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INDUCED PRODUCTION OF TREMULANE SESQUITERPENOIDS IN *Bjerkandera adusta* BY CHEMICAL EPIGENETIC MODIFICATION

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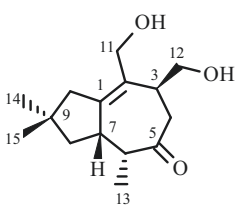
It is well accepted that the secondary metabolism of fungi can be regulated by chemical epigenetics, which involves the modification of chromatin by small molecules targeting epigenome-modifying enzymes such as histone deacetylase (HDACs) and DNA methyltransferases (DNMTs) [1–3]. Since the first report on the activation of silent BGCs in *Aspergillus alternata* and *Penicillium expansum* by the HDACs inhibitor trichostatin A [4], research has exploded in the applications of this concept to mine the fungal genomes, with DNMT and HDAC inhibitors being the most commonly used chemical epigenetic modifiers [5–7]. Herein, as part of our ongoing endeavor in exploring the chemical diversities of Basidiomycetes [8–11], we report the chemical epigenetic regulation of *Bjerkandera adusta* HS-28, an endophyte of the medicinal plant *Huperzia serrata*. Subsequent chemical investigation led to the isolation and characterization of seven compounds **1–7**, including six tremulane sesqiterpenoids **1–6**, as the induced products. To the best of our knowledge, this is the first report on the chemical epigenetic regulation of the fungal genus *Bjerkandera*. In addition, the tremulane sesquiterpenoids were obtained from *Bjerkandera* for the first time.

The fungus *B. adusta* HS-28 was first cultured on shakers at 28°C and 180 rpm in liquid PD medium (potato extracts 200 g, glucose 20 g, distilled water 1 L) supplemented with 300 μM suberoylanilide hydroxamic acid (SAHA) for 6 d, followed by static cultivation for 14 d. The cultures (30 L) were filtered through cheesecloth to separate broth and mycelia. The mycelia were soaked with acetone to afford an extract, which was then suspended in water and partitioned with ethyl acetate. The organic phases were evaporated under reduced pressure to yield a residue (15 g), which was subjected to column chromatography (CC) on MCI CHP20P eluting with a gradient of MeOH–H_2O (20:80→100:0) to give 11 fractions (Frs. A–K). Fraction D (1.5 g) was separated by silica gel CC eluting with a gradient of petroleum ether–acetone (10:1–1:1) to afford five subfractions (Subfrs. D1–D5). Subfraction D3 (150 mg) was further puried by silica gel CC eluting with $CHCl_3$–MeOH (40:1) to yield **2** (5 mg). Compounds **4** (4 mg) and **3** (7 mg) were obtained from Subfrs. D4 and D5, respectively, by silica gel CC eluting with $CHCl_3$–MeOH (30:1). Fraction E (460 mg) was subjected to silica gel CC eluting with $CHCl_3$–MeOH (45:1) to yield **1** (6 mg). Compounds **5** and **6** were obtained from Fr. F (120 mg) as an inseparable mixture (50 mg) by silica gel column chromatography eluting with $CHCl_3$–MeOH (120:1). Fraction G (434 mg) was separated by silica gel CC eluting with a gradient of $CHCl_3$–MeOH (50:1–10:1) to afford **7** (8 mg).



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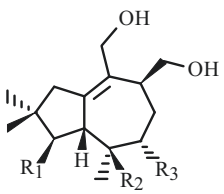
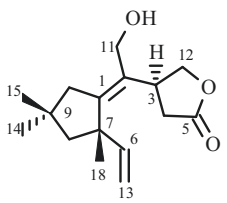


2 - 4

2: $R_1 = R_3 = H$, $R_2 = OH$
3: $R_1 = OH$, $R_2 = R_3 = H$
4: $R_1 = R_2 = H$, $R_3 = OH$



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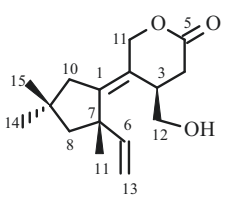

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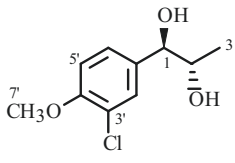

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The structures of the isolates were elucidated on the basis of detailed analyses of their spectroscopic data, including NMR and MS spectra. They were identified as 11,12-dihydroxy-1-tremulen-5-one (**1**) [12], ((3*S*,6*R*,7*R*)-tremul-1-ene-6,11,12-triol (**2**) [13], ceriponol A (**3**) [8], conocenol B (**4**) [14], conocenolide A (**5**) and B (**6**) [14], and (±)-trametol (**7**) [15].

