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Tetrahedron: *Asymmetry* 15 (2004) 15–16
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Asymmetric hydrogenation of *N*-substituted maleimides by cultured plant cells

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Received 12 September 2003; accepted 21 October 2003

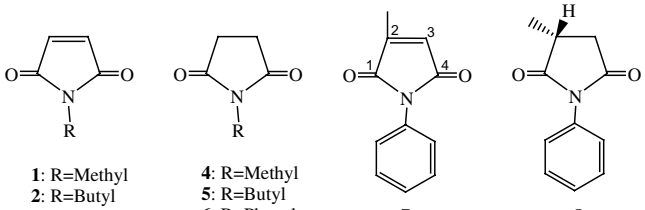
Abstract—The cultured suspension cells of higher plants hydrogenated the C–C double bond of *N*-substituted maleimides to afford the corresponding succinimides. Hydrogenation of *N*-phenyl-2-methylmaleimide by the cultured cells of *Nicotiana tabacum* proved to be highly enantioselective and gave (*R*)-*N*-phenyl-2-methylsuccinimide (99% ee).

The 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] Over the course of developing a new asymmetric reduction, we investigated the enantiofacially selective hydrogenation of maleimides by the cultured cells of *Nicotiana tabacum*.

N-Substituted maleimides **1**–**3** (20 mg each) were administered to flasks containing the cultured suspension cells of *N. tabacum* or *Cathranthus roseus* (20 g)[3] in MS medium[5] (100 mL). The cultures were then 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 bonds of the maleimides **1**–**3** were reduced to give the succinimide derivatives **4**–**6**, respectively, as shown in Table 1. In particular, *N*-phenylmaleimide **3** was completely hydrogenated within one day's incubation to give *N*-phenylsuccinimide **6** with over 99% conversion. These show that the cultured cells of *N. tabacum* have high potential for the reduction of the C–C double bond of the maleimides.

We therefore, examined next the ability of the cultured cells for discriminating the enantiotopic faces of maleimides. *N*-Phenyl-2-methylmaleimide **7**, having a prochiral center at C-2 position, was used as a substrate, and was reduced by the cultured suspension cells of *N. tabacum* under the same analogous conditions as described above. After one day's incubation, (*R*)-*N*-phenyl-2-methylsuccinimide **8**[8,9] was obtained with over 99% conversion. Enantiomeric purity of the product was 99% ee based on the peak analysis of the ^{1}H NMR of the product with Eu(hfc)$_3$.[10] These results demonstrate that the cultured cells have the ability for (i) discriminating the enantiomeric face of the maleimide and (ii) hydrogenating the C–C double bond enantioselectively to give 2-methylsuccinimide with an (*R*)-configuration.

Thus, the asymmetric hydrogenation of 2-methylmaleimide with cultured cells of *N. tabacum* has been reported along with the discrimination of the enantiotopic faces



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

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

7 **8**

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doi:10.1016/j.tetasy.2003.10.033

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

Substrates	Products	Cultured cells	Reaction time (day)	Conversion (%)[a]	Ee (%)[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 expressed as a 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)$_3$.

of the C–C double bond of the maleimide hence producing an optically active 2-substituted succinimide. It is worthy of note that the enantiofacially selective hydrogenation of 2-alkylated maleimide derivatives using cultured plant cells as biocatalysts is one of the more useful methods for chiral generation. The investigation of the enzymes, which catalyze such an asymmetric hydrogenation in *N. tabacum* is currently in progress.

References and Notes

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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 of 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 cell was 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 (*c* 0.56, $CHCl_3$) {lit[9] $[\alpha]_D^{22}$ +8 (*c* 1.2, $CHCl_3$) for (*R*)-enantiomer}; IR (in $CHCl_3$) 1712 cm^{-1} (C=O); CD (*c* 0.52, $CHCl_3$) $[\theta]$ −76.9; ^{1}H 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 ^{1}H 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 a $CDCl_3$ solution of the sample and Eu(hfc)$_3$ (1:1 mol ratio). On the other hand, the ^{1}H NMR of the product **8** under the same conditions 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).