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Letter

Utility of ionic liquid for *Geotrichum candidum*-catalyzed synthesis of optically active alcohols

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ABSTRACT

Ionic liquids have recognized as a solvent for *Geotrichum candidum*-catalyzed optical resolution and/or deracemization of racemic secondary alcohols, giving optically active alcohols. The immobilized *Geotrichum candidum* proceeded the enantioselective oxidation of alcohols, producing chiral alcohols in an ionic liquid. Further, deracemization of racemic alcohols was proceeded to give the corresponding chiral alcohols in high yield with excellent stereoselectivity by the *Geotrichum candidum*–$NaBH_4$ system in the mixture of MES buffer solution and ionic liquid.

1. Introduction

Ionic liquids are having an important impact on organic reactions, and various kinds of reactions in ionic liquids have been reported until now [1–5]. The reaction using biocatalysts in ionic liquids has also been developed since biocatalysts are renewable natural catalysts with high enantio-, regio- and chemoselectivities [6–9]. In our papers [10–16], we have revealed that the advantages of using an ionic liquid as a solvent are the easy separation of the product from the reaction mixture, the easy handling the reaction media and recyclable use of the reaction system.

In general, syntheses of chiral materials as valuable building blocks in organic syntheses have been developed by a various kinds of chemical and/or biological methods [17–19]. Especially, in the synthesis of optically active secondary alcohols, three methods have been known for synthesis of optically active alcohols: optical resolution of alcohols [6], asymmetric reduction of ketones [6], and deracemization of racemic alcohols by chemical and/or biological tools in organic solvents and/or water medium [20–30]. Among these methods, deracemization is the most promising because it permits 100% conversion of the racemate to the corresponding enantiomerically pure material. Nakamura and co-workers have reported enzymatic deracemization of racemic 1-arylethanols, 1-pyridylethanols and β-hydroxyesters in water medium to the corresponding enantiomerically pure alcohol [23,24]. For other example, two biocatalysts were used for the oxidation of (*S*)-mandelic acid to give benzoylformate by *Alcaligenes bronchisepticus*, and then the produced benzoylformate was reduced to give (*R*)-mandelic acid (85% yield; >99% ee) by *Streptococcus faecalis* [31]. Furthermore, deracemization of 3-pentyn-2-ol with *Nocardia fusca* to produce (*R*)-isomer has also reported [32–34]. Recently, tandem biocatalytic oxidation and reduction using two biocatalysts have reported to give the (*S*)-enantiomer of secondary alcohols. Based on the above results, obviously it remains the general method for the biocatalytic synthesis of optically active secondary alcohols which is possible to use various types of aryl-, alkyl- and heterocyclic derivatives.

In this paper, we further describe one of benefits of using an ionic liquid in a chemoenzymatic process, constructing the deracemization of racemic secondary alcohols.

2. Results and discussion

2.1. Optical resolution by biocatalytic oxidation

In our recent report for the utility of ionic liquid in biocatalytic reduction [16], we have revealed that water was not miscible with [bmim][PF_6], while [emim][BF_4] is completely miscible with water, and that the stabilizing effect of the enzyme by keeping water around it using the polymer improves the yield of (*S*)-secondary alcohols in an ionic liquid [emim][BF_4]. For the deracemization of secondary alcohols in an ionic liquid, we have used the immobilized cell produced from *Geotrichum candidum* and water-absorbing polymer (BL-100) at first. In the immobilized cell system holding water and enzyme, the enantioselective oxidation accelerated in an ionic liquid shown in Table 1, yielding the optically active (*R*)-secondary alcohols which were the same stereoisomer obtained from the water medium [23,24]. The above method is one of reasonable general route to obtain the optically active secondary alcohols in an ionic liquid (Scheme 1).

2.2. Deracemization of racemic secondary alcohols with chemoenzymatic method

From the results shown in Table 1, we have found that it is necessary to reduce the produced ketone to the corresponding racemic alcohol to complete the deracemization in an ionic liquid containing water for the stabilizing effect of the enzyme. To realize the above idea, the deracemization of 1-phenylethanol was examined first as a model reaction by the system *Geotrichum candidum*-

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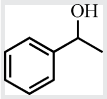
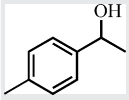
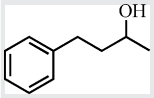
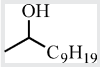
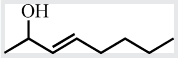
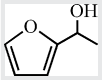
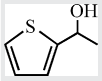
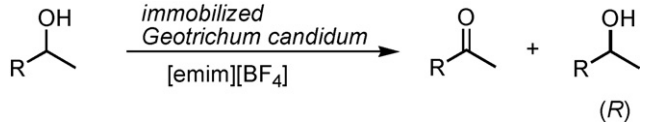
Table 1
Substrate specificity of the oxidation system in [ernirnl[BF_4l by immobilized *G candidum* cell on water-absorbing polymer

