Fitoterapia 109 (2016) 31–38

Contents lists available at ScienceDirect

Fitoterapia

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

Isolation and identification of metabolites of bakuchiol in rats

Pei-le Wang [a], Feng-xiang Zhang [b], Zuo-cheng Qiu [b,c], Zhi-hong Yao [b], Man-sau Wong [c], Xin-sheng Yao [a,b,*], Yi Dai [b,**]

[a] College of Traditional Chinese Materia Medica, Shenyang Pharmaceutical University, Shenyang 110016, China
[b] Institute of Traditional Chinese Medicine and Natural Products, College of Pharmacy, Jinan University, Guangzhou 510632, China
[c] Department of Applied Biology and Chemical Technology, The Hong Kong Polytechnic University, Kowloon, Hong Kong, China

ARTICLE INFO

Article history:
Received 11 September 2015
Received in revised form 10 November 2015
Accepted 13 November 2015
Available online 14 November 2015

Keywords:
Bakuchiol
Psoralea corylifolia
Metabolites
MC3T3-E1 cell ALP activity
HKC-8 cytotoxicity

ABSTRACT

Bakuchiol, the main active component of *Psoralea corylifolia*, showed a range of significant pharmacological activities, including antimicrobial, antiinflammatory, reduction of bone loss and estrogenic activities. In this research, 12 metabolites, including 11 new compounds, were isolated from the urine and feces of rats after oral administration of bakuchiol, and their structures were elucidated by extensive spectroscopic analysis. The possible metabolic pathways of bakuchiol in rats were proposed, and a rare bile acid conjugation reaction was found. In addition, bakuchiol and its metabolites **M1–M3** were studied for their alkaline phosphatase (ALP) activities on MC3T3-E1 cells and cytotoxicity on HKC-8 cells. The data showed that bakuchiol exerted significant effects on ALP activity of MC3T3-E1 cells and cytotoxicity on HKC-8 cells, while **M1–M3** only showed ALP activities at 10^{-5} M on MC3T3-E1 cells and no cytotoxicity on HKC-8 cells.

1. Introduction

Bakuchiol (Fig. 1), a prenylated phenolic monoterpene, is the main active component of *Psoralea corylifolia* that has been widely used in China to treat various diseases, such as psoriasis, vitiligo, osteoporosis, arthralgia and asthma [1]. It has attracted an increasing interest for its antitumor, antimicrobial, inhibition of iNOS expression, antiinflammatory, antipyretic, reduction of bone loss and estrogenic activities [2–8]. Moreover, bakuchiol showed cytotoxicity on HK-2 cells (human renal tubular epithelial cells), and high doses of bakuchiol could induce kidney toxicity in mice [9,10]. Pharmacokinetic studies reported that bakuchiol possessed poor absorption, significant first-pass metabolism, and low bioavailability [10,11]. In our previous in vivo study, one primary oxidized metabolite and 18 metabolites of bakuchiol were identified in biosamples by UPLC/Q-TOF-MS [12]. As the chemical structures of metabolite isomers were difficult to be deduced by liquid chromatography–tandem mass spectrometry analysis [13,14], and bioactivity evaluation needed pure compounds, the isolation of metabolites was necessary. In order to better understand in vivo metabolism and pharmacological activity of bakuchiol, metabolites in feces and urine after oral administration to rats were isolated and identified. The ALP activity on MC3T3-E1 cells and cytotoxicity on HKC-8 cells of bakuchiol as well as its main metabolites were also evaluated.

2. Experimental

2.1. Materials

CD spectra were recorded on JASCO J-810 spectrometer. Optical rotations were measured on a JASCO P-1020 spectrometer. 1D and 2D NMR spectra were measured on a Bruker AV-400/600 spectrometer. HRESIMS data were determined by a Waters Synapt G2 mass spectrometer. Preparative HPLC performed on Shimadzu LC-6AD system equipped with a Shimadzu SPD-20A UV detector (210 nm and 260 nm), and a Welch material XB-C18 (21.2 × 250 mm) column (with a flow rate of 8 mL/min) and a Welch material XB-C18 (10 × 250 mm) column (with a flow rate of 3 mL/min) were used. Silica gel (200–300 mesh, Qingdao Haiyang Chemical Group Corp., Qingdao, China), HP-20 macroporous resin (Diaion, Japan), Sephadex LH-20 (50 μm, Amersham Pharmacia Biotech, Sweden) and ODS (50 μm, YMC, Japan) were used for open column chromatography (CC). Thin-layer chromatography (TLC) was performed using precoated silica gel plates (silica gel GF254, 1 mm, Yantai). Bakuchiol was purchased from Shanghai Ronghe Medical Technological Limited Company (Shanghai, China), and the purity was more than 98% determined by HPLC analysis.

