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Tetrahedron: Asymmetry
Tetrahedron: *Asymmetry* 17 (2006) 1859–1862

Asymmetric hydrogenation of the C–C double bond of 1- and 1,2-methylated maleimides with cultured suspension cells of *Marchantia polymorpha*

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Received 22 May 2006; accepted 20 June 2006
Available online 21 July 2006

Abstract—Suspension cultured cells of *Marchantia polymorpha* have the potential to hydrogenate the C–C double bonds of 2-methyl- and 2,3-dimethylmaleimide derivatives to give enantiomerically pure (2*R*)-2-methyl- and (2*R*,3*R*)-2,3-dimethylsuccinimide derivatives, respectively.

1. Introduction

It is well known that transformation using biocatalysts allows structural modification to provide useful chiral substances under mild and ecologically compatible conditions. We have previously found the ability of cultured plant cells to hydrogenate the C–C double bond of enones with discrimination of its enantiotopic faces to the corresponding optically active ketones.[1–4] In the course of the development of new chiral hydrogenation reactions, we recently found that the hydrogenation of the C–C double bond of *N*-phenyl-2-methylmaleimide by cultured plant cells was highly enantioselective.[5,6] In continuation of this work, we have now studied the reactivity and enantioselectivity in the reduction of 2-methylated and 2,3-dimethylated maleimide derivatives with cultured cells of *Marchantia polymorpha*.

2. Results and discussion

In our previous work, we studied the biotransformation of N-substituted maleimides with several plant cells, such as *Nicotiana tabacum*, *Catharanthus roseus*, *Parthenocissus tricuspidata*, and *M. polymorpha*, and found that the cultured cells of *M. polymorpha* have a high potential for the hydrogenation of the C–C double bond of maleimides.[6] Therefore, we selected the cultured suspension cells of *M. polymorpha* as a biocatalyst and carried out the biotransformation of N-substituted 2-methylmaleimides (**1** and **2**), 2,3-dimethylmaleimides **3**, and *N*-phenylitaconimide **5**. It was found that the endocyclic C–C double bond of **1**–**4** was hydrogenated to give the corresponding succinimides **6**–**9**, respectively, as shown in Table 1. Substrates **2** and **3** were completely transformed to the succinimides **7** and **8**, respectively, by incubation for 5 days. However, the substrate **5** having an exocyclic C–C double bond was not hydrogenated by the cultured cells. In the biotransformation of the dimethylmaleimides **3** and **4**, only *trans*-dimethylsuccinimides **8** and **9** were produced, respectively, but *cis*-dimethylsuccinimides **10** and **11** were not found as a product, in spite of careful GLC analyses of the reaction mixtures. This indicates that *anti*-addition of the hydrogen atoms occurs in hydrogenation of the C–C double bond of the maleimides.

To clarify the effect of the N-substituted groups, such as phenyl, benzyl, and *p*-methoxyphenyl, for reactivity in the reduction of C–C double bond with cultured cells of *M. polymorpha*, the time courses in the biotransformation of N-substituted maleimides, **1**–**4** and **12**, were followed. As shown in Figure 1, it was found that the presence of a *p*-methoxyphenyl group decreases the reactivity slightly.

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doi:10.1016/j.tetasy.2006.06.030

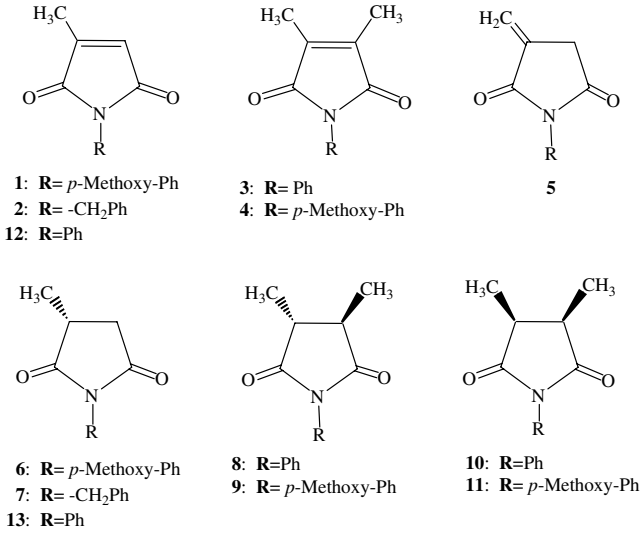

Table 1. Biotransformation of N-substituted maleimides, **1**–**4**, by cultured cells of *M. polymorpha*

