Stereoselective oxidation and reduction by immobilized *Geotrichum candidum* in an organic solvent

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Cells of the fungus, *Geotrichum candidum*, were immobilized on a water-absorbing polymer and used for stereoselective oxidation and reduction in an organic solvent using cyclohexanone, cyclopentanol or alkan-2-ols as additive. Enantiomerically pure (*R*)-1-arylethanols were obtained by the stereoselective oxidation of racemic 1-arylethanols, whereas enantiomerically pure (*S*)-1-arylethanols were obtained by the reduction of the corresponding ketones, in contrast to reduction in water by the free cells in which (*R*)- or (*S*)-1-arylethanols were produced in low ee.

The reaction mechanism was investigated by measuring the partition of the substrates and products between the organic phase and aqueous phase in the polymer around which the cells were immobilized. Deuterated compounds were used to determine the role of the additives.

Introduction

The growing interest in asymmetric synthesis has promoted a great development in biotransformations in organic synthesis and they have been used widely for the syntheses of chiral compounds.[1] The selectivity of reactions is usually high for enzymatic reactions and for microbial reactions when the microbe contains only the necessary enzyme(s). However, when there are many enzymes in a microbe which transform a substrate to products of different configurations, microbial transformation does not afford a product of satisfactory optical purity, and a new method for controlling the enantioselectivity of microbial reactions is awaited. To date, several methods by which to improve the selectivity of microbial reactions such as screening of microorganisms,[2] modification of substrates,[3] and modification of the reaction conditions[4] have been developed. Among these, the last is the most desirable since one can obtain alcohols of excellent optical purity without modifying the kind of microorganisms or substrates by controlling the activities of desired and undesired enzymes. It would be convenient if the reaction conditions could be modified to simplify the experimental manipulation and to make the system suitable for large scale productions, while concurrently controlling the stereoselectivity. For this purpose, the use of organic solvents[5] and immobilization of the microorganism are attractive approaches.

Dry or immobilized bakers' yeast reduction in an organic solvent has been developed. The reduction of various α-keto esters with dry bakers' yeast in benzene[6] or immobilized yeast in polyurethane in hexane[7] affords the corresponding (*R*)-α-hydroxy esters in high enantiomeric excess (ee), whereas that in water gives the (*S*)-α-hydroxy esters. Similarly, β-keto esters are reduced by dry bakers' yeast in petroleum ether to the corresponding (*S*)-β-hydroxy esters.[8] Thus, stereochemical control by the use of organic solvents and/or immobilization in the bakers' yeast reduction have been achieved.

We have found that *Geotrichum candidum* IFO 4597, a fungus for which the cultivation is very simple, can reduce various kinds of ketones with acceptable rates, although the stereoselectivity is not high. Here we report a new system which improves the reactivity and stereoselectivity of *G. candidum*-catalyzed transformations. The system consists of an organic solvent, immobilized microbial cells, and additives such as cyclohexanone, cyclopentanol and alkan-2-ols.[5f-i] The present system increased the reactivity in the oxidation of racemic 1-arylethanols, affording enantiomerically pure (*R*)-1-arylethanols. In the reduction of aryl methyl ketones, this system produced (*S*)-1-arylethanol in excellent ee, in contrast to the reduction in water in which the (*R*)- or (*S*)-1-arylethanol is produced in low ee. The mechanism of improvement in reactivity and enantioselectivity by the use of the system was investigated.

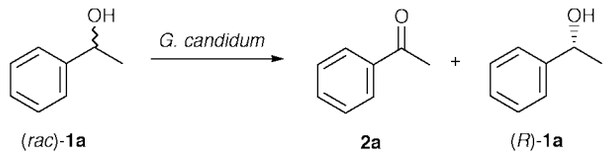
Results and discussion

Oxidation

When racemic 1-phenylethanol (*R*/*S*)-**1a** was incubated with resting free cells of *G. candidum* in water for 24 h, (*S*)-**1a** was oxidized selectively to give the corresponding ketone (**2a**, 12%) leaving (*R*)-**1a** of 17% ee (Table 1, entry 1). Although the oxidation of (*S*)-**1a** but not (*R*)-**1a** took place, the reactivity of (*S*)-**1a** was not strong, so that some (*S*)-**1a** remained after 24 h, which lowered the ee of remaining (*R*)-**1a**. When the reaction was conducted in a biphasic system of water and hexane, the yield increased, giving (*R*)-**1a** of 43% ee (Table 1, entry 2), and when the cell was immobilized on a water-absorbing polymer, the oxidation in hexane proceeded more readily giving (*R*)-**1a** of 86% ee (Table 1, entry 3). To further improve the system, an additive was used. When cyclohexanone (2.5 equivalents with respect to the substrate) was added, (*R*)-**1a** of 99% ee was obtained (Table 1, entry 4). The ee enhancement of the remaining alcohol is a reflection of the degree of conversion because (*S*)-alcohol is selectively oxidized and when all of the (*S*)-alcohol is converted to ketone, only (*R*)-alcohol which does not oxidize at all remains in the reaction mixture.

