Asymmetric reduction of ketones by *Geotrichum candidum* in the presence of Amberlite™ XAD, a solid organic solvent

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A hydrophobic polymer, Amberlite™ XAD, was used as material to control the stereochemical course of microbial reductions. In the presence of XAD, simple aliphatic and aromatic ketones were reduced to the corresponding (*S*)-alcohols in excellent enantiomeric excess (ee) while low enantioselectivities were observed in the absence of the polymer.

Introduction

Asymmetric reduction of carbonyl compounds with biocatalysts is now recognized as a valuable approach to the organic synthesis of optically active compounds.[1] There are two methods of biocatalytic reduction: one is the use of isolated enzymes and the other is the use of microbial whole cells. The former involves the use of alcohol dehydrogenases, such as horse liver alcohol dehydrogenase,[2] yeast alcohol dehydrogenase,[3] *Thermoanaerobium brockii* alcohol dehydrogenase,[4] glycerol dehydrogenase,[5] and secondary alcohol dehydrogenases.[6] These enzymes generally have high stereoselectivities but relatively restricted substrate specificities. On the other hand, microbial whole cells represented by bakers' yeast have broader substrate specificities than alcohol dehydrogenases from the same species. Furthermore, economically the use of microbial whole cells has advantages since isolated dehydrogenases require expensive coenzymes and a recycling system. However, stereoselectivities of microbial reduction are usually low owing to the existance of plural dehydrogenases which show different stereoselectivities in the microbe.[7] Methods to control the stereochemistry of microbial reduction are required for organic synthesis.

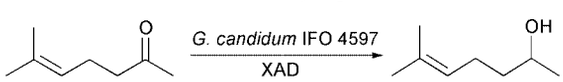
Recently, the hydrophobic polymer, Amberlite™ XAD, has been used to reduce substrate and product inhibitions in microbial reduction, and increase the chemical yield of the reduction.[8] Amberlite™ XAD, made of a hydrophobic polymer such as polystyrene or poly(acryl ester), is a commercially available adsorbent, and has a wide surface area. In this case, the addition of XAD to the reaction system reduced the substrate concentration in the aqueous phase, since the substrate locates mainly in the solid phase (polymer surface) rather than the aqueous phase. Servi and coworkers recently reported that the stereoselectivities of yeast reduction of ethyl 3-oxobutanoate and the C–C double bond of α,β-unsaturated carbonyl compounds were improved by the use of hydrophobic polymers.[9] On the other hand, we found that using a hydrophobic polymer one can control the stereochemistry of the reduction of ketones by the fungus *Geotrichum candidum*, which has been often used for the reduction of ketones and is known to have broad substrate specificities.[10] Approaches such as ours to the reduction of hydrophobic unnatural substrates may be greatly effective, since the inhibition of hydrophobic substrates is known to markedly decrease the yield and the ee of the product in microbial reduction. Here we report that the use of XAD increases enantioselectivities in the reduction of simple aliphatic and aromatic ketones.

Results and discussion

Effect of XAD on reduction of 6-methylhept-5-en-2-one by *G. candidum*

Firstly, several polymers were tested for their abilities to affect the yield and the stereochemical course of microbial reduction of 6-methylhept-5-en-2-one. The results are shown in Table 1 and XAD-7 is found to be the best additive to increase stereoselectivity and yield. XAD-7 is thought to be suitable due to its large surface area and polar structure, and is used throughout the present research.

Table 1 Character of XAD and effect of XAD on the reduction of 6-methylhept-5-en-2-one[a]



Resin	Material	Specific surface area/m^2 g^{-1}	Yield (%)	Ee (%)	(Config.)
XAD-2	Styrene-DVB	280–320	20	50	(*R*)
XAD-4	Styrene-DVB	700–800	17	18	(*R*)
XAD-7	Acrylic ester	350–500	31	86	(*S*)
XAD-8	Acrylic ester	120–300	16	22	(*S*)
None			28	27	(*R*)

[a] The cells (0.5 g wet wt) were added to a mixture of 6-methylhept-5-en-2-one (0.08 mmol) and 100 mg of XAD in water (3.0 mL). The mixture was shaken at 130 rpm for 1 day at 30 °C, put on Extrelut, and eluted with ether. The yield and ee of 6-methylhept-5-en-2-ol were determined by GC analysis as described in the Experimental section.

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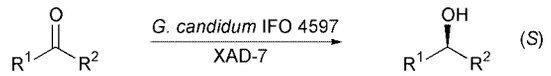
Table 2 Effect of XAD-7 on the reduction of aliphatic ketones by *G. candidum*[a]



		Without XAD-7		With XAD-7		
R^1	R^2	Yield (%)	Ee (Config.) (%)	Yield (%)	Ee (Config.) (%)	XAD-7/mg
Et	Me	98	5.1 (*S*)	98	18 (*S*)	600
Pr	Me	19	27 (*S*)[b]	83	93 (*S*)[b]	600
^{i}Bu	Me	19	86 (*R*)[b]	82	>99 (*S*)[b]	600
Bu	Me	58	18 (*S*)	89	99 (*S*)	600
But-3-enyl	Me	86	9.5 (*S*)	88	93 (*S*)	400
				77	94 (*S*)	600
Pentyl	Me	28	67 (*S*)	84	99 (*S*)	600
4-Methylpentyl	Me	16	61 (*S*)	52	94 (*S*)	400
				34	>99 (*S*)	600
4-Methylpent-3-enyl	Me	58	18 (*R*)	76	97 (*S*)	600
Hexyl	Me	36	60 (*S*)	82	>99 (*S*)	600
Heptyl	Me	44	92 (*S*)	70	>99 (*S*)	600
Octyl	Me	21	97 (*S*)	66	>99 (*S*)	600
Pr	Et	36	60 (*S*)[b]	93	96 (*S*)[b]	400
Bu	Et	88	56 (*S*)[b]	57	93 (*S*)[b]	400
Pentyl	Et	14	33 (*R*)[b]	25	86 (*S*)[b]	200

[a] The reaction conditions are described in the experimental section. [b] Absolute configurations of methyl and ethyl alkanols were determined tentatively. The (*S*)-alcohols were eluted faster than the (*R*)-isomers.

