Enantioselective Microbial Oxidation of Allyl Alcohols

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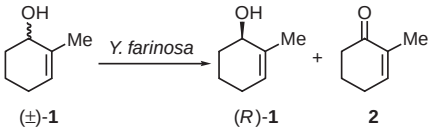
A new route to the optically active allyl alcohols by microbial oxidation is disclosed. *Yamadazyma farinosa* IFO 10896, a yeast, efficiently catalyzes the enantioselective oxidation of allyl alcohols to afford the corresponding optically active alcohols as the remaining substrates. This reaction is applicable to both cyclic and acyclic compounds.

The enzyme-catalyzed oxidation of alcohols is one of the useful tools for obtaining the corresponding ketones and carboxylic acids. Although numerous examples have been reported for the enzymatic reduction of the carbonyl group, little attention has been paid to this oxidation.[1] In particular, there are only a few reports regarding the oxidation of allyl alcohols.[2,3] However, biocatalytic oxidation could be an environmentally benign procedure as a substitute for traditional chemical reactions. Furthermore, the kinetic resolution by the enzymatic enantioselective oxidation of secondary alcohols could produce optically active alcohols with high ee's as the remaining substrates.

In previous studies, *Yamadazyma farinosa* IFO 10896, a yeast, could transform various types of acyclic and cyclic compounds into the corresponding optically active products. For example, the yeast enantiomerically reduced acyclic[4] and cyclic[5] ketones to give the alcohols. The asymmetric reduction of the C=C double bond of α,β-unsaturated ketones occurred using the same yeast to afford the corresponding saturated ketones, and besides, the oxidation of cyclic alcohols was also observed.[5] These results indicate that the yeast includes a plural oxidoreductase, which encourages us to examine the yeast-mediated oxidation. In this paper, we report the enantioselective oxidation of allyl alcohols by the yeast that affords the corresponding optically active alcohols.

We selected the readily available racemic 2-methyl-2-cyclohexenol ((±)-**1**) as the representative substrate (Scheme 1). A typical experimental procedure of the microbial reaction is as follows. A sterilized nutrient medium of pH 7.2 (100 mL) was inoculated with *Y. farinosa* and pre-incubated for 48 h at 30 °C.[6] The grown cells were collected by centrifugation (3000 rpm for 10 min) to afford the resting wet cells (ca. 2.5 g). To a suspension of the cells in 0.1 M phosphate buffer (40 mL, pH 6.5) was added the substrate (±)-**1** (80 μL) and 2.4 g of glucose, and that was shaken at 30 °C. The yields of the products were determined by capillary GLC analysis after



(±)-**1** (*R*)-**1** **2**

Scheme 1.

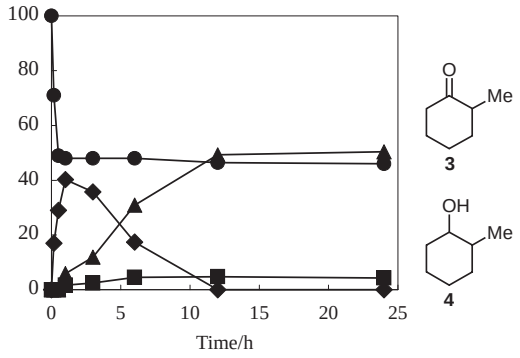


Figure 1. Transformation of allyl alcohol **1** as the substrate using the resting cells of *Y. farinosa* (●, allyl alcohol **1**; ◆, enone **2**; ■, ketone **3**; ▲, alcohol **4**).

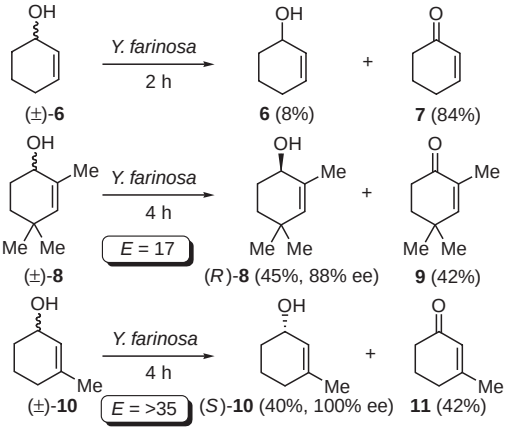
extraction with Et_2O.[7] The time course of the % contents of the products is shown in Figure 1. Interestingly, in this case, the oxidation of the substrate **1** rapidly proceeded, and almost half of the substrate **1** was consumed in only 0.5 h. On the other hand, the corresponding unsaturated ketone, 2-methyl-2-cyclohexenone (**2**), was simultaneously produced, although the yield of **2** gradually decreased in accordance with the reduction of the C=C double bond followed by the reduction of the carbonyl group to afford 2-methylcyclohexanone (**3**) and 2-methylcyclohexanol (**4**) in the same manner as in our previous study.[5] We then focused on the ee of the remaining substrate **1** because the first oxidation step could occur with a high enantioselectivity. After purification by column chromatography on silica gel, the ee was determined by ^{1}H NMR analysis of the corresponding (+)-α-methoxy-α-(trifluoromethyl)phenylacetic (MTPA) ester. These results are shown in Table 1. As expected, the oxidation proceeded with an excellent enantioselectivity, and, after a 0.5-h reaction, the optically active (*R*)-**1** with 93% ee ($[\alpha]_D^{21}$ = +112° (*c* 0.95, MeOH)) was obtained in a 49% yield (*E* value = 60).[8] The chemical reduction of C=C double bond of the obtaining **1** with H_2 and Pd/C followed by separation and MTPA esterification gave the corresponding *cis*-MTPA ester **5**. The absolute configuration of the original **1** was confirmed by comparing the GLC spectrum of **5** with that of the authentic sample.[5] After the reaction for 24 h, the optically pure (*R*)-**1** was afforded, although the saturated alcohol **4** was also detected as an inseparable impurity. The resulting enone **2** can be reduced by use of chemical reagents, for example, $NaBH_4$ with $CeCl_3$, to reproduce the substrate (±)-**1**.