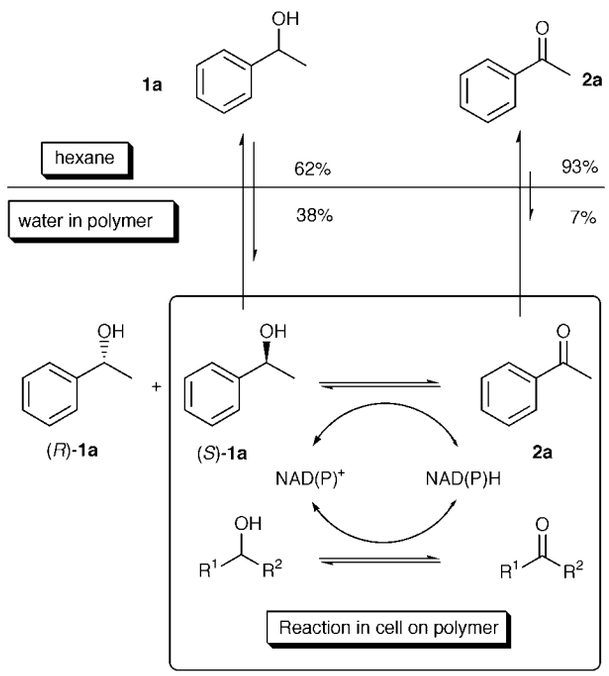
An improvement in the ee of the remaining alcohol (*R*)-**1a** was observed when hexane was employed, the cells were immobilized, and cyclohexanone was added. The improvement by the use of hexane is explained by the partition of the substrates and products between the organic phase and aqueous phase. The solubility of ketones in the aqueous layer is generally much lower than that of alcohols, and as shown in Scheme 1, the substrate-alcohol (*R*/*S*)-**1a** dissolves more easily in the aqueous phase than ketone **2a**: the local concentration of (*R*/*S*)-**1a** around the cells is higher than that of **2a**, which shifts the equilibrium between the oxidation and reduction in the direction more favorable for the oxidation of the alcohol (*R*/*S*)-**1a**. (The process is reversible as for (*S*)-**1a**; the reduction of ketones to (*S*)-**1a** is observed as detailed in the Reduction

Table 1 Oxidation of **1a** with *G. candidum*



Entry	Catalyst	Hexane[a]	Cyclohexanone[b]	Yield[c] (%)	ee of **1a** (%)
1	Free cells in water	—	—	12	17 (*R*)
2	Free cells in water	+	—	29	43 (*R*)
3	Immobilized cells[d]	+	—	44	86 (*R*)
4	Immobilized cells[d]	+	+	51	99 (*R*)

Reaction conditions are described in the Experimental section. [a] 6 mL. [b] 0.2 mmol. [c] Yield of **2a**. [d] The cell was immobilized on water–absorbing polymer (BL-100®).



Scheme 1

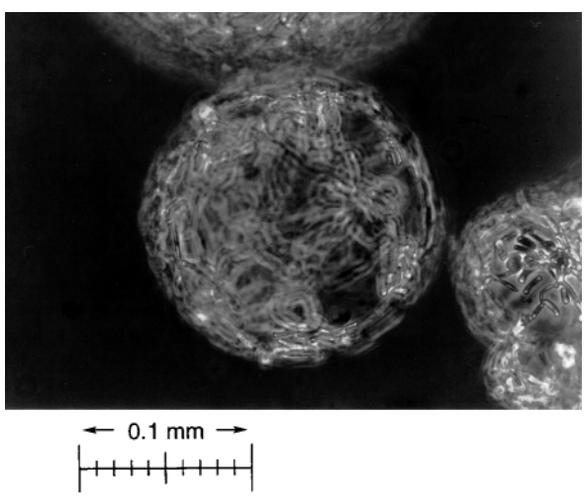


Fig. 1 Picture of immobilized cells on the water-absorbing polymer. The average diameter of the polymer after the absorption of water for immobilization is 0.15 ± 0.05 mm and the number of cells per polymer particle is 50–100.

section.) Organic solvents more polar than hexane are inappropriate because the local concentration of not only **2a** but also (*R*/*S*)-**1a** around the cell is too low for the reaction to occur.

An improvement in the oxidation reactivity was also observed when the cells were immobilized on a water-absorbing polymer. Immobilization was accomplished by adding the water-absorbing polymer (BL-100®) to a suspension of the cells in water. Since the water is absorbed by the polymer instantly and the cells are adsorbed on the polymer, the cells are spread over a large polymer surface (Fig. 1), which increases the surface area between the cells and hexane phase, and results in improvement in the reactivity.

In addition, immobilization of the cells simplifies the workup procedure; the reaction can be quenched easily by filtration of the immobilized cells which is much more straightforward than that of the free cells.

