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Stereoselective reduction of 2-butenolides to chiral butanolides by reductases from cultured cells of *Glycine max*

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Abstract—The stereoselective reduction of 2-butenolides by two reductases, p51 and p83, from cultured plant cells of *Glycine max* was investigated. The reduction of 2-methyl-2-butenolide by p51 reductase produced (*R*)-2-methylbutanolide, whereas the reduction by p83 reductase gave (*S*)-2-methylbutanolide. Both reductases reduced 3-methyl-2-butenolide to (*R*)-3-methylbutanolide. The reduction of 2,3-dimethyl-2-butenolide by p51 reductase gave (2*R*,3*R*)-2,3-dimethylbutanolide, whereas the reduction by p83 reductase produced (2*S*,3*R*)-2,3-dimethylbutanolide. The reduction of 4-alkyl-2-butenolides with these reductases was accompanied by resolution of chiral centers affording (*R*)-4-alkylbutanolides.

Optically active γ-lactones, butanolides, are versatile chiral building blocks for drugs, natural products, and ferroelectric liquid crystals.[1–5] Many studies on the biological production of chiral butanolides by enantiomeric resolution of racemic butanolides with microorganisms have been reported so far.[6–9] Because the maximal 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. Asymmetric reduction of ketones and C–C double bonds catalyzed by reductases from microorganisms and plants has been one of the most important asymmetric induction processes.[10–16] There have been a few studies on the yeast-mediated reduction of 2-butenolides to give chiral butanolides.[17,18] However, little attention has been paid to the enzymatic reduction of 2-butenolides with reductases. Furthermore, there are no reports on the asymmetric reduction of 2,3-disubstituted 2-butenolides to chiral 2,3-disubstituted butanolides and on the reductive resolution of 4-substituted 2-butenolides to give chiral 4-substituted butanolides by a reductase, which contracts the stereocenter remote from the reaction center. This Letter reports, for the first time, the biological production of optically active butanolides including chiral 2,3-disubstituted butanolides and 4-substituted butanolides by the stereoselective reduction of butenolides with two reductases from cultured plant cells of *Glycine max*.

2-Butenolide (0.1 mmol) was administered to a 300 mL conical flask containing the suspension cultured cells of *G. max* (50 g) and 100 mL of MSK-II medium,[19] and the cultures were incubated at 25 °C for one day on a rotary shaker (120 rpm). 2-Butenolide was reduced to butanolide in >99% yield, showing that the cultured *G. max* cells have high potential for the reduction of 2-butenolides.

Next, the reductases were purified from the cultured suspension cells as follows. A crude enzyme fraction extracted from *G. max* with 50 mM Na–phosphate buffer (pH 7.0) was subjected onto diethylaminoethyl-Toyopearl column chromatography, which gave crude reductase fractions. Further purification by chromatography on a AF-Red Toyopearl column and then a Sephadex G-200 column gave homogeneous reductases as judged by SDS-PAGE: p51 reductase was a dimer composed of two identical 25.5 kDa subunits and p83 reductase was a dimer composed of two identical 41.5 kDa subunits. The enzymatic reduction of substituted 2-butenolides **1**–**5** with the isolated p51 reductase was examined. The reaction was carried out at 36 °C for 5 or 12 h in

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a mixture consisting of 10 mL of 50 mM Na–phosphate buffer (pH 7.0), the reductase (ca. 20 μg), 2 mM substrate, and 5 mM NADPH. The products were identified by comparison of their GLC, GC–MS, ^{1}H and ^{13}C NMR data[20] with those of authentic samples,[1,21] and the conversions of the products were determined by GLC analyses. The absolute configuration of the products was determined by comparing the retention times of the resulting butanolide in the chiral GLC on Rt-βDEX with those of authentic chiral samples.[1,21] The enantiomeric purities of the products were determined based on the peak areas of the corresponding enantiomers in the GLC on Rt-βDEX. The p51 reductase reduced enantioselectively the C=C double bond of 2-methylated substrate **1** to give (*R*)-butanolide **6a** with excellent optical purity (>99% ee) and yield (>99%) after 12 h incubation (Fig. 1). On the other hand, 3-methylated substrate **2** was reduced to (*R*)-butanolide **7** (99% ee, 85% yield), suggesting that the steric hindrance of 3-methyl group reduced the yield of the product. The 2,3-dimethyl-2-butenolide (**3**) was reduced to (2*R*,3*R*)-2,3-dimethylbutanolide (**8a**) with high enantiomeric excess (99% ee (99% de)) in 82% yield. Next, 4-substituted 2-butenolides **4** and **5** were subjected to the enzymatic reduction with p51 reductase. After 5 h incubation, racemic 4-propyl-2-butenolide (**4**) was reduced to (*R*)-4-propylbutanolide (**9**) with 53% ee in 43% yield. This suggests that reductive resolution of **4** occurred in the p51 reductase, which contracts the stereocenter remote from the reaction center.[22] When 4-butyl-2-butenolide (**5**) was used as substrate, (*R*)-4-butylbutanolide (**10**) was obtained in 67% ee and 40% yield (see Table 1).

