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Asymmetric reduction of 2-substituted 2-butenolides with reductase from *Marchantia polymorpha*

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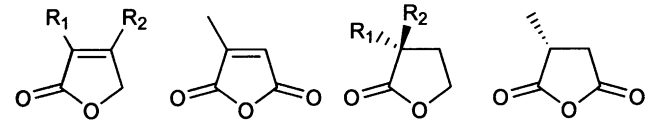
Abstract—A p68 reductase participating in the asymmetric reduction of the C–C double bond of 2-substituted 2-butenolides was isolated from *Marchantia polymorpha*. The enzyme reduced 2-substituted 2-butenolides to give (*R*)-butanolides, and the reduction of citraconic anhydride afforded (*R*)-methylsuccinic anhydride.

Optically active α-substituted γ-lactones such as 2-hydroxybutanolide (HBL) are versatile chiral building blocks for drugs, natural products, and ferroelectric liquid crystals.[1–3] Lactonohydrolase from *Fusarium oxosporum* has already been used for the industrial production of chiral HBLs; enantiomeric resolution of racemic HBL catalyzed by this enzyme gives (*S*)-HBL and (*R*)-2,4-dihydroxybutanoic acid with high enantiomeric purities.[4] Because the maximum yield of chiral products obtained in the resolution process is theoretically 50%, the asymmetric induction process which gives the chiral products directly may be more advantageous.[5,6] A few studies on the yeast-mediated reduction of 3-substituted 2-butenolides to 3-substituted (*S*)-butanolides, that is, β-chiral γ-lactones, have been reported.[6] However, little attention has been paid to the enzymatic production of α-chiral γ-lactones such as optically active HBLs by the reductases participating in the hydrogenation of C–C double bonds. In the course of developing a new asymmetric reduction, we investigated the enantiofacially selective reduction of 2-substituted 2-butenolides and citraconic anhydride by the reductase isolated from plant cell cultures of the liverwort *Marchantia polymorpha*.

The reduction system with cultured cells of *M. polymorpha* was preliminarily tested for its ability to reduce the C–C double bond of 2-butenolide **1**. After 1 day incubation of **1** (30 mg) with 50 g of suspension cells of *M. polymorpha*[7] at 25 °C under illumination (4000 lux), **1** was reduced to butanolide **6** with >99% conversion. To examine the enzymatic reduction of 2-butenolides, the reductase was purified from *M. polymorpha*. A crude enzyme fraction extracted from *M. polymorpha* with 50 mM Na-phosphate buffer (pH 7.0) was subjected onto diethylaminoethyl-Toyopearl column chromatography, which gave a crude reductase fraction that reduces **1** to **6**. Further purification by chromatography on a Blue-Toyopearl column and then a Sephadex G-200 column gave homogeneous reductase as judged by SDS-PAGE: the isolated p68 reductase was a dimer composed of two identical 34 kDa subunits.



1: R_1,R_2=H
2: R_1=OH, R_2=H
3: R_1=Me, R_2=H
4: R_1=H, R_2=Me

5

6: R_1,R_2=H
7: R_1=OH, R_2=H
8: R_1=Me, R_2=H

9

Next, the enzymatic reduction of 2-butenolides with the isolated p68 reductase was examined. The reaction was carried out at 35 °C for 4 or 8 h in a mixture consisting of 10 mL of 25 mM Na-phosphate buffer (pH 7.0), the reductase (ca. 15 μg), 2 mM substrate, and 5 mM NADH. The products were identified by comparison of their TLC, GLC, and GC–MS data with those of authentic samples, and the conversions of the products were determined by GLC analyses. The absolute configuration of the products was confirmed by comparing the