Addition of cyclohexanone also gave an improvement in the reactivity. Among carbonyl compounds such as butyraldehyde, isobutyraldehyde, octanol, benzaldehyde, chloroacetone, 4-methylpentan-2-one, octan-2-one, cyclobutanone, cyclopentanone, cyclohexanone and 4-methylcyclohexanone tested for their ability to promote oxidation, cyclohexanone and chloroacetone were most effective (data not shown). The ee of the remaining alcohol increases according to the amount of cyclohexanone added and at least a 1.2 mol equivalent of cyclohexanone with respect to the substrate is necessary to obtain >99% ee.

Various substrates were applied to the oxidation. The oxidation of other 1-arylethanols is also enhanced in the reaction system with the immobilized cells on the water-absorbing polymer in hexane in the presence of cyclohexanone as shown in Table 2. *meta*- and *para*-Substituted 1-arylethanols are smoothly oxidized to leave (*R*)-1-arylethanol in high ee, however, *ortho*-substituted alcohols are inert to the oxidation. *o*,*p*-Dichlorophenylethanol **1j** is not oxidized at all, even though it is *para*-substituted, due to the *ortho*-substitution. 1-Phenylpropan-2-ol (**1k**) and 4-phenylbutan-2-ol (**1l**) are oxidized smoothly, although 1-phenylpropan-1-ol (**1m**) and 1-phenylbutan-1-ol (**1n**) are hardly oxidized, which means that the small substituent adjacent to the alcohol group must be a methyl.

Reduction

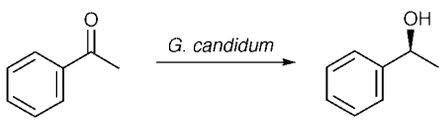
G. candidum also catalyzes the reduction of ketones as well as the oxidation of alcohols, although the reduction of **2a** by the free cells in water afforded only (*R*)-**1a** with 28% ee in 52% yield (Table 3, entry 1). The reduction in hexane by the immobilized cells barely proceeded (Table 3, entry 2), contrary to the oxidation because of the unfavorable partition of **2a** and **1a** between the organic and aqueous phases for the reduction. However, when an alcohol such as cyclopentanol or hexan-2-ol was added to the reaction system, the reduction proceeded smoothly to afford (*S*)-**1a** in excellent ee (Table 3, entry 3, 4). Cyclopentanol or hexan-2-ol worked when they were used in large excess. The yield increased as the amount of cyclopentanol was increased and peaked at 6 mol equivalents with respect to the substrate, whereas alkan-2-ols such as propan-2-ol, butan-2-ol, pentan-2-ol, hexan-2-ol, heptan-2-ol and octan-2-ol exerted saturation on the yield at 10 to 15 mol equivalents.

Table 2 Oxidation of various alcohols with the immobilized cell

Entry	Substrate	Free cells in water		Immobilized cells in hexane with cyclohexanone	
		Yield[a] (%)	Ee[b] (%)	Yield[a] (%)	Ee[b] (%)
1	1-Phenylethanol (**1a**)	12	17 (*R*)	51	99 (*R*)
2	1-(2-Furyl)ethanol (**1b**)	11	23 (*R*)	50	96 (*R*)
3	1-(*o*-Chlorophenyl)ethanol (**1c**)	1	—	13	14 (*R*)
4	1-(*m*-Chlorophenyl)ethanol (**1d**)	8	5 (*R*)	52	97 (*R*)
5	1-(*p*-Chlorophenyl)ethanol (**1e**)	11	20 (*R*)	46	74 (*R*)
6	1-(*o*-Methylphenyl)ethanol (**1f**)	7	6 (*R*)	28	37 (*R*)
7	1-(*m*-Methylphenyl)ethanol (**1g**)	32	40 (*R*)	50	>99 (*R*)
8	1-(*p*-Methylphenyl)ethanol (**1h**)	38	71 (*R*)	50	97 (*R*)
9	1-(*p*-Methoxyphenyl)ethanol (**1i**)	41	75 (*R*)	50	99 (*R*)
10	1-(*o*,*p*-Dichlorophenyl)ethanol (**1j**)	0	—	1	—
11	1-Phenylpropan-2-ol (**1k**)	35	57 (*R*)	44	76 (*R*)
12	4-Phenylbutan-2-ol (**1l**)	48	60 (*R*)	50	97 (*R*)
13	1-Phenylpropan-1-ol (**1m**)	28	41 (*R*)	16	17 (*R*)
14	1-Phenylbutan-1-ol (**1n**)	2	—	0	—

Reaction conditions are described in the Experimental section. [a] Yield of ketones. [b] Ee of the remaining substrate. Preparative scale results: (*R*)-**1a**: ee 98%, $[a]_D$ +53.0 (*c* 0.60, $CHCl_3$) (lit.[13] $[a]_D^{25}$ −57 (*c* 5.12, $CHCl_3$) (*S*)). (*R*)-**1k**: ee 69%, $[a]_D$ −24.4 (*c* 0.97, $CHCl_3$) (lit.[12] $[a]_D^{14}$ +39.7 (*c* 0.515, $CHCl_3$), >99.9% ee (*S*)).

