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Diastereoselective reduction of β-keto carbonyl compounds by cultured plant cells

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Abstract—The diastereoselective reduction of β-keto carbonyl compounds such as 2-benzamidomethyl-3-oxobutanoates and 2-methyl-2-(2-propenyl)cyclopentan-1,3-dione by cultured cells of higher plants was investigated. The reduction of the 2-benzamidomethyl-3-oxobutanoates by *Parthenocissus tricuspidata* diastereoselectively produced the (2*R*,3*S*)-2-benzamidomethyl-3-hydroxybutanoates, whereas the reduction by *Gossypium hirsutum* gave the (2*S*,3*S*)-2-benzamidomethyl-3-hydroxybutanoates. The (2*R*,3*S*)/(2*S*,3*S*) predominance in the reduction with *Nicotiana tabacum*, *Glycine max*, and *Catharanthus roseus* was reversed by the change in the structure of the alkoxyl group in the substrate. On the other hand, the reduction of 2-methyl-2-(2-propenyl)cyclopentan-1,3-dione by *P. tricuspidata* produced (2*R*,3*S*)-3-hydroxy-2-methyl-2-(2-propenyl)cyclopentan-1-one, whereas the reaction by *N. tabacum*, *G. max*, *C. roseus*, and *G. hirsutum* gave (2*S*,3*S*)-3-hydroxy-2-methyl-2-(2-propenyl)cyclopentan-1-one.

Optically active β-hydroxy carbonyl compounds such as 2-substituted 3-hydroxybutanoates and 2,2-disubstituted 3-hydroxycycloalkan-1-ones are versatile chiral synthons in the organic syntheses of biologically active compounds, especially in drug syntheses; chiral 2-benzamidomethyl-3-hydroxybutanoates are useful building blocks for the β-lactam antibiotics such as penems and carbapenems,[1,2] and optically active 3-hydroxy-2-methyl-2-(2-propenyl)cyclopentan-1-one is an important chiral synthon for prostaglandins.[3] Because the asymmetric reduction of the carbonyl compounds with biocatalysts is a very attractive method for the practical preparation of chiral alcohols, many studies on the biological reduction of 2-substituted 3-oxobutanoates and 2,2-disubstituted cyclopentan-1,3-diones by microorganisms, which generally afford a mixture of (2*R*,3*S*)- and (2*S*,3*S*)-isomers of 2-substituted 3-hydroxybutanoates, and (2*S*,3*S*)-isomers of 2,2-disubstituted 3-hydroxycyclopentan-1-ones, respectively, have already been reported.[4–17] However, little attention has been paid to the reduction of the 2-substituted 3-oxobutanoates[18] and the 2,2-disubstituted cyclopentan-1,3-diones by cultured plant cells. Furthermore, a cell-mediated process in which the 2-benzamidomethyl-3-oxobutanoate is reduced to essentially produce one diastereomer, either the (2*R*,3*S*)- or (2*S*,3*S*)-isomer of the 2-benzamidomethyl-3-hydroxybutanoate in enantiomerically pure form, and the 2,2-disubstituted cyclopentan-1,3-dione is transformed to (2*R*,3*S*)-isomer of 2,2-disubstituted 3-hydroxycyclopentan-1-one is still unavailable. Herein, we report the asymmetric reduction of the 2-benzamidomethyl-3-oxobutanoates and 2-methyl-2-(2-propenyl)cyclopentan-1,3-dione by cultured plant cells of *Parthenocissus tricuspidata*, *Nicotiana tabacum*, *Glycine max*, *Catharanthus roseus*, and *Gossypium hirsutum*.

