Fitoterapia 115 (2016) 15–18

Contents lists available at ScienceDirect

Fitoterapia

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

Bioactivity-guided isolation of biphenanthrenes from *Liparis nervosa*

Liang Liu [a,b,c,*,1], Qi-Meng Yin [a,1], Xian-Wen Zhang [a], Wei Wang [a], Xiao-Yun Dong [a,b,c], Xin Yan [a], Rong Hu [a]

[a] Medical School, Yangzhou University, Yangzhou 225009, PR China
[b] Jiangsu Key Laboratory of Integrated Traditional Chinese and Western Medicine for Prevention and Treatment of Senile Diseases, Yangzhou University, Yangzhou 225009, PR China
[c] Jiangsu Key Laboratory of Zoonosis, Jiangsu Co-innovation Center for Prevention and Control of Important Animal Infectious Diseases and Zoonoses, Yangzhou University, Yangzhou 225009, PR China

ARTICLE INFO

Article history:
Received 4 August 2016
Received in revised form 10 September 2016
Accepted 14 September 2016
Available online 15 September 2016

Keywords:
Biphenanthrene
Liparis nervosa
Cytotoxic activity

ABSTRACT

Three new biphenanthrenes, Liparisphenanthrenes A–C (**1**–**3**), along with three known ones were obtained from the ethanolic extract of *Liparis nervosa* (Orchidaceae) by bioactivity-guided fractionation. Their structures were elucidated on the basis of extensive spectroscopic analysis. All the compounds obtained were tested *in vitro* for cytotoxic activities against stomach (HGC-27) and colon (HT-29) cancer cell lines. **1**, **4** and **5** showed potent cytotoxicities to HGC-27 cell line with IC_{50} values of 8.21–9.95 μmol/L, and **1** and **5** also exhibited potent cytotoxic activities to HT-29 cell line with IC_{50} values of 8.53–9.27 μmol/L.

1. Introduction

The medicinal plant *Liparis nervosa* (Thunb.) Lindl. (Orchidaceae) has been widely used in fork medicine for a long time in history, owing to its hemostatic, antihypertensive, hypolipidemic and antitumor effects [1–3]. Previous phytochemical investigations have led to the isolation of various nervogenic acid derivatives and pyrrolizidine alkaloids from *L. nervosa*. But unfortunately, these isolates were proved to be inactive by *in vitro* antitumor activity screening [4–6]. Thus, the antitumor constituents of *L. nervosa* have aroused our interest. We have previously reported one phenanthrene from the petroleum ether extraction of *L. nervosa* [7]. Phenanthrenes, a class of aromatic metabolites, were mainly reported from Orchidaceae family, and its antitumor activity has attracted much attention [8]. Since EtOAc part of *L. nervosa* displayed potent antitumor activity with IC_{50} value of 153.6 μg/mL by *in vitro* antitumor activity screening, it might be a source of active phenanthrenes. In order to find more active phenanthrenes from *L. nervosa*, bioactivity-guided isolation was carried out to isolate constituents from its EtOAc part and three new biphenanthrenes, together with three known ones (Fig. 1) were obtained. In this paper, the structural identification of three previously unreported biphenanthrenes, namely *Liparisphenanthrenes* A–C (**1**–**3**), as well as their cytotoxic activities are reported.

2. Experimental

2.1. General experimental procedures

UV spectra were measured on a Shimadzu U-3900 UV–VIS spectrophotometer. IR spectra were recorded on a Shimadzu IRAffinity-1 spectrometer. HR-ESI-MS were determined by a MAXIS Bruker mass spectrometer. NMR spectra were recorded on a Bruker Avance 600 NMR spectrometer. Semi-preparative HPLC was performed on a CXTH LC3000 instrument equipped with UV3000 Detector and an ODS column (Cosmosil $5C_{18}$-MS-II column, 250 mm × 10 mm, 5 μm). Column chromatography (CC) was performed using silica gel (200–300 mesh, Qingdao Marine Chemistry Ltd., China), Sephadex LH-20 (GE Healthcare, Sweden) and ODS C_{18} (40–63 μm, Merck, Germany). TLC was carried out on precoated silica gel (GF254) plates (Merck). Spots were visualized under UV light and detected by spraying with 10% H_2SO_4 in EtOH followed by heating.

