Asymmetric Hydrogenation of the C-C Double Bond of Enones with the Reductases from *Nicotiana tabacum*

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Three enone reductases, p44, p74, and p90, from the cultured cells of *Nicotiana tabacum* catalyzed the asymmetric hydrogenation of the C-C double bond of enones. Reduction of 2-alkyl-2-cyclohexen-1-ones with the p44 reductase gave highly optically active (*R*)-2-alkylcyclohexanones, whereas reaction with the p90 reductase gave (*S*)-2-alkylcyclohexanones. On the other hand, reduction of 2-alkylidenecyclohexanones with the p74 reductase gave (*S*)-2-alkylcyclohexanones.

Previous studies on the asymmetric hydrogenation of the C-C double bond of enones by plant cell cultures revealed that there are two different types of enone reductase with respect to substrate specificity; one is responsible for the reduction of the endocyclic double bond and the other catalyzes the reduction of the exocyclic double bond.[1–3] We recently isolated several enone reductases from the cultured cells of *N. tabacum* participating in the reduction of the endocyclic C-C double bond of enones. We reported that a 44 kDa enone reductase (p44) was able to reduce only the C-C double bond bearing a hydrogen atom at the β position to the carbonyl group,[4] and that a 90 kDa enone reductase (p90) was capable of reducing a broad range of enones.[5,6] We have now isolated a novel 74 kDa enone reductase (p74) from *N. tabacum* participating in the reduction of the exocyclic C-C double bond of enones. In this paper, we describe the enantioselective formation of chiral ketones by reduction of the C-C double bond of enones with three enone reductases from *N. tabacum* as biocatalysts.

Three reductases, p44, p74 and p90, were isolated from the cultured cells of *N. tabacum* by chromatographic separation.[7] The stereospecificity in the asymmetric reduction of enones with these reductases was examined[9] by using enones **1**–**5** as substrates.[10] In the case of the reduction with the p74 reductase, 2-alkylidenecyclohexanones (**1** and **2**) having an exocyclic C-C double bond were converted to the corresponding saturated ketones, but 2-alkyl-2-cyclohexen-1-ones (**3**–**5**), which have an endocyclic C-C double bond, remained unchanged, as shown in Table 1. In the reduction of 2-alkylidenecyclohexanones (**1** and **2**), hydrogen atoms came from the *re*-face of their C-C double bond to give (*S*)-2-methylcyclohexanone (**6a**) (97% ee) and (*S*)-2-propylcyclohexanone (**8a**) (75% ee), respectively.[14] On the other hand, when the p90 and p44 reductases were used, enones **3**–**5** were converted to the corresponding saturated ketones, but no reduction occurred for enones **1** and **2**, as shown in Table 1. In the reduction with the p90 reductase, a hydrogen atom was stereoselectively added to C-2 from the *re–re* face of the C-C double bond to give highly optically active (*S*)-2-alkylcyclohexanones, **6a**–**8a**.[17] On the contrary, reduction of enones **3**–**5**

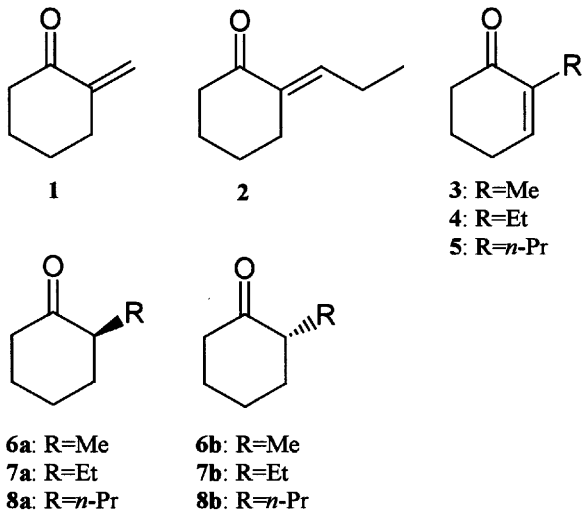

Table 1. Reduction of enones, **1** - **5**, with the enone reductases, p44, p74 and p90, from the cultured cells of *N. tabacum*

Substrate	p74 Reductase				p90 Reductase				p44 Reductase			
	Product	Conv.[a] / %	E.e.[b]	Config.[c]	Product	Conv.[a] / %	E.e.[b]	Config.[c]	Product	Conv.[a] / %	E.e.[b]	Config.[c]
1	**6a**	99	97	*S*	--	0			--	0		
2	**8a**	85	75	*S*	--	0			--	0		
3	--	0			**6a**	95	99	*S*	**6b**	80	>99	*R*
4	--	0			**7a**	57	98	*S*	**7b**	45	>99	*R*
5	--	0			**8a**	35	95	*S*	**8b**	37	>99	*R*

[a]The conversions were expressed as the percentage of the product in the reaction mixture on the basis of GLC. [b]Enantiomeric excess on the basis of GLC on CP-cyclodextrin β 236M-19 column. [c]Preferred configuration at the α-position to the carbonyl group of the products.

with the p44 reductase gave highly optically pure (*R*)-2-alkylcyclohexanones, **6b**–**8b**.[18] These results indicate that the stereospecificity of the hydrogenation of the C-C double bond was opposite between the p90 and p44 reductases.

