Abstract

Microbial transformation of denthyrsinin (1), gigantol (2), and batatasin III (3), the major constituents of *Dendrobium* species (Orchidaceae), was performed using the filamentous fungus *Mucor hiemalis* KCTC 26779. Three glycosylated metabolites were obtained in the biotransformation of 1-3, and their structures were identified as denthyrsinin-6-O-β-D-glucoside (4), gigantol-5-O-β-D-glucoside (5), and batatasin III-3-O-β-D-glucoside (6) by analyzing 1-dimensional and 2-dimensional-nuclear magnetic resonance spectra, as well as high-resolution electrospray ionization mass spectral data. Among them, metabolite 4 has not been previously reported. *Mucor hiemalis* was revealed to catalyze enzymatically glucosylation of the hydroxyl group of phenanthrenes and bibenzyls. This research provides an efficient approach for the glycosylation of phenanthrenes and bibenzyls and can expand the library of available phenanthrene and bibenzyl derivatives for further biological evaluations.

Keywords

microbial transformation, phenanthrenes, bibenzyls, *Mucor hiemalis*, glycosylation

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Dendrobii Herba, typically the dried stems of *Dendrobium* species (Orchidaceae), has been used in Asian countries as a traditional medicine for the treatment of hydrodipsomania, gastrodynia, amblyopia, and myoatrophy due to kidney disorder. Previous phytochemical studies on this plant have revealed that it is rich in phenanthrenes and bibenzyls. Phenanthrenes possess several therapeutic activities such as anti-inflammatory, antibacterial, anti-cancer, and anti-tumor activities. Bibenzyls also display diverse biological activities, including antiplatelet aggregation, anti-inflammatory, anti-mutagenic, anti-retinal neangiogenesis inhibitory, and anti-mutagenic activities.

Microbial transformation is a useful tool for the structural modification of organic compounds, due to the high stereospecificity and regioselectivity resulting from enzymatic reactions. The procedure is also known for easy handling, cost-effectiveness, and for being an eco-friendly tool for the preparation of many natural compounds and their derivatives, as well as for serving as a model of mammalian metabolism. This technique has been widely employed for the production of new chemical derivatives with improvements in physicochemical and pharmaceutical properties. Glycosylation is a well-known method for increasing the water solubility of organic compounds, as well as improving their biological potential and bioavailability. There have been several reports demonstrating that the glycosides of plant polyphenols are more water-soluble than their aglycones. To the best of our knowledge, no microbial biotransformation study has yet been performed to identify the glycosides of phenanthrenes and bibenzyls using microorganisms.

Recently, we have isolated a series of phenanthrenes and bibenzyls from Dendrobii Herba. Among them, denthyrsinin (1) and gigantol (2) were isolated as major compounds. Batatasin III (3) is also a well-known component of *Dendrobium* species. Although 1 and 2 did not show cytotoxic activities on the human pharynx squamous carcinoma (FaDu) cell line in our previous study, it has been reported that compound 1 has cytotoxic activity against several other cancer cell lines and has a hypotensive effect; compound 2 shows diverse biological properties, such as anti-inflammatory, anticancer, anti-osmotic, and antioxidant activities. Batatasin III (3) has been reported to have anticancer and α-glucosidase inhibitory activities. As part of our ongoing research on the biotransformation of natural products in cultures...
of filamentous fungi, three microorganisms were evaluated for their ability to metabolize compounds 1-3. Thin-layer chromatographic (TLC) analyses of the culture extracts during the screening studies indicated that *M. hiemalis* KCTC 26779 was capable of metabolizing 1-3. Scale-up fermentations of 1-3 have resulted in the production of one new (4) and two known metabolites (5 and 6), respectively (Figure 1).