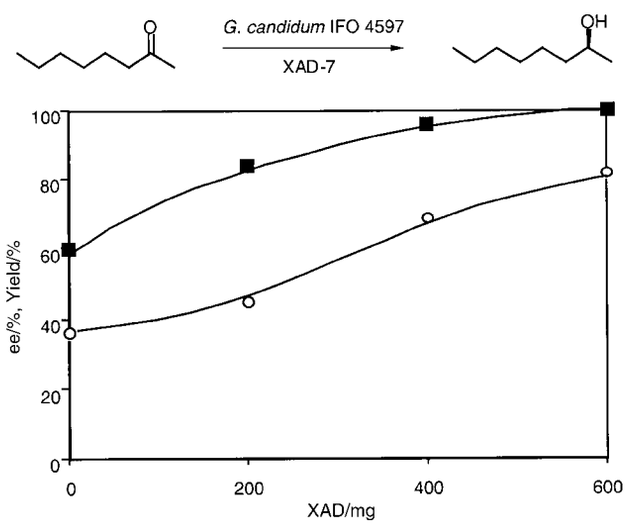


Fig. 1 Effect of the amount of XAD-7 on the stereoselectivity in the reaction of octan-2-one with *G. candidum* IFO 4597; ○ yield, ■ ee.

Reduction of aliphatic ketones

It is difficult to obtain optically pure simple aliphatic secondary alcohols such as octan-2-ol by the reduction of the corresponding ketones with chemical methods in spite of their usefulness as chiral building blocks. For example, the chemical reduction of octan-2-one with an Alpine-borohydride reagent afforded octan-2-ol in only 82% ee,[11] and the reduction with BINAL-H, a well known "super chiral reducing agent", gave octan-2-ol in 24% ee.[12] Yeast reduction of hexan-2-one gives (*S*)-hexan-2-ol in 82% ee.[13]

Fig. 1 shows the effect of XAD-7 on the stereochemical course of the reduction of octan-2-one by *G. candidum*. The reduction without XAD-7 afforded an unsatisfactory result; (*S*)-octan-2-ol was obtained in 36% chemical yield with 60% ee. However, the enantioselectivities and yields of the reduction could be improved by increasing the amount of XAD-7: the addition of 600 mg of XAD-7 gave an excellent ee (>99%) and high yield (82%). Thus, the optically active secondary alcohol was obtained enantioselectively by using XAD-7.

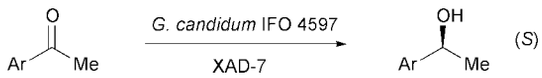
Results from the reduction of other simple aliphatic ketones are summarized in Table 2. In the reduction of alkan-2-ones, the stereochemical course was affected similarly by the addition of XAD-7 and the yield and ee of (*S*)-alcohols increased markedly. Other aliphatic ketones gave similar results; however the stereoselectivity of the reduction of butan-2-one hardly increased. The stereoselectivity of the enzymatic reductions may be worse and/or several enzymes may reduce butan-2-one. The reduction of not only alkyl methyl ketones but also alkyl ethyl ketones afforded the corresponding (*S*)-alcohols in high ee by the use of XAD-7 as illustrated by the reduction of alkan-3-ones.

Reduction of aromatic ketones

Table 3 shows the effect of XAD-7 on the reduction of acetophenone derivatives. Indeed the use of XAD-7 increased the stereoselectivity in the reductions of acetophenone and the *para*-substituted acetophenones, although the ee and yield of the products were still not satisfactory. We think that under aerobic conditions, reoxidation of the (*S*)-alcohol, which is the isomer predominantly produced by the reduction, proceeded to give ketone although that of the corresponding (*R*)-alcohol could not.[14,15] The side reactions were not observed in the reductions and the ketones and the products of the reduction were recovered with high yield.

The reduction of the *ortho*-substituted acetophenone derivative gives the corresponding alcohols in excellent ee both in the presence and absence of XAD-7 and the use of XAD-7 increases the chemical yield, keeping the excellent ee. On the other hand, the reduction of the *meta*-substituted acetophenones barely proceeded. We think that this phenomenon is the result of oxidation of the product alcohol. Previously, we have found that (*S*)-isomers of phenylethanol[14] and *meta*- and *para*-substituted 1-arylethanols are oxidized by *Geotrichum candidum* to the corresponding ketones while the *ortho*-substituted derivatives are inert to the oxidation.[10a] To inhibit oxidation, the reduction was conducted in an argon atmosphere.[16] As shown in Table 4, stereoselectivities and chemical yields of the reduction were increased regardless of the presence of XAD-7 in anaerobic conditions and the use of XAD-7 increased the stereoselectivities though the yields of the reduction changed little since high yields were observed even in the absence of XAD-7. Anyhow, acetophenone derivatives were reduced to the corresponding (*S*)-alcohol at high ee and yield in the presence of XAD-7. The reduction of acetylpyridines afforded the

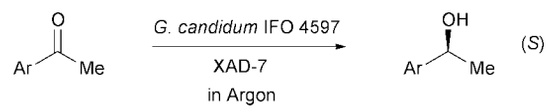
Table 3 Effect of XAD-7 on the reduction of acetophenone derivatives by *G. candidum* IFO 4597[a]



	Without XAD-7		With XAD-7		
Ar	Yield (%)	Ee (Config.) (%)	Yield (%)	Ee (Config.) (%)	XAD-7/mg
Ph	36	10 (*S*)	6	69 (*S*)	400
2-Chlorophenyl	75	>99 (*S*)	90	>99 (*S*)	200
3-Chlorophenyl	0	—	0	—	400
4-Chlorophenyl	77	18 (*R*)	48	77 (*S*)	400
3-Methylphenyl	0	—	0	—	400
4-Methylphenyl	30	87 (*S*)	8	97 (*S*)	300
4-Fluorophenyl	86	32 (*S*)	11	67 (*S*)	200

[a] The reaction conditions are described in the Experimental section.

