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Asymmetric reduction of simple aliphatic ketones with dried cells of *Geotrichum candidum*

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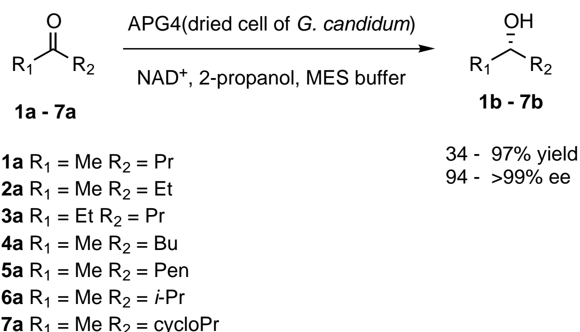
Abstract—Simple aliphatic ketones such as 2-pentanone, 2-butanone, 3-hexanone, etc., were reduced with high yield and excellent enantioselectivity to the corresponding (*S*)-alcohols using dried cells of *Geotrichum candidum*. © 2002 Elsevier Science Ltd. All rights reserved.

1. Introduction

The asymmetric reduction of ketones is one of the most widely studied reactions for the synthesis of chiral compounds. Excellent chemical and biological catalysts[1] have been developed in recent decades and the two substituents at the α-positions of the carbonyl group can be distinguished if one of the groups is not a simple aliphatic substituent, allowing excellent enantioselectivities and yields to be obtained. However, when both substituents are aliphatic groups such as Me, Et, or Pr, none of the reported chemical catalysts can clearly differentiate between groups which are different by one or methylene units. Thus, using non-biological methods, the best results obtained, to our knowledge, in the reduction of 2-butanone and 2-pentanone are 72[2] and 85% e.e.,[3] respectively. Using biological methods, very high enantioselectivities have been reported but the yields are low: 99% e.e. for the reduction of 2-butanone up to 16% conversion using alcohol dehydrogenase from *Psychrophile moraxella* at 0°C[4] and 97% e.e. for that of 3-hexanone using alcohol dehydrogenase from *Thermoanaerobium brockii*, where the reaction was interrupted at 50% conversion to avoid racemization.[5]

Due to environmental concerns, we have been developing methods which use a biocatalyst—a natural, reproducible catalyst. Our system[6–9] consists of APG4 (dried cells of the dimorphic fungus, *Geotrichum candidum* IFO 4597), NAD(P)$^+$, and an alcohol additive such as a 2-alkanol or cyclopentanol. As the ketone substrate is reduced to the alcohol product, NAD(P)$^+$ is formed, which in turn is reduced back to NAD(P)H by the coupled oxidation of 2-alkanol or cyclopentanol. Using the APG4 system, we succeeded in highly enantioselective reduction (>99% e.e.) of various aromatic ketones and β-keto esters with almost quantitative yields, even on gram scale.[6] Aliphatic ketones were also used as the substrate in the APG4 system, and the system was shown to discriminate Me and alkyl groups larger than hexyl.[6] The APG4 system was also tested for its ability to discriminate between two small alkyl groups with very little difference between them: Me versus Et, Et versus Pr, etc., and it was found that the APG4 system could clearly differentiate between the alkyl groups of such compounds; the asymmetric reduction of aliphatic ketones such as 2-butanone and 3-hexanone, etc., proceeded with excellent enantioselectivities, as shown in Scheme 1.



Scheme 1.

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2. Results and discussion

2.1. Reduction of 2-pentanone 1a

First, 2-pentanone **1a** was subjected to the reduction with APG4, NAD^+, and 2-propanol in MES buffer. The yield and enantioselectivity of the resulting 2-pentanol **1b** was determined by GC analysis, and the absolute configuration of **1b** was determined by comparison of the GC retention times with those of the authentic samples. It was found that the product was obtained in 88% yield, with e.e. of >99% (Table 1, entry 1). In this system, the enzyme clearly distinguished between the Me and Pr groups. Other coenzyme and alcohol additives were also tested to see if the high enantioselectivities were steady, and >99% e.e. was observed for any combination of coenzyme (NAD^+ or $NADP^+$) and additive (2-propanol or cyclopentanol). When the amount of 2-propanol was increased, the yield increased without decreasing enantioselectivity. The time course of the reduction shows that the yield of 2-pentanol was increased to 96% in 9 h.

