Bioresource Technology 111 (2012) 496–499

Contents lists available at SciVerse ScienceDirect

Bioresource Technology

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

Short Communication

Biotransformation of sophoricoside in *Fructus sophorae* by the fungus *Schizophyllum commune*

Jianguo Wu, Xialu Yang, Juan Ge, Yiping Zhang, Lin Wu, Jianmiao Liu, Xiaoyu Zhang *

Key Laboratory of Molecular Biophysics of Ministry of Education, College of Life Science and Technology, Huazhong University of Science and Technology, Wuhan 430074, China

ARTICLE INFO

Article history:
Received 26 October 2011
Received in revised form 4 February 2012
Accepted 8 February 2012
Available online 16 February 2012

Keywords:
Schizophyllum commune
Sophoricoside
Fructus sophorae
Biotransformation
4′,7-dihydroxy-5-methoxyisoflavone

ABSTRACT

Biotransformation of sophoricoside in *Fructus sophorae* was performed with *Schizophyllum commune*. Sophoricoside was firstly metabolized to 4′,5,7-trihydroxyisoflavone (2), and then to 4′,7-dihydroxy-5-methoxyisoflavone (3) and 5,7-dihydroxy-4′-methoxyisoflavone (4) as determined by NMR and MS analyzes. The content of compound (2) was increased by more than 30-fold, and compound (3) is a new product that showed good cytotoxic activity with an IC_{50} of 12.1 nmol/ml against MCF-7 cells.

1. Introduction

Flavone-rich *Fructus sophorae* (Kite et al., 2009) is used as Traditional Chinese Medicine (TCM) and is reported to have anti-tumorigenic (DeLemos, 2001), anti-angiogenic (Miura et al., 2002), anti-osteoporotic (Shim et al., 2005), anti-atherosclerosis (Si and Liu, 2007) and anti-oxidant (Han et al., 2009) properties. Sophoricoside is the one flavone in highest concentration in *F. sophorae* and can be hydrolyzed to 4′,5,7-trihydroxyisoflavone, which has three OH groups at C-5, C-7, and C-4′, respectively. Ogawara et al. (1989) found that the OH group at C-5 was critical for the inhibitory of tyrosine protein kinase activity in 4′,5,7-trihydroxyisoflavone, and the OH groups at the C-7 and C-4′ positions increased the inhibitory activity. 7-OH and 4′-OH of 4′,5,7-trihydroxyisoflavone are easily replaced by glucoside, acylated glucoside, or methoxy groups, but 5-OH is difficult to be substituted because of its involvement in a strong intra-molecular hydrogen bond with the C-4 carbonyl group (Rusin et al., 2010).

Biotransformation is an alternative tool to chemical modification of natural products due to its capability to catalyze novel reactions and its regio- and stereo-selectivity (Loughlin, 2000; Riva, 2001); (Baqueiro-Pena et al., 2010; Ye et al., 2010)(Uhnakova et al., 2011).

In the present study, biotransformation of sophoricoside in *F. sophorae* was performed by the fungus *Schizophyllum commune*, which produces cellulase, xylanase, glucosidase and other extra-enzymes (Steiner et al., 1989). The main biotransformation products were also isolated, purified and identified by NMR and MS analyzes in order to find some new products. For studying the activities of the products, cytotoxic activity was evaluated against human breast cancer (MCF-7) cell line using MTT assay.

2. Methods

2.1. General experimental procedures

NMR spectra were recorded on a Bruker AV400 spectrometer (400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR) in DMSO-d_6 with tetramethylsilane as internal standard. LC-MS spectra were obtained on an Agilent 1100 LC/MSD Trap. Ethyl acetate (EtOAc) and methanol (MeOH) were purchased from the National Medicine Chemical Group, Shanghai, People's Republic of China. Sophoricoside, 4′,5,7-trihydroxyisoflavone, 5,7-dihydroxy-4′-methoxyisoflavone and 4′, 5-dihydroxy-7-methoxyisoflavone standards were purchased from Sigma–Aldrich Co. *F. sophorae* was ground into powder which was purchased from the Hospital of Chinese Medicine in Hubei, People's Republic of China. HPLC (LC-10AT vp plus, Shimadzu, Japan) on an ODS C_{18} reverse phase column (250 mm × 4.6 mm, 5 μm) was used to detect the content of sophoricoside (1), (2), (3), and (4), and HPLC with a Hypersil C-18 column (100 mm × 4.6 mm, 5 μm) was used to prepare the compounds (2) and (4) using a 50-μL external sample loop.