Table 3 Reduction of **2a** with *G. candidum*



2a (*S*)-**1a**

Entry	Catalyst	Hexane[c]	Additive	Yield (%)	Ee (%)
1	Free cells in water	—	—	52	28 (*R*)
2	Immobilized cells[a]	+	—	2	—
3	Immobilized cells[a]	+	Cyclopentanol[d]	58	>99 (*S*)
4	Immobilized cells[a]	+	Hexan-2-ol[e]	73	>99 (*S*)
5	Immobilized cells[b]	+	Hexan-2-ol[e]	38	98 (*S*)

Reaction conditions are described in the Experimental section. [a] The cells were immobilized on water absorbing polymer (BL-100®). [b] The cells were immobilized in calcium alginate. [c] 6 mL. [d] 0.5 mmol. [e] 1.0 mmol.

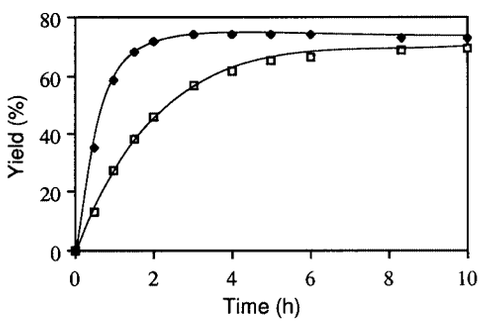


Fig. 2 Effect of immobilization on the reduction rate of **2a**; ◆ with immobilization of the cells, □ without immobilization of the cells.

The present immobilization method is more effective than methods employing calcium alginate,[9] a commonly used immobilizing material, the reduction of **2a** by the immobilized cell in calcium alginate proceeded with low yield (38%) and 98% ee (Table 3, entry 5).

Fig. 2 shows the time-course of the reduction in hexane. Obviously, the reduction by the immobilized cells proceeded more rapidly than that without immobilization. The reduction with the immobilized cells is complete within 3 h. The initial rate of reduction with the immobilized cells is calculated to be 28 mM h^{-1} per 1 g of cells.

Organic solvents other than hexane were also tested for in the reduction by the immobilized cells with cyclopentanol and the yield and ee were the highest with hexane the same as for the oxidation.

Aryl methyl ketones, **2b–2j**, were also reduced smoothly by the same system using 12 equivalents of hexan-2-ol to give the corresponding (*S*)-alcohols in excellent ee (Table 4). The reduction of *ortho*-substituted acetophenones gives relatively better yields than that of *para*-substituted acetophenones probably because the reverse reaction, oxidation, does not proceed for the *ortho*-substituted substrates. The better a substrate is for the stereoselective oxidation, the worse it is for the reduction as for the substituted acetophenone derivatives. However, a different trend was observed for other substrates. Changing the phenyl group in **2a** to a benzyl (**2k**) or 2-phenylethyl (**2l**) group does not interfere with the reduction, whereas the reaction is inhibited by substitution of the methyl group in **1a** with an ethyl or propyl group (Table 4, entries 11–14); which is the same trend as for the oxidation. A methyl group but not an ethyl or a propyl group is suitable as the smaller group adjacent to the keto group for both the reduction and the oxidation.

In the reduction of **2a** by immobilized cells in hexane, alkan-2-ols such as hexan-2-ol and octan-2-ol *etc.* and cyclopentanol are effective additives. To clarify which stereoisomers of alkan-2-ol to use as a reductant, optically active octan-2-ol was prepared and used as an additive. As shown in Table 5, (*S*)-octan-2-ol was more effective than the (*R*)-isomer. Although the effect of (*R*)-octan-2-ol as an additive was small, addition of (*R*)-octan-2-ol undoubtedly increased the yield up to about 9% from 1%. To investigate the role of (*R*)-octan-2-ol, the

Table 4 Reduction of various ketones with immobilized cells

Entry	Substrate	Free cells in water		Immobilized cells in hexane with hexan-2-ol	
		Yield (%)	Ee (%)	Yield (%)	Ee (%)
1	Acetophenone (**2a**)	52	28 (*R*)	73	>99 (*S*)
2	2-Acetylfuran (**2b**)	6	1 (*R*)	81	99 (*S*)
3	*o*-Chloroacetophenone (**2c**)	46	>99 (*R*)	99	99 (*S*)
4	*m*-Chloroacetophenone (**2d**)	22	90 (*R*)	88	>99 (*S*)
5	*p*-Chloroacetophenone (**2e**)	49	46 (*R*)	41	92 (*S*)
6	*o*-Methylacetophenone (**2f**)	4	—	59	>99 (*S*)
7	*m*-Methylacetophenone (**2g**)	50	93 (*R*)	56	>99 (*S*)
8	*p*-Methylacetophenone (**2h**)	10	19 (*R*)	40	>99 (*S*)
9	*p*-Methoxyacetophenone (**2i**)	14	46 (*R*)	12	>99 (*S*)
10	*o*,*p*-Dichloroacetophenone (**2j**)	37	>99 (*S*)	83	>99 (*S*)
11	1-Phenylpropan-2-one (**2k**)	47	88 (*S*)	97	>99 (*S*)
12	Benzylacetone (**2l**)	31	60 (*S*)	88	>99 (*S*)
13	Propiophenone (**2m**)	14	19 (*S*)	2	—
14	Butyrophenone (**2n**)	7	>99	<1	—