Just prior to use for this work, 50 g of cultured cells was transplanted to a 300 mL conical flask containing 100 mL of freshly prepared medium (MS medium for *P. tricuspidata*, *N. tabacum*, and *G. max*; SH medium for *C. roseus* and *G. hirsutum*)[19,20] containing 3% sucrose and 10 mM 2,4-D and grown with continuous shaking for 1 week at 25 °C. A total of 120 mg of each substrate was administered to the 10 flasks (12 mg/flask, none solvent) containing the suspension cultures and the cultures were incubated at 25 °C for 3 days on a rotary shaker (120 rpm). After incubation the cultures were

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harvested and the medium was extracted with ethyl acetate. The yields of the products were determined by HPLC and GLC analyses. Purification of the ethyl acetate extracts by column chromatography on silica gel with pentane–ethyl acetate (95:5, v/v) gave the products. The structures of the products were determined on the basis of their HRFABMS, ^{1}H and ^{13}C NMR, H–H COSY, and C–H COSY.[21] The absolute configurations of the products obtained in the reduction of 2-benzamidomethyl-3-oxobutanoates were determined by comparing the retention times of the corresponding (*R*)-(+)-α-methoxy-α-(trifluoromethyl)phenylacetic acid (MTPA) esters in the HPLC analyses on Deverosil 100-3 column with those of the (*R*)-MTPA esters of authentic chiral samples.[22] The enantiomeric purities of the products were determined based on the peak areas of the corresponding (*R*)-MTPA esters in the HPLC. The absolute configurations and the optical purities of the products obtained in the reduction of 2-methyl-2-(2-propenyl)-cyclopentan-1,3-dione were determined by the procedure reported previously.[7] All of the five plant strains had a high potential for the reduction of methyl 2-benzamidomethyl-3-oxobutanoate (**1**) to give the corresponding β-hydroxy ester **4** (Table 1). The reduction with *P. tricuspidata* gave methyl (2*R*,3*S*)-2-benzamidomethyl-3-hydroxybutanoate (**4a**) (100% de) with an excellent enantioselectivity (>99% ee) in 97% yield. This suggests that the reductive resolution via an enol intermediate occurred in the *P. tricuspidata* cells.[9] The (2*R*,3*S*)-selectivity was also observed in the reduction with *N. tabacum*, *G. max*, and *C. roseus*. The reduction with these cultured cells proceeded with an excellent enantioselectivity to give the corresponding (3*S*)-β-hydroxy esters, for example, the reduction with *N. tabacum* afforded the (2*R*,3*S*)-isomer **4a** (50% de) with >99% ee and minor (2*S*,3*S*)-isomer **4b** with 99% ee. On the other hand, the reduction with *G. hirsutum* exhibited a (2*S*,3*S*)-selectivity to give methyl (2*S*,3*S*)-2-benzamidomethyl-3-hydroxybutanoate (**4b**) (84% de) with a high enantioselectivity [(2*R*,3*S*)-isomer **4a**: 98% ee; (2*S*,3*S*)-isomer **4b**: >99% ee] in 98% yield.

Next, ethyl 2-benzamidomethyl-3-oxobutanoate (**2**), which contained an ethoxyl group, was subjected to the same reduction system. The reduction with *P. tricuspidata* showed a (2*R*,3*S*)-selectivity to give ethyl (2*R*,3*S*)-2-benzamidomethyl-3-hydroxybutanoate (**5a**) (88% de) with an excellent enantioselectivity [(2*R*,3*S*)-isomer **5a**: >99% ee; (2*S*,3*S*)-isomer **5b**: 98% ee] in 82% yield. Interestingly, the reduction with *N. tabacum*, *G. max*, and *C. roseus* exerted a (2*S*,3*S*)-selectivity. The reduction of **2** with *G. hirsutum* exhibited an excellent (2*S*,3*S*)-selectivity to give ethyl (2*S*,3*S*)-2-benzamidomethyl-3-hydroxybutanoate (**5b**) (100% de) with a high enantioselectivity (>99% ee) and yield (97%).

These results demonstrate that the reduction of 2-benzamidomethyl-3-oxobutanoates by the cultured cells of *P. tricuspidata* diastereoselectively gives the (2*R*,3*S*)-2-benzamidomethyl-3-hydroxybutanoates, whereas the reaction with *G. hirsutum* cells affords the (2*S*,3*S*)-2-benzamidomethyl-3-hydroxybutanoates (Fig. 1). It is worth noting that the (2*R*,3*S*)/(2*S*,3*S*) predominance in the reduction with *N. tabacum*, *G. max*, and *C. roseus* is reversed by a change in the structure of the alkoxyl group in the substrate.

