Stereoselective Reduction of Bisdemethoxycurcumin to (R)-Hexahydrobisdemethoxycurcumin by Cultured Morus bombycis Plant Cells

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Abstract
Biotransformation of bisdemethoxycurcumin was examined using cultured plant cells of Morus bombycis, the Moraceae mulberry family, as biocatalysts. The cultured cells converted bisdemethoxycurcumin into (R)-hexahydrobisdemethoxycurcumin in 30% yield in 1 day. The (S)-stereoisomer has been isolated from Betula platyphylla Sukatchev var. japonica.

Keywords
stereoselective reduction, cultured plant cells, Morus bombycis, bisdemethoxycurcumin, (R)-hexahydrobisdemethoxycurcumin

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Cultured plant cells have been studied as useful agents for biotransformation reactions because of their potential to produce specific secondary metabolites such as flavors, pigments, and agrochemicals.¹ The reactions involved in these biotransformations include oxidation, reduction, hydroxylation, esterification, methylation, isomerization, hydrolysis, and glycosylation. Reduction is a characteristic biotransformation reaction in cultured plant cells because oxidoreductases are widespread in plants. Also, it has been the subject of increasing attention, because stereoselective enzymatic reduction is useful for the preparation of chiral building blocks in the synthesis of optically active drugs.²⁻⁵ In addition, the reduction of bioactive compounds can enhance their physicochemical stability, and improve their bio- and pharmacological properties. We recently reported that cultured Marchantia polymorpha cells reduced curcumin [1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadien-3,5-dione] to tetrahydro-curcumin.⁶

Curcumin and its derivatives are yellow pigments found in the rhizomes of Curcuma longa Linn., which have been used for centuries as spices and food colorants. Curcumin compounds have attracted much attention because of their potential to be drugs, and have been widely studied for their anti-oxidative, anticancer, and anti-inflammatory effects.⁷ From the viewpoint of further exploitation by the pharmaceutical industry, it is of importance to enhance the physiological activities of curcumin compounds.

Herein, we report, for the first time, the reduction of bisdemethoxycurcumin into (R)-hexahydrobisdemethoxycurcumin by cultured cells of Morus bombycis.

Bisdemethoxycurcumin (1) was subjected to biotransformation with cultured plant cells of M. bombycis. The biotransformation products were detected by high performance liquid chromatography (HPLC) analysis of the culture medium and the methanol extracts of the cells. The products in the medium were extracted using ethyl acetate. After concentration of the methanol extracts of the cells, the residue was partitioned between water and ethyl acetate. The ethyl acetate fractions were combined, concentrated, and analyzed by HPLC. The cultured cells of M. bombycis converted bisdemethoxycurcumin (1) to hexahydrobisdemethoxycurcumin (2, 30% yield), as shown in Figure 1. The chiral carbon bearing the hydroxy group in 2 was determined as having the (R)-configuration based on its optical rotation $[\alpha]_D^{25} =$
The naturally occurring hexahydro-bisdemethoxycurcumin (2) isolated from *Betula platyphylla* Sukatchev var. *japonica* had the (S)-configuration at the chiral carbon (optical rotation $[\alpha]_{D}^{25} = -1.8^\circ$ (c = 1.02, MeOH)).

Thus, the incubation of bisdemethoxycurcumin with cultured *M. bombycis* cells gave (R)-hexahydrobisdemethoxycurcumin stereoselectively, whereas hexahydrobisdemethoxycurcumin isolated from *B. platyphylla* had the (S)-configuration. This suggests that the reductase responsible for the reduction of the carbonyl group of bisdemethoxycurcumin in the cultured cells of *M. bombycis* has the opposite stereoselectivity to that in *B. platyphylla* cells.

Cultured cells of *Nicotiana tabacum*, *Catharanthus roseus*, and *Gossypium hirsutum* were able to reduce C–C double bonds of exogenous enones. On the other hand, cultured cells of *M. polymorpha* showed high potential for the reduction of C–C double bonds of curcumin to give tetrahydrocurcumin in high yield after a 1-day incubation period. However, cultured cells of *N. tabacum*, *C. roseus*, and *G. hirsutum* could not reduce the C–C double bond of curcumin. These results suggest that the reductases responsible for the reduction of C–C double bonds of curcumin exist only in cultured cells of *M. polymorpha* among these plant cell cultures. It is of interest if the reductase in *M. bombycis* cells can reduce the C–C double bonds of curcumin. This is the first report of the stereoselective reduction of bisdemethoxycurcumin by cultured plant cells. Purification of the reductases, which catalyze the reduction of bisdemethoxycurcumin, is now in progress in our laboratory.

**Experimental**

**General**

HPLC was carried out with a reverse-phase column (Crest Pak C18S [4.6 × 150 mm, JASCO Co., Japan]) (solvent: methanol/water = 35/65; flow rate: 1 mL/min). Bisdemethoxycurcumin used as substrate was purchased from Sigma-Aldrich Co. and was used after purification. The cultured cells of *M. bombycis* were sub-cultured at 4-week intervals on solid Murashige and Skoog medium + 6 μM kinetin-II (MSK-II) medium containing 2% glucose, 1 ppm 2,4-dichlorophenoxyacetic acid, and 1% agar (adjusted to pH 5.8). 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.

**Biotransformation Procedure**

The suspension cells of *M. bombycis* were incubated in 500 mL conical flasks for 3 weeks. The cultured cells in the stationary growth were used for experiments. After the cultivation period, 300 mg of the substrate was added. The transformation was performed by incubating the mixture at 25°C on a rotary shaker. The culture medium was extracted with ethyl acetate. The cells were extracted (×3) by homogenization with methanol. The methanol fraction was concentrated and partitioned between water and ethyl acetate. The ethyl acetate fractions were combined and concentrated. The yield of the products was determined on the basis of the peak area from HPLC and expressed as a relative percentage of the total amount of the whole reaction products extracted. The products were identified by comparisons of their mass spectrometry (MS) and nuclear magnetic resonance (NMR) data with those of authentic samples.

**Declaration of Conflicting Interests**

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