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Hydrogenation of the C–C double bond of maleimides with cultured plant cells

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Abstract

The cultured suspension cells of plants and cyanobacterium, such as *Nicotiana tabacum*, *Catharanthus roseus*, *Marchantia polymorpha*, *Parthenocissus tricuspidata*, *Gossypium hirsutum* and *Cynechococcus* sp. PCC 7942, have a potentiality to hydrogenate the C–C double bond of N-substituted maleimides to afford corresponding succinimides. Hydrogenation of *N*-phenyl-2-methylmaleimide by the cultured cells of *N. tabacum*, *M. polymorpha* and *Cynechococcus* sp. was highly enantioselective to give (*R*)-*N*-phenyl-2-methylsuccinimide.

Keywords: Maleimides; Cultured cells; Hydrogenation

1. Introduction

Asymmetric hydrogenation of compounds with a prochiral face is a useful method for the production of chiral synthons for organic synthesis [1–9]. Recently, we reported the enzymatic hydrogenation of the C–C double bond of enones with discrimination of its enantiotopic faces to afford optically active ketones [10–14]. In the course of the development of new asymmetric reactions, we have investigated the biotransformation of maleimides by the cultured plant cells and found a enantioface selective hydrogenation of the C–C double bond of maleimides [15]. This paper will give a full detail of the results.

2. Experimental

2.1. Analysis

Analytical and preparative TLCs were carried out on glass sheets (0.25 mm and 0.5 mm) coated with silica gel (Merck silica gel 60; GF_{254}). GLC was carried out with FID and a capillary column (0.25 mm × 25 m) coated with 0.25 μm CP cyclodextrin β 236M-19 (WCOT) using N_2 as carrier gas (50 $cm^3\,min^{-1}$) at column temperature 130 °C. NMR spectra were measured on a JEOL LA500 [500 MHz (^1H) and 125 MHz (^{13}C)] NMR spectrometer. GC-MS analyses were performed on a JEOL JMS-700 mass spectrometer combined with a GC instrument under conditions as follows: a glass capillary column (0.20 mm × 50 m) coated with TC-1 (GL Science Ltd.) using N_2 as carrier gas; injector temp. 160 °C; column temperature programming at 2° min^{-1} from 100 to 160 °C. Mass spectra were obtained on the above described

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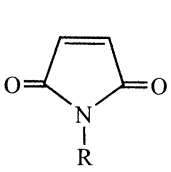
spectrometer conducted with an ionizing energy of 70 eV at 250 °C of the ion source temp.

2.2. Plant materials

Cultured cells of *Nicotiana tabacum* [16], *Parthenocissus tricuspidata*, and *Gossypium hirsutum* have been maintained in our laboratory for over 10 years under subculturing on MS medium [17] containing 3% of sucrose, 10 mM of 2,4-dichlorophenoxyacetic acid (2,4-D) and 1% of agar at 25 °C for every 3–5 weeks. Cultured cells of *Catharanthus roseus* [18] have been maintained in our laboratory for approximately 10 years under subculturing on SH medium [19] containing 3% of sucrose, 10 mM of 2,4-D and 1% of agar at 25 °C for every 3–5 weeks. Cultured cells of *Marthantia polymorpha* [20] have been maintained in our laboratory for approximately 10 years under subculturing on MSK-II medium [21] containing 2% of glucose, 0.1% inositol, 10 mM of 2,4-D and 1% of agar at 25 °C for every 3–5 weeks. Cultured cells of *Cynechococcus* sp. PCC 7942 [22] have been maintained in our laboratory for over 5 years under subculturing on BG-11 medium [23] under illumination (2000 lux) on a rotary shaker (120 rpm) at 25 °C for every 3–5 weeks.