Substrate	Product	Incubation time (d)	Conversion (%)	ee (%)	Configuration
1	**6**	5	89	99	*R*
2	**7**	5	99	>99	*R*
3	**8**	5	99	99	*R*,*R*
4	**9**	5	77	>99	*R*,*R*

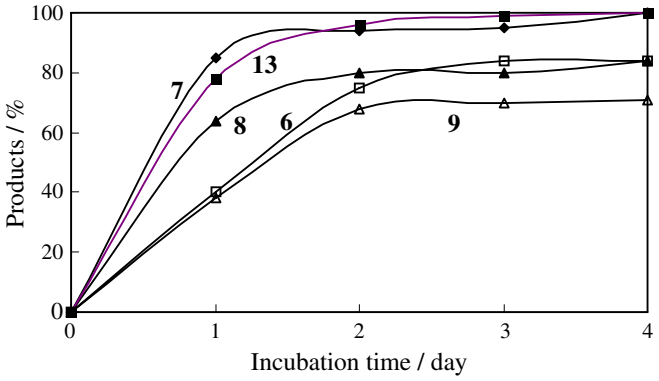

Figure 1. Time-course experiments in the biotransformation of maleimide substrates (**1**–**4** and **12**) into succinimide derivatives with cultured cells of *M. polymorpha*.

Although clear evidences for the difference have not been obtained, it would presumably be a consequence of the electronic effect of the *p*-OMe substituent.

Absolute configuration and enantiomeric purity of the products were determined by their specific rotations, the peak analysis of the ^{1}H NMR with chiral shift reagent, and peak intensity of GLC with a chiral column. Optical rotations of *N*-*p*-methoxyphenyl-2-methylsuccinimide **6** and *N*-benzyl-2-methylsuccinimide **7**, obtained by the biotransformation of N-substituted 2-methylmaleimides, were dextro-rotatory. Since the optical rotation of (*R*)-2-methylsuccinimide derivative was reported to be dextro-rotatory,[6,7] absolute configurations of the products, **6** and **7**, should be *R*. The peak analyses of the ^{1}H NMR spectra with a chiral shift reagent[8] and the GLC with a chiral column showed the enantiomeric purities of **6** and **7** to be 99% ee.

On the other hand, the optical rotations of 2,3-dimethylsuccinimides, **8** and **9**, which were obtained by the biotransformation of N-substituted 2,3-dimethylmaleimides, **3** and **4**, were dextro-rotatory. This indicated that *N*-phenyl-2,3-dimethylsuccinimide **8** and *N*-*p*-methoxyphenyl-2,3-dimethylsuccinimide **9**, had 2*R*,3*R*-configuration, because the optical rotation of (2*R*,3*R*)-2,3-dimethylsuccinimide was reported to be dextro-rotatory.[9] The peak analyses of the ^{1}H NMR spectrum with a chiral shift reagent showed the enantiomeric purities of **8** and **9** to be 99% ee.

Thus, it was found that the cultured suspension cells of *M. polymorpha* have an ability to hydrogenate enantioselectively the C–C double bond of 2- and 2,3-methylated maleimides to afford highly enantiomerically pure succinimide derivatives. Recently, Oishi et al. reported the utilization of a chiral 2,3-dimethylsuccinimide derivative as a model compound for the development of a polymer having chiral recognition ability.[10] It is fascinating to note that the enantioface selective hydrogenation of the 2- and 2,3-alkylated maleimide derivatives with cultured plant cells as a biocatalyst is one of the useful methods for the generation of chirality. In addition, the optically active succinimides, obtained by these biotransformations, could be useful chiral building blocks for the synthesis of biologically active natural products, for example, pyrrolizidine and indolizidine alkaloids.