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retention times of the resulting butanolide or the corresponding acetyl ester in the GLC with chiral stationary phase with those of authentic optically active samples along with determination of specific rotation of the products.[8,9] The enantiomeric purities of the products were determined based on the peak areas of the corresponding enantiomers or acetates in the GLC on Rt-β DEX.[8] As a result, it was found that the C–C double bond of **1** was reduced to give **6** with >99% conversion by the p68 reductase after 4 h (Table 1), showing that the p68 reductase has potential for the reduction of **1**. To examine the enantioselectivity of the reduction at the 2-position of 2-butenolides by the p68 reductase, **2** and **3** were used as substrates. After 8 h incubation, **2** was reduced to (*R*)-HBL **7** in >99% conversion and the chiral GLC analysis of acetates of the product **7** showed that the hydrogenation at the 2-position occurred with excellent enantioselectivity (>99% ee) (Fig. 1b). This suggests that the asymmetric reduction with the p68 reductase is very useful for the practical preparation of (*R*)-HBL in enantiomerically pure form. The reduction of **3** likewise gave **8** of >99% ee in 99% conversion. On the other hand, no reduction occurred in the case of 3-substituted 2-butenolide **4**, indicating that the p68 reductase isolated from *M. polymorpha* is a novel enzyme apparently different from the reductase in yeast with respect to the substrate specificity. A challenging substrate for the asymmetric reduction, citraconic anhydride **5**, was reduced to give enantiomerically pure (*R*)-methylsuccinic anhydride **9** (100% ee)[10,11] which is a potentially useful chiral building block for organic and polymer syntheses.[11,12] These results demonstrate that the p68 reductase from *M. polymorpha* is able to catalyze the enantioface-discriminating hydrogenation of the C–C double bond of 2-substituted 2-butenolides to give (*R*)-butanolides and capable of reducing citraconic anhydride to give (*R*)-methylsuccinic anhydride.

Thus, a novel plant reductase which catalyzes the hydrogenation of 2-substituted 2-butenolides has been isolated from *M. polymorpha*. It should be emphasized that the new asymmetric reduction of 2-substituted 2-butenolides and citraconic anhydride using the reductase from *M. polymorpha* as a biocatalyst is one of the most useful methods for chiral generation; this method is useful for practical preparation of 2-substituted (*R*)-butanolides, such as (*R*)-HBL, and (*R*)-methylsuccinic anhydride in enantiomerically pure forms.

Table 1. Reduction of 2-butenolides and citraconic anhydride with the p68 reductase from *M. polymorpha*

Substrates	Products	Reaction time (h)	Conversion (%)[a]	Ee (%)	Configuration
1	**6**	4	>99	—	—
2	**7**	8	>99	>99	*R*
3	**8**	8	99	>99	*R*
4	—	8	0	—	—
5	**9**	4	>99	100	*R*

[a] Percentage of the products in the reaction mixture on the basis of GLC analyses.

References

1. (a) Ohfune, Y.; Tomita, M.; Nomoto, K. *J. Am. Chem. Soc.* **1981**, *103*, 2409; (b) Walba, D. M. In *Advances in the Synthesis and Reactivity of Solids*; Lallouk, T. E., Ed.; JAI: London, 1991; Vol. 1, p 173; (c) Vidari, G.; Lanfranchi, G.; Sartori, P.; Serra, S. *Tetrahedron: Asymmetry* **1995**, *6*, 2977; (d) Mendlik, M. T.; Cottard, M.; Rein, T.; Helquist, P. *Tetrahedron Lett.* **1997**, *38*, 6375; (e) Dioubankova, N. N.; Malakhov, A. D.; Stetsenko, D. A.; Korshun, V. A.; Gait, M. J. *Org. Lett.* **2002**, *4*, 4607; (f) Storer, R. I.; Takemoto, T.; Jackson, P. S.; Brown, D. S.; Baxendale, I. R.; Ley, S. V. *Chem. Eur. J.* **2004**, *10*, 2529; (g) Rodeschini, V.; Boiteau, J. G.; Van, W. P.; Tarnus, C.; Eustache, J. *J. Org. Chem.* **2004**, *69*, 357.
2. Shiuey, S. J.; Partridge, J. J.; Uskokovic, M. R. *J. Org. Chem.* **1988**, *53*, 1040.
3. Leuenberger, G. W.; Boguth, W.; Barner, R.; Schmid, M.; Zell, R. *Helv. Chim. Acta* **1979**, *62*, 455.
4. (a) Shimizu, S.; Kataoka, M. In *Encyclopedia of Bioprocess Technology: Fermentation, Biocatalysis, and Bioseparation*; Flickinger, M. C., Drew, S. W., Eds.; John Wiley & Sons: New York, 1999; p 1571; (b) Shimizu, S.; Kataoka, M. In *Encyclopedia of Bioprocess Technology: Fermentation, Biocatalysis, and Bioseparation*; Flickinger, M. C., Drew, S. W., Eds.; John Wiley & Sons: New York, 1999; p 1923.
5. For example: (a) Kergomard, A.; Renard, M. F.; Veschambre, H. *Tetrahedron Lett.* **1978**, 5197; (b) Kergomard, A.; Renard, M. F.; Veschambre, H. *J. Org. Chem.* **1982**, *47*, 792; (c) Desrut, M.; Kergomard, A.; Renard, M. F.; Veschambre, H. *Biochem. Biophys. Res. Commun.* **1983**, *110*, 908; (d) Kergomard, A.; Renard, M. F.; Veschambre, H.; Courtois, D.; Petiard, V. *Phytochemistry* **1988**, *27*, 407; (e) Hirata, T.; Izumi, S.; Shimoda, K.;