2.2. Plant material

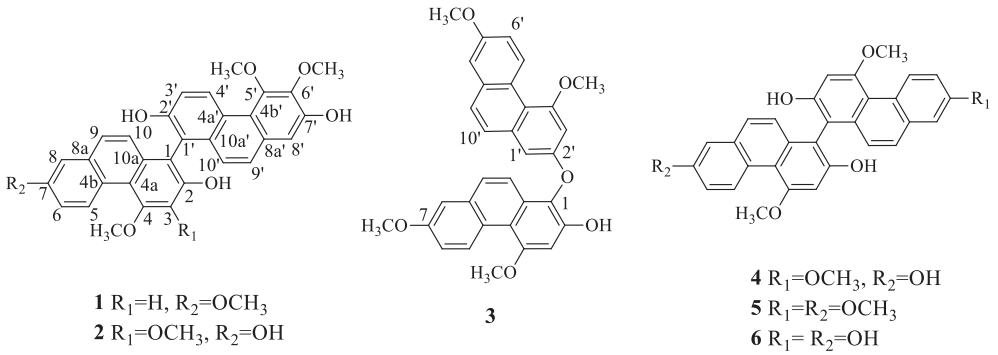
The whole grasses with roots of *L. nervosa* were collected in Chongqing city of China in Feb. 2014. The plant materials were identified by Prof. Hu-Yin Huai, School of Biological Science and Technology of Yangzhou University, and a voucher specimen (No. JXQ20140205) was deposited at the Herbarium of Pharmacy Department, Medical School of Yangzhou University.

2.3. Extraction and isolation

The powdered, dry whole grasses with roots of *L. nervosa* (10 kg) were extracted with 95% EtOH (150 L) four times at 85 °C, each for

* Corresponding author at: Medical School, Yangzhou University, Yangzhou 225009, PR China.
E-mail address: enjoyyz@163.com (L. Liu).
[1] Co-first author.

http://dx.doi.org/10.1016/j.fitote.2016.09.006
0367-326X/



1 R_1=H, R_2=OCH_3
2 R_1=OCH_3, R_2=OH

3

4 R_1=OCH_3, R_2=OH
5 R_1=R_2=OCH_3
6 R_1= R_2=OH

Fig. 1. Structures of compounds **1–6**.

2 h. After removal of EtOH under reduced pressure, the dark residue was suspended in distilled water and partitioned successively with petroleum ether (PE, 20 L), EtOAc (20 L), and *n*-BuOH (20 L). EtOAc fraction (193.1 g) was separated by a silica gel column chromatography (CC) eluted with a gradient of CH_2Cl_2-MeOH (50:1–0:100, v/v) to give 16 fractions (Fr.1–Fr.16) based on TLC analyses. Fr.2 (6.3 g) was isolated by a silica gel CC washed with a gradient of PE- EtOAc (4:1–0:100) to get 13 fractions (Fr. 2-1–Fr.2-13). Fr.2-8 (229.2 mg) was then subjected to a Sephadex LH-20 CC using $CHCl_3$-MeOH (2:1) as eluent to yield 3 fractions (Fr.2-8-1–Fr.2-8-3). Fr.2-8-3 (34.6 mg) was purified by semi-preparative HPLC with MeOH:H_2O (17:3, 2.0 mL/min) to get compound **3** (4.5 mg). Fr. 2-11 (359.6 mg) was chromatographed on a Sephadex LH-20 CC eluted with $CHCl_3$-MeOH (2:1) to yield 4 fractions (Fr.2-11-1–Fr.2-11-4). Compound **5** (13.5 mg) was obtained from Fr.2–11-4 by semi-preparative HPLC, using MeOH:H_2O (79:21, 2.0 mL/min) as mobile phase. Fr.2-12 (153.2 mg) was separated by a Sephadex LH-20 CC eluted with $CHCl_3$-MeOH (2:1) to get 5 fractions (Fr.2-12-1–Fr.2-12-5). Compound **1** (2.2 mg) was then purified from Fr.2-12-5 (18.4 mg) by semi-preparative HPLC, using MeOH-H_2O (79:21, 2.0 mL/min) as mobile phase. Fr.3 (14.5 g) was isolated by a silica gel CC eluted with a gradient of *n*-hexane: EtOAc (8:1–0:100) to give 12 fractions (Fr.3-1–Fr.3-12) based on TLC analyses. Seven fractions (Fr.3-8-1–Fr.3-8-7) were afforded from Fr. 3-8 (1.3 g) chromatographed on a Sephadex LH-20 column washed by MeOH:H_2O (2:1). Compound **4** (21.5 mg) was purified from Fr.3-8-6 (38.5 mg) by semi-preparative HPLC with MeOH:H_2O (18:7, 2.0 mL/min) as mobile phase. Fr.3-9 (750.0 mg) was subjected to CC on Sephadex LH-20 washed with $CHCl_3$:MeOH (2:1) to yield 5 fractions (Fr.3-9-1–Fr.3-9-5). Fr.3-9-4 was separated by a RP-C18 silica gel CC eluted with a gradient of MeOH-H_2O (4:1–100:0) to yield 8 fractions (Fr.3-9-4-1–Fr.3-9-4-8). Fr.3-9-4-2 (58.5 mg) was purified by semi-preparative HPLC, using MeOH:H_2O (7:3, 2.0 mL/min) as mobile phase to yield compound **2** (1.8 mg). Fr.5 (32.6 g) was separated into 3 fractions (Fr.5-1–Fr.5-3) by a Sephadex LH-20 CC washed with $CHCl_3$-MeOH (1:1). Fr.5-3 (2.7 g) was applied to a silica gel CC eluted with a gradient of PE- EtOAc (2:1–0:100) to yield 13 fractions (Fr.5-3-1–Fr.5-3-13). Compound **6** (7.8 mg) was finally obtained from Fr.5-3-6 (19.0 mg) through semi-preparative HPLC using MeOH:H_2O (11:9, 2.0 mL/min) as mobile phase.