Substrates **1**–**5** were next subjected to the enzymatic reduction by p83 reductase. The p83 reductase reduced **1** to (*S*)-butanolide **6b** with 98% ee in >99% yield, showing that the enantioselectivity at 2-position of 2-butenolide in the reduction with p83 reductase was opposite to that with p51 reductase. The substrate **2** was reduced to (*R*)-product **7** (99% ee, 99% yield). The p83 reductase reduced 2,3-dimethyl substrate **3** to (2*S*,3*R*)-product **8b** with 96% ee (98% de) in 90% yield. 4-Alkyl-2-butenolides **4** and **5** were reduced to (*R*)-products **9** and **10**, enantiomeric purities of which were ees of 80% and 87%.

Thus, the enantioselective reduction of 2-butenolides has been accomplished by two reductases from cultured plant cells of *G. max*. It is worth noting that the enantioselective formation of each enantiomer of 2-substituted butanolide has been achieved by selective use of these enzymes, which are different in enantioselectivity. Earlier, it has been reported that horse liver alcohol dehydrogenase-catalyzed oxidation of *cis*-2,3-dimethylbutane-1,4-diol gave (2*S*,3*R*)-2,3-dimethylbutanolide with 100% ee in 15% yield.[21] This Letter reports, for the first time, the enantioselective reduction of 2,3-dimethyl-2-butenolide to give both (2*R*,3*R*)- and (2*S*,3*R*)-2,3-dimethylbutanolide with high optical purity and yield, and the reductive resolution of 4-substituted 2-butenolides to afford chiral 4-substituted butanolides

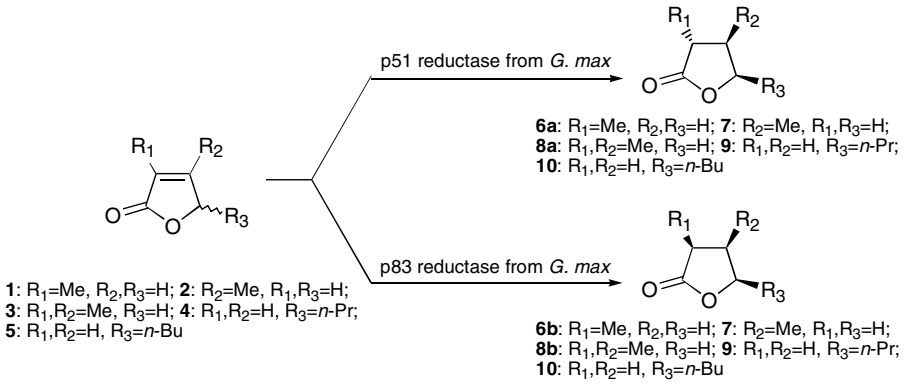

Figure 1. Stereoselective reduction of 2-butenolides **1**–**5** by the reductases from cultured cells of *G. max*.

Table 1. Stereoselective reduction of 2-butenolides to chiral butanolides with reductases from cultured plant cells of *G. max*

Reductase	Substrates	Products	Reaction time (h)	Conversion[a] (%)	ee (%)
p51 reductase	**1**	**6a**	12	>99	>99
	2	**7**	12	85	99
	3	**8a**	12	82	99
	4	**9**	5	43	53
	5	**10**	5	40	67
p83 reductase	**1**	**6b**	12	>99	98
	2	**7**	12	99	99
	3	**8b**	12	90	96
	4	**9**	5	47	80
	5	**10**	5	38	87

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

by reductases, which contracts the stereocenter remote from the reaction center. Further studies on the whole sequences of the reductases using molecular biological techniques to clarify the role of the reductases in *G. max* and to apply the expressed enzymes for large scale synthesis of chiral butanolides are currently in progress.

