Microbial Transformation of the Eudesmane Sesquiterpene Plectranthone

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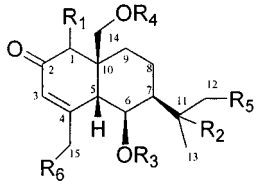
Microbial transformation studies of plectranthone (**1**) have revealed that it was metabolized by a number of microorganisms. Using a standard two-stage fermentation technique, *Beauvaria bassiana* (ATCC 7159) produced five metabolites, **2**−**6**. These metabolites were characterized on the basis of spectral data.

Plectranthone (**1**) is a diacetate *cis*-eudesmane sesquiterpene isolated, along with desacetylplectranthone and isodesacetylplectranthone, from the aerial parts of *Plectranthus cylindraceus* [1] Hochst. Ex Benth (Lamiaceae). *P. cylindraceus* is a folk medicine widely used in the southern and western region of Saudi Arabia as a disinfectant and a deodorant.[2]



1: R_1=R_2=R_5=R_6=H, R_3= $\overset{18}{C}O\overset{16}{C}H_3$, R_4= $\overset{19}{C}O\overset{17}{C}H_3$

2: R_1=R_5=R_6=H, R_2=OH, R_3= $\overset{18}{C}O\overset{16}{C}H_3$, R_4= $\overset{19}{C}O\overset{17}{C}H_3$

3: R_1=R_2=R_4=R_5=R_6=H, R_3= $\overset{18}{C}O\overset{16}{C}H_3$

4: R_1=OH, R_2=R_4=R_5=R_6=H, R_3= $\overset{18}{C}O\overset{16}{C}H_3$

5: R_1=R_2=R_3=R_6=H, R_4= $\overset{19}{C}O\overset{17}{C}H_3$, R_5=OH

6: R_1=R_2=R_4=R_5=H, R_3= $\overset{18}{C}O\overset{16}{C}H_3$, R_6=OH

Microorganisms, particularly fungi, have been used successfully as in vitro models for the prediction of mammalian drug metabolism.[3−8] It was anticipated that the microbial metabolism of **1** would produce significant quantities of metabolites that would be difficult to obtain from either animal systems or chemical synthesis. The results from this method often parallel those obtained from human biotransformation and, thus, can be predictive. This report relates the microbial transformation of **1** and the isolation and characterization of its metabolites.

Of 20 microbial cultures screened for their ability to catalyze bioconversion of plectranthone (**1**), *Absidia glauca* ATCC 22752, *Aspergillus flavipes* ATCC 11013, *Beauvaria bassiana* ATCC 7159, *Cladosporium resinae* ATCC 22712, and *Penicillium frequentans* ATCC 10444 showed definite metabolism. These cultures produced several more polar metabolites, some of which were produced by more than one culture. It was shown (TLC basis) that *B. bassiana* ATCC 7159 was the most efficient microorganism to metabolize plectranthone (**1**) into five metabolites and, therefore, was selected for preparative-scale fermentation. Metabolites **2**−**6** were isolated by solvent extraction, purified by chromatography, and subjected to spectral analyses. Complete unambiguous assignments of ^{1}H and ^{13}C resonances of plectranthone were previously reported. [1]

Metabolite **2** was isolated as colorless oil in a 2.1% yield. The molecular formula was found to be $C_{19}H_{28}O_6$ on the basis of the ion peaks at *m/z* 375.1810 [M + Na]$^+$, *m/z* 353.1999 [M + 1] $^+$, and *m/z* 293.1749 [M + 1 − CH_3COOH]$^+$, and NMR data. Most ^{1}H and ^{13}C NMR (see Experimental Section) data of **2** were similar to those of **1** except for resonances attributed to the isopropyl side chain. The ^{13}C NMR spectra revealed the presence of an oxygenated quaternary signal at δ 72.1, which was concluded to be attached to a hydroxyl group, as suggested by the IR spectrum (ν_{max} 3440 cm^{-1}) and by the presence of a D_2O exchangeable proton at δ 1.90. This hydroxyl group was determined from HMBC spectra to be at C-11 due to the presence of long-range correlations between C-11 (δ 72.1, s) and the two methyl group protons H-12 and H-13, which resonated as singlets at δ 1.20 and 1.22. This was further supported by the expected downfield shifts for C-7 (3.5 ppm), C-12 (6.8 ppm), and C-13 (8.1 ppm) and the upfield shift for C-8 (2.7 ppm) relative to those of **1**. The unambiguous assignment of C-19 was possible due to the presence of ^3J cross-peaks with H-14a (δ 3.92, d, J = 11.1 Hz) and H-14b (δ 4.48, d, J = 11.1 Hz). Likewise, C-18 (δ 170.7, s) showed ^3J cross-peak with H-6 (δ 5.82, br s), thus confirming its assignment. Therefore, **2** was established as 11-hydroxyplectranthone.