*2.3.1. Liparisphenanthrene A (**1**)*

Brown amorphous powder; IR (KBr) ν_{max} (cm^{-1}) 3244, 2931, 2837, 1614, 1573, 1469, 1354, 1273, 1205, 1018 and 831; UV(MeOH) λ_{max} (logε) 264 (0.598) nm; negative ion HR-ESI-MS *m/z* 521.1603 $[M-H]^-$ (calcd for $C_{32}H_{25}O_7$, 521.1606); ^1H NMR and ^{13}C NMR data: Table 1.

*2.3.2. Liparisphenanthrene B (**2**)*

Brown amorphous powder; IR (KBr) ν_{max} (cm^{-1}) 3327, 2933, 1610, 1571, 1544, 1458, 1282, 1209, 1020, 952 and 829; UV(MeOH) λ_{max} (logε) 264 (0.386) nm; positive ion HR-ESI-MS *m/z* 561.1520 $[M+Na]^+$ (calcd for $C_{32}H_{26}O_8Na$, 561.1520); ^1H NMR and ^{13}C NMR data: Table 1.

*2.3.3. Liparisphenanthrene C (**3**)*

Brown amorphous powder; IR (KBr) ν_{max} (cm^{-1}) 3361, 2924, 2852, 1618, 1465, 1361, 1271, 1228, 1168, 1134, 1041 and 823; UV(MeOH) λ_{max} (logε) 263 (0.514) nm; negative ion HR-ESI-MS *m/z* 505.1665 $[M-H]^-$ (calcd for $C_{32}H_{25}O_6$, 505.1657); ^1H NMR and ^{13}C NMR data: Table 1.

Table 1
^1H and ^{13}C NMR data of compounds **1–3**.

	1		2		3	
Position	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C
1		112.2		116.7		128.6
1′		118.2		119.2	6.52 d (3.0)	103.2
2		153.4		148.8		147.1
2′		153.1		154.1		156.8
3	7.04 s	99.8		143.3	7.07 s	100.6
3′	7.33 d (9.0)	116.8	7.32 d (9.6)	117.6	7.19 d (2.4)	99.7
4		157.9		152.4		155.6
4′	9.33 d (9.0)	126.5	9.51 d (9.6)	129.0		159.0
4a		113.9		120.2		113.9
4a′		122.9		125.4		115.3
4b		124.5		125.1		124.2
4b′		117.6		119.9		123.9
5	9.46 d (9.6)	128.7	9.44 d (9.6)	129.6	9.42 d (9.6)	128.7
5′		151.1		153.0	9.39 d (9.6)	128.7
6	7.22 dd (9.0, 3.0)	116.2	7.13 dd (9.0, 3.0)	117.8	7.26 dd (9.6, 3.0)	116.7
6′		142.0		143.6	7.23 dd (9.6, 3.0)	116.4
7		156.0		156.1		156.3
7′		149.1		150.5		156.4
8	7.28 d (2.4)	108.4	7.07 (2.4)	112.4	7.38 d (2.4)	108.8
8′	7.00 s	108.8	6.99 s	110.1	7.37 d (2.4)	108.8
8a		132.2		135.1		132.5
8a′		128.2		130.7		133.0
9	7.45 d (9.0)	127.1	7.24 d (9.0)	127.2	7.70 d (9.6)	128.2
9′	7.26 d (9.6)	126.1	7.26 d (9.6)	127.8	7.63 d (9.0)	127.8
10	6.93 d (9.0)	124.9	6.95 d (9.0)	126.0	7.73 d (9.6)	119.9
10′	6.86 d (9.0)	123.9	6.91 d (9.0)	125.4	7.45 d (9.0)	127.1
10a		133.3		130.4		127.5
10a′		132.6		134.4		133.6
3-OCH_3			4.12 s	61.7		
4-OCH_3	4.13 s	55.5	4.08 s	60.5	4.11 s	55.8
7-OCH_3	3.86 s	55.0			3.88 s	55.1
4′-OCH_3					4.12 s	55.9
5′-OCH_3	3.98 s	59.8	4.03 s	60.6		
6′-OCH_3	3.93 s	60.5	4.05 s	61.6		
7′-OCH_3					3.88 s	55.0

^1H NMR data were measured at 600 MHz in DMSO-d_6 for **1** and **3**, in CD_3OD for **2**; ^{13}C NMR data were measured at 150 MHz in DMSO-d_6 for **1** and **3**, in CD_3OD for **2**.