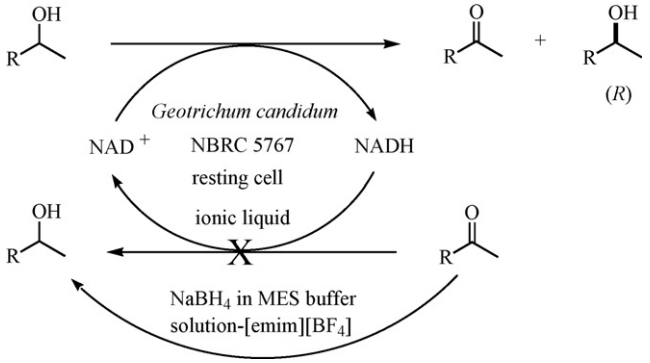
Substrate	Yield of ketone (%)	Yield of alcohol (%)	% ee of substrate (*R*)-alcohol[a]
	52	49	97
	52	48	94
	51	42	95
	43	39	97
	41	48	97
	30	67	41
	42	55	81
	39	46	>99

Reaction conditions 24 h at 30 °C. [a]Determined by GC analysis with chiral column (Chirasil-DEX CB or CP-Cyclodextrin-B-2,3,6-M-19. The absolute configuration was determined to be *R* for all samples examined by comparing the GC retention times.

reductive reagent ($NaBH_4$ and/or $NaBH_3CN$) in an ionic liquid to test the improvement for the yield of deracemization and/or the optical purity of secondary alcohol in the various ratios of reductive reagent, wet cell and MES buffer solution to create the most convenient reaction condition (Scheme 2). From the results of entries 6 and 7 shown in Table 2, deracemization proceeded drastically to give (*R*)-2-phenylethanol with high optical purity (99% ee). However, $NaBH_3CN$ as a reductive reagent (entry 3) was not improving the reduction of ketone. Obviously, in the case of other alcohols (entries 9 and 10), deracemization does not complete in the absence of ionic liquid. Furthermore, the important factor for deracemization with high optical purity is the ratio of MES buffer solution and ionic liquid shown in Table 2.



Scheme 1. Asymmetric oxidation of racemic alcohols by immobilized *Geotrichum candidum*.



Scheme 2. Deracemization process in MES buffer solution-[emim][BF_4].

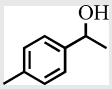
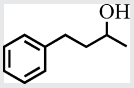
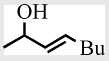
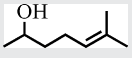
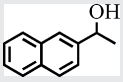
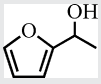
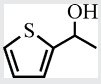
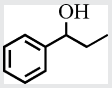
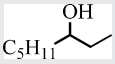
To aim for the construction of the wide substrate specificity with the excellent enantioselectivity for a valuable synthetic practical route using an ionic liquid as media, we examined the deracemization with the complex system derived from *Geotrichum candidum* NBRC 5767 and $NaBH_4$ in the mixture of MES buffer and ionic liquid [emim][BF_4]. Various types of secondary alcohols such as 1-aryl ethanol, 4-phenyl-2-butanol (*E*)-3-octen-2-ol, 2-octanol, 1-(2-furyl)ethanol, 1-(thiophen-2-yl)ethanol and/or 2-naphtylethanol were used as substrates, and it was found that all of them were deracemizated with moderate to excellent yield, and the enantioselectivity was excellent for all of the substrates tested from the results shown in Table 3. In the cases of 1-phenylpropanol and 3-octanol, as we have found that the first step (oxidation process) is not proceed smoothly from the results shown in Table 3 (entries 14, 15 and 17), the reaction condition such as excess of *Geotrichum candidum* and/or extension time was used to obtain the corresponding chiral alcohol. In our previous report [16], we have reported that the reduction of ketones with *Geotrichum candidum* produced (*S*)-secondary alcohols. In this system, $NaBH_4$ in the mixture solution of [emim][BF_4]–MES buffer reduced the ketones to the corresponding

Table 2
Effect of reaction conditions on the deracemization[a]

Entry	Substrate	Ionic liquid (3 ml)	$NaBH_4$ or $NaBH_3CN$	Reaction time (h)	Wet cell (g)	MES buffer (ml)	Yield (%)		% ee of alcohol
							Ketone	Alcohol	
1	1-Phenylethanol	[emim][BF_4]	$NaBH_4$(3.2 equiv.)	24	0.25	(1.5, H_2O)	4	89	19
2		[emim][BF_4][b]	$NaBH_4$(3.0 equiv.)	24	0.25	1.5	10	81	12
3		[emim][BF_4]	$NaBH_3CN$(3.7 equiv.)	24	0.25	1.5	31	60	51
4		[emim][BF_4]	$NaBH_4$(3.0 equiv.)	24	0.50	3.0	20	65	98
5		[emim][BF_4]	$NaBH_4$(3.1 equiv.)	48	0.50	3.0	31	60	92
6		[emim][BF_4]	$NaBH_4$(6.4 equiv.)	48	0.50	3.0	0	95	99
7		[emim][BF_4]	$NaBH_4$(9.0 equiv.)	24	0.50	3.0	1	96	99
8		[emim][BF_4]	None	24	0.25	(1.5, H_2O)	38	57	64
9	2-Octanol	None	$NaBH_4$(9.0 equiv.)	24	0.50	6.0	5	36	74
10	6-Methyl-5-hepten-2-ol	None	$NaBH_4$(9.0 equiv.)	24	0.50	6.0	99	10	86