2.2. Animals

Male Sprague–Dawley rats (250 ± 20 g) were obtained from Medical Experimental Animal Center of Guangdong Province (Guangzhou, China). They were housed at ambient temperature of 20 ± 2 °C

* Correspondence to: X.-s. Yao, College of Traditional Chinese Materia Medica, Shenyang Pharmaceutical University, China.
** Corresponding author.
E-mail addresses: tyaoxs@jnu.edu.cn (X. Yao), daiyi1004@163.com (Y. Dai).

http://dx.doi.org/10.1016/j.fitote.2015.11.006
0367-326X/

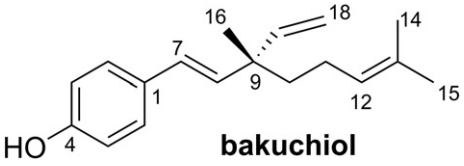

Fig. 1. The chemical structure of bakuchiol.

with 12-h light/dark cycles for two weeks before experiment and were fed a standard diet and water ad libitum. The animals were fasted with free access to water in metabolic cages separately over night before experiment. Bakuchiol (12 g) was given to rats (32 animals) by gavage at the dose of 100 mg/kg/day in turn (three days gavaged and three days fed with a standard diet, six days in cycle). Urine and feces samples were collected for 24 h after each administration from animals housed in stainless steel metabolism cages equipped with a urine and feces separator. These samples were stored at −20 °C. The animal protocols were approved by the Guide for the Care and Use of Laboratory Animals of Jinan University. All procedures were in accordance with Guide for the Care and Use of Laboratory Animals (National Institutes of Health).

2.3. Extraction and isolation

A total of 12 L urine was collected, after being condensed in vacuum, the urinary sample was chromatographed over a HP-20 macroporous resin column (5.0 × 120 cm) eluted with water, 30% EtOH–H_2O, 60% EtOH–H_2O and 95% EtOH–H_2O, successively. The 60% EtOH–H_2O eluate (C, 3.0 g) was subjected to an ODS column (3.0 × 35.0 cm), eluted with an EtOH–H_2O gradient solvent system (15%, 20%, 30% v/v) to be given in three fractions (C1-3). Fraction C3 (532 mg) was subjected to preparative HPLC with 30% ACN–H_2O (t_R = 35 min), then subfraction C3C (4.3 mg) was further purified by Sephadex LH-20 column (0.8 × 20.0 cm) eluted with EtOH to yield **M4** (0.5 mg). The 95% EtOH–H_2O eluate (D, 0.6 g) was subjected to a Sephadex LH-20 column (3.0 × 37.0 cm) and then further purified by preparative HPLC (30% ACN–H_2O, 8 mL/min) as the mobile phase to yield **M1** (132.1 mg, t_R = 85 min), **M2** (4.6 mg, t_R = 80 min) and **M3** (8.2 mg, t_R = 103 min).

The collected feces (490 g) were dried naturally, and were then extracted with EtOAc (1500 mL × 3). The extracts (31.2 g) were successively separated by silica gel CC (5.5 × 70 cm, 200–300 mesh, 350 g), eluted with a cyclohexane-EtOAc (20:1, 10:1, 10:2, 10:3, 2:1, 1:1, 0:1, v/v) to yield 7 fractions (F1-7). Fraction F1 (651.3 mg) was isolated by preparative HPLC (80% ACN–H_2O, 3 mL/min) as the mobile phase to yield **M9** (0.6 mg, t_R = 25 min) and bakuchiol (98.2 mg, t_R = 27 min). Fraction F3 (1.9 g) was subjected to a Sephadex LH-20 column (3.0 × 35.0 cm) and then further purified by preparative HPLC (52% ACN–H_2O, 3 mL/min) to yield **M5** (1.6 mg, t_R = 94 min), and **M6** (2.3 mg, t_R = 100 min). Fraction F4 (2.5 g) was also subjected to a Sephadex LH-20 column (3.0 × 37 cm) eluted with EtOH and preparative HPLC (49% ACN–H_2O, 8 mL/min) to afford **M7** (1.5 mg, t_R = 92 min), and **M8** (2.1 mg, t_R = 104 min). Fraction F5 (1.5 g) was separated by Sephadex LH-20 (3.0 × 35.0 cm) eluted with EtOH and preparative HPLC (78% ACN–H_2O, 8 mL/min) to afford **M11** (3.2 mg, t_R = 70 min) and **M10** (3.7 mg, t_R = 90 min). Fraction F6 (572.2 mg) was purified by preparative HPLC (79% ACN–H_2O, 3 mL/min) as the mobile phase to yield **M12** (3.9 mg, t_R = 30 min).