The absolute configuration of the products was determined by comparison of the sign of the optical rotation with that reported previously or by comparison of the GC retention times with those of authentic samples. (*S*)-**1b**: Isolated yield 63%, ee >99%, $[\alpha]_D$ −54.7 (*c* 1.05, $CHCl_3$) (lit.[13] $[\alpha]_D^{25}$ −57 (*c* 5.12, $CHCl_3$), (*S*)). **1b**: Product decomposed during purification. (*S*)- **1c**: Isolated yield 88%, ee >99%, $[\alpha]_D$ −62.8 (*c* 1.07, $CHCl_3$) (lit.[10] $[\alpha]_D$ −56.5 (*c* 0.0463, $CHCl_3$) 90% ee (*S*)). (*S*)-**1d**: Isolated yield 75%, ee >99%, $[\alpha]_D$ −40.5 (*c* 1.19, $CHCl_3$) (lit.[11] $[\alpha]_D^{20}$ +36.7 (*c* 1.0, $CHCl_3$) 84.6% ee (*R*)). (*S*)-**1e**: Isolated yield 32%, ee 92%, $[\alpha]_D$ −65.7 (*c* 1.15, $CHCl_3$). (*S*)-**1f**: Isolated yield 25%, ee >99%, $[\alpha]_D$ −58.9 (*c* 1.01, EtOH) (lit.[10] $[\alpha]_D$ −58.6 (*c* 0.0665, EtOH) 95% ee (*S*)). (*S*)-**1g**: Isolated yield 43%, ee 99% $[\alpha]_D$ −39.4 (*c* 1.05, EtOH) (lit.[12] $[\alpha]_D^{14}$ −41.9 (*c* 0.500, EtOH), >99.9%, ee (*S*)). (*S*)-**1h**: Isolated yield 21%, ee 99%, $[\alpha]_D$ −54.4 (*c* 1.02, $CHCl_3$) (lit.[14] $[\alpha]_D$ +39 (*c* 1, $CHCl_3$), 76% ee (*R*)). (*S*)-**1i**: Isolated yield 10%, ee >99%, $[\alpha]_D$ −23.5 (*c* 1.00, $CHCl_3$) (lit.[10] $[\alpha]_D$ −46.2 (*c* 0.0273, $CHCl_3$) 87% ee (*S*)). (*S*)-**1j**: Isolated yield 71%, ee >99%, $[\alpha]_D$ −58.7 (*c* 1.03, $CHCl_3$); absolute configuration was determined after dehalogenation of the product to **1a**, $[\alpha]_D$ −48.7 (*c* 1.13, $CHCl_3$). (*S*)-**1k**: Isolated yield 65%, ee >99%, $[\alpha]_D$ +42.6 (*c* 1.01, $CHCl_3$) (lit.[12] $[\alpha]_D^{14}$ +39.7 (*c* 0.515, $CHCl_3$), >99.9% ee (*S*)). (*S*)-**1l**: Isolated yield 71%, ee 98%, $[\alpha]_D$ +17.4 (*c* 1.80, $CHCl_3$).

Table 5 Effect of chirality of additives on the reduction of **2a** with immobilized cells in hexane

Additive	Yield (%)	Ee (%)
None	1	—
rac-Octan-2-ol[a]	46	>99 (*S*)
(*S*)-Octan-2-ol[b]	58	>99 (*S*)
(*R*)-Octan-2-ol[c]	9	89 (*S*)
Tetrahydrofuran[d]	10	>99 (*S*)
1,2-Dimethoxyethane[e]	8	>99 (*S*)

[a] 0.4 mmol [b] 98% ee, 0.4 mmol [c] 99.98% ee, 0.4 mmol. [d] 1.23 mmol. [e] 0.96 mmol.