Compound 4 was obtained as a brown solid with a molecular ion peak at \( m/z \) 485.1420 [M + Na]+ in its high-resolution electrospray ionization mass spectrum (HR-ESI-MS), which is consistent with an elemental formula of C\(_{23}\)H\(_{26}\)O\(_{10}\)Na. The \(^1\)H and \(^{13}\)C-nuclear magnetic resonance (NMR) spectra of 4 were identical to those of dethyrisinin, except for the difference in signals of the sugar. The \(^1\)H NMR spectrum of 4 displayed a signal for an anomic proton at \( \delta_{\text{H}} \) 5.22 (d, \( J = 7.7 \) Hz, H-1'), indicating a \( \beta \) configuration. In addition, proton signals at \( \delta_{\text{H}} \) 3.76 (1H, dd, \( J = 12.1, 2.4 \) Hz, H-6'a), 3.66 (1H, dd, \( J = 12.1, 5.1 \) Hz, H-6'b), 3.59 (1H, m, H-2'), 3.50 (1H, d, \( J = 8.8 \) Hz, H-3'), 3.47 (1H, m, H-4'), 3.25 (1H, ddd, \( J = 9.3, 5.1, 2.4 \) Hz, H-5'), and carbon signals at \( \delta_{\text{C}} \) 105.0 (C-1'), 78.41 (C-5'), 77.9 (C-3'), 75.91 (C-2'), 71.35 (C-4'), and 62.4 (C-6') for a sugar moiety were observed. The \(^1\)H-\(^1\)H-correlated spectroscopy (COSY) correlations of H-1'/H-2', H-4'/H-3', H-5', and H-5'/H-6' and the \(^1\)H-\(^1\)H nuclear Overhauser enhancement spectroscopy (NOESY) correlations of H-1'/H-3', H-2'/H-4' identified the presence of a glucoside. The \( \beta \)-d-glucoside was positioned to C-6 in the phenathrene aglycone, supported by the \(^1\)H-\(^{13}\)C heteronuclear multiple bond correlation (HMBC) NMR correlations of H-9/C-8, H-8/C-6, and H-1'/C-6. Therefore, compound 4 was elucidated as dethyrisinin-6-O-\( \beta \)-d-glucoside (Figure 2).

Two known compounds were identified as gigantol-5-O-\( \beta \)-d-glucoside (5) and batatasin III-3-O-\( \beta \)-d-glucoside (6) by comparing their spectroscopic data with the published data.  

![Figure 1. Biotransformation of 1-3 by *Mucor hiemalis* KCTC 26779.](image-url)
Further detailed analyses of $^1$H-$^1$H COSY, $^1$H-$^1$H NOESY, and $^1$H-$^13$C HMBC NMR data revealed that the $\beta$-d-glucoside was positioned at C-5 and C-3 via glycosidic linkages in the structures of 5 and 6, respectively (Figure 2).

There is little chemical and biological literature on compounds 5 and 6. Compound 5 was isolated from the stems of *Dendrobium fimbriatum* as a new compound, but no activity has been reported. Compound 6 was found in *Pimelea bulbocodioides* for the first time and has since also been isolated from *Pinus yunnanensis* and *Bletilla striata*. Compound 6 has been reported to possess kinase inhibitory activity in HeLa cervical carcinoma cells and antioxidant activity against hydrogen peroxide-induced toxicity in SK-N-SH cells. Therefore, we suggest that compounds 4-6 may be effective in improving the therapeutic effects or intestinal absorption. However, further studies on biological evaluation, water-solubility tests, and bioavailability of these compounds are required.

In conclusion, a microbial transformation study of dentityrinsin (1), gigantol (2), and batatasin III (3) with the filamentous fungus *M. biemalis* afforded three metabolites (4-6), respectively. One novel metabolite, dentityrinsin-6-O-$\beta$-d-glucoside (4), and two known metabolites, gigantol-5-O-$\beta$-d-glucoside (5) and batatasin III-3-O-$\beta$-d-glucoside (6), were produced through their glycosylation, which process was postulated to be biocatalyzed by an enzyme glycotransferase. Thus, biotransformation by *M. biemalis* can be used as a promising method for glycosylation of phenanthrenes and bibenzyls.

**Experimental**

**General**

The optical rotations were measured on a 343 Plus polarimeter (Perkin Elmer, Waltham, MA, USA). One-dimensional (1D) and 2-dimensional (2D) NMR spectroscopic experiments were performed on a JNM-ECA 500 MHz NMR instrument (JEOL Ltd., Tokyo, Japan) with tetramethylsilane (TMS) as the internal standard. HR-ESI-MS were recorded on a Waters SYNAPT G2 mass spectrometer (Waters, Milford, MA, USA). TLC analysis was performed on Kieselgel 60 F254 (Merck, Darmstadt, Germany) and Kieselgel 60 RP-18- F254S (Merck, Darmstadt, Germany), with visualization under ultraviolet light (254 and 365 nm) and 10% (v/v) sulfuric acid spray, followed by heating at 180 °C for 2 minutes. Silica gel (70-230 mesh, Merck, Darmstadt, Germany), RP-18 (YMC gel ODS-A, 12 nm, S-75 μm, YMC Co., Tokyo, Japan), and Sephadex LH-20 (GE Healthcare Bio-Sciences, Uppsala, Sweden) were used for column chromatography (CC). Medium pressure liquid chromatography (MPLC) was performed on a CombiFlash Rf200 system (Teledyne ISCO, Lincoln, NE, USA) with RediSep Rf normal phase silica columns.