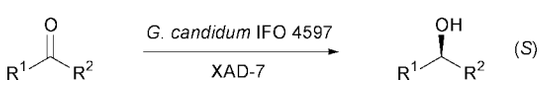
Table 4 Effect of XAD-7 on the reduction of acetophenone derivatives by *G. candidum* IFO 4597 in an argon atmosphere[a]



	Without XAD-7		With XAD-7		
Ar	Yield (%)	Ee (Config.) (%)	Yield (%)	Ee (Config.) (%)	XAD-7/mg
Ph	94	81 (*S*)	79	99 (*S*)	400
3-Chlorophenyl	78	98 (*S*)	98	>99 (*S*)	600
4-Chlorophenyl	61	38 (*S*)	51	94 (*S*)	400
3-Methylphenyl	70	98 (*S*)	92	>99 (*S*)	600
4-Methylphenyl	61	15 (*S*)	55	99 (*S*)	600
4-Fluorophenyl	99	74 (*S*)	80	97 (*S*)	200
3-Bromo-2-pyridyl	99	89 (*S*)	99	99 (*S*)	400
3-Pyridyl	99	93 (*S*)	99	99 (*S*)	600
4-Pyridyl	99	92 (*S*)	99	98 (*S*)	600

[a] The reaction conditions are described in the Experimental section. [b] The reaction proceeded in an argon atmosphere.

Table 5 Effect of XAD-7 on the reduction of aromatic ketones by *G. candidum* IFO 4597[a]



			Without XAD-7		With XAD-7		
R^1	R^2	Argon	Yield (%)	Ee (Config.) (%)	Yield (%)	Ee (Config.) (%)	XAD-7/mg
Ph	Me	No	64	86 (*S*)	90	>99 (*S*)	600
Benzyl	Me	No	50	74 (*S*)	74	>99 (*S*)	600
Phenoxymethyl	Me	No	99	3.6 (*S*)	86	99 (*S*)	600
Phenylthiomethyl	Me	No	97	51 (*S*)	81	84 (*S*)	600
Phenylthiomethyl	Me	Yes	76	68 (*S*)	82	99 (*S*)	600
Ph	Et	Yes	35	94 (*S*)	32	>99 (*S*)	600

[a] The reaction conditions are described in the Experimental section.

corresponding alcohols in high enantioselectivities (98–99%) and chemical yields (99%).

Next, other ketones which have a phenyl moiety in the vicinity of the carbonyl group were reduced. The use of XAD-7 also increased the stereoselectivities in the reduction of these substrates (Table 5).

Reduction on a preparative scale

Next, reduction on a preparative scale was attempted. Several ketones, which afforded high enantioselectivities for the reduction on a small scale, were reduced on 300 mg scale. The isolated yields and enantioselectivities of the reduction are summarized in Table 6. The isolated yield of the reduction of hexan-2-one was not good compared to the results obtained on a small scale: hexan-2-ol and heptan-2-ol are easily evaporated during the process of purification because of their relatively low boiling points. The other substrates afforded the corresponding alcohols in moderate yields with excellent ee (98 to >99%).

Use of an aqueous–organic biphasic reaction system is known to affect the stereochemistry in the reduction of α-ketoesters with bakers' yeast[17] as well as in the reduction of aromatic ketones with *Geotrichum candidum*.[10a] When a water-insoluble organic solvent is added to an enzymatic–aqueous reaction mixture, the substrate concentration in the aqueous phase decreases due to the partition of the substrate between the two phases if the substrate is more soluble in the organic phase than in the aqueous phase. Since the enzymatic reaction proceeds in the aqueous phase, where the substrate concentration is lowered by the addition of an organic phase, stereo-

Table 6 Reduction of ketones on a preparative scale[a]

Substrate	Isolated yield (%)	GC yield (%)	Ee (%)	(Config.)
Hexan-2-one	30	89	99	(*S*)
Heptan-2-one	55	84	99	(*S*)
Octan-2-one	45	82	>99	(*S*)
Nonan-2-one	70	70	>99	(*S*)
6-Methylheptan-2-one	35	52	>99	(*S*)
6-Methylhept-5-en-2-one	64	66	98	(*S*)
Acetophenone	76	79	99	(*S*)
4-Methylacetophenone	63	92	>99	(*S*)
Phenylacetone	74	90	>99	(*S*)
4-Phenylbutan-2-one	90	90	>99	(*S*)
Phenoxyacetone	65	86	>99	(*S*)
Phenylthioacetone	88	88	95	(*S*)

[a] The reaction conditions are described in the Experimental section.

chemical control of microbial reduction can be achieved when plural enzymes with different stereoselectivities and different K_m values catalyze the reaction. An enzyme with the smallest K_m value reacts preferentially in the aqueous–organic biphasic system over other enzymes with larger K_m values because of the low substrate concentrations in the aqueous phase.[17a] Thus, stereochemical control is achieved by the use of nonpolar organic solvents.

However, the yield of reduction often decreased markedly in our preliminary experiments. It is well known that organic solvents often cause serious damage to microbial cells. They may destroy the lipid layer and other structures of microbial cells, and diminish their activities of reduction. Since organic solvents are known to denature the enzyme structure, they can seldom be used in an enzymatic reaction, except for the reaction with hydrolytic enzymes such as lipases. An alternative to the organic–aqueous system is the use of a solid–aqueous biphasic system. The surface of a hydrophobic polymer can be used to concentrate the hydrophobic substrate on it and to decrease the substrate concentration in the aqueous phase. The advantage of using the polymer is that unlike organic solvents, a solid polymer does not penetrate into the organic cell membrane of a microbe or damage it. Therefore, the use of polymers in microbial reactions rather than organic solvents is strongly recommended. For example, in a highly reactive reaction such as the reduction of acetophenone with *Geotrichum candidum*, organic solvents such as hexane can be used[10a] to improve both the enantioselectivity and chemical yield, but not in the case of a poorly reactive substrate such as propiophenone (the yield decreases from 14% in an aqueous system to 2% in a hexane–aqueous system[10a]). On the contrary, the use of a solid polymer such as XAD-7 increased the enantioselectivity (from 94% ee to >99% ee) while keeping the chemical yield unchanged. Therefore, solid polymers can be used for the stereochemical control without damaging the cell activity.

Conclusion

The application of stereochemical control of microbial reduction to organic synthesis has several advantages, namely, ease of handling, cost effectiveness and wide substrate specificities. Unlike organic solvents, a solid polymer can not penetrate into the organic cell membrane of a microbe and the use of polymers in microbial reactions is recommended.[18] The two-phase reaction using a hydrophobic polymer can effectively control the stereoselectivity of microbial reduction, because the hydrophobic polymer reduces the substrate and the product concentration in the aqueous phase. The use of XAD-7 in the reduction of ketones by *G. candidum* could increase both the enantioselectivities and the chemical yield of the reduction. Furthermore, excellent enantioselectivities are achieved in the reductions of simple aliphatic ketones such as octan-2-one and several aromatic ketones. Our system is especially suitable for the synthesis of simple alkan-2-ols which are difficult to obtain by chemical methods.

