Biotransformation of Resveratrol to Piceid by *Bacillus cereus*

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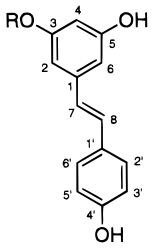
Microbial transformation of resveratrol (**1**), *trans*-3,4′,5-trihydroxystilbene, was studied. Preparative scale biotransformation of **1** with whole-cell suspensions of *Bacillus cereus* UI 1477 resulted in the production of metabolite **2** which was identical in all respects to an authentic sample of piceid, resveratrol 3-*O*-*β*-D-glucoside.

Resveratrol (**1**) (*trans*-3,4′,5-trihydroxystilbene), a constituent of several food products and medicinal plants, is a potent phytoalexin[1] which displays an array of pharmacological[2] and antimicrobial[3] activities. It exhibits strong in vitro antioxidant activity;[4] however, its in vivo role is questionable.[5] In addition, studies have demonstrated that resveratrol inhibits platelet aggregation in vitro and in vivo[6] which may contribute to its potential cardioprotective properties. Other investigations have suggested that resveratrol is capable of inhibiting certain steps in arachidonate metabolism[7] and may possess antiinflammatory activity. Further studies indicate that **1** is an inhibitor of monoamine oxidase A[8] and gastric H^+, K^+-ATPase.[9] Resveratrol (**1**) has also demonstrated cancer chemopreventive properties.[10]



1 R = H
2 R = *β*-D-Glucose

Despite the widespread human exposure to **1**, there have been no reports on the mammalian metabolism of this bioactive stilbene. A study showed that **1** is likely to undergo metabolic transformation as it accumulates in the livers of rats following oral administration.[11] To facilitate the mammalian metabolism studies of **1**, microorganisms are being utilized in our laboratory as in vitro models to mimic and predict the metabolic fate of resveratrol in mammalian systems. Furthermore, we are interested in utilizing microbial cultures as in vitro biocatalysts to prepare new and potentially active analogues of **1** since many of its known analogues have demonstrated equal or greater bioactivity.[12,13] In the present study we describe the isolation and structure elucidation of piceid (**2**), resveratrol 3-*O*-*β*-D-glucoside, as a microbial metabolite of resveratrol (**1**) from whole-cell suspensions of *Bacillus cereus* UI 1477.

A total of 12 microbial cultures were screened for their ability to biotransform resveratrol (**1**). On the basis of comparative TLC analyses of test cultures and controls, *B. cereus* UI 1477 was the only organism capable of biotransforming **1** to a more polar metabolite. A preparative scale biotransformation of **1** using whole-cell suspensions of *B. cereus* afforded metabolite **2** as a pure compound in 11% yield. Metabolite **2** was isolated and purified by preparative TLC. The FAB mass spectrum of metabolite **2**, MH^+ at *m*/*z* 391, indicated that it was a glucoside conjugate of **1**. The physical and spectral data of **2** were consistent with the reported data for piceid.[14−17] In addition, metabolite **2** was identical in all respects to an authentic sample of piceid confirming that **2** is the 3-*O*-*β*-D-glucoside conjugate of **1**.

Piceid (**2**) has previously been isolated from a variety of plant species[14] and synthesized.[18] Piceid has also been reported to exhibit a number of biological activities.[4,7,8,14,18] Recently, a dehydrodimer has been reported as a transformation product of **1** by the grapevine pathogen *Botrytis cinerea*.[19] This is the first report of preparing piceid (**2**) from **1** using a microorganism as a biocatalyst. Further microbial transformation studies of **1** are in progress.

Experimental Section

General Experimental Procedures. Melting points were determined in open capillary tubes with a Thomas-Hoover capillary melting point apparatus and are uncorrected. Optical rotations were measured with a Perkin-Elmer 241 digital polarimeter. IR spectra were recorded in KBr using a Nicolet Impact 400D FT-IR spectrophotometer. UV spectra were recorded in MeOH using a Gilford spectrophotometer. Centrifugation of whole-cell suspensions and microbial cultures was carried out using a Heraeus Megafuge 2.0R centrifuge at 4 °C and 2724*g*. The term in vacuo refers to removal of solvent with a rotary evaporator under water aspirator vacuum (15−30 mmHg). ^{1}H and ^{13}C NMR spectra were obtained in acetone-d_6 on a JEOL-Eclipse 400 FT-NMR spectrometer operating at 400 and 100 MHz, respectively. Standard pulse sequences were used for COSY, HETCOR, DEPT, and APT experiments. Low- and high-resolution FAB mass spectra were obtained using a VG 70-SEQ mass spectrometer (VG Analytical, Manchester, England) at the Mass Spectrometry Facility, Department of Medicinal Chemistry, University of Washington, Seattle, WA 98195.

Chromatographic Conditions. TLC analyses were carried out on precoated Silica G-25 UV_{254} plates (E. Merck, Darmstadt). Visualization of TLC plates was performed using 10% phosphomolybdic acid in absolute ethanol as a spray reagent. Spots were visualized by spraying the plates and then heating them at 110 °C for 3 min in an oven. The solvent system used for developing TLC plates was a solution of EtOAc−hexane−acetic acid (8:1:1 v/v). Preparative TLC separations were carried out on 20 × 20 cm precoated Silica Gel 60 F_{254} plates with 2 mm layer thickness (E. Merck,

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Darmstadt). Visualization of preparative TLC plates was performed under UV light (254 nm).