2.3. Biotransformation of N-substituted maleimides (1–3 and 7) with cultured plant cells

Prior to use for biotransformation experiments, each suspension cells of *N. tabacum*, *P. tricuspidata*, *G. hirsutum*, *C. roseus*, and *M. polymorpha* were cultured in 300 ml conical flasks containing 100 ml of the proper medium for these cells on a rotary shaker (75 rpm) at 25 °C for 3 weeks under illumination (4000 lux). The culture media used were a MS medium supplemented with 3% sucrose and 10 mM 2,4-2,4-D for the cells of *N. tabacum*, *P. tricuspidata* and *G. hirsutum*, a SH medium supplemented with 3% sucrose and 10 mM 2,4-D for that of *C. roseus*, and a MSK-II medium supplemented with 2% glucose, 0.1% inositol and 10 mM 2,4-D for of *M. polymorpha*.



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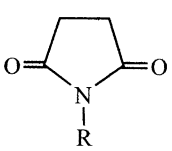
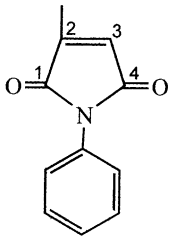
2: R=Butyl
3: R=Phenyl



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



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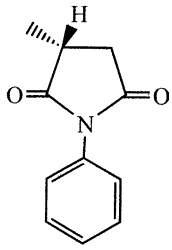

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To the flask containing the suspension cells (about 20 g) in the medium (100 ml), N-substituted maleimides **1**–**3** and *N*-phenyl-2-methylmaleimide (**7**) (each 20 mg) in dimethylsulfoxide (0.2 ml) was administered, and the cultures were incubated at 25 °C on a rotary shaker (75 rpm) under illumination (4000 lux). After the incubation, the cells and medium were separated by filtration with suction. The cells were extracted with MeOH and the extract was concentrated by evaporation under reduced pressure. The methanolic fraction was partitioned between H_2O and diethyl ether. The filtered medium was extracted with diethyl ether. The diethyl ether extracts from the cells and the culture medium were combined and the solvent was evaporated under reduced pressure to give crude products. The crude products were subjected to preparative TLC with hexane:EtOAc (3:2) to give corresponding N-substituted succinimides. The conversion rates were determined by GLC, as shown in Table 1. The structure of each product was confirmed by direct comparison of GLC and MS, and/or ^{1}H and ^{13}C NMR spectral data with their authentic samples, which were prepared by the catalytic hydrogenation with Pd-C from the corresponding N-substituted maleimides (**1**–**3** and **7**) (Sigma).

N-Methylsuccinimide (**4**): MS *m/z* (rel. int.) 113 [100, M^+], 85 (21), 84 (19), 56 (100); ^{1}H NMR ($CDCl_3$) δ 2.71 (s, ^{4}H, $-CH_2-CH_2-$) and 2.99 (s, ^{3}H, Me); ^{13}C NMR ($CDCl_3$) δ 24.8 (Me), 28.2 (CH_2) and 177.3 (C=O).

N-Buthylsuccinimide (**5**): MS *m/z* (rel. int.) 155 [45, M^+], 126 (15), 100 (100), 84 (25).

N-Phenylsuccinimide (**6**): MS *m/z* (rel. int.) 175 [100, M^+], 147 (50), 119 (100), 93 (95), 77 (50), 55 (56); ^{1}H NMR ($CDCl_3$) δ 2.91 (s, ^{4}H, $-CH_2-CH_2-$) and 7.2–7.5 (5H, phenyl proton); ^{13}C NMR ($CDCl_3$) δ 28.2 (CH_2), 126.2 (3′- and 5′-C), 128.0 (2′- and 6′-C), 129.0 (1′-C), 131.8 (4′-C), and 176.5 (C=O).