Experimental

Instruments

Gas chromatographic analysis was performed using a Shimadzu GC-14A gas chromatograph equipped with a Shimadzu CR-6A Chromatopac. ^{1}H-NMR spectra were recorded on a Varian VXR-200 spectrometer in $CDCl_3$. Optical rotation was measured with a Perkin-Elmer 241 polarimeter. IR spectra were obtained from a JASCO FT/IR-5300. Elemental analyses were done with a Yanako MT-3 Elemental Analyzer.

Materials

Organic reagents were purchased from Nacalai Tesque, Inc., Kanto Chemical, Inc. or Wako Pure Chemical Industries, Ltd. unless otherwise indicated.

XAD-7 was washed successively with 1 M hydrochloric acid, 1 M aq. sodium hydroxide, water, methanol, and ether before use. The authentic samples of racemic alcohols were obtained by the reduction of the corresponding ketones with sodium borohydride.

Cultivation of *G. candidum*

Glycerol (30 g), yeast extract (10 g), polypeptone (5 g), KH_2PO_4 (11.18 g), and K_2HPO_4 (3.12 g) were dissolved in water and the volume was adjusted to 1.0 L with water. A portion of the resulting solution (30 mL) was placed in a 100 mL test tube, and the rest was placed in a 2 L Sakaguchi flask which was covered with a silicone cap and sterilized (121 °C, 20 min). The solution in the test tube was incubated with stored microbes of *G. candidum* IFO 4597 and stirred for 24 h at 30 °C and 130 rpm. The resulting mixture was transferred to the flask and stirred for 24 h at 30 °C and 130 rpm. The mixture was then filtered to obtain the cells (18 g wet weight).

General procedure for reduction of ketone by *G. candidum*

The cells (1.5 g wet wt) were added to a mixture of ketone (0.08 mmol) and various amounts of XAD-7 (0, 200, 400, and 600 mg) in water (3.0 mL), and shaken at 130 rpm for 1 day at 30 °C. The resulting mixture was put on Extrelut (Merck) and eluted with ether. The chemical yield and the ee of the product were determined by GLC analysis using undecane or pentadecane as an internal standard. The ee of the product which could not be analyzed directly was determined by GLC analysis after acetylation. The conditions and the retention time of GLC analysis are outlined below. The absolute configuration was determined by comparing the retention time with that of authentic chiral compounds.

1-Phenylethanol: CP-Cyclodextrin-B-236-M-19, 0.25 mm × 25 m, He: 2 mL min^{-1}, 100 °C, (*S*)-1-phenylethanol 15.6 min, (*R*)-phenylethanol 14.6 min.

1-(*o*-Chlorophenyl)ethanol:[19] CP-Cyclodextrin-B-236-M-19, 0.25 mm × 25 m, He: 2 mL min^{-1}, 130 °C, (*S*)-1-(*o*-chlorophenyl)ethanol 13.9 min, (*R*)-phenylethanol 13.0 min.

1-(*m*-Chlorophenyl)ethanol:[19] CP-Cyclodextrin-B-236-M-19, 0.25 mm × 25 m, He: 2 mL min^{-1}, 130 °C, (*S*)-1-(*m*-chlorophenyl)ethanol 13.9 min, (*R*)-1-(*m*-chlorophenyl)-ethanol 13.0 min.

1-(*p*-Chlorophenyl)ethanol:[19] CP-Cyclodextrin-B-236-M-19, 0.25 mm × 25 m, He: 2 mL min^{-1}, 130 °C, (*S*)-1-(*p*-chlorophenyl)ethanol 14.7 min, (*R*)-1-(*p*-chlorophenyl)ethanol 13.6 min.

1-(*m*-Methylphenyl)ethanol:[19] CP-Cyclodextrin-B-236-M-19, 0.25 mm × 25 m, He: 2 mL min^{-1}, 120 °C, (*S*)-1-(*m*-methylphenyl)ethanol 13.7 min, (*R*)-1-(*m*-methylphenyl)-ethanol 13.3 min.

1-(*p*-Methylphenyl)ethanol:[19] CP-Cyclodextrin-B-236-M-19, 0.25 mm × 25 m, He: 2 mL min^{-1}, 115 °C, (*S*)-1-(*p*-methylphenyl)ethanol 12.1 min, (*R*)-1-(*p*-methylphenyl)ethanol 11.2 min.

1-Phenylpropan-2-ol: CP-Cyclodextrin-B-236 M-19, 0.25 mm × 25 m, He: 2 mL min^{-1}, 105 °C, (*S*)-2-acetoxy-1-phenylpropane 15.8 min, (*R*)-2-acetoxy-1-phenylpropane 16.8 min.

1-Phenylbutan-3-ol: Chiraldex-G-TA, 0.25 mm × 30 m, He: 2 mL min^{-1}, 105 °C, (*S*)-1-phenylbutan-3-ol 21.4 min, (*R*)-1-phenylbutan-3-ol 22.2 min.

1-Phenoxypropan-2-ol: CP-Cyclodextrin-B-236-M-19, 0.25 mm × 25 m, He: 2 mL min^{-1}, 105 °C, (*R*)-2-acetoxy-1-phenoxypropane 13.9 min, (*S*)-2-acetoxy-1-phenoxypropane 14.4 min.

1-Phenylthiopropan-2-ol: Chiraldex-G-TA, 0.25 mm × 30 m, He: 2 mL min^{-1}, 130 °C, (*S*)-1-phenylthiopropan-2-ol 21.1 min, (*R*)-1-phenylthiopropan-2-ol 21.9 min.

Butan-2-ol: Chiraldex-G-TA, 0.25 mm × 30 m, He: 2 mL min^{-1}, 32 °C, (*S*)-butan-2-ol 3.9 min, (*R*)-butan-2-ol 4.1 min.