3. Experimental

3.1. General experimental procedures

Analytical and preparative TLCs were carried out on glass sheets coated with silica gel (0.25 mm or 0.5 mm; Merck silica gel 60 GF_{254}). The NMR spectra were measured with a JEOL LA-500 (500 MHz, ^{1}H; 125 MHz, ^{13}C) spectrometer with TMS as an internal standard. Optical rotations were measured with a JASCO DIP-370 digital polarimeter. GLCs with FID were carried out on a capillary column (0.25 mm × 30 m) coated with 0.25 μm ZB-5 (Zebron) using N_2 as carrier gas (60 cm^3 min^{-1}) at column temperature programmed from 180 to 250 °C at 5 °C/min; injector temperature 250 °C and on a chiral capillary column (0.25 mm × 20 m) coated with 0.25 μm CHIRALDEX G-TA (Tokyokasei) using N_2 as carrier gas (60 cm^3 min^{-1}) at column temperature programmed from 150 to 180 °C at 5 °C/min; injector temperature 180 °C. HPLCs were carried out on Puresil C_{18} column (Waters) using CH_3CN/H_2O = 2:3 (v/v) as the eluent.

3.2. Substrates

N-*p*-Methoxyphenyl-2-methylmaleimide **1**, *N*-benzyl-2-methylmaleimide **2**, *N*-phenyl-2,3-dimethylmaleimide **3**, *N*-*p*-methoxyphenyl-2,3-dimethylmaleimide **4**, and *N*-phenylitaconimide **5** were prepared by use of a combination of acid anhydrides such as 2,3-dimethylmalonic anhy-

dride, citraconic anhydride, and itaconic anhydride and amines such as *p*-methoxy aniline and benzyl amine, according to the reported procedure.[11]

An amine and acid anhydride were mixed in high pressure vessel, which was capped with a teflon screw. The vessel was submerged in oil bath at 120 °C overnight. The reaction mixture (yellow solid) was dissolved with ethyl acetate and filtrated through a short silica gel column. The ethyl acetate solution was concentrated after addition of 10% NaOH solution. The residue was purified by column chromatography on silica gel eluted with ethyl acetate and *n*-hexane (1:9) to give the substrates, **1**, **2**, **3**, **4**, and **5**.

3.2.1. *N*-*p*-Methoxyphenyl-2-methylmaleimide 1. ^{1}H NMR ($CDCl_3$) δ 2.18 (3H, d, $J = 1.5$ Hz, 2-Me), 3.84 (3H, s, OMe), 6.48 (1H, q, $J = 1.5$ Hz, 3-H), 6.98 (2H, d, $J = 8.5$ Hz, 2′- and 6′-H), 7.25 (2H, d, $J = 8.5$ Hz, 3′- and 5′-H); ^{13}C NMR ($CDCl_3$) δ 11.1 (2-Me), 55.5 (OMe), 114.4 (C-2′ and C-6′), 124.2 (C-1′), 127.3 (C-3), 127.5 (C-3′ and C-5′), 145.7 (C-2), 158.9 (C-4′), 169.9 (C-1), 170.3 (C-4).

3.2.2. *N*-Benzyl-2-methylmaleimide 2. ^{1}H NMR ($CDCl_3$) δ 2.01 (3H, d, $J = 1.5$ Hz, 2-Me), 6.48 (1H, q, $J = 1.5$ Hz, 3-H), 4.60 (2H, s, 7′-H_2), 7.20 (2H, d, $J = 8.5$ Hz, 2′- and 6′-H), 7.26 (3H, m, 3′-, 4′-, and 5′-H); ^{13}C NMR ($CDCl_3$) δ 10.8 (Me), 41.3 (C-7′), 127.3 (C-2′ and C-6′), 127.6 (C-3′ and C-5′), 128.2 (C-4′), 128.8 (C-3), 136.4 (C-1′), 145.6 (C-2), 170.0 (C-1), 171.3 (C-4).

3.2.3. *N*-Phenyl-2,3-dimethylmaleimide 3. ^{1}H NMR ($CDCl_3$) δ 2.03 (6H, s, 2- and 3-Me), 7.25–7.35 (3H, m, 2′-, 4′- and 6′-H), 7.45 (2H, t, $J = 8.5$ Hz, 3′- and 5′-H); ^{13}C NMR ($CDCl_3$) δ 8.8 (2- and 3-Me), 125.7 (C-2′ and C-6′), 127.4 (C-1′), 128.9 (C-3′ and C-5′), 131.9 (C-4′), 137.4 (C-2 and C-3) 170.4 (C-1 and C-4).

