Fitoterapia 109 (2016) 201–205

Contents lists available at ScienceDirect

Fitoterapia

journal homepage: www.elsevier.com/locate/fitote

Biotransformation of patchoulol by *Cunninghamella echinulata* var. *elegans*

Fangfang Xu [a,1], Kangsheng Liao [a,1], Yuhong Liu [b], Zhenbiao Zhang [b], Dean Guo [c,*], Ziren Su [b,*], Bo Liu [a,*]

[a] The Second Affiliated Hospital of Guangzhou University of Chinese Medicine, Guangdong Provincial Academy of Chinese Medical Sciences, Guangzhou 510006, China
[b] College of Chinese Medicines, Guangzhou University of Chinese Medicine, Guangzhou 510006, China
[c] Shanghai Research Center for Modernization of Traditional Chinese Medicine, National Engineering Laboratory for TCM Standardization Technology, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, 501 Haike Road, Shanghai 201203, China

ARTICLE INFO

Article history:
Received 7 November 2015
Received in revised form 31 December 2015
Accepted 4 January 2016
Available online 8 January 2016

Keywords:
Biotransformation
Patchoulol
Cunninghamella echinulata var. *elegans*
X-ray crystallography

ABSTRACT

Biocatalysis of patchoulol (PA) was performed by the fungus *Cunninghamella echinulata* var. *elegans*. Eight metabolites (**1**–**8**) including four new compounds were obtained, and their structures were elucidated as (5*R*,8*S*)-5,8 dihydroxypatchoulol (**1**), (5*R**,9*R**)-5,9 dihydroxypatchoulol (**2**), (6*S**, 9*S**)-6,9 dihydroxypatchoulol (**3**), and (4*R**)-4 hydroxypatchoulol (**4**) by spectroscopic analysis. The absolute configuration of **1** was determined by single crystal X-ray diffraction.

1. Introduction

Patchoulol (PA, Fig. 1) is a bioactive tricyclic sesquiterpene obtained from the dried aerial part of *Pogostemon cablin* (Blanco) Benth. (Labiatae), which is commonly known as "Guang-huo-xiang" in traditional Chinese medicine used for the treatment of common cold, nausea and diarrhea [1].

Previous pharmacological studies have revealed PA to be associated with inhibitory activities on neurotoxicity of β-amyloid peptide [2], enhancing cognition in scopolamine-induced learning and memory impairment of mice [3], anti-inflammatory effects in LPS-stimulated RAW 264.7 macrophages and animal models [4–5] and anti-influenza effects in *in vitro* and *in vivo* [6–7]. However, the utilization of PA as a herbal medicine is greatly limited by its poor hydrophilicity and low bioavailability [8]. The utmost stability of the patchoulol skeleton and the existence of a sterically hindered hydroxyl group bring about tremendous difficulty for structural modification of PA. Conventional chemical synthesis methods fail to give feasible practice. To overcome this difficulty, we developed a biotransformation method trying to acquire the derivations of PA.

Biotransformation is a biochemical reaction that is catalyzed by whole cells (microorganisms, plant cells and animal cells) or by isolated enzymes, with the ability of refined region- and stereoselectivity for the oxidation of remote, unactivated C–H sites in a complex skeleton [9]. Many studies have reported that sesquiterpenoids are susceptible to biotransformation by microorganisms [10–12].

Early reports confirmed that PA could be hydroxylated and acetylated by *Botrytis cinerea* [13], *Absidia coerulea* and *Mucor hiemalis* [14]. In our present work, the fungus strain *Cunninghamella echinulata* var. *elegans* was found to display the hydroxylated ability to PA. Eight metabolites, four of which have not been reported previously, were isolated and identified in the biotransformation processes of PA.