Pentan-2-ol: Chiraldex-G-TA, 0.25 mm × 30 m, He: 2 mL min^{-1}, 40 °C, (*S*)-pentan-2-ol 7.9 min, (*R*)-pentan-2-ol 8.5 min.

Hexan-2-ol: Chiraldex-G-TA, 0.25 mm × 30 m, He: 2 mL min^{-1}, 40 °C, (*S*)-hexan-2-ol 14.0 min, (*R*)-hexan-2-ol 16.0 min.

Heptan-2-ol: Chiraldex-G-TA, 0.25 mm × 30 m, He: 2 mL min^{-1}, 50 °C, (*S*)-heptan-2-ol 17.1 min, (*R*)-heptan-2-ol 17.8 min.

Octan-2-ol: HR-20M, 0.25 mm × 25 m, He: 2 mL min^{-1}, 100 °C, octan-2-ol 5.5 min; CP-Cyclodextrin-B-236-M-19, 0.25 mm × 25 m, He: 2 mL min^{-1}, 80 °C, (*S*)-2-acetoxyoctane 14.4 min, (*R*)-2-acetoxyoctane 17.6 min.

Nonan-2-ol: HR-20M, 0.25 mm × 25 m, He: 2 mL min^{-1}, 110 °C, nonan-2-ol 5.5 min; CP-Cyclodextrin-B-236-M-19, 0.25 mm × 25 m, He: 2 mL min^{-1}, 105 °C, (*S*)-2-acetoxynonane 12.7 min, (*R*)-2-acetoxynonane 14.0 min.

Decan-2-ol: HR-20M, 0.25 mm × 25 m, He: 2 mL min^{-1}, 130 °C, decan-2-ol 4.1 min; CP-Cyclodextrin-B-236-M-19, 0.25 mm × 25 m, He: 2 mL min^{-1}, 105 °C, (*S*)-2-acetoxydecane 12.9 min, (*R*)-2-acetoxydecane 14.1 min.

Hexan-3-ol: HR-20M, 0.25 mm × 25 m, He: 2 mL min^{-1}, 80 °C, hexan-3-ol 3.3 min; Chiraldex-G-TA, 0.25 mm × 30 m, He: 2 mL min^{-1}, 60 °C, (*S*)-3-acetoxyhexane 10.1 min, (*R*)-3-acetoxyhexane 10.7 min.

Heptan-3-ol: HR-20M, 0.25 mm × 25 m, He: 2 mL min^{-1}, 80 °C, heptan-3-ol 4.4 min; Chiraldex-G-TA, 0.25 mm × 30 m, He: 2 mL min^{-1}, 60 °C, (*S*)-3-acetoxyheptane 17.0 min, (*R*)-3-acetoxyheptane 18.3 min.

Octan-3-ol: HR-20M, 0.25 mm × 25 m, He: 2 mL min^{-1}, 80 °C, octan-3-ol 7.3 min; CP-Cyclodextrin-B-236-M-19, 0.25 mm × 25 m, He: 2 mL min^{-1}, 80 °C, (*S*)-3-acetoxyoctane 12.0 min, (*R*)-3-acetoxyoctane 13.2 min.

2-Methylhept-2-en-6-ol: Chiraldex-G-TA, 0.25 mm × 30 m, He: 2 mL min^{-1}, 70 °C, (*S*)-2-methylhept-2-en-6-ol 12.9 min, (*R*)-2-methylhept-2-en-6-ol 14.4 min.

6-Methylheptan-2-ol: Chiraldex-G-TA, 0.25 mm × 30 m, He: 2 mL min^{-1}, 50 °C, (*S*)-6-methylheptan-2-ol 26.7 min, (*R*)-6 methylheptan-2-ol 27.3 min.

4-Methylpentan-2-ol: Chiraldex-G-TA, 0.25 mm × 30 m, He: 2 mL min^{-1}, 60 °C, (*S*)-4-methylpentan-2-ol 5.8 min, (*R*)-4-methylpentan-2-ol 6.2 min.

Hex-5-en-2-ol: Chiraldex-G-TA, 0.25 mm × 30 m, He: 2 mL min^{-1}, 50 °C, (*S*)-hex-5-en-2-ol 10.0 min, (*R*)-hex-5-en-2-ol 11.0 min.

1-(4′-Pyridyl)ethanol:[20] Chirasil-DEX CB, 0.25 mm × 25 m, He: 2 mL min^{-1}, 105 °C, (*S*) 1-(4′-pyridyl)ethanol 9.8 min, (*R*)-1-(3′-pyridyl)ethanol 9.6 min.

1-(3′-Pyridyl)ethanol:[20] Chirasil-DEX CB, 0.25 mm × 25 m, He: 2 mL min^{-1}, 130 °C, (*S*) 1-(3′-pyridyl)ethanol 13.4, (*R*)-1-(3′-pyridyl)ethanol 12.6 min.

1-(3′-Bromo-2′-pyridyl)ethanol:[21] Chirasil-DEX CB, 0.25 mm × 25 m, He: 2 mL min^{-1}, 140 °C, (*S*)-1-(3′-bromo-2′-pyridyl)ethanol 11.7, (*R*)-1-(3′-bromo-2′-pyridyl)ethanol 14.0 min.

Reductions of ketones by *G. candidum* on a preparative scale

(*S*)-1-Phenylethanol. The cells (15 g wet wt) were added to a mixture of acetophenone (302 mg, 2.51 mmol) and XAD-7 (12 g) in water (90 mL), and shaken at 130 rpm for 1 day at 30 °C. The mixture was filtered, and the resin and the filtrate were extracted with ether separately. The ethereal solutions were collected, dried over anhydrous magnesium sulfate, and concentrated *in vacuo*. The residue was subjected to column chromatography (silica gel, hexane–AcOEt = 7 : 1), and the crude product was subjected to bulb-to-bulb distillation (25 mmHg, 130 °C) to afford (*S*)-1-phenylethanol (233 mg, 1.91 mmol, 76% yield, 99% ee). The ee was determined by GLC analysis, and the absolute configuration was determined by comparing its optical rotations with that reported. $[\alpha]_D$ −53.5 (*c* = 1.13, $CHCl_3$) (lit.[19] $[\alpha]_D$ +58.7 (*c* = 1.13, $CHCl_3$)), >99% ee (*R*); ^{1}H-NMR (200 MHz, $CDCl_3$) δ 1.49 (d, 3H, *J* = 6.8 Hz), 1.85 (s, 1H), 4.90 (q, 1H, *J* = 6.8 Hz), 7.25–7.37 (5H, *J* = 6.4 Hz); IR (neat) ν 3356, 3086, 3063, 3030, 2974, 2928, 2882, 1950, 1881, 1809, 1602, 1493, 1452, 1369, 1302, 1203, 1097, 1079, 1030, 1010, 997, 899, 760, 700 cm^{-1}. Anal. Calcd. for $C_8H_{10}O$: C 78.65, H 8.25, O 13.20%. Found C 78.44, H 8.41, O 13.15%.