**Materials and Microorganisms**

Dentityrinsin (1) and gigantol (2) were isolated from dried stems of *Dendrobiium Herba*, as described in the previous study. Batatasin III (3) was purchased from Wuhan ChemFaces Biochemical Co., Ltd., Hubei, China. All the microorganisms

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**Figure 2.** Key heteronuclear multiple bond correlation (→), correlation spectroscopy (↔), and nuclear Overhauser enhancement spectroscopy (↔↔) correlations of compounds 4-6.
were obtained from the Korean Collection for Type Cultures (KCTC). Three cultures were used for the preliminary screening process as follows: *A. coerulea* KCTC 6936, *Cunninghamella elegans* var. *elegans* 6992, and *Mucor hiemalis* 26779. The ingredients for microbial media, including dextrose, peptone, and malt extract, were purchased from Becton, Dickinson and Company (Sparks, MD, USA). *A. coerulea* and *M. hiemalis* were cultured on malt medium (malt extract 20 g/L, dextrose 20 g/L, peptone 1 g/L). *Cunninghamella elegans* var. *elegans* was cultured on potato dextrose medium (24 g/L).

**Screening Procedures**

Microbial metabolism studies were performed according to the standard 2-stage procedure. The actively growing microbial cultures were inoculated in 250 mL flasks containing 50 mL of malt medium for *A. coerulea* and *M. hiemalis* and potato dextrose medium for *C. elegans* var. *elegans* and incubated with gentle agitation (200 rpm) at 25 °C in a temperature-controlled shaking incubator. After inoculation for 24 hours, the dimethyl sulfoxide (DMSO) solutions (1.5 mg/200 μL) of 1-3 were added to each flask, and the incubation was continued under the same conditions for another 7 days. Sampling and TLC monitoring were performed at an interval of 24 hours. Culture controls consisted of fermentation media to which the microorganisms were grown without the addition of 1-3.

**Scale-Up Fermentations of 1-3**

Preparative scale-up fermentations were carried out in 3 (500 mL) flasks each containing 150 mL of malt medium, and each 20 mg of 1-3, dissolved in DMSO, which were distributed evenly among the flasks, respectively. After incubation for 7 days, the microbial culture broth was extracted with ethyl acetate (EtOAc; 400 mL × 3), and the organic layers were combined and concentrated in vacuo. The EtOAc extract (378 mg) of 1 from *M. hiemalis* culture broth was chromatographed by silica gel CC with chloroform (CHCl₃)-methanol (MeOH) (9:1, v/v) as mobile phase to give metabolite 4 (15 mg). The EtOAc extract (473 mg) of 2 from *M. hiemalis* culture broth was chromatographed by MPLC with CHCl₃-MeOH (10:9 to 9:1, v/v) as mobile phase, affording 3 subfractions (F0201 and F0203). Sub-fraction F0202 (200 mg) was separated by reverse-phase CC with MeOH-water (H₂O) (1:1 to 7:3, v/v) as mobile phase to give metabolite 5 (5.3 mg). The EtOAc extract (285 mg) of 3 from *M. hiemalis* culture broth was processed by silica gel CC with CHCl₃-MeOH (8:1, v/v) as mobile phase to afford metabolite 6 (8 mg).

**Dentherzinin-6-O-β-D-glucoside (4).** Brown solid.

\[\alpha^\text{D} + 39.2^\circ (c 0.03, \text{CH}_3\text{OH}).\]

**Batatasin III-3-O-β-D-glucoside (5).** White solid.

\[\alpha^\text{D} + 30.4^\circ (c 0.03, \text{CH}_3\text{OH}).\]

**Batatasin III-3-O-β-D-glucoside (6).** Red solid.

\[\alpha^\text{D} + 44.2^\circ (c 0.01, \text{CH}_3\text{OH}).\]

**Declaration of Conflicting Interests**

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