* Corresponding author at: Key Laboratory of Molecular Biophysics of Ministry of Education, College of Life Science and Technology, Huazhong University of Science and Technology, Luoyu load No. 1034, Wuhan 430074, China. Tel.: +86 027 87792108; fax: +86 027 87792128.
E-mail address: xyzhang.imer@gmail.com (X. Zhang).

0960-8524/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biortech.2012.02.038

2.2. Microorganisms and culture media

S. commune was obtained from the China General Microbiological Culture Collection Center in Beijing, People's Republic of China and grown on potato medium prepared as follows: 200 g of husked potato were cut into pieces, boiled in de-ionized water for 0.5 h, and filtered by filter cloth. The filtrate was combined with water to 1000 mL, and 20 g of glucose was added. Biotransformation medium was prepared by adding 50 g of *F. sophorae* powder to 1000 mL water. All media were sterilized by autoclaving before usage.

2.3. Biotransformation, extraction and isolation

Mycelia of *S. commune* from agar slants (2 cm^2) were transferred with an inoculation needle to 250-mL Erlenmeyer flasks containing 100 mL of potato medium and cultured at 27 °C and 150 r/min for 72 h to make a stock inoculum. A 10-mL volume of the culture was added to a 500-mL flask containing 200 mL of biotransformation medium, and the flasks were placed on a rotary shaker operating at 150 r/min at 27 °C. After 2 d, 100 mL of the 2-day fermentation broth was extracted twice with EtOAc. The EtOAc extract was separated and purified by semi-preparative liquid chromatography (MeOH-0.15% formic acid, 35:65) to obtain (2) (21 mg, 95% purity). After 16 d, 1000 mL of the 16-day fermentation broth was extracted twice with EtOAc. The EtOAc extract was concentrated by vacuum and about 800 mg residue was collected and re-crystallized with EtOAc-water (50:50) three times. The new residue was washed with pure water, then (3) (about 500 mg, 95% purity) was obtained. The water extract in the 16-day fermentation extracted broth was concentrated by vacuum and separated and purified by semi-preparative liquid chromatography (MeOH-0.15% formic acid, 35:65) to obtain (4) (5 mg, 95% purity).

2.4. Cytotoxic assay

Cytotoxic activities of (2) and (3) were evaluated against human breast cancer (MCF-7) cell line by a colorimetric assay using 5 mg/ml of [3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide] (MTT) (Deng and Qin, 2010).

2.5. Enzyme assays

Fifty milliliter of fermentation broth was centrifuged in a low-speed large capacity centrifuge (TDL-5-A, Anting, ShangHai), and the supernatant was utilized as the crude enzyme preparation. Cellulase activity in the crude enzyme was detected as described by Desrochers et al. (1981).

3. Results and discussion

3.1. Biotransformation results

During the biotransformation process, the fermentation medium became more viscous because of the production of schizophyllan (Li et al., 2011), and some biotransformation enzymes were also detected such as β-glucosidase. Three new products were found in *F. sophorae* along with the biotransformation, and listed in Table 1. By day 2, the content of compound (2) had increased more than 30-fold at the expense of sophoricoside (1). After biotransformation for 16 d, two new compounds were obtained. The concentration of compound (3) was 2740 nmol/ml, and the concentration of compound (4) was 120 nmol/ml.

Table 1
Biotransformation of sophoricoside (1) in *F. sophorae* (50 g/1000 mL water) by *S. commune*.

Biotransformation time (days)	Yield of products in fermentation culture (nmol/ml)			
	1	2	3	4
0	2770	93	n.d.	n.d.
2	n.d.	2960	15	n.d.
16	n.d.	150	2740	120

n.d.: no detected.

3.2. Identification of biotransformation compounds

Compound (2) was a yellow powder with a molecular ion $[M+H]^+$ at *m/z* 271 and $[M-H]^-$ at *m/z* 269, which were the same of the ion fragment of (1) $[M-162]^+$ and $[M-162]^-$, respectively. These data suggested that (2) was a deglycoside derivative of (1). On the basis of the molecular structure of (1), the compound (2) might be 4′,5,7-trihydroxyisoflavone. The ^1H NMR spectra of (2) showed the presence of 4′-OH (signal at δ 9.60 (1H, br s, HO-4′)) (Hosny and Rosazza, 1999). On the basis of these spectral data, compound (2) was assigned as 4′,5,7-trihydroxyisoflavone.