11,12-Dihydroxy-1-tremulen-5-one (1), $C_{15}H_{24}O_3$, colorless oil. ESI-MS *m/z* 275 $[M + Na]^+$. ^1H NMR (500 MHz, $CDCl_3$, δ, ppm, J/Hz): 4.30 (1H, d, J = 11.0, H_a-11), 3.94 (1H, d, J = 11.5, H_b-11), 3.66 (1H, dd, J = 10.5, 5.5, H_a-12), 3.56 (1H, dd, J = 10.0, 7.5, H_b-12), 3.08 (1H, m, H-7), 2.84 (1H, m, H_a-4), 2.82 (1H, m, H-3), 2.62 (1H, m, H_b-4), 2.53 (1H, m, H-6), 2.40 (1H, dd, J = 15.5, 2.5, H_a-10), 2.09 (1H, d, J = 15.0, H_b-10), 1.61 (1H, ddd, J = 12.0, 12.0, 2.0, H_a-8), 1.50 (1H, dd, J = 11.0, 11.0, H_b-8), 1.13 (3H, s, H-14), 1.04 (3H, d, J = 7.0, H-13), 0.92 (3H, s, H-15). ^{13}C NMR (125 MHz, $CDCl_3$, δ, ppm): 214.9 (C-5), 145.7 (C-1), 131.6 (C-2), 65.1 (C-11), 63.1 (C-12), 49.8 (C-6), 48.2 (C-10), 43.9 (C-8), 43.7 (C-3), 41.7 (C-7), 41.0 (C-4), 37.9 (C-9), 28.4 (C-14), 26.8 (C-15), 11.1 (C-13).

(3*S*,6*R*,7*R*)-Tremul-1-ene-6,11,12-triol (2), $C_{15}H_{26}O_3$, colorless oil. ESI-MS *m/z* 277 $[M + Na]^+$. ^1H NMR (500 MHz, CD_3OD, δ, ppm, J/Hz): 4.00 (1H, d, J = 11.0, H_a-11), 3.88 (1H, d, J = 11.0, H_b-11), 3.71 (1H, dd, J = 11.0, 8.0, H_a-12), 3.58 (1H, dd, J = 11.0, 7.5, H_b-12), 3.03 (1H, dd, J = 11.0, 8.0, H-7), 2.53 (1H, dddd, J = 8.0, 7.0, 4.0, 4.0, H-3), 2.26 (1H, dd, J = 15.0, 2.5, H_a-10), 1.93 (1H, br.d, J = 15.0, H_b-10), 1.92 (1H, m, H_a-5), 1.87 (1H, m, H_a-4), 1.67 (2H, m, H_a-4, 5), 1.59 (1H, ddd, J = 13.0, 8.0, 2.5, H_a-8), 1.51 (1H, dd, J = 13.0, 11.0, H_b-8), 1.06 (3H, s, H-15), 1.04 (3H, s, H-13), 0.83 (3H, s, H-14). ^{13}C NMR (125 MHz, CD_3OD, δ, ppm): 144.0 (C-1), 134.6 (C-2), 73.2 (C-6), 66.1 (C-11), 61.5 (C-12), 53.4 (C-7), 47.9 (C-10), 44.2 (C-3), 43.9 (C-8), 42.1 (C-5), 36.8 (C-9), 28.8 (C-15), 27.0 (C-14), 25.1 (C-4), 19.7 (C-13).

Ceriponol A (3), $C_{15}H_{26}O_3$, colorless oil. ESI-MS *m/z* 277 $[M + Na]^+$. ^1H NMR (500 MHz, $CDCl_3$, δ, ppm, J/Hz): 4.21 (1H, d, J = 11.0, H_a-11), 3.92 (1H, dd, J = 9.0, 9.0, H_a-12), 3.78 (1H, d, J = 11.0, H_b-11), 3.56 (1H, dd, J = 9.0, 4.0, H_b-12), 3.52 (1H, d, J = 9.0, H-8), 2.66 (1H, br.d, J = 8.5, H-7), 2.53 (1H, m, H-3), 2.37 (1H, d, J = 15.5, H_a-10), 2.09 (1H, m, H-6), 1.87 (1H, d, J = 15.0, H_b-10), 1.81 (1H, m, H_a-4), 1.79 (1H, m, H_a-5), 1.69 (1H, m, H_b-5), 1.62 (1H, m, H_b-4), 1.06 (3H, s, H-15), 0.86 (3H, d, J = 7.0, H-13), 0.82 (3H, s, H-14). ^{13}C NMR (125 MHz, $CDCl_3$, δ, ppm): 139.9 (C-1), 133.6 (C-2), 82.2 (C-8), 64.5 (C-11), 63.2 (C-12), 53.6 (C-7), 45.2 (C-3), 44.6 (C-10), 39.4 (C-9), 32.4 (C-5), 29.2 (C-6), 26.0 (C-15), 22.4 (C-4), 19.6 (C-14), 12.3 (C-13).