2.2. Reduction of 2-butanone 2a

Next, 2-butanone **2a** was reduced using the same system to obtain (*S*)-2-butanol. The result is shown in Table 2. Unfortunately, under the same conditions as for the reduction of 2-pentanone **1a**, the enantioselectivity of the reduction was only moderate (77% e.e., Table 2, entry 1). This is probably due to the presence of more than two competing enzymes with different enantioselectivities ((*R*)-enzyme(s) and (*S*)-enzyme(s)). Screening of the dried cells of other microorganisms resulted in even lower e.e.[†] The reaction conditions were optimized using other coenzyme or additive alcohol. The use of $NADP^+$ instead of NAD^+ did not affect the enantioselectivity (Table 2, entry 2). When cyclopentanol was used instead of 2-propanol, an improvement was observed (Table 2, entries 3 and 4). When a larger amount of cyclopentanol or 2-propanol was used, the enantioselectivity was greatly improved to e.e.s of 93 and 94%, respectively (Table 2, entries 5 and 6). This improvement is probably due to the relatively stronger inhibition of the (*R*)-enzyme(s) than that of the (*S*)-enzyme(s) with the large amount of the additive alcohols.

[†] Reduction of 2-butanone **2a** (20 μL, 0.22 mmol) by the dried cell of various organisms (10 mg) and four different combinations of a coenzyme (5 mg) and an additive alcohol (100 mg), NAD^+ and 2-propanol (Condition A), NAD^+ and cyclopentanol (Condition B), $NADP^+$ and 2-propanol (Condition C), $NADP^+$ and cyclopentanol (Condition D), in MES buffer (pH 7.0, 0.1 M, 3 mL) at 30°C for 20 h gave the following results:
Endomyces magnusii IFO 4600: Conditions A 90% yield, 19% e.e./Conditions B 66% yield, 57% e.e/Conditions C 85% yield, 40% e.e./Conditions D 41% yield, 66% e.e.;
Endomyces geotrichum IFO 9541: Conditions A 87% yield, 42% e.e./Conditions B 92% yield, 71% e.e./Conditions C 85% yield, 59% e.e./Conditions D 89% yield, 78% e.e.;
Geotrichum candidum IFO 5767: Conditions A 86% yield, 46% e.e./Conditions B 63% yield, 78% e.e./Conditions C 88% yield, 61% e.e./Conditions D 92% yield, 83% e.e.;
Geotrichum candidum ATCC 34614: Conditions A 85% yield, 54% e.e./Conditions B 91% yield, 80% e.e./Conditions C 86% yield, 52% e.e./Conditions D 92% yield, 80% e.e.;
Galactomyces reessii IFO 1112: Conditions A 89% yield, 44% e.e./Conditions B 92% yield, 74% e.e./Conditions C 86% yield, 52% e.e./Conditions D 88% yield, 78% e.e.

Table 1. Reduction of 2-pentanone **1a** with the APG4 system

Entry	Coenzyme	Additive alcohols (μL)[a]	Yield (%)	E.e. (%)
1	NAD^+	2-Propanol (100)	88	>99
2	$NADP^+$	2-Propanol (100)	85	>99
3	NAD^+	Cyclopentanol (100)	88	>99
4	$NADP^+$	Cyclopentanol (100)	86	>99
5	NAD^+	2-Propanol (500)	97	>99

The reaction conditions are described in Section 4.
[a] Amount of an alcohol additive to reduce 10 μL (0.093 mmol) of **1a**.

Table 2. Reduction of 2-butanone **2a** with the APG4 system

Entry	Coenzyme	Additive alcohols (μL)[a]	Yield (%)	E.e. (%)
1	NAD^+	2-Propanol (100)	85	77
2	$NADP^+$	2-Propanol (100)	85	76
3	NAD^+	Cyclopentanol (50)	88	84
4	$NADP^+$	Cyclopentanol (50)	89	83
5	NAD^+	Cyclopentanol (150)	79	93
6	NAD^+	2-Propanol (500)	73	94

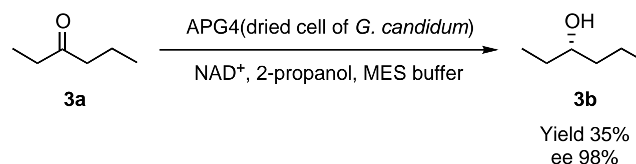
The reaction conditions are described in Section 4.
[a] Amount of an additive alcohol to reduce 10 μL (0.11 mmol) of **2a**.

2.3. Reduction of 3-hexanone 3a

A considerably challenging substrate for asymmetric reduction, 3-hexanone **3a**, was also reduced by the APG4 system. Surprisingly, the enantioselectivity of the reduction was 98%, as shown in Scheme 2. The GC chromatograph of the propionate of the product **3b** is shown in Fig. 1. Thus, the system could clearly distinguish the Et and Pr groups.

2.4. Substrate specificity

To examine the generality of these excellent enantioselectivities, other aliphatic ketones were also reduced by the APG4 system (Table 3). As expected, most of the



Scheme 2.