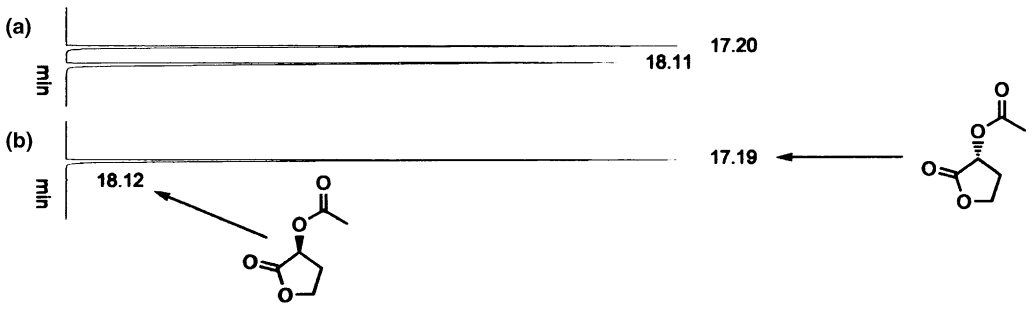

Figure 1. Chiral GLC analyses of acetates of (a) racemic 2-hydroxybutanolide and (b) the product **7** obtained in the reduction of **2** for the determination of enantioselectivity of enzymatic reduction.

Hayashi, M. *J. Chem. Soc., Chem. Commun.* **1993**, 1426; (f) Shimoda, K.; Ito, D. I.; Izumi, S.; Hirata, T. *J. Chem. Soc., Perkin Trans. 1* **1996**, 355; (g) Shimoda, K.; Hirata, T.; Noma, Y. *Phytochemistry* **1998**, *49*, 49; (h) Kawai, Y.; Hayashi, M.; Inaba, Y.; Saitou, K.; Ohno, A. *Tetrahedron Lett.* **1998**, *39*, 5225; (i) Shimoda, K.; Hirata, T. *J. Mol. Catal. B: Enzym.* **2000**, *8*, 255; (j) Kawai, Y.; Inaba, Y.; Tokitoh, N. *Tetrahedron: Asymmetry* **2001**, *12*, 309; (k) Kawai, Y.; Hayashi, M.; Tokitoh, N. *Tetrahedron: Asymmetry* **2001**, *12*, 3007; (l) Shimoda, K.; Izumi, S.; Hirata, T. *Bull. Chem. Soc. Jpn.* **2002**, *75*, 813; (m) Shimoda, K.; Kubota, N.; Hamada, H.; Kaji, M.; Hirata, T. *Tetrahedron: Asymmetry* **2004**, *15*, 1677; (n) Shimoda, K.; Kubota, N.; Hamada, H. *Tetrahedron: Asymmetry* **2004**, *15*, 2443; (o) Kurata, A.; Kurihara, T.; Kamachi, H.; Esaki, N. *Tetrahedron: Asymmetry* **2004**, *15*, 2837; (p) Fronza, G.; Fuganti, C.; Pinciroli, M.; Serra, S. *Tetrahedron: Asymmetry* **2004**, *15*, 3073.