Compound (3) was a white powder with a molecular ion $[M+H]^+$ at *m/z* 285 and $[M-H]^-$ at *m/z* 283 respectively. These data suggested that (3) was a monomethylation product of (2). When comparing the NMR spectra of (3) with that of (2), two new signals were observed at δ 3.79 (3H, s, H_3C-O) and δ 56.34 (CH_3-O), and one signal disappeared at δ 12.97 (1H, br s, HO-5). These data suggested that the position of methylation was at 5-OH. On the basis of these spectral data, compound (3) was assigned as 4′,7-dihydroxy-5-meth oxyisoflavone.

Compound (4) was a light yellow power, that showed a $[M+H]^+$ at *m/z* 285 and $[M-H]^-$ at *m/z* 283, respectively. These data suggested that (4) was an isomer of (3). When comparing the NMR spectra of (4) with that of (2), the signal of present existed, but the signal of 4′-OH was not. On the basis of these spectral data, compound (4) was assigned as 5,7-dihydroxy-4′-methoxyisoflavone.

3.2.1. 4′,5,7-Trihydroxyisoflavone

yellow powder; UV (MeOH) λ_{max} (nm): 260; ^1H NMR (DMSO-d_6, 400 MHz) δ 8.33(1H, s, H-2), 6.22(1H, d, *J* = 2.4 Hz, H-6), 6.39 (1H, d, *J* = 2.4 Hz, H-8), 7.37 (2H, dd, *J* = 8.8, 2.0 Hz, H-2′, H-6′), 6.82 (2H, dd, *J* = 8.4, 2.0 Hz, H-3′, H-5′), 12.96 (1H, br s, HO-5). 10.90 (1H, br s, HO-7), 9.60 (1H, br s, HO-4′); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 154.46 (C-2), 122.75 (C-3), 180.68 (C-4), 162.46 (C-5), 99.43 (C-6), 164.73 (C-7), 94.13 (C-8), 158.05 (C-9), 104.93 (C-10), 121.66 (C-1′), 130.62 (C-2′), 115.52 (C-3′), 157.88 (C-4′), 115.52 (C-5′), 130.62 (C-6′); (+)-ESI-MS *m/z* 271.3 (calcd for $C_{15}H_{11}O_5$, 271.2); (−)-ESI-MS *m/z* 269.2 (calcd for $C_{15}H_9O_5$, 269.2).

3.2.2. 4′,7-Dihydroxy-5-methoxyisoflavone

white powder; UV(MeOH) λ_{max} (nm): 256; ^1H NMR (DMSO-d_6, 400 MHz) δ 8.06(1H, s, H-2), 6.38(1H, d, *J* = 2.4 Hz, H-6), 6.40 (1H, d, *J* = 2.4 Hz, H-8), 7.28 (2H, dd, *J* = 8.8, 2.0 Hz, H-2′, H-6′), 6.76 (2H, dd, *J* = 8.4, 2.0 Hz, H-3′, H-5′), 10.70 (1H, br s, HO-7), 9.49 (1H, br s, HO-4′), 3.79 (3H, s, MeO-5); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 150.81(C-2), 125.12 (C-3), 174.21 (C-4), 161.64 (C-5), 99.43 (C-6), 162.63 (C-7), 96.94 (C-8), 159.58 (C-9), 108.33 (C-10), 123.27 (C-1′), 130.67(C-2′), 115.52 (C-3′), 157.46 (C-4′), 115.25 (C-5′), 130.67(C-6′), 56.34 (O-CH_3); (+)-ESI-MS *m/z* 285.3 (calcd for $C_{16}H_{13}O_5$, 285.3); (−)-ESI-MS *m/z* 283.3 (calcd for $C_{16}H_{11}O_5$, 283.3).

3.2.3. 5,7-Dihydroxy-4′-methoxyisoflavone

white powder; UV(MeOH) λ_{max} (nm): 258; ^1H NMR (DMSO-d_6, 400 MHz) δ 8.09(1H, s, H-2), 6.36(1H, d, *J* = 2 Hz, H-6), 6.40 (1H, d, *J* = 2 Hz, H-8), 7.29 (2H, dd, *J* = 8.4, 2.0 Hz, H-2′, H-6′), 6.78 (2H, dd, *J* = 8.8, 2.0 Hz, H-3′, H-5′), 10.72 (1H, br s, HO-7), 12.86 (1H, br s, HO-5), 3.84 (3H, s, MeO-4′); ^{13}C NMR (DMSO-d_6, 100 MHz) δ