Further, 2-methyl-2-(2-propenyl)cyclopentan-1,3-dione (**3**) was used as the substrate. The reduction by *P. tricuspidata* exerted a (2*R*,3*S*)-selectivity to produce (2*R*,3*S*)-3-hydroxy-2-methyl-2-(2-propenyl)cyclopentan-1-one (**6a**) (58% de) with an excellent enantioselectivity [(2*R*,3*S*)-isomer **6a**: 99% ee; (2*S*,3*S*)-isomer **6b**: 95% ee] in 91% yield (Fig. 2). On the other hand, a (2*S*,3*S*)-selectivity was observed in the reduction with *N. tabacum*, *G. max*, *C. roseus*, and *G. hirsutum*, for example, the reduction with *G. hirsutum* gave (2*S*,3*S*)-3-hydroxy-2-methyl-2-(2-propenyl)cyclopentan-1-one (**6b**) with a high (2*S*,3*S*)-selectivity (96% de) [(2*R*,3*S*)-isomer **6a**: 78% ee; (2*S*,3*S*)-isomer **6b**: >99% ee]. These results suggest that **3** is held and reduced by the reductases from each cultured cells in the same manner as in the case of the reduction of **2**.

Table 1. Diastereoselective reduction of β-keto carbonyl compounds by the cultured plant cells

Substrates	Products	Cultured cells	Conversion (%)[a]	(2*R*,3*S*)/(2*S*,3*S*)	ee (%)	
					(2*R*,3*S*)	(2*S*,3*S*)
1	**4a**	*P. tricuspidata*	97	100/0	>99	—
		N. tabacum	95	75/25	>99	99
		G. max	>99	57/43	>99	98
		C. roseus	92	58/42	>99	98
	4b	*G. hirsutum*	98	8/92	98	>99
2	**5a**	*P. tricuspidata*	82	94/6	>99	98
	5b	*N. tabacum*	>99	7/93	91	>99
		G. max	>99	45/55	99	99
		C. roseus	86	10/90	99	>99
		G. hirsutum	97	0/100	—	>99
3	**6a**	*P. tricuspidata*	91	79/21	99	95
	6b	*N. tabacum*	99	4/96	84	>99
		G. max	96	35/65	99	99
		C. roseus	67	11/89	96	99
		G. hirsutum	93	2/98	78	>99

[a] The conversions were expressed as the percentage of the product in the reaction mixture on the basis of HPLC (**4** and **5**) and GLC (**6**).

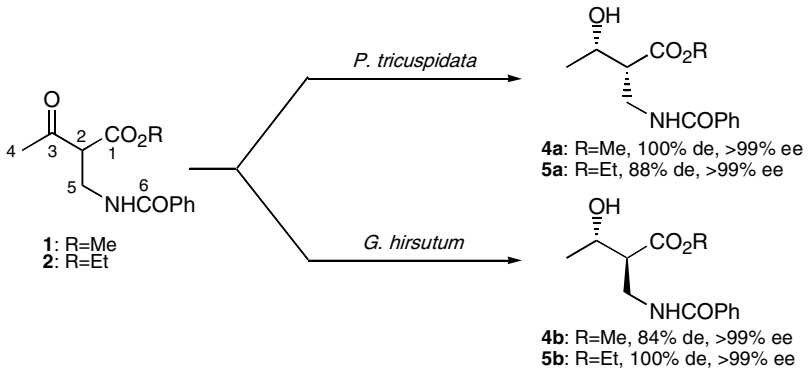

Figure 1. Diastereoselective reduction of 2-benzamidomethyl-3-oxobutanoates **1** and **2** by the cultured cells of *P. tricuspidata* and *G. hirsutum*.

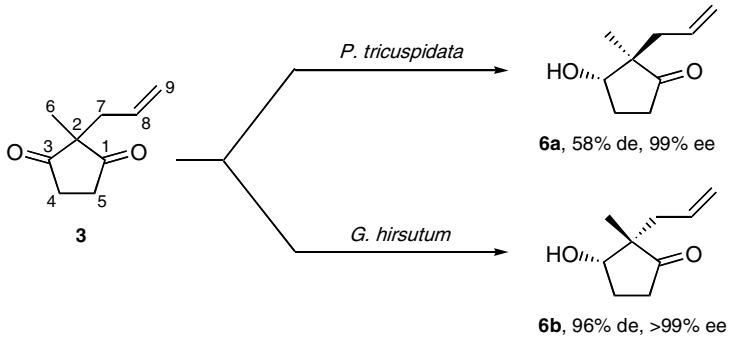

Figure 2. Diastereoselective reduction of 2-methyl-2-(2-propenyl)cyclopentan-1,3-dione (**3**) by the cultured cells of *P. tricuspidata* and *G. hirsutum*.