(*S*)-1-Phenylpropan-2-ol. The cells (15 g wet wt) were added to a mixture of phenylacetone (315 mg, 2.35 mmol) and XAD-7 (18 g) in water (90 mL), and shaken at 130 rpm for 1 day at 30 °C. The mixture was filtered, and the resin and the filtrate were extracted with ether separately. The ethereal solutions were collected, dried over anhydrous magnesium sulfate, and concentrated *in vacuo*. The residue was subjected to column chromatography (silica gel, hexane–AcOEt = 8 : 1), and the crude product was subjected to bulb-to-bulb distillation (27 mmHg, 150 °C) to afford (*S*)-1-phenylpropan-2-ol (236 mg, 1.73 mmol, 74% yield, >99% ee). After a part of the 1-phenylpropan-2-ol was acetylated with acetyl chloride and triethylamine, the ee was determined by GLC analysis. The absolute configuration was determined by comparing its optical rotation with that reported. $[\alpha]_D$ +38.7 (*c* = 1.11, $CHCl_3$) (lit.[19] $[\alpha]_D$ +39.7 (*c* = 0.515, $CHCl_3$)), >99.9% ee (*S*); ^{1}H-NMR (200 MHz, $CDCl_3$) δ 0.91 (d, 3H, *J* = 6.4 Hz), 3.95 (m, 1H), 4.16 (q, 2H, *J* = 6.4 Hz), 7.18–7.35 (m, 5H); IR (neat) ν 3376, 3086, 3029, 2970, 2930, 1709, 1603, 1497, 1454, 1373, 1209, 1119, 1080, 1040, 941, 839, 743, 700 cm^{-1}. Anal. Calcd. for $C_9H_{12}O$: C 79.37, H 8.88, O 11.75%. Found C 79.15, H 9.04, O 11.81%.

(*S*)-1-(*p*-Methylphenyl)ethanol. The cells (15 g wet wt) were added to a mixture of *p*-methylacetophenone (300 mg, 2.24 mmol) and XAD-7 (18 g) in water (90 mL), and shaken at 130 rpm for 1 day at 30 °C. The mixture was filtered, and the resin

and the filtrate were extracted with ether separately. The ethereal solutions were collected, dried over anhydrous magnesium sulfate, and concentrated *in vacuo*. The residue was subjected to column chromatography (silica gel, hexane–AcOEt = 7 : 1), and the crude product was subjected to bulb-to-bulb distillation (25 mmHg, 130 °C) to afford (*S*)-1-phenylethanol (193 mg, 1.45 mmol, 63% yield, >99% ee). The ee was determined by GLC analysis, and the absolute configuration was determined by comparing its optical rotations with that reported. $[\alpha]_D$ +56.8 (c = 1.00, $CHCl_3$) (lit.[19] $[\alpha]_D$ +57.3 (c = 0.190, $CHCl_3$)), >99.9% ee (*S*);^{1}H-NMR (200 MHz, $CDCl_3$) δ 1.41 (d, 3H, J = 6.4 Hz), 1.78 (s, 1H), 2.27 (s, 3H), 4.88 (q, 1H, J = 6.4 Hz), 7.09 (d, 2H, J = 8.4 Hz), 7.20 (d, 2H, J = 8.4 Hz); IR (neat) ν 3358, 3022, 2972, 2926, 2870, 1514, 1450, 1369, 1304, 1202, 1180, 1117, 1088, 1011, 941, 899, 818, 729 cm^{-1}.

(*S*)-1-Phenylbutan-3-ol. The cells (15 g wet wt) were added to a mixture of 1-phenylbutan-3-one (301 mg, 2.03 mmol) and XAD-7 (18 g) in water (90 mL), and shaken at 130 rpm for 1 day at 30 °C. The mixture was filtered, and the resin and the filtrate were extracted with ether separately. The ethereal solutions were collected, dried over anhydrous magnesium sulfate, and concentrated *in vacuo*. The residue was subjected to column chromatography (silica gel, hexane–AcOEt = 10 : 1), and the crude product was subjected to bulb-to-bulb distillation (23 mmHg, 150 °C) to afford (*S*)-1-phenylbutan-3-ol (273 mg, 1.82 mmol, 90% yield, >99% ee). The ee was determined by GLC analysis. The absolute configuration was determined by comparing its optical rotation with that reported. $[\alpha]_D$ +21.1 (c = 1.00, C_6H_6) (lit.[19] $[\alpha]_D$ +22 (c = 0.38, C_6H_6)), >99.9% ee (*S*); ^{1}H-NMR (200 MHz, $CDCl_3$) δ 1.24 (d, 3H, J = 6.2 Hz), 1.70 (s, 1H), 1.77 (m, 2H), 2.69 (t, 2H, J = 8.2 Hz), 3.83 (m, 1H), 7.22 (m, 5H); IR (neat) ν 3358, 3086, 3063, 3027, 2967, 2928, 2863, 1603, 1495, 1454, 1375, 1312, 1181, 1128, 1082, 1055, 1030, 955, 936, 909, 856, 747, 698 cm^{-1}. Anal. Calcd. for $C_{10}H_{14}O$: C 79.96, H 9.39, O 10.65%. Found C 79.94, H 9.43, O 10.63%.