[a]Reaction conditions: 24 h at 30 °C. Determined by GC analysis with chiral column (Chirasil-DEX CB or CP-Cyclodextrin-B-2,3,6-M-19. The absolute configuration was determined to be *R* for all samples examined by comparing the GC retention times with those of the authentic samples in the references. [b]Immobilized system prepared from water-absorbing polymer was used.

Table 3
Deracemization of secondary alcohols

Entry	Substrate	$NaBH_4$	Wet cell (g)	Yield (%)		% ee alcohol[a]
				Ketone	Alcohol	
1		$NaBH_4$(9.0 equiv.)	0.50	1	96	99
2		$NaBH_4$(9.0 equiv.)	0.50	5	87	95
3		$NaBH_4$(9.0 equiv.)	0.50	15	79	96
4		$NaBH_4$(9.0 equiv.)	0.50	0	54	>99
5		$NaBH_4$(9.0 equiv.)[b]	0.75	0	58	>99
6		$NaBH_4$(9.1 equiv.)	0.50	8	68	97
7		$NaBH_4$(9.0 equiv.)	0.50	10	67	89
8		$NaBH_4$(9.0 equiv.)[b]	0.75	10	74	99
9		$NaBH_4$(9.0 equiv.)[c]	1.00	14	85	98
10		$NaBH_4$(9.0 equiv.)[b]	0.75	21	56	>99
11		$NaBH_4$(9.0 equiv.)	0.50	18	60	97
12		$NaBH_4$(9.1 equiv.)	0.50	16	66	>99
13		$NaBH_4$(8.9 equiv.)	0.50	12	73	>99
14		$NaBH_4$(9.0 equiv.)	0.50	4	96	19
15		$NaBH_4$(9.0 equiv.)	6.00[d]	17	33	66
16		$NaBH_4$(9.0 equiv.)	6.00[e]	32	49	>99
17		$NaBH_4$(9.0 equiv.)	0.50	3	90	16
18		$NaBH_4$(9.0 equiv.)	3.00[f]	9	41	97

Reaction conditions: 24 h at 30 °C in the mixture of MES buffer (3 ml) and [emim][BF_4] (3 ml). [a]Determined by GC analysis with chiral column (Chirasil-DEX CB or CP-Cyclodextrin-B-2,3,6-M-19. The absolute configuration was determined to be *R* for all samples examined by comparing the GC retention times with those of the authentic samples in the references. [b]MES buffer solution (4.5 ml) [emim][BF_4] (4.5 ml). [c]MES buffer solution (6.0 ml) [emim][BF4] (6.0 ml). [d]MES buffer solution (36.0 ml) [emim][BF4] (36.0 ml). [e]MES buffer solution (18.0 ml) [emim][BF_4] (18.0 ml), 51 h. [f]MES buffer solution (18.0 ml) [emim][BF4] (18.0 ml), 24 h.

alcohol more quickly than the reduction of ketone with *Geotrichum candidum*.

3. Conclusion

We have found the utility of ionic liquid for the optical resolution by the biocatalytic oxidation and deracemization of racemic secondary alcohols with chemoenzymatic method. Especially, the stabilizing effect of the enzyme by keeping water around it using the MES buffer solution improves the oxidation of alcohol, and $NaBH_4$ in the mixture solution of [emim][BF_4]–MES buffer accelerates the reduction of ketone to the corresponding alcohol. Further, we have found that the effective practical method using the system derived from MES buffer and ionic liquid [emim][BF_4] with *Geotrichum candidum* and $NaBH_4$ is developed for the deracemization of secondary alcohols to produce the optically active materials with excellent enantioselectivity.

4. Experimental procedure

4.1. Geotrichum candidum

NBRC 5767 was cultivated, and the wet cell was prepared as reported previously [16]. The wet cell (0.50 g) was suspended in MES buffer solution (3 ml), and then followed by addition of an ionic liquid (3 ml), the substrate (0.041 mmol), and $NaBH_4$ (9 equiv.). The whole was shaken at 280 rpm for 24 h at 30 °C. The products were extracted with diethyl ether (5 ml 5×), and analyzed GC with a

chiral column (Chirasil-DEX CB or CP-Cyclodextrin-B-2,3,6-M-19) using dodecane as an internal standard.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.molcatb.2008.07.011.

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