Microorganism. The *B. cereus* UI 1477 culture was obtained from the Department of Medicinal and Natural Products Chemistry, College of Pharmacy, University of Iowa, Iowa City, IA.

Media. All preliminary screening experiments were carried out in a medium consisting of dextrose, 20 g; yeast extract, 5 g; peptone, 5 g; NaCl, 5 g; K_2HPO_4, 5 g; and distilled H_2O, 1000 mL. Stock cultures of fungi and bacteria were stored on slants of Mycophil and Eugon (Difco, Detroit, Michigan) agar, respectively, at 4 °C. The 0.1 M phosphate buffer (pH 7.2) used for whole-cell suspensions of *B. cereus* consists of K_2HPO_4, 10.6 g; KH_2PO_4, 4.08 g; dextrose, 20 g; and distilled H_2O, 1000 mL.

Fermentation Procedures. Microbial metabolism studies were carried out by incubating the cultures with shaking on an Innova 5000 Gyrotory shaker (New Brunswick Scientific Co., NJ), operating at 150 rpm and 25 °C. Preliminary screening experiments were carried out in 125-mL stainless steel capped DeLong culture flasks containing 25 mL of medium. The media were sterilized at 121 °C and 18 psi for 15 min. Fermentations were carried out according to a standard two-stage protocol. In general, the substrate was added in DMF (1 mg/10 μL) to the incubation media 24 h after inoculation of stage II cultures at a concentration of 0.2 mg/mL. The fermentations were sampled at 24 h intervals by extracting 3 mL of the culture broth with 3 × 3 mL of EtOAc. The extracts were concentrated and chromatographed on TLC plates. Substrate controls were composed of sterile media to which the substrate was added and incubated without microorganisms. Culture controls consisted of fermentation blanks in which the microorganisms were grown under identical conditions without the addition of the substrate. Substrate-autoclaved culture controls consisted of microbial cultures that were grown under the usual conditions to maturity (usually 5−7 days), autoclaved for 30 min, and then incubated after the substrate was added.

Biotransformation of 1 to 2. Resveratrol (**1**) was purchased from Sigma Chemical Co. (St. Louis, MO) and Pharmascience Inc. (Montreal, QC). The physical and spectral data of **1** have been widely reported in the literature.[14,17] A standard two-stage fermentation protocol was followed for the bioconversion of **1** to **2**. Twenty 2-L flasks, each containing 400 mL of sterile medium, were inoculated with 3 mL of 72 h stage I *B. cereus* culture and incubated on a shaker for 72 h. Five hundred milliliter aliquots of stage II cultures were then centrifuged at 2724*g* for 18 min at 4 °C. The supernatant portions were decanted and discarded. Each pellet from the aliquots was gently rinsed with 10 mL of sterile phosphate buffer before being resuspended in 200 mL of dextrose-enriched phosphate buffer (filter-sterilized, 0.22 μm). Whole-cell suspensions of *B. cereus* from each aliquot were then placed separately into 16 1-L flasks. Forty milligram portions (in 400 μL DMF) of resveratrol were added to each flask before incubating on the shaker. All incubations and extraction procedures were conducted in covered vessels in order to avoid UV-induced isomerization of **1** and **2**.

Following a 72 h incubation, 500 mL aliquots of the whole-cell suspensions were centrifuged under the previously outlined conditions. The pellets were each rinsed twice with 50 mL of EtOAc. One-liter portions of the supernatant (the buffer) were extracted with 3 × 330 mL of EtOAc. The EtOAc extract was dried over Na_2SO_4, filtered, and evaporated in vacuo yielding 662 mg of a yellow residue. The residue was dissolved in a minimal amount of EtOAc:MeOH (3:1) and chromatographed on 2 preparative TLC plates. Metabolite **2** was visualized under UV light (254 nm) as an intense violet band (R_f 0.15), and the silica from this region was removed from the plate. The silica (8 g) was crushed into a fine powder and extracted with EtOAC:MeOH (3:1) (8 × 4 mL/1 g silica). The solvent was then aspirated, filtered, and evaporated in vacuo. The resulting 454 mg of light yellow residue was again chromatographed on 2 preparative TLC plates under identical conditions, yielding 298 mg of off-white foamy powder. Crystallization from H_2O:Me_2CO (9:1) yielded 121 mg of **2** as white needles (11% yield): mp 136−138 °C (lit.[14] mp 135−137 °C); $[\alpha]^{25}_D$ −51.8°(*c* 0.5 g/100 mL, MeOH) (lit.[16] $[\alpha]^{25}_D$ −60°); FABMS (positive) *m*/*z* 391 $[MH]^+$; HR FABMS (positive) *m*/*z* 391.1403 (calcd for $C_{20}H_{23}O_8$, 391.1392); spectral data (IR, UV, ^{1}H and ^{13}C NMR) of **2** were in agreement with those reported in the literature for piceid.[14,15] Metabolite **2** was identical in all respects to an authentic sample of piceid.

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