6. (a) Takabe, K.; Tanaka, M.; Sugimoto, M.; Yamada, T.; Yoda, H. *Tetrahedron: Asymmetry* **1992**, *3*, 1385; (b) Takabe, K.; Hiyoshi, H.; Sawada, H.; Tanaka, M.; Miyazaki, A.; Yamada, T.; Katagiri, T.; Yoda, H. *Tetrahedron: Asymmetry* **1992**, *3*, 1399.
7. Katoh, K.; Ishikawa, M.; Miyake, K.; Ohta, Y.; Hirose, Y.; Iwamura, T. *Physiol. Plant.* **1980**, *49*, 241.
8. The chiral GLC analyses were performed with FID and a capillary column of Rt-βDEX (0.25 mm × 50 m) using N_2 as a carrier gas (injector, 180 °C; detector, 180 °C; make up, 50 mL min^{-1}). The absolute configuration and enantiomeric composition of the product **7** were determined by chiral GLC analysis of the corresponding acetates obtained quantitatively in the acetylation of **7** with acetic anhydride and pyridine. Retention times for (*R*)- and (*S*)-2-acetoxybutanolides in the GLC at the oven temperature of 140 °C were 17.2 and 18.1 min, respectively. The absolute configuration and optical purity of the product **8** were determined by chiral GLC analysis of **8**. Retention times for (*R*)- and (*S*)-2-methylbutanolides in the GLC at the oven temperature of 120 °C were 22.6 and 23.7 min, respectively.
9. In order to obtain the products adequate for measurement of optical rotation, the reaction was performed in a similar condition to the standard assay system except that the scale was enlarged; 0.50 mmol of **2** and 0.40 mmol of **3** was administered. Extraction from the reaction mixture with ether followed by purification using column chromatography on silica gel with pentane–ethyl acetate (95:5, v/v) gave the products (0.35 mmol of **7** and 0.29 mmol of **8**). The optical rotation data of the products **7** and **8** are as follows; **7**: $[\alpha]_D^{25} = +68.3$ (*c* 0.37, $CHCl_3$) {lit.[2] $[\alpha]_D^{25} = -65.2$ for (*S*)-enantiomer}; **8**: $[\alpha]_D^{25} = +24.9$ (*c* 0.34, $CHCl_3$) {lit.[3] $[\alpha]_D^{20} = -22.9$ for (*S*)-enantiomer}.
10. The absolute configuration of the product **9** (0.22 mmol of **9** was obtained in the reduction of 0.30 mmol of **5**) was determined to be *R* by the measurement of specific rotation of **9**; $[\alpha]_D^{25} = +34.8$ (*c* 0.28, MeOH) {lit.[11] $[\alpha]_D^{25} = -36.5$ for (*S*)-enantiomer}. The enantiomeric excess of **9** was determined by chiral GLC analysis of **9** on Rt-βDEX. Retention times for (*R*)- and (*S*)-methylsuccinic anhydrides in the GLC at the oven temperature of 120 °C were 35.5 and 36.3 min, respectively.
11. Blagbrough, I. S.; Coates, P. A.; Hardick, D. J.; Lewis, T.; Rowan, M. G.; Wonnacott, S.; Potter, B. L. *Tetrahedron Lett.* **1994**, *35*, 8705.
12. (a) Arason, K. M.; Bergmeier, S. C. *Org. Prep. Proc. Int.* **2002**, *34*, 339; (b) Getzler, Y. L.; Kundnani, V.; Lobkovsky, E. B.; Coates, G. W. *J. Am. Chem. Soc.* **2004**, *126*, 6842.