Table 6 Effect of deuterium content in **1a** on the reduction of **2a** with immobilized cells in hexane

Additive	D in water (%)[d]	D in **1a** (%)[e]
rac-[2-^{2}H]Octan-2-ol[a]	0	40
(*S*)-[2-^{2}H]Octan-2-ol[b]	0	36
(*R*)-[2-^{2}H]Octan-2-ol[c]	0	0
rac-Octan-2-ol[a]	33	16
rac-Octan-2-ol[a]	66	24
rac-Octan-2-ol[a]	99	45
rac-Octan-2-ol[a]	99	>99

[a] 0.4 mmol. [b] 92% ee, 0.2 mmol. [c] >99% ee, 0.2 mmol. [d] Deuterium content in the water used for immobilization of the cells. [e] Deuterium content in **1a**.

effect of other organic substances (tetrahydrofuran and 1,2-dimethoxyethane) on reduction of **2a** was studied. When these additives were used, the yield of **1a** increased to about 10%, regardless of the kind and the amount, which indicates that the yield of **1a** increases as a result of the inhibition of the oxidation of product, **1a**. When (*R*)-octan-2-ol or these organic compounds were used for the oxidation of **1a**, they also inhibited the oxidation (data not shown).

Further experiments clarified the function of the additives as a reductant. Deuterated additives, [2-^{2}H]octan-2-ol, and/or deuterated water were employed for the reduction of **2a** with the immobilized cells to search for the source of the hydrogen transferred to C1-H of 1-phenylethanol (*S*)-**1a**. As shown in Table 6, the deuterium at CD-OH in the additive alcohol was transferred onto the product except for the case of (*R*)-[2-^{2}H]-octan-2-ol.

Since the product was only partially deuterated in spite of starting with completely deuterated additives, other sources of the hydrogen in the C1 position were investigated. When deuterated water was used in the immobilization step, the C1 position of the product was deuterated. According to the deuterium content of the water, the deuterium content of the product was increased (Table 6). The hydrogen at C*H*-OH of the product comes either from the additive (octan-2-ol) or the solvent (water) since when both deuterated additive and deuterated water were used for the reduction, the deuterium content in the product at C*H*-OH was >99%. This suggests that there are more than two routes for the reduction; (1) the additive such as octan-2-ol reduces the $NAD(P)^+$ which reduces the main substrate directly, (2) initially the additive such as octan-2-ol reduces the $NAD(P)^+$, then the NAD(P)H reduces other cofactors like flavin, followed by exchange of the hydrogen on flavin *etc*. with one from water, and finally the reduction of the main substrate occurs. To investigate the reaction mechanisms further the enzymes reducing the substrates need to be isolated.

Conclusion

Geotrichum candidum was immobilized on a water-absorbing polymer and used for stereoselective oxidation and reduction in hexane using cyclohexanone, cyclopentanol, and alkan-2-ols as additives. Enantiomerically pure (*R*)-1-arylethanols were obtained by the oxidation of racemic 1-arylethanols, whereas enantiomerically pure (*S*)-1-arylethanols were obtained by the reduction of the corresponding ketones. Using only (*S*)-selective enzyme(s), the stereoselective synthesis of both enantiomers with excellent ee was achieved because alcohol dehydrogenase(s) catalyze both oxidation and reduction. This system is suitable for large scale synthesis because expensive coenzymes are not necessary, and the use of the organic solvent simplifies the work-up procedure.

The system utilizing immobilized cells and hexane favors the oxidation over the reduction reaction due to the partitioning of the ketones and alcohols between the organic and aqueous phases. However, by choosing the right additives the reactivity can be controlled in both directions; cyclohexanone is suitable for the oxidation of the main substrate, whereas alkan-2-ols and cyclopentanol are suitable for the reduction. To elucidate the role of the additive, deuterated alkan-2-ol was employed for the reduction of the substrate and it was clarified that alkan-2-ols act as a hydride source for the reduction.

Experimental

General

^{1}H NMR spectra were recorded at 200 MHz on a Varian VXR-200 spectrometer in $CDCl_3$. Capillary gas chromatograms were recorded on a Shimadzu GC-9A gas chromatograph with Shimadzu C-R6A Chromatopac. GC-MS spectra were obtained with a JEOL JMS-DX300 spectrometer and analyzed with a JMA-3500 spectrometer. Optical rotations were measured with a JASCO DIP-181 digital polarimeter and are given in units of 10^{-1} deg cm^2 g^{-1}.

Commercially available reagents were purchased from Nacalai Tesque Co., Ltd., Tokyo Kasei Co., Ltd. and Aldrich Chemical Co., Ltd. unless otherwise indicated. Solvents and purchased reagents were generally used without additional purification unless otherwise indicated. BL-100® (water-absorbing polymer) was kindly supplied by Osaka Yuki Kagaku Kogyo Co., Ltd. *Geotrichum candidum* IFO 4597 was cultivated as described previously.[4]

Immobilization of the cells on the water-absorbing polymer, BL-100®

To a suspension of the resting cells (15 g wet wt) in water (90 mL), BL-100® (water-absorbing polymer, 15 g) was added and the mixture was stirred with a spatula. The resulting powder (120 g) was used for the reaction.