Metabolite **3** was isolated as colorless needles in 21.2% yield. It was shown to possess the molecular formula $C_{17}H_{26}O_4$ from the presence of an ion peak at *m/z* 234 [M − CH_3COOH]$^+$ and from NMR data. Moreover, the NMR data showed that **3** was desacetylplectranthone and identical to that previously isolated from *P. cylindraceous*.[1] Other physical and spectral data were indistinguishable from those previously reported.[1]

Metabolite **4** was isolated as colorless oil in 2.5% yield. HRESIMS of **4** gave a characteristic ion peak at *m/z* 333.1669 [M + Na]$^+$ and was consistent with the molecular formula $C_{17}H_{26}O_5$. The ^{13}C NMR revealed the loss of one acetate moiety and the oxygenation of one methylene carbon (C-1). The HMBC spectra showed strong three-bond correlation between H-6 (δ 5.50, br s) and C-18 (δ 171.0, s), suggesting that **4** was a desacetylplectranthone derivative. The oxygenated methine proton (H-1, δ 3.93, s) showed a two-bond cross-peak with C-2 (δ 197.8, s). On the other hand, three-bond correlations between H-3 (δ 5.95, s) and C-1 (δ 78.5, d), and H-1 and C-14 (δ 68.1, t), ensured the position of the microbially added hydroxyl group as C-1. Therefore, **4** is 1-hydroxydesacetylplectranthone. Due to the

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low yield (2.5%) and the progressive decomposition of **4**, the stereochemistry around C-1 was left ambiguous.

Metabolite **5** gave a HRESIMS spectrum similar to that of **4**, consistent with the molecular formula $C_{17}H_{26}O_5$, suggesting a hydroxydesacetyl derivative of **1**. The ^{13}C NMR and HMBC spectra unambiguously determined that **5** was 12-hydroxyisodesacetylplectranthone. The HMBC spectra showed three-bond correlations between H-14a (δ 3.37, d, J = 10.6 Hz) and H-14b (δ 4.04, d, J = 10.6 Hz) and C-19 (δ 170.9, s), H-12a (δ 3.61, dd, J = 10.6, 4.8 Hz) and H-12b (δ 3.66, dd, J = 10.6, 4.8 Hz) and C-7 (δ 38.7, d), H-13 (δ 1.00, d, J = 6.9 Hz) and C-12 (δ 65.9, t), and H-12a & H-12b and C-13 (δ 15.2, q).

Metabolite **6**, isolated in 1% yield, gave a HRESIMS spectrum similar to those of **4** and **5**. It was concluded that **6** was another hydoxydesacetyl derivative of **1**. However, ^{13}C NMR and HMBC spectra of **6** indicated its structure to be 15-hydroxydesacetylplectranthone. The HMBC spectra presented important correlations between H-6 (δ 5.34, br s) and C-18 (δ 171.7, s), and H-15a (δ 4.28, d, J = 15.9 Hz) and H-15b (δ 4.52, d, J = 15.9 Hz), and C-3 (δ 125.5, d) and C-4 (δ 160.6, s).