N-Phenyl-2-methylsuccinimide (**8**): $[\alpha]_D^{25} + 6.6 \pm 0.8$ (c 0.56, $CHCl_3$) (lit. [24] $[\alpha]_D^{22} + 8 \pm 0.4$ (c 1.2, $CHCl_3$) for *R*-enantiomer); IR ($CHCl_3$) 1712 cm^{-1} (C=O); CD (c 0.52, $CHCl_3$) $[\theta]_{272}$ −76.9; ^{1}H NMR ($CDCl_3$) δ 1.46 (^{3}H, d, J = 7.1 Hz, 2-Me), 3.04 (^{1}H, ddq, J = 9.3, 4.6, and 7.3 Hz, 2-H), 2.51 (^{1}H, dd, J = 17.7 and 4.5 Hz, 3-Ha), 3.10 (^{1}H, dd, J = 17.6 and 9.3 Hz, 3-Hb), 7.29 (^{2}H, d, J = 8.3 Hz, *o*-H), 7.39 (^{1}H, t, J = 7.4 Hz, *p*-H), 7.47 (^{2}H, t, J = 7.7 Hz, *m*-H); ^{13}C NMR ($CDCl_3$) 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). Optical purity of the product was calculated based on the peak analysis of the methyl proton signals of the ^{1}H NMR with chiral shift reagent, Eu(hfc)$_3$ (Sigma Ltd.) [25]. The methyl proton signals of racemic *N*-phenyl-2-methylsuccinimide were observed 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

Table 1
Hydrogenation of N-substituted maleimides (**1**–**3**) by the cultured suspension cell

Substrates	Products	Cultured cells	Incubation time (day)	Conversion (%)[a]
1	**4**	*N. tabacum*	5	49
		C. roseus	5	19
		P. tricuspidata	5	16
		G. hirsutum	5	25
		M. polymorpha	5	>99
			1	>99
		Cynechococcus sp.	0.5	>99
2	**5**	*N. tabacum*	5	84
		C. roseus	5	86
		P. tricuspidata	5	50
		G hirsutum	5	29
		M. polymorpha	5	>99
		Cynechococcus sp.	0.5	>99
3	**6**	*N. tabacum*	5	>99
			1	>99
		C. roseus	5	>99
			1	95
		P. tricuspidata G	5	99
		P. tricuspidata G	5	>99
		P. tricuspidata G	5	>99
			1	>99
		Cynechococcus sp.	0.5	>99

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

of the sample and $Eu(hfc)_3$ (1:1 mol. ratio). On the other hand, the relative integral values of the methyl proton signals at δ 2.64 (d, $J = 7.0$ Hz) to that of 2.56 (d, $J = 7.0$ Hz) were 0.55–100 for the incubation product with *N. tabacum* cells and 2.28–100 for the incubation product with *M. polymorpha* cells.

2.4. Biotransformation of N-phenyl-2-methymaleimide (7) with cultured suspension cells of Cynechococcus sp.

Suspension cells of *Cynechococcus* sp. PCC 7942 were cultured in 500 ml conical flask containing 300 ml of BG-11 medium [20] under illumination (2000 lux) on a rotary shaker (120 rpm) at 25 °C for 3 weeks. To the flask (about 2 g of the cells), *N*-phenyl-2-methylmaleimide (**7**) (10 mg) in dimethylsulfoxide (0.1 ml) was administered, and the cultures were incubated at 25 °C for 30 min on a rotary shaker (120 rpm) under illumination (2000 lux). After incubation, the cultures were extracted with EtOAc. The extract was concentrated under reduced pressure and then subjected to preparative TLC with hexane:EtOAc (3:2) to give *N*-phenyl-2-methylsuccinimide (6 mg). The yield of the product was determined by GLC analysis of the EtOAc extract by use of a standard curve prepared with authentic sample. Optical purity of the product was determined on the peak analysis of the ^{1}H NMR with chiral shift reagent, $Eu(hfc)_3$ under the same conditions as above. The relative integral value of the methyl proton signals at δ 2.64 (d, $J = 7.0$ Hz) to that of 2.56 (d, $J = 7.0$ Hz) was 0.45–100.