3.2.4. *N*-*p*-Methoxyphenyl-2,3-dimethylmaleimide 4. ^{1}H NMR ($CDCl_3$) δ 2.07 (6H, s, 2- and 3-Me), 3.84 (3H, s, OMe), 6.98 (2H, d, $J = 9.0$ Hz, 2′- and 6′-H), 7.25 (2H, d, $J = 9.0$ Hz, 3′- and 5′-H); ^{13}C NMR ($CDCl_3$) δ 8.8 (2- and 3-Me), 55.5 (OMe) 114.3 (C-2′ and C-6′), 124.6 (C-1′), 127.3 (C-3′ and C-5′), 137.3 (C-2 and C-3), 158.7 (C-4′), 171.2 (C-1 and C-4).

3.2.5. *N*-Phenylitaconimide 5. ^{1}H NMR ($CDCl_3$) δ 3.43 (2H, t, $J = 2.3$ Hz, 3-H), 5.67 (1H, t, $J = 2.1$ Hz, 5-Ha), 6.37 (1H, t, $J = 2.3$ Hz, 5-Hb), 7.23 (2H, d, $J = 7.5$ Hz, 2′- and 6′-H), 7.32 (2H, t, $J = 7.5$ Hz, 4′-H), 7.40 (2H, t, $J = 7.5$ Hz, 3′- and 5′-H); ^{13}C NMR ($CDCl_3$) 41.0 (C-3), 123.7 (C-2′ and C-6′), 124.1 (C-5), 125.2 (C-4′), 127.5 (C-3′ and C-5′), 131.5 (C-1′), 179.1 (C-1) and 187.6 (C-4).

3.3. Plant material

The cells of *M. polymorpha*[12] have been subcultured every 3 weeks using MSK-II medium[13] containing 2% glucose, 0.1% inosoitol, 10 mM of 2,4-dichrolophenoxyacetic acid (2,4-D) for more than 10 years in our laboratory. Prior to use for biotransformation experiments, the cultured cells were transplanted to a 300 ml conical flask containing 100 ml of MSK-II medium with 2% glucose, 0.1% inositol, 10 mM of 2,4-D, and cultured on a rotary shaker (110 rpm) for 10 days at 25 °C under illumination (4000 lx).

3.4. Biotransformation of maleimides 1–5 with cultured plant cells of *M. polymorpha*

To the flask containing the suspended cells (about 20 g) of *M. polymorpha* in MSK-II medium (100 ml), each substrate **1**–**5** (20 mg) in DMSO (0.2 ml) was administered, and the cultures were incubated at 25 °C on a rotary shaker (110 rpm) under illumination (4000 lx). After the incubation, the cell and medium were separated by filtration with suction. The filtrated medium was extracted with diethyl ether and the extract was concentrated by rotary evaporator to give crude extracts. These crude extracts were subject to preparative TLC with ether and *n*-hexane (3:1) to give products **6**–**9** in a pure form. The conversion yields of the products were determined by GLC analysis of the crude extracts, as shown in Table 1. The structure of each product was identified by NMR and MS analyses.