Experimental Section

General Experimental Procedures. Melting points were determined in open capillary tubes, using a Mettler 9100 electrothermal melting point apparatus, and were uncorrected. The IR spectra were recorded in a KBr disk using an ATI Mattson Genesis Series FTIR spectrophotometer. UV spectra were measured in methanol using a UV-160 IPC UV−visible dual-beam spectrophotometer. Optical rotations were taken with a Perkin-Elmer 241 MC polarimeter. The ^1H and ^{13}C NMR were obtained on a Bruker DRX-500 spectrometer operating at 500 and 125 MHz, respectively. Both ^1H and ^{13}C NMR spectra were recorded in $CDCl_3$, and the chemical shift values were expressed in δ (ppm) relative to the internal standard TMS. For the ^{13}C NMR spectra, the number of attached protons was determined by DEPT 135°. 2D NMR data were obtained using the standard pulse sequence of the Bruker DRX-500 for COSY, HMQC, HMBC, and NOESY. Low-resolution EIMS were obtained using a Shimadzu QP5000 gas chromotography/mass spectrometer. HRMS was carried out using a Bruker Bioapex FTMS with an electrospray ionization spectrometer.

Cultures and Fermentation Screening Procedure. The microbial cultures were originally obtained from the American Type Culture Collection (ATCC), Rockville, MD, or from the USDA Northern Regional Research Laboratories (NRRL), Peoria, IL, and are maintained in King Saud University, Department of Pharmacognosy Culture Collection. *Helicostylum piriforme* QM 6945 was obtained from Quartermaster Culture Collection, Quartermaster Research and Engineering Commands, United States Army Natick Laboratories, Natick, MA. Stock cultures were maintained on agar slants of media recommended by the ATCC and were stored at 4 °C.

The microorganisms used were *Absidia glauca* ATCC 22752, *Aspergillus alliaceus* NRRL 315, *Aspergillus flavipes* ATCC 11013, *Aspergillus flavus* NRRL 501, *Aspergillus niger* ATCC 16888, *Beauvaria bassiana* ATCC 7159, *Bullera alba* ATCC 18568, *Caldariomyces fumago* ATCC 11925, *Candida albicans* B311, *Cladosporium resinae* ATCC 22712, *Cunninghamella echinulata* NRRL 3655, *Cunninghamella echinulata* ATCC 9244, *Debaryomyces polymorphus* ATCC 20280, *Doratomyces microsporus* ATCC 16225, *Eupenicillium javanicum* ATCC 26879, *Geotrichum amycelicum* ATCC 24658, *Hansenula anomala* ATCC 20170, *Helicostylum piriforme* QM 6945, *Kluyveromyces marxianus var. lactis* ATCC 2628, and *Penicillium frequentans* ATCC 10444.

All the preliminary screening and preparative-scale experiments were carried out as reported before[9] and according to a standard two-stage protocol.[3,7] Substrate **1** was prepared as a 15% solution in *N*,*N*-dimethylformamide (DMF) and added to the 24-h-old stage II culture medium of the microorganism at a concentration 0.3 mg/mL of medium. Substrate controls were composed of sterile medium to which the substrate (4 mg/100μL DMF) was added and incubated without microorganisms. Culture controls consisted of fermentation blanks in which the microorganisms were grown under identical conditions but without the substrate addition. After 2 weeks of incubation, each control was harvested and analyzed by TLC.

Fermentation Sampling and Chromatographic Conditions. The fermentations were sampled by withdrawing 5 mL of culture and extracting it with 5 mL of $CHCl_3$. The concentrated organic phase was analyzed by TLC for the presence of metabolites. TLC analyses were performed on precoated silica gel 60 F_{254} (Merck) plates using 4% MeOH in $CHCl_3$ as the solvent system. Visualization was accomplished by exposure to short-wavelength UV (λ_{max} 254) and spraying with *p*-anisaldehyde spray reagent. The adsorbent used for column chromatography was Si gel 60/230−400 mesh (EM Science). Plectranthone (**1**), the substrate used in this project, was isolated from *P. cylindraceus* as previously reported.[1] All solvents used for chromatographic purposes were reagent grade.

Preparative-Scale Fermentation of 1 by *Beauvaria bassiana*. *B. bassiana* ATCC 7159 was grown in 21 250-mL culture flasks each containing 50 mL of medium α. A total of 315 mg of **1** (in 2.1 mL of DMF) was evenly distributed among the 24-h-old stage II culture. After 2 weeks, the incubation mixtures were checked by TLC. TLC revealed that most of **1** was transformed and five metabolites were produced.