2.5. Time course experiments in the biotransformation of N-substituted maleimides

Each 20 g of the cultured plant cells, such as *N. tabacum* and *M. polymorpha*, was portioned to five flasks containing 100 ml of the medium. *N*-Phenylmaleimide (**3**) (10 mg) was administered to the each flask and the mixtures were incubated on a rotary shaker (75 rpm) at 25 °C under illumination (4000 lux). In the case of the biotransformation with *Cynechococcus* sp. PCC 7942, the suspension cells (about 2 g) were portioned to six flasks containing 100 ml of BG-11 medium. *N*-Phenylmaleimide (**3**) (10 mg) was administered to the flasks and the mixtures were incubated on a rotary shaker (120 rpm) at 25 °C under illumination (2000 lux).

At a regular time interval, one of the cultures was extracted with EtOAc. The conversion rates of the product were evaluated on the basis of the peak ratio of the substrate and product in the GLC analyses of the EtOAc extract.

3. Results and discussion

N-Substituted maleimides **1**–**3** was incubated with cultured suspension cells of plants and cyanobacterium, such as *N. tabacum*, *C. roseus*, *M. polymorpha*, *P. tricuspidata*, *G. hirsutum* and *Cynechococcus* sp.. 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 transformed by the incubation for 5 days with those cultured

Table 2
Hydrogenation of *N*-phenyl-2-methylmaleimide (**7**) by the cultured suspension cells

Substrate	Product	Cultured cells	Incubation time (h)	Yield (%)[a]	e.e. (%)[b]	Configuration
7	**8**	*N. tabacum*	24	75	99	*R*
7	**8**	*M. polymorpha*	12	86	98	*R*
7	**8**	*Cynechococcus* sp.	l	61	99	*R*

[a] The yield of the product was determined by GLC analysis of the reaction mixture by use of a standard curve prepared with authentic sample.
[b] The enantiomeric excess was calculated on the peak analysis of the ^{1}H NMR of the product with chiral shift reagent, Eu(hfc)$_3$.

plant cells and for 12 h with *Cynechococcus* sp. to give *N*-phenylsuccinimide (**6**) in over 99% conversion yield. These show that the cultured plant cells have an ability to reduce the C–C double bond of the maleimides. To determine the potentiality of these cultured cells for the reduction of maleimides, the time course in the biotransformation was followed. It was found that the potentiality of the cultured cells for the biotransformation of the maleimides was very high and was higher in the order of *Cynechococcus* sp., *M. polymorpha* and *N. tabacum*, as shown in Fig. 1.

Therefore, we next examined the ability of the cultured cells for discriminating enantiotopic face of the C–C double bond of maleimides. *N*-Phenyl-2-methylmaleimide (**7**) was used as a substrate, and was reduced by the cultured suspension cells of *N. tabacum*, *M. polymorpha* and *Cynechococcus* sp. (*R*)-*N*-Phenyl-2-methylsuccinimide (**8**) was obtained as a product in 61–86% yields, as shown in Table 2. Enantiomeric purity of the product was 98–99% e.e. on the basis of the peak analysis in ^{1}H NMR spectra of the product with Eu(hfc)$_3$ [25]. The result demonstrates that the cultured cells have the ability for discriminating the enantiomeric face of the maleimide.

Thus, it was found that the cultured suspension cells of plants and cyanobacterium have a potentiality to hydrogenate the C–C double bond of N-substituted maleimides to afford the corresponding succinimides. The hydrogenation of maleimides has been realized with discrimination of the enantiotopic face of the C–C double bond of 2-methylmaleimide derivative to give optically pure (*R*)-2–methylsuccinimide derivative. 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.

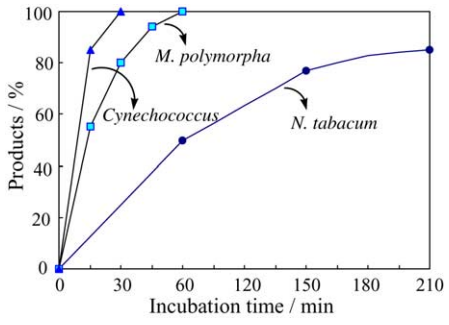

Fig. 1. Time course in the biotransformation of *N*-phenyl-maleimide (**3**) with the cultured cells of *Cynechococcus* sp., *M. polymorpha* and *N. tabacum*.

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