(*S*)-1-Phenoxypropan-2-ol. The cells (15 g wet wt) were added to a mixture of phenoxyacetone (310 mg, 2.06 mmol) and XAD-7 (18 g) in water (90 mL), and shaken at 130 rpm for 1 day at 30 °C. The mixture was filtered, and the resin and the filtrate were extracted with ether separately. The ethereal solutions were collected, dried over anhydrous magnesium sulfate, and concentrated *in vacuo*. The residue was subjected to column chromatography (silica gel, hexane–AcOEt = 6 : 1), and the crude product was subjected to bulb-to-bulb distillation (25 mmHg, 180 °C) to afford (*S*)-1-phenoxypropan-2-ol (205 mg, 1.35 mmol, 65% yield, 99% ee). After a part of the 1-phenoxypropan-2-ol was acetylated with acetyl chloride and triethylamine, the ee was determined by GLC analysis. The absolute configuration was determined by comparing its optical rotation with that reported. $[\alpha]_D$ −3.6 (c = 0.58, EtOH) (lit.[22] $[\alpha]_D$ −2.7 (c = 1.80, EtOH)), >99% ee (*S*); ^{1}H-NMR (200 MHz, $CDCl_3$) δ 1.28 (d, 3H, J = 6.8 Hz), 2.47 (s, 1H), 3.79 (m, 1H), 3.94 (m, 1H), 4.19 (m, 1H), 7.29 (5H, m); IR (neat) ν 3040, 2975, 2930, 1711, 1599, 1497, 1458, 1385, 1331, 1292, 1246, 1173, 1152, 1078, 1043, 1001, 936, 864, 812, 754, 692 cm^{-1}. Anal. Calcd. for $C_9H_{12}O_2$: C 71.03, H 7.95, O 21.02%. Found C 70.81, H 8.06, O 21.13%.

(*S*)-1-Phenylthiopropan-2-ol. The cells (15 g wet wt) were added to a mixture of phenylthioacetone (300 mg, 1.81 mmol) and XAD-7 (18 g) in water (90 mL), and shaken at 130 rpm for 1 day at 30 °C. The mixture was filtered, and the resin and the filtrate were extracted with ether separately. The ethereal solutions were collected, dried over anhydrous magnesium sulfate, and concentrated *in vacuo*. The residue was subjected to column chromatography (silica gel, hexane–AcOEt = 6 : 1), and the crude product was subjected to bulb-to-bulb distillation (25 mmHg, 180 °C) to afford (*S*)-1-phenylthiopropan-2-ol (266 mg, 1.58 mmol, 88% yield, 99% ee). The absolute configuration was determined by comparing its optical rotation with that reported. $[\alpha]_D$ +8.8 (c = 1.02, MeOH) (lit.[23] $[\alpha]_D$ +8.20 (MeOH)), 94% ee (*S*); ^{1}H-NMR (200 MHz, $CDCl_3$) δ 1.26 (d, 3H, J = 6.8 Hz), 2.51 (s, 1H), 2.84 (dd, 1H, J = 13.6, 8.4 Hz), 3.10 (dd, 1H, J = 13.6, 3.6 Hz), 3.85 (m, 1H), 7.31 (5H, m); IR (neat) ν 3383, 3059, 2970, 2924, 1584, 1480, 1439, 1373, 1302, 1267, 1240, 1192, 1125, 1071, 1038, 1026, 936, 876, 818, 741, 691 cm^{-1}.

(*S*)-Hexan-2-ol. The cells (15 g wet wt) were added to a mixture of hexan-2-one (314 mg, 3.14 mmol) and XAD-7 (18 g) in water (90 mL), and shaken at 130 rpm for 1 day at 30 °C. The mixture was filtered, and the resin and the filtrate were extracted with ether separately. The ethereal solutions were collected, dried over anhydrous magnesium sulfate, and concentrated *in vacuo*. The residue was subjected to column chromatography (silica gel, hexane–AcOEt = 8 : 1) to afford (*S*)-hexan-2-ol (94 mg, 0.93 mmol, 30% yield, 99% ee). The ee was determined by GLC analysis. The absolute configuration was determined by comparing its optical rotation with that reported. $[\alpha]_D$ +11.3 (c = 0.58, EtOH) (lit.[5] $[\alpha]_D$ +10.39 ($CHCl_3$)), 96% ee (*S*); ^{1}H-NMR (200 MHz, $CDCl_3$) δ 0.87 (m, 3H), 1.17 (d, 3H, J = 6.6 Hz), 1.20–1.70 (m, 7H), 3.79 (m, 1H); IR (neat) ν 3358, 2963, 2932, 2863, 1462, 1375, 1327, 1146, 1113, 1051, 1017, 941 cm^{-1}.

(*S*)-Heptan-2-ol. The cells (15 g wet wt) were added to a mixture of heptan-2-one (300 mg, 2.63 mmol) and XAD-7 (18 g) in water (90 mL), and shaken at 130 rpm for 1 day at 30 °C. The mixture was filtered, and the resin and the filtrate were extracted with ether separately. The ethereal solutions were collected, dried over anhydrous magnesium sulfate, and concentrated *in vacuo*. The residue was subjected to column chromatography (silica gel, hexane–AcOEt = 10 : 1), and the crude product was subjected to bulb-to-bulb distillation (25 mmHg, 150 °C) to afford (*S*)-heptan-2-ol (169 mg, 1.48 mmol, 55% yield, 99% ee). The ee was determined by GLC analysis. The absolute configuration was determined by comparing its optical rotation with that reported. $[\alpha]_D$ +9.0 (c = 0.83, $CHCl_3$) (lit.[5] $[\alpha]_D$ +10.21 ($CHCl_3$)), 99% ee (*S*); ^{1}H-NMR (200 MHz, $CDCl_3$) δ 0.87 (m, 3H), 1.17 (d, 3H, J = 6.6 Hz), 1.20–1.55 (m, 8H), 1.67 (s, 1H), 3.77 (m, 1H); IR (neat) ν 3356, 2961, 2930, 2860, 1462, 1375, 1310, 1261, 1200, 1113, 1061, 1030, 951, 908, 806 cm^{-1}.