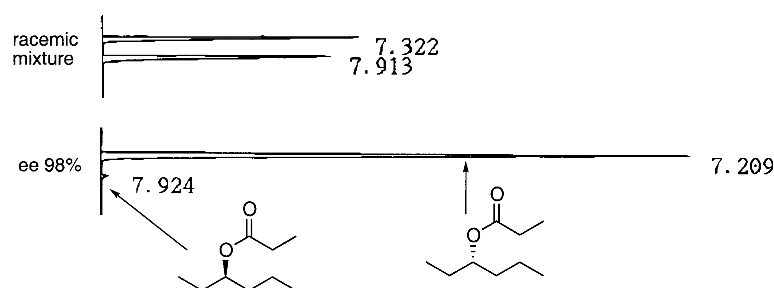


Figure 1. GC chromatograph of propionate of (a) (*R*/*S*)-3-hexanol and (b) reduction product (*S*)-3-hexanol, for the determination of enantioselectivity.

substrate tested could be reduced to enantiomerically pure alcohols (e.e. >99%). The examination of a series of small chain aliphatic ketones proved high flexibility in the substrate specificities and excellent discriminating ability between two small alkyl groups. The enzyme usually shows very high enantioselectivity when the natural substrate was used, but it was also the case when man-made substrates were used.

3. Conclusion

Simple aliphatic ketones such as 2-pentanone, 2-butanone, 3-hexanone, etc., were reduced with excellent enantioselectivity to the corresponding (*S*)-alcohols by using the dried cells of *Geotrichum candidum* (APG4). The APG4 system, which is very useful practically for the reduction of various ketones, can distinguish between two alkyl groups with a difference of a single methylene unit.

4. Experimental

4.1. General

Chemicals including ketones and racemic and optically active alcohols used as substrate or authentic samples for GC analysis were purchased from Nacalai Tesque, Inc., Wako Pure Chemical Industries Ltd., or Aldrich Chemical Co. unless otherwise indicated and used without further purification. *Geotrichum candidum* IFO 4597 was cultivated as described previously.[6] APG4 (acetone powder, a microbial dried-cell preparation dehydrated using acetone, of *G. candidum* IFO 4597) was prepared as described previously.[6] Gas chromatographic analyses were performed using chiral GC-columns (Chirasil-DEX CB: 25 m; He 2 mL/min, or Chiraldex G-TA: 30 m or 40 m) equipped on Shimadzu GC-14B with C-R7A plus or Shimadzu GC-9A with C-R6A. Yields were determined by GC analysis using decane, undecane, or dodecane as an internal standard. E.e. of the product was determined by GC analysis after acetylation or propionylation. The absolute configurations of the product alcohols were determined by comparing the GC retention times with those of authentic samples.

Table 3. Reduction of various aliphatic ketones with the APG4 system

Substrate	Yield (%)	E.e. (%)
4a	89	>99
5a	89	>99
6a	96	98
7a	34	>99

The reaction conditions are described in Section 4.

4.2. General procedure of the reduction of ketones with APG4

The ketone (10 μL), $NAD(P)^+$ (5 mg), and 2-propanol or cyclopentanol were added to a suspension of APG4 (10 mg) in MES (2-(*N*-morpholino)ethanesulfonic acid) buffer (pH 7.0, 0.1 M, 3.0 mL). The mixture was shaken at 130 rpm and 30°C for 20 h and the resulting mixture was eluted from Extrelut with ether. The chemical yield was determined by GC analysis. The e.e. of the product after esterification with propionyl chloride or acetyl chloride and pyridine in CH_2Cl_2 was determined by GC analysis. The GC conditions and retention times of the internal standards are as follows; Column: Chirasil-DEX CB: 25 m; decane: 60°C: 14.6 min, 70°C: 9.2 min; undecane: 80°C: 11.9 min; dodecane: 100°C: 9.3 min.

4.3. Time course of the reduction of 2-pentanone 1a

Substrate **1a** (10 μL, 0.093 mmol), NAD^+ (5 mg, 7.0 μmol) and 2-propanol (500 μL, 6.53 mmol) were added to a suspension of APG4 (10 mg) in MES (2-(*N*-morpholino)ethanesulfonic acid) buffer (pH 7.0, 0.1 M, 3 mL). The mixture was shaken at 130 rpm and 30°C and the resulting mixture was eluted from Extrelut with ether. The chemical yield of the product were determined by GC analysis; 1 h: 22%; 2 h: 39%; 3 h: 55%; 4 h: 71%; 5 h: 79%; 6 h: 88%; 7 h: 92%; 8 h: 94%; 9 h: 96%.

