Chinese Chemical Letters 27 (2016) 757–760

Contents lists available at ScienceDirect

Chinese Chemical Letters

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

Original article

Three new pyrrolizidine alkaloids derivatives from *Liparis nervosa*

Shuai Huang, De-Xin Zhong, Lian-Hai Shan, Ye-Zi Zheng, Zhi-Kuan Zhang, Ya-Heng Bu, Hong-Wen Ma, Xian-Li Zhou *

School of Life Science and Engineering, Southwest Jiaotong University, Chengdu 610031, China

ARTICLE INFO

Article history:
Received 19 August 2015
Received in revised form 17 September 2015
Accepted 23 December 2015
Available online 8 January 2016

Keywords:
Orchidaceae
Liparis nervosa
Pyrrolizidine alkaloids
Nervogenic acid derivatives

ABSTRACT

Three new pyrrolizidine alkaloids, nervosine VII (**1**), nervosine VIII (**2**) and nervosine IX (**3**) were isolated from the whole plant extract of *Liparis nervosa*. Their structures were elucidated by extensive spectroscopic analyses (including 1D, 2D NMR, and HR-ESI-MS) and chemical methods. Compounds **1–3** were evaluated for their cytotoxic activity against A549, MCF-7and H460 human cancer cell lines.

1. Introduction

Liparis nervosa (Thunb. ex A. Murray) Lindl. is widely used in folk medicine for detoxicating and hemostatic function [1]. Plants belonging to the genus *Liparis*, are herbaceous plants widely distributed in China. Previous phytochemical and biological investigations of *L. nervosa* led to the isolation of a series of pyrrolizidine alkaloids, nervogenic acid derivatives, triterpenoids, steroids and flavonoids [2–7]. Notably, numerous pyrrolizidine alkaloids with significant anti-inflammatory activity were isolated [5].

In our ongoing study to find active natural products, chemical study was carried out on the ethanol extract of *L. nervosa*, and obtained three new pyrrolizidine alkaloids, nervosine VII (**1**), nervosine VIII (**2**) and nervosine IX (**3**) (Fig. 1). Their structures and absolute configurations were elucidated by use of various spectral methods (IR, HR-ESI-MS, 1D and 2D NMR) and chemical methods. All the compounds were evaluated for their cytotoxic activity against A549, MCF-7 and H460 human cancer cell lines. Herein, we report the isolation and structural elucidation of these compounds, as well as their cytotoxicities.

2. Experimental

2.1. General experimental procedures

Optical rotations were measured on a Perkin-Elmer 341 polarimeter. 1D and 2D NMR spectra were recorded on a Bruker AV 600 NMR spectrometer, and IR spectra on a ThermoFisher Nicolet 6700 spectrometer (KBr discs, cm^{-1}). HR-ESI-MS were carried out on a Q-TOF micro mass spectrometer (Waters, USA). Silica gel (Qingdao Haiyang Chemical Co., Ltd., China). TLC plates precoated with silica gel GF254 (Qingdao Haiyang Chemical Co., Ltd., China) were visualized under a UV lamp at 254 nm or by spraying the Dragendorff's reagent or by iodine.

2.2. Plant material

The whole plants of *L. nervosa* were collected in Zunyi, Guizhou Province, China in July 2014. The plant was identified by Professor Liang-Ke Song in School of Life Science and Engineering, Southwest Jiaotong University, Sichuan, China, where a voucher specimen is deposited (No. ZN361520140801).

2.3. Extraction and isolation

The whole plants of *L. nervosa* (14 kg) were extracted with 95% ethanol at room temperature (50 L × 3, each 7 days). After removal of the solvent by evaporation, the ethanol extract (750 g) was

* Corresponding author.
E-mail address: xxbiochem@163.com (X.-L. Zhou).

http://dx.doi.org/10.1016/j.cclet.2016.01.003

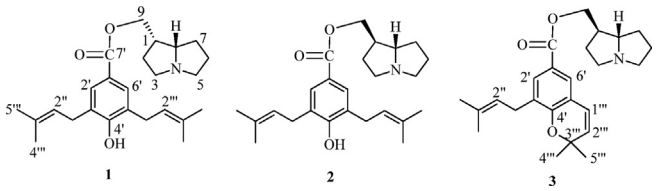

Fig. 1. Compounds **1–3** from whole plant of *Liparis nervosa*.

recovered. The extract was then suspended in H_2O (2 L) and extracted successively with petroleum ether (60–90 °C) (1 L × 4), EtOAc (1 L × 4), and *n*-butanol (1 L × 4) to obtain the petroleum ether extract (260 g), EtOAc extract (160 g) and *n*-butanol extract (180 g) successively.

The EtOAc extract (120 g) was subjected to a silica gel (100–200 mesh) column eluted in a step gradient manner with CH_2Cl_2:CH_3OH (50:1–0:1) to afford fractions (A–F) based on TLC analysis. Fraction E (7 g) was subjected to silica gel column, eluted with petroleum ether:Me_2CO:Et_2N (20:1:1) to yield compounds **1** (80 mg), **2** (50 mg) and **3** (3 mg).