Oxidation of alcohols 1a–1n by the free cells in water

To a suspension of the cells (0.5 g) in water (3.0 mL), **1a–1n** (0.08 mmol) was added and the mixture was shaken at 130 rpm at 30 °C for 24 h. The mixture was extracted with ether (3 × 2.0 mL), dodecane as an internal standard for GC analysis was added, and the resulting ether solution was dried over anhydrous magnesium sulfate and subjected to GC analysis to determine the yield of **2a–2n** and the ee of **1a–1n**. The GC conditions are as described previously.[4]

Oxidation of alcohols 1a–1n by the immobilized cells in hexane with cyclohexanone at GC scale

To a suspension of the immobilized cells on BL-100® (4.0 g) in hexane (6 mL), **1a–1n** (0.08 mmol), cyclohexanone (0.2 mmol) and dodecane as an internal standard for GC analysis were added and the resulting suspension was shaken at 130 rpm and 30 °C for 24 h. The organic layer was subjected to GC analysis to determine the yield of **2a–2n** and ee of **1a–1n**.

Oxidation of alcohol 1a at preparative scale

To a suspension of the immobilized cells (120 g) in hexane (180 mL), **1a** (1.5 mmol) and cyclohexanone (0.30 mL) were added and the resulting suspension was shaken at 130 rpm at 30 °C for 24 h. The resulting mixture was filtered and the immobilized cell was washed with ether, then the combined organic layer was evaporated to obtain the crude product. (*R*)-**1a** was purified with silica gel column chromatography: 31%, ee 98%, $[a]_D$ +53.0 (*c* 0.60, $CHCl_3$) (lit.[13] $[a]_D^{25}$ −57 (*c* 5.12, $CHCl_3$) (*S*)). The spectral data are in accord with those reported previously.[4]

Reduction of 2a–2n by the free cells in water

2a–**2n** was reduced by the free cells as described previously.[4]

Reduction of 2a–2n by the immobilized cells in hexane at GC scale

To a suspension of the immobilized cells on BL-100® (4.0 g) in hexane (6 mL), **2a–2n** (0.08 mmol), hexan-2-ol (1.0 mmol) and dodecane as an internal standard for GC analysis were added. The resulting suspension was shaken at 130 rpm at 30 °C for 24 h. The organic layer was subjected to GC analysis to determine the yield and ee.

Reduction of ketones 2a–2l by the immobilized cells in hexane at preparative scale

To a suspension of the immobilized cells (80 g) in hexane (120 mL), **2a–2l** (1.7 mmol) and hexan-2-ol (20 mmol) were added. The resulting suspension was shaken at 130 rpm at 30 °C for 24 h. The suspension was filtered and the immobilized cells were washed with ether. The combined organic layer was washed with brine, dried over anhydrous magnesium sulfate and the solvent was evaporated under reduced pressure. Hexan-2-ol was removed from the residual oil under reduced pressure (60 °C/25 mmHg, (*R*)-hexan-2-ol exerted $[a]_D$ −1.72 (*c* 9.8, Et_2O)). The product was purified by silica gel column chromatography (ethyl acetate–hexane, 1 : 5) and by distillation with a Kugelrohr apparatus. The spectral data are in accord with those reported previously.[4]

Determination of absolute configuration of (−)-1-(2′,4′-dichlorophenyl)ethanol; dehalogenation of (−)-1j

(−)-1-(2′,4′-Dichlorophenyl)ethanol obtained from the reduction of **2j** by the immobilized cells was converted into 1-phenylethanol (**1a**) to compare the sign of optical rotation with that reported in the literature.[13] A mixture of (−)-**1j** (0.13 g, 0.68 mmol), ethanol (13 mL), sodium hydroxide (0.4 g) and 5% palladium on carbon (45 mg) was stirred overnight under an atmosphere of hydrogen at room temperature. The catalyst was removed by filtration, and the filtrate was neutralized by 2 M HCl and the solvent evaporated. Brine was added and the mixture was extracted with ether. The ether solution was dried over anhydrous magnesium sulfate and the solvent was evaporated. The residue was distilled under reduced pressure to afford a colorless oil (0.080 g). The NMR spectrum of the product was identical to that of 1-phenylethanol and the product exerted $[a]_D$ −48.6 (*c* 1.13, $CHCl_3$), which corresponds to (*S*)-**1a**: yield 97%, ee 91%.

Determination of the partition of 1a and 2a between hexane and water absorbed in BL-100®

Water (3.0 mL) was absorbed on BL-100® (0.5 g) and the polymer was placed in a test tube. A hexane solution (6.0 mL) of **1a** or **2a** (0.08 mmol) and dodecane (4.30 mM) as an internal standard was added to the test tube and the mixture was shaken at 30 °C for 24 h. The hexane layer was subjected to GC analysis to determine the partition of **1a** or **2a** between the hexane and water phase.

Reduction of 2a with immobilized cells on calcium alginate

Cells (2.0 g wet wt) were suspended in 12 mL of 1% sodium alginate and the suspension was extruded slowly into a $CaCl_2$ solution (0.05 M) through a syringe. The immobilized cells were washed with distilled water and wiped with a Kimwipe®. The total weight of the immobilized cells was 7.3 g.