We next examined the substrate specificity of the reaction (Scheme 2). As a result, the reaction rate of the oxidation of (±)-2-cyclohexenol (**6**) without a methyl group was extremely fast. After a 2-h reaction, the racemic alcohol **6** was recovered

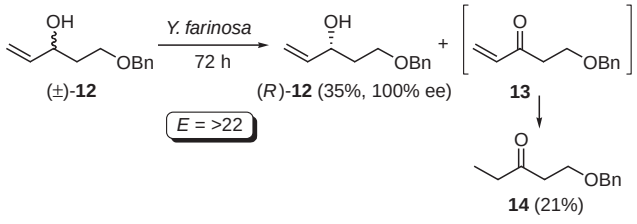
Table 1. Microbial oxidation of cyclic allyl alcohol (±)-**1**[a]

Time/h	Allyl alcohol (*R*)-**1**		Enone **2**	*E* value
	Yield/%[b]	Ee/%	Yield/%[b]	
0.2	71	32	17	11
0.5	49	93	29	60
24	40	100	0	>35

[a]The reaction was performed using 18 mM of the substrate. [b]Determined by GLC analysis with dodecane as the internal standard.



Scheme 2.



Scheme 3.

in only 8% yield. On the other hand, (±)-2,4,4-trimethyl-2-cyclohexenol (**8**) was enantioselectively oxidized to afford (*R*)-**8** (45%) with 88% ee and **9** (42%); (*R*)-**8**, $[\alpha]_D^{25} = +65.7$ (*c* 1.09, MeOH), lit.[9] $[\alpha]_D^{23} = +87.9$ (*c* 1.00, MeOH) (94% ee (*R*)) (*E* value = 17). Surprisingly, the oxidation of (±)-3-methyl-2-cyclohexenol (**10**) proceeded with an excellent enantioselectivity, although the enantioselective mode was different from that in other cases, and the optically pure (*S*)-**10** with the opposite absolute configuration and the corresponding enone **11** were obtained in 40% and 42%, respectively; (*S*)-**10**, $[\alpha]_D^{22} = -42.4$ (*c* 0.37, $CHCl_3$), lit.[10] $[\alpha]_D^{23} = -26.7$ (*c* 1.5, $CHCl_3$) (29% ee (*S*)) (*E* value = >35). These results indicated that the methyl group at the C-2 position on the substrates is very important for the S-selective oxidation, but the substrate bearing the C-3 methyl group could be captured into the enzyme active site with a different angle and/or direction mode. It is noteworthy that this reaction is applicable to not only cyclic compounds but also an acyclic compound, (±)-5-benzyloxy-1-penten-3-ol (**12**) (Scheme 3). The S-enantioselective oxidation of (±)-**12** proceeded to afford the optically pure (*R*)-**12** in a 35% yield (*E* value = >22).[11] In this case, the intermediate enone **13** was quickly reduced in the microbial system to give the saturated ketone **14** in a 21% yield.

In conclusion, we have established the microbial enantioselective oxidation of allyl alcohols as a new route to the optically active allyl alcohols and the corresponding enones. In particular, this reaction is applicable to the preparation of both cyclic and acyclic optically active compounds. This procedure is expected to be a useful tool for organic synthesis, and further investigations for applying this method and the study of the mechanism are now in progress.

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6 The medium contained 1.0% glucose, 0.7% polypeptone, 0.5% yeast extracts, and 0.5% K_2HPO_4 in distilled water.

7 Conditions for capillary GLC analysis: column, TC-WAX (0.25 mm × 50 m, GL Sciences Inc.); injection, 130 °C; detector, 130 °C; oven, 100 °C; carrier gas, He; head presuure, 2.4 kg/cm^2; dodecane as the internal standard (6.7 min), **3** (10.1 min), *cis*-**4** (13.5 min), *trans*-**4** (13.9 min), **2** (15.9 min), **1** (22.5 min).

8 C. S. Chen, Y. Fujimoto, G. Girdaukas, C. J. Sih, *J. Am. Chem. Soc.* **1982**, *104*, 7294. The *E* values were calculated by ln[(1 − conv.)(1 − ee(alcohol))]/ln[(1 − conv.)(1 + ee(alcohol))] in all cases. The conversions were calculated by 1 − yield(alcohol). In the cases of **1** (24-h reaction), **10** and **12**, we considered that the ee's of the alcohols were over 0.999.

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11 The absolute configuration and the ee of **12** were determined by the same methods as that in our previous paper. See: M. Nogawa, M. Shimojo, K. Matsumoto, M. Okudomi, Y. Nemoto, H. Ohta, *Tetrahedron* **2006**, *62*, 7300.

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