2. Materials and methods

2.1. General experimental procedures

Melting points were measured on an X-5 μ-melting point apparatus. Optical rotations were determined with a Rudolph Autopol I polarimeter in methanol (MeOH). IR spectra were recorded on a Perkin Elmer Spectrum 400 infrared spectrometer. NMR experiments were performed on a Bruker AV-400 spectrometer. Single Crystal Diffractometer was performed on a Bruker Smart 1000 CCD. HPLC

* Corresponding authors.
E-mail addresses: daguo@simm.ac.cn (D. Guo), suziren@gzucm.edu.cn (Z. Su), doctliu@263.net (B. Liu).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.fitote.2016.01.001
0367-326X/

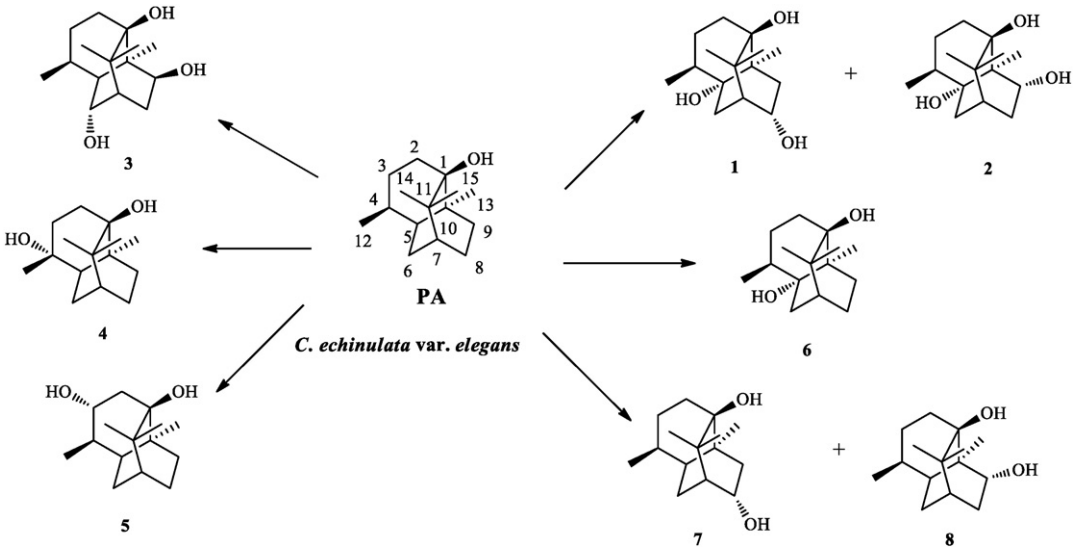

Fig. 1. Structure of biotransformed products **1–8** of Patchoulol.

was measured on Agilent 1260 chromatograph with evaporative light-scattering detector.

2.2. Substance

C. echinulata var. *elegans* ATCC 9245 (American Type Culture Collection) was used. Patchoulol (PA, purity ≥99%) was provided by Professor Su Ziren (Guangzhou University of Chinese Medicine).

2.3. Microorganism and biotransformation of patchoulol by C. echinulata var. elegans

Well-developed *C. echinulata* var. *elegans* was removed from the surface of Potato Dextrose Agar (Medium), suspended in 5 mL sterilized water, and inoculated in a sterile complex medium (20.0 g Sabouraud-dextrose, 15.0 g sucrose and 10.0 g peptone, in 1000 mL deionized water, the pH was adjusted to 6.50 using 0.1 N sodium hydroxide). Cultures were grown in twenty four flasks (500 mL medium in 2.0 L) in rotary shakers at 28 °C with shaking speed at 180 rotations per minutes for 48 h. 10 mL patchoulol (25 mg/mL, in methanol) was filter-sterilized and added to each flask, and the fermentation continued for a further period of 14 days.

