# Asymmetric Reduction of α,β-Unsaturated Cyclic Ketones by a Yeast

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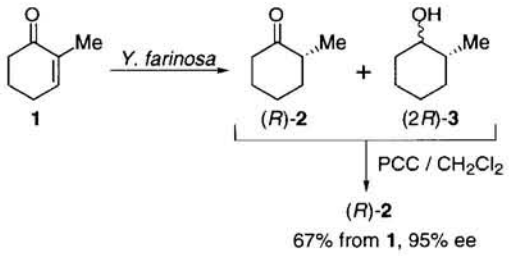
The asymmetric reduction of the carbon-carbon double bond of α,β-unsaturated cyclic compounds proceeds with high enantioselectivity when catalyzed by *Yamadazyma farinosa* IFO 10896. Microbial reduction of 2-methyl-2-cyclohexen-1-one followed by PCC oxidation affords the optically active (*R*)-2-methyl-1-cyclohexanone of 95% ee. On the other hand, the sequential reactions of (*E*)-2-propylidene-1-cyclohexanone gives the unsaturated ketone possessing an *S*-configuration at the C-2 position.

The enzyme-mediated enantioselective reduction of substrates having a prochiral face is a widely used and very efficient procedure in asymmetric synthesis.[1] Although numerous examples have been reported for the enzymatic reduction of the carbonyl group, much attention has not been paid to the reduction of the carbon-carbon double bond of an α,β-unsaturated carbonyl group. In particular, relatively few studies on the asymmetric reduction of α,β-unsaturated cyclic compounds have been reported despite the usefulness of the functionalized cyclic molecules as building blocks in organic synthesis.[2,3] In previous studies, *Yamadazyma farinosa* IFO 10896 (a yeast which was re-classified from *Pichia farinosa* IAM 4682) could transform many types of cyclic compounds into the corresponding chiral products. For example, the yeast hydrolyzed cyclic enol esters to afford optically active ketones,[4] and reduced cyclic ketones to give alcohols.[4-6] These results encouraged us to try the yeast-mediated reduction of cyclic compounds bearing a carbon-carbon double bond. In this paper, we report that the reduction of α,β-unsaturated cyclic ketones by the yeast and the subsequent oxidation affords optically active saturated ketones.

We selected readily available 2-methyl-2-cyclohexen-1-one (**1**) as the representative substrate.[7] A typical experimental procedure of the microbial reaction is as follows. One hundred ml of sterilized nutrient medium of pH 7.2 was inoculated with *Y. farinosa* and incubated for 48 h at 30 °C.[8] The grown cells were collected by centrifugation to give ca. 3.5 g of wet cells. The substrate **1** (80 μl) and 2.4 g of glucose were added to 40 ml of a suspension of the resting cells in 0.1 M Na-phosphate buffer (pH 6.5) and incubated at 30 °C. The yields of the products were determined by capillary GLC analysis with dodecane as the internal standard.[9] Extraction of the broth with $Et_2O$ followed by purification using column chromatography on silica-gel and Kugelrohr distillation gave the products. The time course of contents % of the products is shown in Figure 1a. As expected, the reduction of the carbon-carbon double bond of **1** with *Y. farinosa* smoothly proceeded (Scheme 1). For the reaction




**Scheme 1.**

after 24 h, the substrate **1** completely disappeared and the unsaturated alcohols, *cis*- and *trans*-2-methyl-1-cyclohexanol (**3**), were formed as major products in 53% and 33% yield, respectively,[10] while the saturated ketone, 2-methyl-1-cyclohexanone (**2**), was produced as a minor product in 7% yield while an unsaturated alcohol, 2-methyl-2-cyclohexen-1-ol (**4**, Figure 2), could not be detected.[11] PCC oxidation of the combined mixture of the products gave optically active **2** as a single product in 67% yield based on **1**, $[\alpha]_D^{23}$ -11.8° (c 0.62, MeOH). The stereochemistry of the resulting **2** was then identified as the *R* configuration by comparing its sign of optical rotation with the reported one; lit.[12] $[\alpha]_D$ +14° (c 0.23, MeOH),

