Production of (2*R*,3*S*)-2-Benzamidomethyl-3-Hydroxybutanoates by Immobilized Plant Cells of *Parthenocissus Tricuspidata*

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Abstract: Incubation of methyl 2-benzamidomethyl-3-oxobutanoate with cultured plant cells of *Parthenocissus tricuspidata* for 2 days afforded methyl (2*R*,3*S*)-2-benzamidomethyl-3-hydroxybutanoate with 100% de and >99% ee in 51% yield. The use of immobilized cells of *P. tricuspidata* in calcium alginate gel improved the yield of the product (95% yield) with 100% de and >99% ee. The immobilized cells of *P. tricuspidata* maintained the high potential for the reduction of methyl 2-benzamidomethyl-3-oxobutanoate (85% yield) after 5 times of usage.

Keywords: stereoselective reduction, methyl 2-benzamidomethyl-3-oxobutanoate, methyl (2*R*, 3*S*)-2-benzamidomethyl-3-hydroxybutanoate, *Parthenocissus tricuspidata*, immobilized cells

Introduction

Chiral 2-benzamidomethyl-3-hydroxybutanoates are useful chiral building blocks for asymmetric synthesis of biologically active compounds; optically active 2-benzamidomethyl-3-hydroxybutanoates are chiral synthons for carbapenems.[1,2] Diastereoselective reduction of 2-benzamidomethyl-3-oxobutanoates is an attractive method for the production of optically enriched 2-benzamidomethyl-3-hydroxybutanoates. Recently, microbial reduction of 2-methyl-3-oxobutanoate to give a mixture of *syn*- and *anti*- 3-hydroxy-2-methylbutanoate.[3–6] However, little attention has been paid to the diastereo- and enantioselectivity in the reduction of 2-benzamidomethyl-3-oxobutanoates by cultured plant cells. Furthermore, there have been no reports on the large scale production of 2-benzamidomethyl-3-hydroxybutanoates by immobilized plant cells.[7] We report herein the high production of methyl (2*R*,3*S*)-2-benzamidomethyl-3-hydroxybutanoate by the reduction of methyl 2-benzamidomethyl-3-oxobutanoate with immobilized cells of *P. tricuspidata* in calcium alginate gel.

Materials and Methods

General

HPLC was carried out with a Deverosil 100-3 column (Nomura Chemical Co. Ltd.) (solvent: hexane/THF/MeOH=1000/100/1; flow rate: 1 ml/min). The ^{1}H and ^{13}C NMR, H-H COSY, C-H COSY, and HMBC spectra were recorded using a Varian XL-400 spectrometer in $CDCl_3$ solution and the chemical shifts were expressed in δ (ppm) referring to TMS. The FABMS spectra were measured using a JEOL MStation JMS-700 spectrometer.

Methyl 2-benzamidomethyl-3-oxobutanoate and methyl (2*R*, 3*S*)-2-benzamidomethyl-3-hydroxybutanoate were purchased from Aldrich or Wako Pure Chemical Co. Ltd.

Cultured *P. tricuspidata* cells were subcultured at 4-week intervals on solid MS medium containing 2% glucose, 1 ppm 2,4-dichlorophenoxyacetic acid, and 1% agar (adjusted to pH 5.7) under illumination

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(4000 lux). A suspension culture was started by transferring 20 g of the cultured cells to 300 ml of liquid MS medium in a 500 ml conical flask.

Reduction of methyl 2-benzamidomethyl-3-oxobutanoate (1) by cultured cells of *P. tricuspidata*

Cultured cells of *P. tricuspidata* were incubated in 500 ml conical flasks for 3 weeks. To a 500 ml conical flask containing 100 g of suspension cultures, 50 mg of the substrate was added. The mixture was incubated at 25 °C for 2 days on a rotary shaker (70 rpm) under illumination (4000 lux). After the incubation period, products were extracted from the medium with ethyl acetate. The chemical yields of the products were determined by HPLC analyses. The ethyl acetate fraction was subjected to chromatography on silica gel with hexane-ethyl acetate (95:5, v/v) to separate the products. The products were identified by comparisons of their HPLC, FABMS, and NMR data with those of authentic samples. The absolute configurations, diastereomeric excess (de), and enantiomeric excess (ee) of the resulting methyl 2-benzamidomethyl-3-hydroxybutanoate were determined by comparing the HPLC retention times of their (*R*)-MTPA esters on Deverosil 100-3 column with those of the (*R*)-MTPA esters of authentic chiral alcohols. The retention times of (*R*)-MTPA esters of (2*S*,3*R*)-, (2*R*,3*S*)-, (2*R*,3*R*)-, and (2*S*,3*S*)-isomers in the HPLC were 25.1, 26.4, 31.9, and 38.5 min, respectively.

Spectral data for methyl (2*R*,3*S*)-2-benzamidomethyl-3-hydroxybutanoate (**2**): FABMS m/z 252 $[M + H]^+$; ^{1}H NMR(400MHz, $CDCl_3$) δ 1.25 (3H, d, *J* = 6.4, H-4), 2.62 (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), 7.45 (1H, td, *J* = 7.0, 1.2 Hz, *p*-H), 7.76 (2H, dd, *J* = 7.0, 1.2 Hz, *o*-H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 20.9 (C-4), 38.1 (C-5), 52.1 (OCH_3), 52.6 (C-2), 65.7 (C-3), 127.3 (*o*-C in Ph), 128.5 (*m*-C in Ph), 131.7 (*p*-C in Ph), 168.7 (C-6), 174.1 (C-1).