4.4. Reduction of 3-hexanone 3a

Substrate **3a** (10 μL, 0.081 mmol), NAD^+ (5 mg, 7.0 μmol), and 2-propanol (100 μL, 1.31 mmol) were added to a suspension of APG4 (10 mg) in MES (2-(*N*-morpholino)ethanesulfonic acid) buffer (pH 7.0, 0.1 M, 3.0 mL). The mixture was shaken at 130 rpm and 30°C for 20 h and the resulting mixture eluted from Extrelut with ether. The chemical yield was determined by GC

analysis. E.e. of the product after esterification with propionyl chloride and pyridine in CH_2Cl_2 were determined by GC analysis; 35% yield, 98% e.e. (Column: Chirasil-DEX CB, Column temp.: 80°C, Retention times: **3a**: 2.7 min, **3b**: 4.9 min, (*S*)-3-hexyl propionate: 7.3 min, (*R*)-3-hexyl propionate: 7.9 min).

4.5. Reduction of ketones 1a, 2a, 4a–7a

These ketones were reduced at 130 rpm and 30°C for 20 h in the same manner as described above. The results are shown in Tables 1–3. GC conditions for the analysis and retention times of **1a**, **2a**, **4a**–**7a**, **1b**, **2b**, **4b**–**7b**, and their acetyl or propionyl esters are as follows; Reduction of **1a**: Column: Chirasil-DEX CB, Column temp.: 100°C, **1a**: 1.7 min, **1b**: 2.2 min, Column temp.: 60°C, (*S*)-2-pentyl acetate: 5.8 min, (*R*)-2-pentyl acetate: 9.4 min; Reduction of **2a**: Column: Chirasil-DEX CB, Column temp.: 60°C, **2a**: 1.8 min, **2b**: 2.9 min, (*S*)-2-butyl propionate: 6.0 min, (*R*)-2-butyl propionate: 6.8 min; Reduction of **4a**: Column: Chirasil-DEX CB, Column temp.: 80°C, **4a**: 2.9 min, **4b**: 5.3 min, (*S*)-2-hexyl propionate: 7.5 min, (*R*)-2-hexyl propionate: 9.0 min; Reduction of **5a**: Column: Chirasil-DEX CB, Column temp.: 100°C, **5a**: 2.9 min, **5b**: 4.4 min, (*S*)-2-heptyl propionate: 6.1 min, (*R*)-2-heptyl propionate: 6.8 min; Reduction of **6a**: Column: Chirasil-DEX CB, Column temp.: 60°C, **6a**: 2.6 min, **6b**: 6.2 min, Column temp.: 40°C, (*R*)-**6b** 14.9 min, (*S*)-**6b** 15.8 min; Reduction of **7a**: Column: Chirasil-DEX CB, Column temp.: 70°C, **7a**: 2.7 min, **7b**: 4.9 min, (*S*)-(1-cyclopropyl)ethyl acetate: 5.5 min, (*R*)-(1-cyclopropyl)ethyl acetate: 6.5 min.

References

1. Fehring, V.; Selke, R. *Angew. Chem., Int. Ed.* **1998**, *37*, 1827–1830.
2. Harada, T.; Osawa, T. In *Chiral Reactions in Heterogeneous Catalysis*; Jannes, G.; Dubois, V., Eds. Enantio-differentiating hydrogenation of 2-butanone: distinction between CH_3 and C_2H_5 with a modified nickel catalyst; Plenum Press: New York, 1995; pp. 83–88.
3. Schmitzer, A. R.; Franceschi, S.; Perez, E.; Rico-Lattes, I.; Lattes, A.; Thion, L.; Erard, M.; Vidal, C. *J. Am. Chem. Soc.* **2001**, *123*, 5956–5961.
4. Velonia, K.; Tsigos, I.; Bouriotis, V.; Smonou, I. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 65–68.
5. Keinan, E.; Hafeli, E. K.; Seth, K. K.; Lamed, R. *J. Am. Chem. Soc.* **1986**, *108*, 162–169.
6. Nakamura, K.; Matsuda, T. *J. Org. Chem.* **1998**, *63*, 8957–8964.
7. Nakamura, K.; Kitano, K.; Matsuda, T.; Ohno, A. *Tetrahedron Lett.* **1996**, *37*, 1629–1632.
8. Matsuda, T.; Harada, T.; Nakajima, N.; Itoh, T.; Nakamura, K. *J. Org. Chem.* **2000**, *65*, 157–163.
9. Nakamura, K.; Matsuda, T.; Itoh, T.; Ohno, A. *Tetrahedron Lett.* **1996**, *37*, 5727–5730.