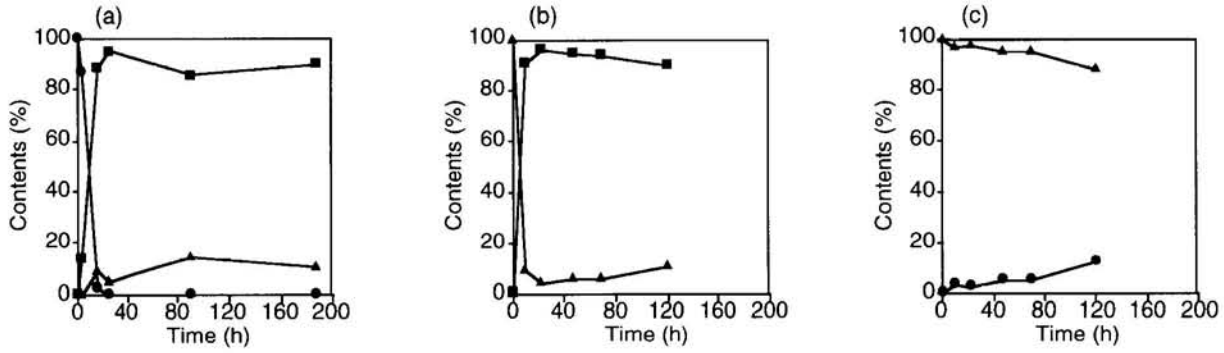



**Figure 1.** Transformations of unsaturated ketone **1** (a), saturated ketone **2** (b), and saturated alcohol **3** (c) as the substarates using resting cells of *Y. farinosa*. The substrates **2** and **3** were racemic, and **3** consisted of the mixture of diastereomers (**3a** : **3b** = 5 : 1). The reactions were carried out at 30 °C. (●, **1**; ▲, **2**; ■, **3**)

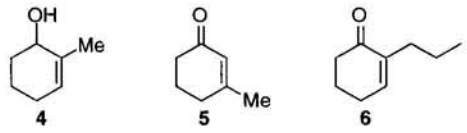
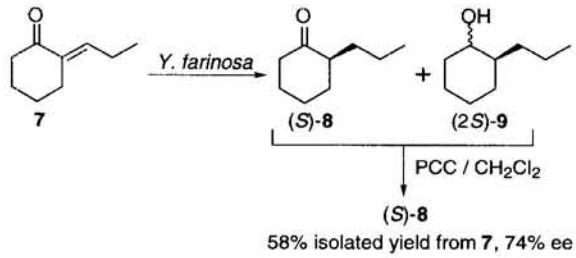



**Figure 2.**

(*S*)-form. The ee of **2** was determined to be 95% ee by capillary GLC analysis of the corresponding (+)-α-methoxy-α-(trifluoromethyl)phenylacetic (MTPA) ester which was derived from **2** *via* reduction with Li(*sec*-Bu)$_3$BH (L-Selectride®) and esterification.[13] The enantioselectivity of the C-2 position of the products strictly corresponds to the enantioface selectivity of the enzyme.

In the microbial step, it is clear that the reduction of the carbon-carbon double bond of **1** firstly occurred with high enantioselectivity, and then the carbonyl group of resulting **2** was reduced with rather law diastereoselectivity to afford **3**. Interestingly, the diastereomeric ratio of **3** gradually changed (*cis*/*trans*: 2.9/1 after 3 h; 1.6/1 after 24 h; 1/4.5 after 188 h) and **2** did not completely disappear even after 188 h. In order to make it clear, microbial transformations of *dl*-**2** and *dl*-**3** (epimeric pair of diastereomers) as substrates were performed. As shown in Figure 1b, the reduction of *dl*-**2** rapidly proceeded. Furthermore, *trans*-isomer gradually increased with reaction time (*cis*/*trans*: 1.6/1 after 3 h; 1/3.8 after 120 h). Figure 1c shows that *dl*-**3** was slightly oxidized to form *dl*-**2**. Even in this case, the ratio of *cis*/*trans* of **3** also changed from 1/2.8 to 1/4.9 after 120 h. These results clearly indicates that diastereoselectivity of the reduction of **2** is essentially low, and the oxidation of **3** to **2** causes changing the ratio of the diastereomers of **3** in the original reaction.