3.4.1. *N*-*p*-Methoxyphenyl-2-methylsuccinimide 6. $[\alpha]_D^{25} = +4.0 \pm 0.3$ (*c* 0.13, $CHCl_3$) {lit.[7] $[\alpha]_D^{25} = +8 \pm 0.4$ (*c* 1.2, $CHCl_3$) for (*R*)-*N*-phenyl-2-methylsuccinimide}; IR ($CHCl_3$) 1718 cm^{-1} (CO); ^{1}H NMR ($CDCl_3$) δ 1.48 (3H, d, $J = 7.0$ Hz, 2-Me), 3.06 (1H, ddq, $J = 11.5$, 3.5, and 7.0 Hz, 2-H), 2.53 (1H, dd, $J = 13.5$ and 4.0 Hz, 3-Ha), 3.12 (1H, dd, $J = 13.5$ and 11.5 Hz, 3-Hb), 3.86 (3H, s, OMe), 7.01 (2H, d, $J = 8.5$ Hz, 3′- and 5′-H), 7.23 (2H, d, $J = 8.5$ Hz, 2′- and 6′-H); ^{13}C NMR ($CDCl_3$) δ 16.9 (2-Me), 34.8 (C-3), 36.4 (C-2), 55.5 (OMe), 114.4 (C-2′ and C-6′), 124.6 (C-1′), 127.1 (C-3′ and C-5′), 176.0 (C-4), 179.7 (C-1). The enantiomeric purity of the product was determined on the basis of the peak analysis of the methyl proton signals of the ^{1}H NMR with chiral shift reagent, Eu(hfc)$_3$ (Sigma Ltd.).[8] The methyl proton signals of racemic *N*-*p*-methoxyphenyl-2-methylsuccinimide were observed at δ 2.15 (d, $J = 7.5$ Hz; relative integral value = 100) and 2.22 (d, $J = 7.5$ Hz; integral value = 100) in the $CDCl_3$ solution of the sample and Eu(hfc)$_3$ (1:1 mol ratio). In the NMR spectrum of the product **6** with Eu(hfc)$_3$, the ratio of relative integral value of the methyl proton signals at δ 2.15 (d, $J = 7.5$ Hz) and 2.22 (d, $J = 7.5$ Hz) was about 99.5–0.5.

3.4.2. *N*-Benzyl-2-methylsuccinimide 7. $[\alpha]_D^{25} = +14.0 \pm 0.7$ (*c* 0.18, $CHCl_3$); IR ($CHCl_3$) 1703 cm^{-1} (C=O); ^{1}H NMR ($CDCl_3$) δ 1.27 (3H, d, $J = 6.5$ Hz, 2-Me), 2.76 (1H, ddq, $J = 11.5$, 3.5 and 6.5 Hz, 2-H), 2.25 (1H, dd, $J = 12.5$ and 3.5 Hz, 3-Ha), 2.78 (1H, dd, $J = 12.5$ and 11.5 Hz, 3-Hb), 4.58 (2H, s, 7′-H), 7.25 (3H, m, 3′-, 4′- and 5′-H), 7.32 (2H, d, $J = 6.5$ Hz, 2′- and 6′-H); ^{13}C NMR ($CDCl_3$) δ 16.6 (2-Me), 34.7 (C-3), 36.4 (C-2), 42.3 (C-5), 127.8 (C-4′), 128.6 (C-2′ and C-6′), 128.7 (C-3′ and C-5′), 135.8 (C-1′), 176.0 (C-4), 180.2 (C-1). The methyl proton signals of racemic *N*-benzyl-2-methylsuccinimide were observed at δ 2.07 (d, $J = 6.5$ Hz; relative integral value = 100) and 2.31 (d, $J = 6.5$ Hz; integral value = 100) in the $CDCl_3$ solution of the sample and Eu(hfc)$_3$ (1:1 mol ratio). The relative intensity of the methyl proton signal in the ^{1}H NMR of the product **7** with

$Eu(hfc)_3$ was over 99.5% in the signal at δ 2.07 (d, $J = 6.5$ Hz).

3.4.3. *N*-Phenyl-2,3-dimethylsuccinimide 8. $[\alpha]_D^{25} = +21.0 \pm 0.8$ (c 0.03, $CHCl_3$) (lit.[9] $[\alpha]_D^{20} = +53.6 \pm 0.4$ (c 1.4, $CHCl_3$) for (2*R*,3*R*)-2,3-dimethylsuccinimide); IR ($CHCl_3$) 1709 cm^{-1} (C=O); ^1H NMR ($CDCl_3$) δ 1.42 (6H, d, $J = 7.0$ Hz, 2- and 3-Me), 2.35 (2H, dq, $J = 12.5$ and 6.0 Hz, 2- and 3-H), 7.28 (2H, d, $J = 7.5$ Hz, 2′- and 6′-H), 7.35 (2H, t, $J = 7.5$ Hz, 4′-H), 7.45 (2H, t, $J = 7.5$ Hz, 3′- and 5′-H); ^{13}C NMR ($CDCl_3$) δ 15.2 (2- and 3-Me), 43.2 (C-2 and C-3), 126.3 (C-2′ and C-6′), 128.4 (C-1′), 129.0 (C-3′ and C-5′), 132.0 (C-4′), 178.4 (C-1 and C-4). The methyl proton signals of racemic *N*-phenyl-2,3-dimethylsuccinimide were observed at δ 2.36 (d, $J = 5.5$ Hz; relative integral value = 100) and 2.49 (d, $J = 5.5$ Hz; integral value = 100) in the $CDCl_3$ solution of the sample and $Eu(hfc)_3$ (1:1 mol ratio). The relative intensity of the methyl proton signal in the ^1H NMR of the product **8** with $Eu(hfc)_3$ was over 99.5% in the signal at δ 2.36 (d, $J = 5.5$ Hz). The enantiomeric purity of the product was confirmed on the basis of peak analysis using GLC on chiral column: retention times in the GLC were 12.60 for (2*R*,3*R*)-**8** and 12.37 for (2*S*,3*S*)-**8**, and the peak ratio indicated that the enantiomeric excess of (2*R*,3*R*)-**8** is about 99%.