Preparation of immobilized cells of *P. tricuspidata* in calcium alginate gel

Sodium alginate (2%) was suspended in water (1 l) by vigorous stirring at 50 °C for 1 h. The cultured cells (100 g) in the stationary growth phase have been used for experiments. Cultured *P. tricuspidata* cells were added to this solution and the mixture was stirred for additional 2 h until it became homogeneous. The suspension was added dropwise from a dropping funnel with a glass tube into a 10% $CaCl_2$ solution (1 l) with stirring to form pieces of spherical calcium alginate gel with 5 mm diameter immediately. Washing with water gave immobilized *P. tricuspidata* cells which were used for the large scale production of 2-benzamidomethyl-3-hydroxybutanoate.

Large scale synthesis of methyl (2*R*,3*S*)-2-benzamidomethyl-3-hydroxybutanoate (2) by immobilized cells of *P. tricuspidata* in calcium alginate gel

Substrate (50 mg) was added to the immobilized *P. tricuspidata* cells with 300 ml of MS medium containing 1% glucose in a 1 l conical flask, and the flask was incubated for 2 days. Repetitive use of the immobilized *P. tricuspidata* cells was investigated. The used culture medium was changed to the freshly prepared medium containing 50 mg of substrate and continuous four batch reactions have been carried out at 2 days intervals. Products were extracted from the medium and were purified by the same method as described above. The yields of the product obtained after each batch reactions were 95, 94, 91, 89, and 85%, respectively.

Results

Methyl 2-benzamidomethyl-3-oxobutanoate (**1**) was subjected to the reduction by cultured plant cells of *P. tricuspidata*. After incubation of **1** with cultured cells of *P. tricuspidata* for 2 days, reduction products were isolated from ethyl acetate fraction by chromatography on silica gel column. The HPLC and NMR analyses of the reduction products showed that only *syn*-isomer was obtained in 51% yield (100% de). The absolute configuration of the conversion products was determined by HPLC analyses of their (*R*)-MTPA esters to be (2*R*,3*S*). So the reduction product was identified as methyl (2*R*,3*S*)-2-benzamidomethyl-3-hydroxybutanoate (Fig. 1). The enantioselectivity of the reduction by *P. tricuspidata* was excellent (>99% ee). A time-course of the biotransformation of **1** was carried out to investigate the ability of cultured plant cells of *P. tricuspidata* to reduce **1** to **2** (Table 1).

Next, reduction of **1** by the immobilized cells of *P. tricuspidata* in calcium alginate gel was

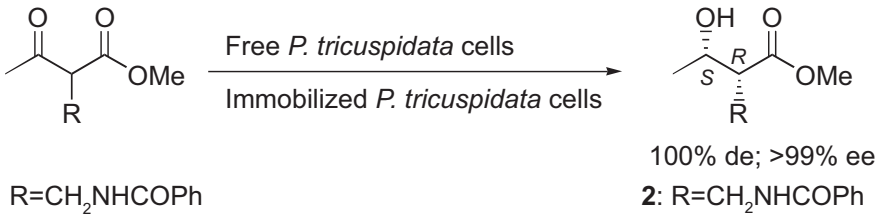

Figure 1. Stereoselective reduction of methyl 2-benzamidomethyl-3-oxobutanoate (**1**) by free and immobilized cells of *P. tricuspidata*.

investigated. The extracellular product **2** was obtained after incubation of the substrate **1** with the immobilized cells of *P. tricuspidata* for 2 days. The immobilized *P. tricuspidata* cells had high potential to reduce **1** to **2** in 95% yield. A time-course of the reduction of **1** with immobilized *P. tricuspidata* cells was examined (Table 1). The reduction with immobilized *P. tricuspidata* cells showed excellent diastereoselectivity and enantioselectivity, 100% de and >99% ee. Repetitive use of the immobilized *P. tricuspidata* cells was then carried out. In repetitive batch use, the immobilized cells of *P. tricuspidata* cells maintained the high potential for the reduction of **1** (85% yield) after 5 times of usage.

Discussion

The biological reduction of methyl 2-benzamidomethyl-3-oxobutanoate (**1**) was investigated using the cultured plant cells of *P. tricuspidata* as biocatalysts. It was found that cultured *P. tricuspidata* cells were able to reduce **1** to methyl (2*R*,3*S*)-2-benzamidomethyl-3-hydroxybutanoate (**2**), with excellent diastereo- and enantioselectivities. There have been several studies on the reduction of 2-alkyl-3-oxobutanoates by microorganisms as biocatalysts.[3–6] Most microorganisms reduce 2-alkyl-3-oxobutanoates to a mixture of *syn*- and *anti*-products. There have been no reports on the selective production of *syn*-isomer of methyl 2-benzamidomethyl-3-hydroxybutanoate by biocatalysts. The results obtained here suggested that the plant suspension culture of *P. tricuspidata* acts as a good biocatalyst to give *syn*-product, methyl (2*R*,3*S*)-2-benzamidomethyl-3-hydroxybutanoate (**2**). The use of immobilized *P. tricuspidata* cells drastically improved the yield of the product (95%). The repetitive use of immobilized *P. tricuspidata* had advantage of excreting the products into the used medium and the immobilized cells remained high potential for the reduction of **1** (85% yield) even after five batch reactions. These results demonstrated that the repetitive use of immobilized cells of *P. tricuspidata* is useful for the large scale production of **2** rather than the normal suspension culture.

Table 1. The production of **2** by free or immobilized cells of *P. tricuspidata.*

Incubation time (h)	Yield of 2 (%)	
	Free cells	Immobilized cells
6	15	27
12	25	46
18	31	60
24	37	70
30	41	78
36	45	85
42	48	90
48	51	95

Disclosure

The authors report no conflicts of interest.

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