Next, the substrate specificity of the reaction was examined. As a result, *Y. farinosa* could not reduce β-substituted substrate, 3-methyl-2-cyclohexen-1-one (**5**, Figure 2). Displacement of the methyl with a n-propyl group, 2-propyl-2-cyclohexen-1-one (**6**, Figure 2), also significantly affected the reaction system and the reduction of **6** did not proceed. On the other hand, the exo carbon-carbon double bond of (*E*)-2-propylidene-1-cyclohexanone (**7**)[14] was smoothly reduced using a large amount of the resting cells (ca. 14 g) to afford the corresponding saturated products 2-propyl-1-cyclohexanone (**8**) and 2-propyl-1-




**Scheme 2.**

cyclohexanol (**9**, Scheme 2).[15] PCC oxidation of the mixture of **8** and **9** gave the optically active ketone (*S*)-**8** of 74% ee in 58% total isolated yield; lit.[16] $[\alpha]_D$ +27.9° (c 4, MeOH), (*S*)-form.[17] It is noteworthy that the resulting **8** had the opposite configuration to that of **2** obtained by the enzymatic reaction as mentioned above. This is probably due to a different hydrophobic fitting of the side chain between the enzyme and the substrates, although the details are not yet clear. 2-Methylidene-1-cyclohexanone was not suitable for the substrate because the compound was likely to polymerize. Further investigations of this system using other 2-alkylidene-1-cycloalkanones are now in progress.

In conclusion, we found that *Y. farinosa* reduced the carbon-carbon double bond of the α,β-unsaturated cyclic ketones and the following oxidation afforded optically active saturated ketones. The enzyme system has a high and unique structure specificity for the substrates.

## References and Notes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7 E. W. Warnhoff, D. G. Martin, and W. Johnson, "*Organic Synthesis*," Coll. Vol. IV, 162 (1963).

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

9 Conditions for capillary GLC analysis: column, TC-WAX (0.25 mm x 50 m, GL Sciences Inc.); injection, 130 °C; detector, 130 °C; oven, 100 °C; carrier gas, He; head pressure, 2.4 kg/cm$^2$; dodecane (6.7 min), **2** (10.1 min), *cis*-**3** (13.5 min), *trans*-**3** (13.9 min), **1** (15.9 min).

10 *cis*- (1*S*,2*R*)-**3**: 98% ee, $[\alpha]_D^{23}$ +18.6° (c 1.00, MeOH), lit.[18] $[\alpha]_D^{20}$ +24.3° (c 1, MeOH); *trans*-(1*R*,2*R*)-**3**: 89% ee, $[\alpha]_D^{23}$ -34.3° (c 1.08, MeOH), lit.[18] $[\alpha]_D^{20}$ +42.9° (c 1, MeOH). The diastereomers of **3** were easily separated by column chromatography on silica-gel. The ee of *cis*-**3** was determined by the method described in Ref. 13. On the other hand, the ee of *trans*-**3** was determined by HPLC analysis of the corresponding MTPA ester. Conditions of HPLC analysis: column, Zorbax-Sil (0.46 mm x 25 cm, DuPont Instruments); eluent, hexane/AcOEt = 200/1; flow rate, 0.5 ml/min.

11 Changes in the pH (5.5 - 8.0) of the reaction media did not affect the reaction.

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13 Reduction of **2** with Li(*sec*-Bu)$_3$BH gave *cis*-**3** as a single product. The ee of the original **2** was determined by capillary GLC analysis of MTPA ester derived from the resulting *cis*-**3**. Conditions of capillary GLC analysis: column, TC-WAX (0.25 mm x 50 m, GL Sciences Inc.); injection, 200 °C; detector, 200 °C; oven, 170 °C; carrier gas, He; head pressure, 2.4 kg/cm$^2$.

14 The configuration of **7** at the alkylidene site was assigned to be *E* based on a 270 MHz $^1$H NMR experiment (solvent, $CDCl_3$; TMS as an internal standard). The chemical shift of the olefin proton signal comparatively shifted downfield to δ 6.61 (tt, $J_1$ = 2.0 Hz, $J_2$ = 7.5 Hz, 1H) due to the placement of this proton in the deshielding region of the neighboring carbonyl group. See, J. K. Crandall, J. P. Arrington, and J. Hen, *J. Am. Chem. Soc.*, **89**, 6208 (1967).

15 The microbial reaction for 24 h afforded the mixture of **8** and **9** (cis/trans = 1/2.0). The product ratio of **8**/**9** was 1/11.

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17 The ee of **8** was determined by capillary GLC analysis using TC-WAX of the corresponding MTPA ester derived from **8** *via* reduction with Li(*sec*-Bu)$_3$BH and esterification.

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