Thus, the diastereoselective reduction of 2-benzamidomethyl-3-oxobutanoates and 2-methyl-2-(2-propenyl)-cyclopentan-1,3-dione has been accomplished by the cultured plant cells of *P. tricuspidata*, *N. tabacum*, *G. max*, *C. roseus*, and *G. hirsutum*. Recently, it has been reported that the reduction of methyl 2-benzamidomethyl-3-oxobutanoate with BINAP catalyst gave the (2*S*,3*R*)-isomer of methyl 2-benzamidomethyl-3-hydroxybutanoate (93% de, 99% ee).[23–25] It should be emphasized that the diastereoselective formation of each of the (2*R*,3*S*)- and (2*S*,3*S*)-2-benzamidomethyl-3-hydroxybutanoates in enantiomerically pure forms has been achieved by the selective use of these plant cells. On the other hand, it is worth noting that the (2*R*,3*S*)-isomer of 2,2-disubstituted 3-hydroxycyclopentan-1-one was preferentially produced by the cultured plant cells of *P. tricuspidata*. Further investigations using the enzyme preparation from these cultured plant cells are now in progress.

Acknowledgments

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21. Spectral data for the products; product **4a** (obtained in the reduction of **1** with *P. tricuspidata*): HRFABMS *m*/*z* 252.1933 $[M+H]^+$; ^1H NMR (400 MHz, $CDCl_3$) δ 1.24 (3H, d, $J = 6.4$ Hz, H-4), 2.63 (1H, m, 2-H), 3.62 (1H, m, H-5a), 3.69 (3H, s, OCH_3), 4.02 (2H, m, H-3, 5b), 4.53 (1H, d, $J = 4.0$ Hz, OH), 7.37 (2H, td, $J = 6.4$, 1.6 Hz, *m*-H), 7.48 (1H, td, $J = 7.2$, 1.2 Hz, *p*-H), 7.76 (2H, dd, $J = 6.4$, 1.2 Hz, *o*-H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 20.9 (C-4), 38.0 (C-5), 52.0 (OCH_3), 52.6 (C-2), 65.7 (C-3), 127.3 (*o*-C in Ph), 128.6 (*m*-C in Ph), 131.7 (*p*-C in Ph), 168.7 (C-6), 174.1 (C-1). Product **4b** (obtained in the reduction of **1** with *G. hirsutum*): HRFABMS *m*/*z* 252.1928 $[M+H]^+$; ^1H NMR (400 MHz, $CDCl_3$) δ 1.26 (3H, d, $J = 7.2$ Hz, H-4), 2.63 (1H, m, 2-H), 3.57 (1H, m, H-5a), 3.74 (3H, s, OCH_3), 4.00 (1H, m, H-3), 4.12 (1H, m, H-5b), 4.29 (1H, d, $J = 4.0$ Hz, OH), 7.04 (1H, br s, NH), 7.44 (2H, td, $J = 7.2$, 1.2 Hz, *m*-H), 7.48 (1H, td, $J = 7.2$, 1.2 Hz, *p*-H), 7.76 (2H, dd, $J = 7.2$, 1.2 Hz, *o*-H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 20.6 (C-4), 37.7 (C-5), 51.9 (OCH_3), 52.4 (C-2), 65.4 (C-3), 126.9 (*o*-C in Ph), 128.5 (*m*-C in Ph), 131.7 (*p*-C in Ph), 168.6 (C-6), 174.1 (C-1). Product **5a** (obtained in the reduction of **2** with *P. tricuspidata*): HRFABMS *m*/*z* 266.2056 $[M+H]^+$; ^1H