(*S*)-Octan-2-ol. The cells (15 g wet wt) were added to a mixture of octan-2-one (310 mg, 2.50 mmol) and XAD-7 (18 g) in water (90 mL), and shaken at 130 rpm for 1 day at 30 °C. The mixture was filtered, and the resin and the filtrate were extracted with ether separately. The ethereal solutions were collected, dried over anhydrous magnesium sulfate, and concentrated *in vacuo*. The residue was subjected to column chromatography (silica gel, hexane–AcOEt = 8 : 1), and the crude product was subjected to bulb-to-bulb distillation (27 mmHg, 150 °C) to afford (*S*)-octan-2-ol (145 mg, 115 mmol, 45% yield, 99% ee). After a part of the octan-2-ol was acetylated with acetyl chloride and triethylamine, the ee was determined by GLC analysis, and the absolute configuration was determined by comparing its optical rotations with that reported. $[\alpha]_D$ +8.5 (c = 0.86, $CHCl_3$) (lit.[5] $[\alpha]_D$ +8.78 ($CHCl_3$)), 97% ee (*S*); ^{1}H-NMR (200 MHz, $CDCl_3$) δ 0.87 (m, 3H), 1.17 (d, 3H, J = 6.2 Hz), 1.20–1.55 (m, 10H), 1.76 (s, 1H), 3.77 (m, 1H); IR (neat) ν 3364, 2961, 2930, 2859, 1460, 1375, 1262, 1215, 1111, 1067, 802, 760 cm^{-1}.

(*S*)-Nonan-2-ol. The cells (15 g wet wt) were added to a mixture of nonan-2-one (316 mg, 2.22 mmol) and XAD-7 (18 g) in water (90 mL), and shaken at 130 rpm for 1 day at 30 °C.

The mixture was filtered, and the resin and the filtrate were extracted with ether separately. The ethereal solutions were collected, dried over anhydrous magnesium sulfate, and concentrated *in vacuo*. The residue was subjected to column chromatography (silica gel, hexane–AcOEt = 10 : 1), and the crude product was subjected to bulb-to-bulb distillation (23 mmHg, 150 °C) to afford (*S*)-nonan-2-ol (211 mg, 1.46 mmol, 66% yield, >99% ee). After a part of the nonan-2-ol was acetylated with acetyl chloride and triethylamine, the ee was determined by GLC analysis, and the absolute configuration was determined by comparing its optical rotations with that reported. $[a]_D$ +7.9 (c = 0.86, $CHCl_3$) (lit.[5] $[a]_D$ +7.96 ($CHCl_3$)), 97% ee (*S*); ^{1}H-NMR (200 MHz, $CDCl_3$) δ 0.87 (m, 3H), 1.17 (d, 3H, J = 6.2 Hz), 1.21–1.55 (m, 12 H), 1.84 (s, 1H), 3.77 (m, 1H); IR (neat) ν 3353, 2961, 2880, 2857, 1464, 1375, 1306, 1211, 1115, 1076, 930, 849 cm^{-1}.

(*S*)-2-Methylhept-2-en-6-ol. The cells (15 g wet wt) were added to a mixture of 2-methylhept-2-en-6-one (308 mg, 2.44 mmol) and XAD-7 (18 g) in water (90 mL), and shaken at 130 rpm for 1 day at 30 °C. The mixture was filtered, and the resin and the filtrate were extracted with ether separately. The ethereal solutions were collected, dried over anhydrous magnesium sulfate, and concentrated *in vacuo*. The residue was subjected to column chromatography (silica gel, hexane–AcOEt = 10 : 1), and the crude product was subjected to bulb-to-bulb distillation (25 mmHg, 150 °C) to afford (*S*)-2-methylhept-2-en-6-ol (200 mg, 1.58 mmol, 65% yield, 98% ee). The ee was determined by GLC analysis, and the absolute configuration was determined by comparing its optical rotations with that reported. $[a]_D$ +11.2 (c = 0.84, $CHCl_3$) (lit.[5] $[a]_D$ +10.76 ($CHCl_3$)), 99% ee (*S*); ^{1}H-NMR (200 MHz, CDCl3) δ 1.17 (d, 3H, J = 6.2 Hz), 1.45 (m, 2H), 1.60 (s, 3H), 1.67 (d, 3H, J = 1.2 Hz), 1.70 (m, 1H), 2.05 (m, 2H), 3.78 (m, 1H), 5.11 (m, 1H); IR (neat) ν 3358, 2969, 2926, 2361, 1451, 1377, 1173, 1127, 1074, 1061, 990, 953, 907, 856, 826 cm^{-1}. Anal. Calcd. for $C_8H_{16}O$ C 74.94, H 12.58, O: 12.48%. Found C 74.94, H 12.58, O 12.68%.

(*S*)-6-Methylheptan-2-ol. The cells (15 g wet wt) were added to a mixture of 6-methylheptan-2-one (310 mg, 2.42 mmol) and XAD-7 (18 g) in water (90 mL), and shaken at 130 rpm for 1 day at 30 °C. The mixture was filtered, and the resin and the filtrate were extracted with ether separately. The ethereal solutions were collected, dried over anhydrous magnesium sulfate, and concentrated *in vacuo*. The residue was subjected to column chromatography (silica gel, hexane–AcOEt = 10 : 1), and the crude product was subjected to bulb-to-bulb distillation (25 mmHg, 150 °C) to afford (*S*)-6-methylheptan-2-ol (109 mg, 0.837 mmol, 35% yield, >99% ee). The ee was determined by GLC analysis. The absolute configuration was determined by comparing its retention time with that of authentic sample which was obtained by palladium-black mediated hydrogenation of (*S*)-2-methylhept-2-en-6-ol. $[a]_D$ +8.7 (c = 0.72, $CHCl_3$); ^{1}H-NMR (200 MHz, $CDCl_3$) δ 0.86 (d, 6H, J = 6.6 Hz), 1.11–1.60 (m, 9H), 3.78 (m, 1H); IR (neat) ν 3345, 2957, 2932, 2870, 1466, 1367, 1337, 1202, 1148, 1117, 1017, 972, 931, 856 cm^{-1}.

Hydrogenation of (*S*)-2-methylhept-2-en-6-ol

Palladium-black (10 mg) was added to a solution of (*S*)-2-methylhept-2-en-6-ol (0.16 mmol, 98% ee) in acetic acid (1 mL), and the resulting mixture was stirred for 6 h at room temperature under a hydrogen atmosphere at atmospheric pressure. The mixture was put on to silica gel, and eluted with ether. The yield and ee of the product were determined by GLC analysis; (*S*)-6-methylheptan-2-ol (87% yield, 98% ee) was assigned as the first fraction (26.7 min) by comparing its retention time with that of a racemic sample. The absolute configuration of hex-5-en-2-ol was determined by the same method.

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