The incubation mixtures were combined and filtered to remove the mycelia, and the filtrate (1.05 L) was extracted with $CHCl_3$ (1 L × 4). The combined extracts were dried over anhydrous Na_2SO_4 and evaporated to dryness in vacuo at 40 °C to afford a brownish residue (0.51 g), which was purified by column chromatography over silica gel (51 g, 2 × 40 cm) using MeOH−$CHCl_3$ (3:17) as the eluting solvent system. Fractions of 20 mL each were collected and pooled on the basis of TLC analyses. Fractions 33−35 yielded semipure 11-hydroxyplectranthone (**2**) (38 mg), which upon further purification afforded 7 mg of pure **2** (2.1% yield) with R_f = 0.29. Moreover, fractions 43−61 yielded desacetylplectranthone (**3**) (R_f = 0.24, 58 mg, 21.2% yield), fractions 81−110 yielded 1-hydroxydesacetylplectranthone (**4**) (R_f = 0.17, 8 mg, 2.5% yield), fractions 114−145 yielded 12-hydroxyisodesacetylplectranthone (**5**) (R_f = 0.12, 1.4 mg, 0.5% yield), and fractions 158−214 yielded 15-hydroxydesacetylplectranthone (**6**) (R_f = 0.08, 2.7 mg, 1% yield).

11-Hydroxyplectranthone (2): colorless oil; $[\alpha]^{25}_D$ −8.0° (*c* 0.14, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 237 (3.78) nm; IR (neat) ν_{max} 3440, 2920, 1740, 1670 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 5.96 (1H, br s, H-3), 5.82 (1H, br s, H-6), 4.48, 3.92 (each 1H, d, J = 11.1 Hz, H-14), 3.08 (1H, br s, H-5), 2.61, 2.06 (each 1H, d, J = 15.8 Hz, H-1), 2.11 (3H, s, CH_3-16 or 17), 2.08 (3H, s, CH_3-16 or 17), 2.06 (3H, s, CH_3-15), 1.90 (1H, br s, OH), 1.77 (1H, m, H-8a), 1.66 (1H, m, H-9a), 1.64 (1H, m, H-8b), 1.51 (1H, m, H-9b), 1.33 (1H, m, H-7), 1.22 (3H, s, CH_3-12 or 13), 1.20 (3H, s, CH_3-12 or 13); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 198.3 (s, C-2), 171.2 (s, C-19), 170.7 (s, C-18), 157.8 (s, C-4), 128.8 (d, C-3), 72.1 (s, C-11), 71.4 (d, C-6), 69.7 (t, C-14), 47.9 (t, C-1), 46.7 (d, C-7), 44.6 (d, C-5), 38.7 (s, C-10), 29.3 (q, C-12 or 13), 29.2 (t, C-9), 27.9 (q, C-12 or 13), 23.0 (q, C-15), 21.7 (q, C-16 or 17), 21.1 (q, C-16 or 17), 18.2 (t, C-8); HRESIMS *m*/*z* 353.1999 (calcd for $C_{19}H_{29}O_6$ [M + H]$^+$, 353.1964).

Desacetylplectranthone (3): colorless needles ($CHCl_3$): mp 213−214 °C. Other spectral data were indistinguishable from those previously reported.[1]

1-Hydroxydesacetylplectranthone (4): colorless oil; $[\alpha]^{25}_D$ −27.7° (*c* 0.15, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 240 (3.70) nm; IR (neat) ν_{max} 3440, 2900, 1730, 1670 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 5.95 (1H, br s, H-3), 5.50 (1H, br s, H-6), 3.93 (1H, s, H-1), 3.90, 3.59 (each 1H, d, J = 11.3 Hz, H-14), 3.24 (1H, br s, H-5), 2.14 (3H, s, CH_3-15), 2.10 (3H, s, CH_3-16), 1.72 (1H, m, H-8a), 1.64 (1H, m, H-11), 1.62 (1H, m, H-8b), 1.48, 1.37 (each 1H, m, H-9), 1.27 (2H, s, OH-1 and OH-14), 1.14 (1H,

m, H-7), 0.94 (3H, d, J = 6.2 Hz, CH_3-12 or 13), 0.93 (3H, d, J = 6.2 Hz, CH_3-12 or 13); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 197.8 (s, C-2), 171.0 (s, C-18), 162.6 (s, C-4), 125.5 (d, C-3), 78.5 (d, C-1), 72.5 (d, C-6), 68.1 (t, C-14), 43.7 (s, C-10), 43.3 (d, C-7), 42.4 (d, C-5), 28.7 (d, C-11), 25.2 (t, C-9), 24.2 (q, C-15), 22.4 (q, C-12 or 13), 21.7 (q, C-16), 21.3 (q, C-12 or 13), 21.3 (t, C-8); HRESIMS m/z 333.1669 (calcd for $C_{17}H_{26}O_5Na$ $[M + Na]^+$, 333.1677).