To a suspension of immobilized cells on calcium alginate (1.8 g) in hexane (6.0 mL), **2a** (0.08 mmol), hexan-2-ol (1.0 mmol) and dodecane as an internal standard for GC analysis were

added. The resulting suspension was shaken at 130 rpm at 30 °C for 24 h. The organic layer was subjected to GC analysis to determine yield and ee.

Preparation of racemic and chiral [2-^{2}H]octan-2-ol

(*R*/*S*)-[2-^{2}H]Octan-2-ol. $NaBD_4$ (7.9 mmol, 0.33 g) was added to 50 mL of an ethanol solution of octan-2-one (15.8 mmol, 2.0 g) at 0 °C, and the mixture was stirred at rt for 4 h. After neutralization with 1 M HCl, ethanol was removed under reduced pressure, and the product was extracted with ether (30 mL × 3), washed with water, aqueous sodium hydrogen carbonate, and water, successively, and dried over anhydrous sodium sulfate. Then, the ether was evaporated under reduced pressure, and the residual oil was purified by distillation with a Kugelrohr apparatus to give (*R*/*S*)-[2-^{2}H]octan-2-ol (yield 89%, 1.84 g). ^{1}H NMR ($CDCl_3$) δ 0.86 (t, 3H, CH_3, J = 6.4 Hz), 1.15 (s, 3H, CH_3), 1.26–1.40 (m, 10H, CH_2), 1.54 (s, 1H, OH); FTIR ν(NaCl neat) 2130 cm^{-1}.

(*S*)-[2-^{2}H]Octan-2-ol. Vinyl acetate (41 mmol, 3.6 g) was added to a mixture of (*R*/*S*)-[2-^{2}H]octan-2-ol (7.6 mmol, 1.0 g), lipase (CAL SP 435, 0.2 g), and molecular sieves (0.5 g) in benzene (50 mL), the resulting mixture was stirred for 3 h then filtered through Extrelute®, and the solvent was evaporated under reduced pressure. The resulting (*S*)-octan-2-ol was separated from the (*R*)-2-octyl acetate by silica gel column chromatography (eluent: hexane–ethyl acetate, 9 : 1). (*S*)-Octan-2-ol was purified by distillation with a Kugelrohr apparatus, and the ee was determined by GC analysis (yield 36%, ee 92%). The spectral data are in accord with those for the racemic one.

(*R*)-[2-^{2}H]Octan-2-ol. The (*R*)-2-octyl acetate (4.3 mmol, 0.75 g, ee 93%) obtained above was hydrolyzed by stirring with lithium aluminum hydride (3.4 mmol, 0.13 g) in ether (25 mL) for 1 h, then the mixture was neutralized with 1 M HCl. The organic layer was separated from the aqueous layer, and the product was extracted with ether from the aqueous layer. The combined organic portion was evaporated under reduced pressure to obtain (*R*)-[2-^{2}H]octan-2-ol, which was re-acetylated by CAL and vinyl acetate and hydrolyzed as described above for further enhancement of ee. After the second acetylation and hydrolysis, the residual oil was purified by distillation with a Kugelrohr apparatus (yield 23%, ee >99%). The spectral data are in accord with those for the racemic one.

Reduction of 2a by deuterated additive, [2-^{2}H]octan-2-ol

To a suspension of the immobilized cell on BL-100® (4.0 g) in hexane (6 mL), **2a** (0.08 mmol), [2-^{2}H]octan-2-ol and dodecane as an internal standard for GC analysis were added. The resulting suspension was shaken at 130 rpm and 30 °C for 24 h. The organic layer was subjected to GC analysis to determine yield and ee and to GC-MS analysis (Thermon-3000 (Shincarbon A) 60–80 mesh, 5 mm × 1 m, 80–150 °C min^{-1}) to determine the deuterium content of the product.

Reduction of 2a by immobilized cells with deuterated water

For immobilization of the cells with 33% or 66% deuterated water, to a suspension of cells (0.5 g wet wt) in deuterated water (3 mL, D_2O : H_2O = 1 : 2 or 2 : 1), BL-100® (0.5 g) was added and the mixture was stirred with a spatula. For immobilization of the cells with >99% deuterated water, the cells (0.5 g wet wt) were washed with deuterated water (6 mL × 4) and suspended in deuterated water (3 mL), then BL-100® (0.5 g) was added and the mixture was stirred with a spatula. To a suspension of the immobilized cells on BL-100® (4.0 g) in hexane (6 mL), **2a** (0.08 mmol), octan-2-ol or [2-^{2}H]octan-2-ol (0.4 mmol) and dodecane as an internal standard for GC analysis were added. The resulting suspension was shaken at 130 rpm at 30 °C for 24 h. The organic layer was washed with water and subjected to GC analysis to determine yield and ee and to GC-MS analysis (Thermon-3000 (Shincarbon A) 60–80 mesh, 5 mm × 1 m, 80–150 °C min^{-1}) to determine the deuterium content of the product.

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