2.4. Isolation and purification of metabolites

The cultures were harvested, the broth and mycelia were separated in a Buchner funnel. The mycelia were discarded while the culture broth (12.0 L) was extracted with three equal volumes of ethyl acetate (EtOAc) and concentrated by atmospheric distillation. Then the dark brown residues (2.5 g) were subjected to silica gel column chromatography eluted with a petroleum ether/EtOAc gradient to afford 18 fractions. Fraction 8 (120 mg) was purified using semi-preparative HPLC (MeOH: H_2O, 40: 60, flow rate, 3.0 mL/min) to yield compounds **1** and **2** (169 mg, **1**:**2** = 7.5: 1, t_R 10.5 min), **3** (3.3 mg, purity >90%, t_R 13.3 min) and **4** (8.0 mg, purity >90%, t_R 16.5 min). Fraction 10 was loaded onto Sephadex LH-20 CC (2.5 cm * 50 cm) and eluted with $CHCl_3$/MeOH (1:1, 300 mL) to give **5** (8.2 mg). Fraction 5 (305 mg) was further purified using semi-prepared HPLC (MeOH: H_2O, 70:30, flow rate, 3.0 mL/min) to afford **6** (45 mg, purity >90%, t_R 15.4 min), **7** (11 mg, purity >90%, t_R 19.0 min) and **8** (12.1 mg, purity >90%, t_R 23.7 min).

2.4.1. (5R, 8S)-5, 8 dihydroxypatchoulol (**1**) and (5R*, 9R*)-5, 9 dihydroxypatchoulol (**2**)

White solid; mp: 148–151 °C; $[\alpha]^{25}_D$ + 34.67 (*c* 0.30, MeOH); IR ν_{max} (film): 3427, 2956, 1458, 1336, 1221, 1021 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 2; HREIMS *m/z* = 254.1876 $[M]^+$ ($C_{15}H_{26}O_3$, calcd for 254.1876).

2.4.2. (6S*, 9S*)-6, 9 dihydroxypatchoulol (**3**)

White solid; mp 144–146 °C; $[\alpha]^{25}_D$ + 45.33 (*c* 0.21, MeOH); IR ν_{max} (film) 3428, 2947, 1627, 1456, 1363, 1203, 1034 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 2; HREIMS *m/z* = 254.1877 $[M]^+$ ($C_{15}H_{26}O_3$, calcd for 254.1876).

2.4.3. (4R*)-4 hydroxypatchoulol (**4**)

White solid; mp 156–158 °C; $[\alpha]^{25}_D$ + 85.33 (*c* 0.18, MeOH); IR ν_{max} (film) 3303, 2908, 1650, 1457, 1361, 1220, 1050 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 2; HREIMS *m/z* = 238.1926 $[M]^+$ ($C_{15}H_{26}O_2$, calcd for 238.1927).

2.4.4. (3R*)-3 hydroxypatchoulol (**5**)

White solid; mp 112–114 °C; $[\alpha]^{25}_D$ − 62.33 (*c* 0.24, MeOH); IR ν_{max} (film) 3351, 2945, 1455, 1366, 1289, 1079 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 2; HREIMS *m/z* = 238.1926 $[M]^+$ ($C_{15}H_{26}O_2$, calcd for 238.1927).

2.4.5. (5R*)-5 hydroxypatchoulol (**6**)

White solid; mp 105–107 °C; $[\alpha]^{25}_D$ − 15.76 (*c* 0.34, MeOH); IR ν_{max} (film) 3502, 2955, 1630, 1455, 1323, 1203, 1090, 974 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 2; HREIMS *m/z* = 238.1929 $[M]^+$ ($C_{15}H_{26}O_2$, calcd for 238.1927).

2.4.6. (8S*)-8 hydroxypatchoulol (**7**)

White solid; mp 156–158 °C; $[\alpha]^{25}_D$ − 12.33 (*c* 0.24, MeOH); IR ν_{max} (film) 3369, 2929, 1464, 1386, 1298, 1033, 977, 931 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 2; HREIMS *m/z* = 238.1925 $[M]^+$ ($C_{15}H_{26}O_2$, calcd for 238.1927).

2.4.7. (9R*)-9 hydroxypatchoulol (**8**)

White solid; mp 157–159 °C; $[\alpha]^{25}_D$ − 35.81 (*c* 0.21, MeOH); IR ν_{max} (film) 3369, 2925, 1454, 1366, 1183, 1054, 986, 822 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 2; HREIMS *m/z* = 238.1926 $[M]^+$ ($C_{15}H_{26}O_2$, calcd for 238.1927).