3.4.4. *N*-*p*-Methoxyphenyl-2,3-dimethylsuccinimide 9. $[\alpha]_D^{25} = +15.0 \pm 0.4$ (c 0.08, $CHCl_3$); IR ($CHCl_3$) 1709 cm^{-1} (C=O); ^1H NMR ($CDCl_3$) δ 1.41 (6H, d, $J = 6.0$ Hz, 2- and 3-Me), 2.55 (2H, dq, $J = 12.5$ and 6.0 Hz, 2- and 3-H), 3.80 (3H, s, OMe), 6.94 (2H, d, $J = 8.5$ Hz, 2′- and 6′-H), 7.17 (2H, d, $J = 8.5$ Hz, 3′- and 5′-H); ^{13}C NMR ($CDCl_3$) δ 15.1 (2- and 3-Me), 43.2 (C-2 and C-3), 55.4 (OMe) 114.4 (C-2′ and C-6′), 124.6 (C-1′), 127.6 (C-3′ and C-5), 158.0 (C-4′), 178.4 (C-1 and C-4). The methyl proton signals of racemic *N*-*p*-methoxyphenyl-2,3-dimethylsuccinimide were observed at δ 2.07 (d, $J = 6.0$ Hz; relative integral value = 100) and 2.16 (d, $J = 6.0$ Hz; integral value = 100) in the $CDCl_3$ solution of the sample and $Eu(hfc)_3$ (1:1 mol ratio). The relative intensity of the methyl proton signal in the 1H NMR of the product **9** with $Eu(hfc)_3$ was over 99.5% in the signal at δ 2.07 (d, $J = 6.0$ Hz).

3.5. Time-course experiments

Cultured cells of *M. polymorpha* (about 20 g) were transferred to several 300 ml Erlenmeyer flasks containing 100 ml MSK-II medium and each (20 mg) of the substrates, **1**–**4** and **12**, was added to the suspension cultures and incubated at 25 °C in rotary shaker (110 rpm) under illumination. At a regular time interval, one of the flasks was taken out and filtrated. The filtrate was extracted with diethyl ether. The conversion yield of the product was determined on the basis of the peak ratio in the GLC and HPLC analyses of the ether extract.

3.6. Preparation of racemic N-substituted *trans*-2,3-dimethylsuccinimides

N-Substituted *trans*-2,3-dimethylsuccinimides were prepared by the isomerization of N-substituted *cis*-2,3-dimethylsuccinimides, according to the reported procedures.[10] To a solution of *N*-phenyl-*cis*-2,3-dimethylsuccinimide **10** (15 mg, 0.073 mmol) in DMSO (0.4 ml), three drops of Sp [L-(−)-sparteine] (Aldrich) were added and the mixture was heated at 60 °C for 8 days in oil bath. The reaction mixture was subjected to silica gel column chromatography (hexane/ethyl acetate = 1/1, v/v) to give racemic *N*-phenyl-*trans*-2,3-dimethylsuccinimide. The conversion of the *cis*-2,3-dimethylsuccinimide to the *trans*-isomer was 85%, on the basis of the peak ratio of the GLC analysis of the crude product.

Racemic *N*-*p*-methoxyphenyl-*trans*-2,3-dimethylsuccinimide was also prepared from *N*-*p*-methoxyphenyl-*cis*-2,3-dimethylsuccinimide **11** in a yield of 83% by a similar procedure as above.

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