Thus, it was demonstrated that the enone reductases from *N. tabacum* were able to reduce enantiotropically the C-C double bond of enones to afford optically active 2-alkylated ketones. It is worth noting that each enantiomer of 2-alkylated ketones can be synthesized by selective use of these enone reductases from *N. tabacum*.

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References and Notes

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7 The suspension cells of *N. tabacum*[1] were cultivated at 25 °C for 3 weeks in Murashige and Skoog's medium[8] on a rotary shaker (75 rpm). The cells (200 g) were homogenized in 0.1 M Na–Pi buffer (pH 6.8) containing 10 mM 2-mercaptoethanol and 5 mM dithiothreitol. After centrifugation at 10000 *g* for 15 min, the supernatant was fractionated by treatment with $(NH_4)_2SO_4$ (40 to 60% satn.). The crude enzyme soln obtained was desalted and then applied to a DEAE–Toyopearl column with a 0–0.5 M linear gradient of NaCl in 50 mM Tris-HCl buffer (pH 8.0) containing 1.0 mM 2-mercaptoethanol and 1.0 mM dithiothreitol (buffer A) to give three enone reductase fractions, which contained the 44, 74 and 90 kDa proteins, respectively. Each of the fractions was further purified on a hydroxylapatite column with buffer A and a Red–Toyopearl column with buffer A containing a 0–1.0 M linear gradient of NaCl.
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9 The mixture (2 mL) of enone (100 μmol), NADPH (200 μmol), Triton X-100 (0.1%), and enzyme preparation (5 μg) in 50 mM Tris-HCl buffer (pH 8.0) containing 1.0 mM 2-mercaptoethanol and 1.0 mM dithiothreitol was incubated at 35 °C for 8 h. After incubation, the reaction mixture was subjected to GLC and GC-MS analyses, and then the products were purified by column chromatography on silica gel. The absolute configurations and enantiomeric purities of the products were determined by circular dichroism (CD) spectra and the peak area of the corresponding enantiomers in the GLC analyses on CP cyclodextrin β 236M-19 column.
10 2-Alkylidenecyclohexanones (**1** and **2**) were prepared from the α-alkylidene acetals of cyclohexanone according to the reported procedure.[11,12] **1**: MS (EI) *m/z* 110 (M^+); ^{1}H NMR (500 MHz; $CDCl_3$) δ 6.01 (1H, t, *J* = 1.9 Hz, >C=CH_2), 6.12 (1H, t, *J* = 1.9 Hz, >C=CH_2); **2**: MS (EI) *m/z* 138 (M^+), ^{1}H NMR ($CDCl_3$) δ 1.05 (3H, t, *J* = 7.6 Hz, Me), 2.11 (2H, q, *J* = 7.6 Hz, –C$\underline{H}_2$–Me), 6.61 (1H, tt, *J* = 7.4 and 2.1 Hz, >C=CH–). 2-Alkyl-2-cyclohexen-1-ones (**3**–**5**) were prepared from *O*-methoxybenzoic acid by reductive alkylation according to the reported precedure.[13] **3**: MS (EI) *m/z* 110 (M^+), ^{1}H NMR ($CDCl_3$) δ 1.77 (3H, q, *J* = 1.5 Hz, Me), 6.74 (1H, tq, *J* = 4.3 and 1.5 Hz, >C=CH–); **4**: MS (EI) *m/z* 124 (M^+), ^{1}H NMR ($CDCl_3$) δ 1.00 (3H, t, *J* = 7.5 Hz, Me), 2.20 (2H, qq, *J* = 7.5 and 1.5 Hz, –C$\underline{H}_2$–Me), 6.69 (1H, tt, *J* = 4.1 and 1.5 Hz, >C=CH–); **5**: MS (EI) *m/z* 138 (M^+), ^{1}H NMR ($CDCl_3$) δ 0.89 (3H, t, *J* = 7.4 Hz, Me), 1.41 (2H, se, *J* = 7.5 Hz, –C$\underline{H}_2$–Me), 2.15 (2H, tq, *J* = 7.4 and 1.2 Hz, –C$\underline{H}_2$–CH_2–Me), 6.69 (1H, t, *J* = 4.3 Hz, >C=CH–).
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14 **6a:** $[\theta]_{288}$ +960 (*c* 0.25, MeOH) {lit.[15] $[\theta]_{288}$ –987 for *R* enantiomer}; **8a**: $[\theta]_{288}$ +1860 (*c* 0.25, MeOH) {lit.[16] $[\theta]_{288}$ +2480}.
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17 **6a**: $[\theta]_{288}$ +993 (*c* 0.3, MeOH); **7a**: $[\theta]_{288}$ +2293 (*c* 0.1, MeOH); **8a**: $[\theta]_{288}$ +2351 (*c* 0.12, MeOH).
18 **6b**: $[\theta]_{288}$ –989 (*c* 0.2, MeOH); **7b**: $[\theta]_{288}$ –2341 (*c* 0.1, MeOH) {lit.[15] $[\theta]_{288}$ +2200 for *S* enantiomer}; **8b**: $[\theta]_{288}$ –2483 (*c* 0.1, MeOH).