wide substrate specificity, preservability of the biocatalyst, high ratio of substrate to biomass, redox coupler system by 2-alkanol, flexibility in the choice of coenzyme and reductant, easiness of preparation, experimental reproducibility and freedom from asepsis. First and most important of all, the substrate specificity of the acetone powder system is very wide in spite of the high enantioselectivity of the reduction. As shown in Table 2, the reduction of ketones from β-ketoesters and acetophenone derivatives to aliphatic ketones results in high yield and excellent ee. Next, preservation of the acetone powder for extended periods is possible. Storage of this powder in a freezer preserves the enzyme activity for more than 1 year, whereas the resting cell of the microbe is usually active only for a few days. The use of APG4 also resolves the problem of the excessively high weight of the biocatalyst compared to the substrate, usually required in biocatalysis.[14] The ratio of the resting cell mass / substrate (50/1) is largely improved to the ratio of APG4 / substrate (1/1). Although the system requires a coenzyme, only a small amount is sufficient for the reduction, since the redox coupler system can recycle it more than 100 times (data not shown) with the aid of easily available and cheap alcohols such as 2-alkanol. The flexibility in the structures of the reductant and coenzyme are also advantages. The reductant can be any 2-alkanol from isopropanol to 2-octanol, and both NAD^+ and $NADP^+$ are effective as coenzymes. Other advantages are easiness of the preparation of APG4, which does not require any special equipment or professional technique, and the powder is available within an hour. Once

APG4 is prepared, the catalyst from the same batch can be used for many experiments over a long time, which leads to experimental reproducibility. Since some of the cell components are destroyed during the preparation of APG4, asepsis is not required.

Table 2. Reduction of Ketones by the Acetone Powder System[a]

Substrate	Yield / %	Ee / % (Config.)
Methyl 3-oxobutanoate	>99	>99(*S*)
Ethyl 3-oxobutanoate	99	>99(*S*)
Neopentyl 3-oxobutanoate	>99	>99(*S*)
Acetophenone	89	99(*S*)
o-Chloroacetophenone	>99	>99(*S*)
m-Chloroacetophenone	95	>99(*S*)
p-Chloroacetophenone	62	96(*S*)
6-Methyl-5-heptene-2-one	92	96(*S*)

[a] The reaction conditions are described in the text.

In a typical experiment, the acetone powder of *Geotrichum candidum* IFO4597(APG4) was prepared as follows. The cells were mixed with cold acetone (-20 °C), and the resulting suspension was filtered. The procedure was repeated three times, then the powder was dried under reduced pressure. A ketone (0.08 mmol), NAD^+ (1.5 μmol) and 2-pentanol (100 μL) were added to a suspension of APG4 (10 mg) in MES (2-(*N*-morpholino)ethanesulfonic acid) buffer (pH 7.1, 0.1 M, 3 mL). The mixture was shaken at 130 rpm at 30 °C for 1 day, and the mixture was put on Extrelut and eluted with ether. Chemical yield and ee were measured using a chiral GC-column (G-TA, 0.25 mm x 30 m, He: 2 mL/min).

On a preparative scale, *m*-chloroacetophenone (1 mmol, 155 mg), 2-propanol (800 mg), NAD^+ (50 mg) and APG4 (200 mg) were added to 30 ml of MES buffer (0.1 M, pH=7.1). The mixture was stirred at room temperature for 1 day in an argon atmosphere in the dark. After the addition of Extrelut, the product was extracted with ether and concentrated under reduced pressure. The yield and ee were determined to be 95% and >99% (*S*), respectively, from GC analysis. The residue was subjected to silica gel column chromatography (eluent, hexane : ethyl acetate = 5 : 1), giving (*S*)-1-*m*-chlorophenylethanol (143 mg, 91%): $[\alpha]_D$ -43.5°(*c* 1.08, $CHCl_3$)(ref [15] $[\alpha]_D$ +36.7°, 85% ee(*R*)).

The present method is facile and convenient for organic chemists to treat a biocatalyst because of the easiness of the preparation of the acetone powder, capability of extended preservation of the biocatalyst, and feasible manipulation of the reaction. Therefore, the method can be developed for a wide variety of applications. The present reduction system may also be applicable to industrial preparations of chiral alcohols.

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