NMR (400 MHz, $CDCl_3$) δ 1.27 (6H, m, H-4, 2′), 2.60 (1H, m, 2-H), 3.61 (1H, m, H-5a), 4.06–4.21 (4H, m, H-3, 5b, 1′), 4.36 (1H, d, $J = 4.0$ Hz, OH), 7.14 (1H, br s, NH), 7.42 (2H, td, $J = 7.2$, 1.2 Hz, *m*-H), 7.48 (1H, td, $J = 7.2$, 1.2 Hz, *p*-H), 7.77 (2H, dd, $J = 7.2$, 1.2 Hz, *o*-H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.2 (C-2′), 20.8 (C-4), 38.0 (C-5), 52.6 (C-2), 61.0 (C-1′), 65.7 (C-3), 127.0 (*o*-C in Ph), 128.6 (*m*-C in Ph), 131.8 (*p*-C in Ph), 168.7 (C-6), 173.7 (C-1). Product **5b** (obtained in the reduction of **2** with *G. hirsutum*): HRFABMS *m*/*z* 266.2104 $[M+H]^+$; ^1H NMR (400 MHz, $CDCl_3$) δ 1.26 (3H, t, $J = 7.2$ Hz, H-2′), 1.28 (3H, d, $J = 6.4$ Hz, H-4), 2.85 (1H, m, 2-H), 3.74 (1H, m, H-5a), 3.88–4.22 (4H, m, H-3, 5b, 1′), 7.18 (1H, br s, NH), 7.41 (2H, td, $J = 6.4$, 1.4 Hz, *m*-H), 7.47 (1H, td, $J = 7.2$, 1.2 Hz, *p*-H), 7.76 (2H, dd, $J = 6.4$, 1.6 Hz, *o*-H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.1 (C-2′), 20.9 (C-4), 38.8 (C-5), 51.0 (C-2), 60.9 (C-1′), 67.1 (C-3), 126.9 (*o*-C in Ph), 128.7 (*m*-C in Ph), 131.6 (*p*-C in Ph), 168.7 (C-6), 173.7 (C-1). Product **6a** (obtained in the reduction of **3** with *P. tricuspidata*): HRFABMS *m*/*z* 155.1715 $[M+H]^+$; ^1H NMR (400 MHz, $CDCl_3$) δ 1.01 (3H, s, H-6), 1.68 (1H, br s, OH), 1.88–2.48 (6H, m, H-4, 5, 7), 4.23 (1H, t, $J = 6.2$ Hz, H-3), 5.13 (2H, m, H-9), 5.77 (1H, m, H-8); ^{13}C NMR (100 MHz, $CDCl_3$): δ 15.2 (C-6), 27.4 (C-7), 35.0 (C-5), 39.9 (C-4), 53.2 (C-2), 75.6 (C-3), 118.6 (C-9), 133.5 (C-8), 220.0 (C-1). Product **6b** (obtained in the reduction of **3** with *G. hirsutum*): HRFABMS *m*/*z* 155.1712 $[M+H]^+$; ^1H NMR (400 MHz, $CDCl_3$) δ 1.01 (3H, s, H-6), 1.77 (1H, d, $J = 2.8$ Hz, OH), 1.98–2.51 (6H, m, H-4, 5, 7), 4.13 (1H, m, H-3), 5.16 (2H, m, H-9), 5.88 (1H, m, H-8); ^{13}C NMR (100 MHz, $CDCl_3$): δ 19.9 (C-6), 27.8 (C-7), 34.2 (C-5), 35.6 (C-4), 53.2 (C-2), 77.3 (C-3), 118.2 (C-9), 134.6 (C-8), 220.5 (C-1).
22. HPLC was carried out with Deverosil 100-3 (Nomura Chemical Co. Ltd.) column [eluent: hexane–THF–MeOH (1000:100:1); flow rate: 1 mL/min; detection: UV 254 nm]. Retention times for the (*R*)-MTPA esters of (2*S*,3*R*)-**4**, (2*R*,3*S*)-**4**, (2*R*,3*R*)-**4**, (2*S*,3*S*)-**4**, (2*S*,3*R*)-**5**, (2*R*,3*S*)-**5**, (2*R*,3*R*)-**5**, and (2*S*,3*S*)-**5** in the HPLC were 25.1, 26.4, 31.9, 38.5, 26.7, 28.3, 34.4, and 40.1 min, respectively.
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