Asymmetric Hydrogenation of *N*-substituted maleimides by the Cultured Plant Cells

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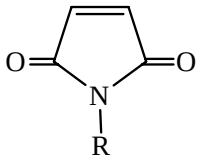
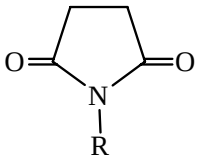
Abstract: The cultured suspension cells of higher plants hydrogenated the C-C double bond of *N*-substituted maleimides to afford corresponding succinimides. Hydrogenation of *N*-phenyl-2-methylmaleimide by the cultured cells of *Nicotiana tabacum* was highly-enantiospecific to give (*R*)-*N*-phenyl-2-methylsuccinimide (99% e.e.).

Asymmetric reduction of compounds with a prochiral center is a useful method for the production of chiral synthons for organic synthesis.[1] Recently, we reported the enzymatic hydrogenation of enones with discrimination of its enantiotopic faces to afford optically active ketones.[2] In the course of the development of new asymmetric reduction, we have now investigated the enantioface selective hydrogenation of maleimides by the cultured cells of *Nicotiana tabacum*.

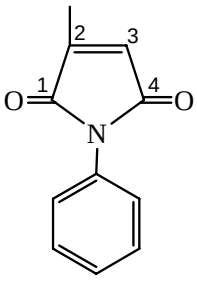
N-Substituted maleimides **1**~**3** (each 20 mg) was administered to the flask containing the cultured suspension cells of *N. tabacum* or *Cathranthus roseus* (20 g)[3] in MS medium[5] (100 ml), and the cultures were incubated at 25 °C for 1 or 5 days. The yields of products were determined by GLC of the product. It was found that the C-C double bond of the maleimides **1**~**3** was reduced to give succinimide derivatives **4**~**6**, respectively, as shown in Table 1. Especially *N*-phenylmaleimide (**3**) was completely hydrogenated in one day's incubation to give *N*-phenylsuccinimide (**6**) in over 99% conversion. These show that the cultured cells of *N. tabacum* have high potentiality for the reduction of the C-C double bond of the maleimides.



1: R=Methyl
2: R=Butyl
3: R=Phenyl



4: R=Methyl
5: R=Butyl
6: R=Phenyl



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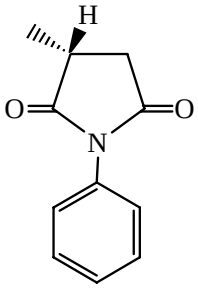

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Therefore, we next examined the ability of the cultured cells for discriminating enantiotopic face of maleimides. *N*-Phenyl-2-methylmaleimide (**7**), having a prochiral center at C-2 position, was used as a

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substrate, and was reduced by the cultured suspension cells of *N. tabacum* under analogous condition as above. After one day's incubation, (*R*)-*N*-phenyl-2-methylsuccinimide (**8**)[8,9] was obtained in over 99% conversion. Enantiomeric purity of the product was 99% *e.e.* on the basis of the peak analysis in ^{1}H NMR spectra of the product with Eu(hfc)$_3$.[10] The result demonstrates that the cultured cells have the ability for discriminating the enantiomeric face of the maleimide and hydrogenating the C-C double bond enantiospecifically to give 2-methylsuccinimide having *R*-configuration.

Table 1. Hydrogenation of maleimides by the cultured cells of higher plants

Substrates	Products	Cultured cells	Reaction time / day	Conversion / %[a)]	E.e./ %[b)]	Configuration
1	**4**	*N. tabacum*	5	49		
		C. roseus	5	19		
2	**5**	*N. tabacum*	5	84		
		C. roseus	5	86		
3	**6**	*N. tabacum*	5	>99		
		N. tabacum	1	>99		
		C. roseus	5	>99		
7	**8**	*N. tabacum*	1	>99	99	*R*

a) The conversions were expressed as the percentage of the products in the reaction mixture on the basis of GLC analysis.

b) The enantiomeric excess was calculated on the peak analysis of the ^{1}H NMR of the product with chiral shift reagent, Eu(hfc)$_3$.

Thus, asymmetric hydrogenation of 2-methylmaleimide with cultured cells of *N. tabacum* has been realized with discrimination of the enantiotopic face of the C-C double bond of the maleimide and optically active 2–substituted succinimide was prepared. It is fascinating to note that the enantioface selective hydrogenation of 2-alkylated maleimide derivatives with cultured plant cells as a biocatalyst is one of the useful methods for the chiral generation. The investigation of enzymes which catalyze such an asymmetric hydrogenation in *N. tabacum* is now in progress.

References

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3. Suspension cells of *N. tabacum*[4] were cultured in 500 ml conical flasks containing 200 ml Murashige and Skoog's (MS) medium[5] supplemented with 3% sucrose and 10 mM 2,4-dichlorophenoxyacetic acid (2,4-D) under illumination (4000 lux). On the other hand, suspension cells of *C. roseus*[6] were cultured in 500 ml conical flasks containing 200 ml of SH medium[7] supplemented with 3% sucrose and 10 mM 2,4-D under illumination (4000 lux). Each suspension cells were cultivated on a rotary shaker (75 rpm) at 25 °C for 3 weeks prior to use for biotransformation experiments.
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8. Product **8**: $[\alpha]_D^{25}$ +6.6±0.8 (c 0.56, $CHCl_3$) (lit.[9] $[\alpha]_D^{22}$ +8±0.4 (c 1.2, $CHCl_3$) for *R*-enantiomer); IR (in $CHCl_3$) 1712 cm^{-1} (C=O); CD (c 0.52, $CHCl_3$) [θ] -76.9; ^1H NMR (500 MHz, $CDCl_3$) δ 1.46 (3H, d, *J*=7.1 Hz, 2-Me), 3.04 (1H, ddq, *J*=9.3, 4.6, and 7.3 Hz, 2-H), 2.51 (1H, dd, *J*=17.7 and 4.5 Hz, 3-Ha), 3.10 (1H, dd, *J*=17.6 and 9.3 Hz, 3-Hb), 7.29 (2H, d, *J*=8.3 Hz, *o*-H), 7.39 (1H, t, *J*=7.4 Hz, *p*-H), 7.47 (2H, t, *J*=7.7 Hz, *m*-H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 16.9 (Me), 34.9 (CH), 36.7 (CH_2), 126.4 (*o*-C in Ph), 128.6 (*p*-C in Ph), 129.1 (*m*-C in Ph), 132.0 (N-C in Ph), 175.4 (C=O), 179.5 (C=O).
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10. Methyl proton signals of racemic *N*-phenyl-2-methylsuccinimide in the ^1H NMR spectrum were revealed at δ 2.64 (d, *J*=7.0 Hz; relative integral value=100) and 2.56 (d, *J*=7.0 Hz; integral value=100) in the $CDCl_3$ solution of the sample and $Eu(hfc)_3$ (1:1 mol. ratio). On the other hand, the ^1H NMR of the product **8** under the same condition showed the methyl proton signals at δ 2.64 (d, *J*=7.0 Hz; integral value=0.55) and 2.56 (d, *J*=7.0 Hz; integral value=100).