Conocenol B (4), $C_{15}H_{26}O_3$, colorless oil. ESI-MS *m/z* 255 $[M + H]^+$. ^1H NMR (500 MHz, CD_3OD, δ, ppm, J/Hz): 4.05 (1H, d, J = 11.5, H_a-11), 3.98 (1H, m, H-5), 3.96 (1H, d, J = 11.5, H_b-11), 3.63 (2H, m, H-12), 2.89 (1H, br.t, J = 9.0, H-7), 2.61 (1H, m, H-3), 2.28 (1H, dd, J = 15.0, 2.5, H_a-10), 1.98 (1H, br.d, J = 15.0, H_b-10), 1.95 (1H, m, H_a-4), 1.88 (1H, m, H-6), 1.70 (1H, ddd, J = 13.0, 13.0, 3.5, H_b-4), 1.57 (1H, ddd, J = 12.0, 12.0, 2.5, H_a-8), 1.48 (1H, dd, J = 12.0, 12.0, H_b-8), 1.09 (3H, s, H-14), 0.88 (3H, s, H-15), 0.79 (3H, d, J = 7.0, H-13). ^{13}C NMR (125 MHz, CD_3OD, δ, ppm): 145.2 (C-1), 133.5 (C-2), 73.2 (C-5), 66.2 (C-11), 62.8 (C-12), 49.1 (C-10), 46.7 (C-8), 44.2 (C-7), 43.7 (C-3), 40.8 (C-6), 38.6 (C-9), 30.6 (C-4), 29.1 (C-14), 27.5 (C-15), 6.3 (C-13).

Conocenolides A (5) and **B (6)**, $C_{15}H_{22}O_3$, colorless oil. The two compounds were obtained as an inseparable mixture in the ratio of approximately 2:1 deduced from the intensity of NMR resonances, based on which the ^{13}C NMR signals were assigned. ^{13}C NMR for **5** (125 MHz, $CDCl_3$, δ, ppm): 177.4 (C-5), 148.2 (C-1), 141.9 (C-6), 128.9 (C-2), 113.3 (C-13), 71.0 (C-12), 60.3 (C-11), 48.1 (C-8), 46.0 (C-7), 45.8 (C-10), 37.6 (C-9), 36.9 (C-3), 32.5 (C-4), 28.4 (C-14), 27.1 (C-15). ^{13}C NMR for **6** (125 MHz, $CDCl_3$, δ, ppm): 172.3 (C-5), 143.0 (C-1), 139.7 (C-6), 123.3 (C-2), 114.3 (C-13), 69.1 (C-11), 63.1 (C-12), 48.2 (C-8), 46.3 (C-10), 45.8 (C-7), 37.6 (C-9), 35.2 (C-3), 31.9 (C-4), 28.5 (C-14), 27.2 (C-15).

(±)-Trametol (7), $C_{10}H_{13}ClO_3$, yellowish powder. $[\alpha]_D^{25}$ 0°. ^1H NMR (500 MHz, $CDCl_3$, δ, ppm, J/Hz): 7.37 (1H, d, J = 2.0, H-2′), 7.19 (1H, dd, J = 7.0, 2.0, H-6′), 6.89 (1H, d, J = 7.0, H-5′), 4.58 (1H, d, J = 3.5, H-1), 3.95 (1H, m, H-2), 3.86 (3H, s, H-7′), 1.05 (3H, d, J = 5.5, H-3). ^{13}C NMR (125 MHz, $CDCl_3$, δ, ppm): 154.6 (C-4′), 133.7 (C-1′), 128.7 (C-2′), 126.2 (C-6′), 122.5 (C-3′), 111.9 (C-5′), 76.6 (C-1), 71.3 (C-2), 56.3 (C-7′), 17.4 (C-3).

To date, the vast majority of research in epigenetic mining of fungal metabolites has focused on Ascomycetes, especially the genus *Aspergillus* and *Fusarium*. Members of the Basidiomycetes and Mucorales, which are rich in various secondary metabolites, have largely been ignored [16]. The fungus *B. adusta* was known as an important decomposer in the fungal community [17] and was also reported to degrade highly recalcitrant xenobiotics such as industrial dyes [18, 19]. However, little is known about its secondary metabolites, except for several simple phenolic chlorides. In the present study, *B. adusta* HS-28, a fungal endophyte belonging to the division of Basidiomycetes, was subjected to chemical epigenetic regulation by SAHA. Seven compounds **1–7**, including six tremulane sesqiterpenoids **1–6**, were isolated and identified from the mycelium. The tremulane sesquiterpenoids **1–6** were determined to be induced by SAHA by comparing with our previous chemical investigation on the fungus cultured without SAHA [20]. To the best of our knowledge, this is the first report on the occurrence of tremulane sesquiterpenoids in the fungal genus *Bjerkandera*. Tremulane sesquiterpenopids were first isolated from the

aspen rotting fungus *Phellinus tremulae* [21]. As compared with other types of sesquiterpenoids, the tremulane sesquiterpenopids were not extensively studied, with limited distribution in *Ceriporia lacerate* [8, 9], *Conocybe siliginea* [12, 14], *Phellinus igniarius* [13], and *Marasmius cladophyllus* [22]. Results of the present study provided an alternative source for this type of sesquiterpenoids and verified the feasibility of employing chemical epigenetic methods in activating the silent BGCs of Basidiomycetes.

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