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20. In order to obtain the products adequate for spectroscopic analyses, the reaction was performed in a similar condition to the standard assay system except that the scale was 10–20-fold enlarged. 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. *Spectral data for the products*. Product **6a** (obtained in the reduction of **1** with p51 reductase): HREIMS *m/z* 100.0252 $[M]^+$; ^1H NMR (400 MHz, $CDCl_3$) δ 1.27 (3H, d, $J = 7.2$, CH_3), 1.93 (1H, m, CH), 2.43–2.63 (2H, m, CH_2), 4.17–4.37 (2H, m, CH_2); ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.7 (CH_3), 30.3 (CH), 33.7 (CH_2), 65.9 (CH_2), 179.7 (C=O). Product **6b** (obtained in the reduction of **1** with p83 reductase): HREIMS *m/z* 100.0255 $[M]^+$; ^1H and ^{13}C NMR data were entirely consistent with those for **6a**. Product **7** (obtained in the reduction of **2** with p51 reductase): HREIMS *m/z* 100.0251 $[M]^+$; ^1H NMR (400 MHz, $CDCl_3$) δ 1.15 (3H, d, $J = 6.8$, CH_3), 2.14–2.22 (2H, m, CH_2), 2.60–2.69 (1H, m, CH), 4.09–4.28 (2H, m, CH_2); ^{13}C NMR (100 MHz, $CDCl_3$): δ 17.7 (CH_3), 36.5 (CH), 37.2 (CH_2), 69.9 (CH_2), 180.1 (C=O). Product **8a** (obtained in the reduction of **3** with p51 reductase): HREIMS *m/z* 114.0681 $[M]^+$; ^1H NMR (400 MHz, $CDCl_3$) δ 1.16 (3H, d, $J = 6.2$ Hz, CH_3), 1.24 (3H, d, $J = 6.4$ Hz, CH_3), 1.91–2.27 (2H, m, 2CH), 3.73 (1H, t, $J = 9.0$ Hz, H_a–CH_2), 4.36 (1H, t, $J = 9.0$, H_b–CH_2); ^{13}C NMR (100 MHz, $CDCl_3$): δ 13.2 (CH_3), 15.8 (CH_3), 38.7 (CH), 41.6 (CH), 72.2 (CH_2), 180.0 (C=O). Product **8b** (obtained in the reduction of **3** with p83 reductase): HREIMS *m/z* 114.0680 $[M]^+$; ^1H NMR (400 MHz, $CDCl_3$) δ 0.95 (3H, d, $J = 6.8$ Hz, CH_3), 1.10 (3H, d, $J = 6.8$ Hz, CH_3), 2.52–2.65 (2H, m, 2CH), 3.87 (1H, d, $J = 9.0$ Hz, H_a–CH_2), 4.24 (1H, dd, $J = 9.0$, 5.6 Hz, H_b–CH_2); ^{13}C NMR (100 MHz, $CDCl_3$): δ 9.8 (CH_3), 13.2 (CH_3), 33.7(CH), 38.1 (CH), 73.1 (CH_2), 179.5 (C=O). Product **9** (obtained in the reduction of **4** with p83 reductase): HREIMS *m/z* 128.0837 $[M]^+$; ^1H NMR (400 MHz, $CDCl_3$) δ 1.05 (3H, t, $J = 6.4$ Hz, CH_3), 1.28–1.74 (4H, m, $2CH_2$), 2.11–2.72 (4H, m, $2CH_2$), 4.03 (1H, m, OCH); ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.0 (CH_3), 27.5 (CH_2), 33.6 (CH_2), 35.5 (CH_2), 36.9 (CH_2), 87.2 (CH), 176.6 (C=O). Product **10** (obtained in the reduction of **5** with p83 reductase): HREIMS *m/z* 142.0998 $[M]^+$; ^1H NMR (400 MHz, $CDCl_3$) δ 0.98 (3H, t, $J = 6.4$ Hz, CH_3), 1.31–1.73 (6H, m, $3CH_2$), 2.10–2.75 (4H, m, $2CH_2$), 4.01 (1H, m, OCH); ^{13}C NMR (100 MHz, $CDCl_3$): δ 13.9 (CH_3), 22.5 (CH_2), 27.9 (CH_2), 33.5 (CH_2), 36.3 (CH_2), 37.1 (CH_2), 87.0 (CH), 176.5 (C=O).
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