M1: white colorless oil; $[\alpha]_D^{25}$ + 27.6 (c 0.5 MeOH); UV (MeOH) λ_{max} (log ε): 205 (4.64), 262 (3.51) nm; IR (KBr) ν_{max}: 3426, 2926, 2861, 1727 cm^{-1}; ^1H and ^{13}C NMR data (see Table 1); HRESIMS *m/z* 245.1177 $[M–H]^-$ (calcd for $C_{15}H_{17}O_3$, 245.1178).

M2: white colorless oil; $[\alpha]_D^{25}$ + 6.7 (c 0.5 MeOH); UV (MeOH) λ_{max} (log ε): 205 (4.34), 263 (3.30) nm; IR (KBr) ν_{max}: 3438, 2926, 2861, 1739 cm^{-1}; ^1H and ^{13}C NMR data (see Table 1); HRESIMS *m/z* 275.1288 $[M–H]^-$ (calcd for $C_{16}H_{19}O_4$, 275.1283).

M3: colorless oil; $[\alpha]_D^{25}$ + 3.2 (c 0.75, MeOH); UV (MeOH) λ_{max} (log ε): 204 (4.28), 262 (3.51) nm; IR (KBr) ν_{max}: 3437, 2923, 1712 cm^{-1}; ^1H and ^{13}C NMR data (see Tables 1 and 2); HRESIMS *m/z* 275.1288 $[M–H]^-$ (calcd for $C_{16}H_{19}O_4$, 275.1283).

Table 1
1D NMR data for **M1–M4** (**M1–M3** were measured in $CDCl_3$, and **M4** was measured in DMSO-d_6).

Position	Norbakuchinic acid (**M1**)[a]		**M2**[a]		**M3**[a]		**M4**[b]	
	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C
1		128.2		130.2		131.5		128.5
2	7.22 (1H, d, 8.4)	127.2	6.88 (1H, overlapped)	108.3	6.99 (1H, brs)	111.8	7.18 (1H, d, 8.6)	127.1
3	6.69 (1H, d, 8.4)	115.3		146.8		145.9	6.69 (1H, d, 8.6)	115.3
4		156.7		145.3		146.2		156.5
5	6.69 (1H, d, 8.4)	115.3	6.85 (1H, overlapped)	114.6	6.78 (1H, d, 8.0)	110.8	6.69 (1H, d, 8.6)	115.3
6	7.22 (1H, d, 8.4)	127.2	6.86 (1H, overlapped)	119.9	6.82 (1H, brd, 8.0)	118.8	7.18 (1H, d, 8.6)	127.1
7	6.20 (1H, d, 16.3)	126.9	6.26 (1H, d, 16.3)	128.1	6.24 (1H, d, 16.2)	127.8	6.16 (1H, d, 16.5)	127.5
8	6.01 (1H, d, 16.3)	133.4	5.99 (1H, d, 16.3)	134.4	6.00 (1H, d, 16.2)	135.1	6.03 (1H, d, 16.5)	131.8
9		41.6		42.2		42.2		42.1
10	1.71 (2H, t, 7.8)	35.4	1.87 (2H, t, 8.2)	35.4	1.85 (2H, t, 8.2)	35.4	1.79 (1H, m) 1.68 (1H, m)	32.2
11	2.15 (2H, t, 7.8)	29.5	2.35 (2H, t, 8.2)	29.4	2.34 (2H, t, 8.2)	29.5	2.23 (1H, m) 2.12 (1H, m)	39.0
12		174.7		178.0		178.1		173.4
16	1.12 (3H, s)	22.9	1.21 (3H, s)	23.5	1.19 (3H, s)	23.5	0.99 (3H, s)	19.2
17	5.86 (1H, dd, 17.5, 10.8)	145.4	5.85 (1H, dd, 17.4, 10.8)	145.0	5.84 (1H, dd, 17.6, 10.8)	144.9	3.25 (1H, m)	78.0
18	5.00 (1H, d, 17.5) 5.03 (1H, d, 10.8)	112.2	5.06 (1H, d, 17.4) 5.09 (1H, d, 10.8)	113.1	5.04 (1H, d, 17.6) 5.08 (1H, d, 10.8)	113.1	3.49 (1H, m) 3.18 (1H, m)	62.8
3-OCH_3			3.91 (3H, s)	56.1				
4-OCH_3					3.88 (3H, s)	56.2		
4-OH							9.38 (1H, brs)	
17-OH							4.63 (1H, d, 4.7)	
18-OH							4.35 (1H, brs)	
–CH_2CH_3							3.98 (2H, m) 1.12 (3H, t, 7.1)	59.6 14.0

[a] Recorded at 400 MHz for ^1H.
[b] Recorded at 600 MHz for ^1H.