12-Hydroxyisodesacetylplectranthone (5): colorless oil; $[\alpha]^{25}_D$ −10.2° (c 0.02, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 242 (3.94) nm; IR (neat) ν_{max} 3400, 2920, 1730, 1660 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 5.94 (1H, br s, H-3), 5.61 (1H, br s, H-6), 4.04 (1H, d, J = 10.6 Hz, H-14a), 3.66, 3.61 (each 1H, dd, J = 10.6, 4.8 Hz, H-12), 3.37 (1H, d, J = 10.6 Hz, H-14b), 3.16 (1H, br s, H-5), 2.67 (1H, d, J = 15.8 Hz, H-1a), 2.12 (3H, s, CH_3-17), 2.11 (3H, s, CH_3-15), 2.04 (1H, d, J = 15.8 Hz, H-1b), 1.64 (1H, m, H-8a), 1.62 (1H, m, H-9a), 1.59 (1H, m, H-11), 1.58 (1H, m, H-8b), 1.58 (1H, br s, OH), 1.37 (1H, m, H-7), 1.36 (1H, m, H-9b), 1.28 (1H, s, OH), 1.00 (3H, d, J = 6.9 Hz, CH_3-13); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 199.4 (s, C-2), 170.9 (s, C-19), 159.0 (s, C-4), 128.5 (d, C-3), 71.6 (d, C-6), 68.1 (t, C-14), 65.9 (t, C-12), 47.7 (t, C-1), 43.3 (d, C-5), 40.6 (s, C-10), 38.7 (d, C-7), 38.0 (d, C-11), 28.6 (t, C-9), 23.2 (q, C-15), 21.7 (q, C-17), 21.1 (t, C-8), 15.2 (q, C-13); HRESIMS m/z 333.1673 (calcd for $C_{17}H_{26}O_5Na$ $[M + Na]^+$, 333.1677).

15-Hydroxydesacetylplectranthone (6): colorless oil; $[\alpha]^{25}_D$ −27.9° (c 0.06, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 238 (3.72) nm; IR (neat) ν_{max} 3400, 2920, 1730, 1660 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 6.21 (1H, br s, H-3), 5.34 (1H, br s, H-6), 4.52, 4.28 (each 1H, d, J = 15.9 Hz, H-15), 3.95, 3.37 (each 1H, d, J = 10.6 Hz, H-14), 3.28 (1H, br s, H-5), 2.65 (1H, d, J = 15.8 Hz, H-1a), 2.13 (3H, s, CH_3-16), 2.11 (1H, d, J = 15.8 Hz, H-1b), 1.67 (1H, m, H-8a), 1.66 (1H, m, H-9a), 1.59 (1H, m, H-11), 1.54 (1H, br s, OH), 1.52 (1H, m, H-8b), 1.44 (1H, m, H-9b), 1.27 (1H, s, OH), 1.08 (1H, m, H-7), 0.93 (3H, d, J = 6.7 Hz, CH_3-12 or 13), 0.92 (3H, d, J = 6.7 Hz, CH_3-12 or 13); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 199.8 (s, C-2), 171.7 (s, C-18), 160.6 (s, C-4), 125.5 (d, C-3), 72.1 (d, C-6), 68.2 (t, C-14), 64.6 (t, C-15), 47.2 (t, C-1), 43.4 (d, C-7), 40.7 (s, C-10), 40.4 (d, C-5), 29.0 (d, C-11), 28.8 (t, C-9), 21.7 (q, C-16), 21.2 (q, C-12 or 13), 21.1 (q, C-12 or 13), 21.0 (t, C-8); HRESIMS m/z 333.1671 (calcd for $C_{17}H_{26}O_5Na$ $[M + Na]^+$, 333.1677).

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