Nervosine VII (**1**): Amorphous solid; $[\alpha]^{20}_D$ +23.0 (*c* 0.860 $CHCl_3$); IR (KBr, cm^{-1}): ν_{max} 3411, 2966, 2921, 1709, 1600, 1452, 1383, 1309, 1258, 1245, 1184, 1102, 992, 770. ^1H NMR and ^{13}C NMR data see Table 1. HR-ESI-MS at *m*/*z* 398.2710 $[M + H]^+$ (calcd. for $C_{25}H_{36}NO_3$, 398.2695).

Nervosine VIII (**2**): Amorphous solid; $[\alpha]^{20}_D$ −7.6 (*c* 0.696 $CHCl_3$); IR (KBr, cm^{-1}): ν_{max} 3403, 2964, 2918, 2951, 1711, 1601, 1452, 1375, 1308, 1284, 1245, 1184, 1098, 905, 770. ^1H NMR and ^{13}C NMR data see Table 1. HR-ESI-MS at *m*/*z* 398.2703 $[M + H]^+$ (calcd. for $C_{25}H_{36}NO_3$, 398.2695).

Nervosine IX (**3**): Amorphous solid; $[\alpha]^{20}_D$ −13.6 (*c* 0.174 $CHCl_3$); IR (KBr, cm^{-1}): ν_{max} 3418, 2960, 2918, 2950, 1714, 1641, 1602, 1463, 1375, 1361, 1310, 1284, 1247, 1193, 1166, 1124, 1101, 1056, 991, 953, 905, 769, 726. ^1H NMR and ^{13}C NMR data see Table 1. HR-ESI-MS at *m*/*z* 396.2536 $[M + H]^+$ (calcd. for $C_{25}H_{34}NO_3$, 396.2539).

Lindelofidine: $[\alpha]^{20}_D$ +70.2 (*c* 0.600, EtOH); HR-ESI-MS at *m*/*z* 142.1223 $[M + H]^+$ (calcd. for $C_8H_{16}NO$, 142.1232).

Laburnine: $[\alpha]^{20}_D$ +12.3 (*c* 0.025, EtOH); HR-ESI-MS at *m*/*z* 142.1248 $[M + H]^+$ (calcd. for $C_8H_{16}NO$, 142.1232).

2.4. Alkaline hydrolysis of compounds *1* and *2*

A solution of compound **1** (30 mg) in 0.5 mL of MeOH was refluxed with 1 mol/L NaOH (0.5 mL) for 1 h. After cooling, the reaction mixture was acidified with 1 mol/L HCl and extracted with $CHCl_3$ (3 × 4 mL). The aqueous solution was made alkaline with 1 mol/L NaOH and extracted with $CHCl_3$ (3 × 4 mL), evaporation of the organic phase yielded lindelofidine (8.0 mg) [5]. The process was repeated for compound **2** (40 mg) to get laburnine (10 mg).

Table 1
^1H NMR and ^{13}C NMR spectroscopic data[a,b] for compounds **1–3**.

No.	**1**		**2**		**3**	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1	*β* 2.68 m	40.7	*α* 2.11 m	45.3	*α* 2.13 m	45.3
2	*α* 1.61 m *β* 1.87 m	27.2	*α* 2.07 m *β* 1.72 m	30.9	*α* 2.07 m *β* 1.72 m	30.9
3	*α* 2.67 m *β* 3.06 m	54.0	*α* 2.57m *β* 3.21 m	54.6	*α* 2.58 m *β* 3.23 m	54.6
5	*α* 2.50 m *β* 3.18 m	55.9	*α* 2.60 m *β* 2.98 m	54.9	*α* 2.61 m *β* 3.01 m	54.9
6	*α* 1.87 m *β* 1.72 m	26.6	*α* 1.87 m *β* 1.79 m	26.1	*α* 1.86 m *β* 1.80 m	26.1
7	*α* 1.47 m *β* 1.72 m	26.5	*α* 1.63 m *β* 2.02 m	31.9	*α* 1.63 m *β* 2.00 m	31.9
8	*β* 3.60 m	66.2	*β* 3.32 m	68.4	*β* 3.36 m	68.3
9	4.25 dd (8.4, 11.2) 4.34 dd (6.8, 11.2)	64.8	4.17 dd (8.3, 10.9) 4.38 dd (5.9, 10.9)	66.9	4.20 dd (8.8, 11.2) 4.36 dd (6.4, 11.2)	66.9
1′	–	121.4	–	121.5	–	121.9
2′	7.64 s	129.3	7.67 s	129.5	7.67 br.s	131.0
3′	–	127.9	–	127.4	–	129.2
4′	–	157.9	–	157.7	–	154.8
5′	–	127.9	–	127.4	–	120.4
6′	7.64 s	129.3	7.67 s	129.5	7.52 br.s	125.9
7′	–	166.8	–	166.7	–	166.5
1″	3.34 d (6.8)	29.3	3.34 d (6.8)	29.4	3.28 d (7.8)	28.1
2″	5.30 t (6.8)	121.7	5.30 t (6.8)	121.6	5.27 t (7.8)	122.0
3″	–	134.3	–	134.6	–	132.5
4″	1.73 s	17.9	1.75 s	17.9	1.73 s	17.9
5″	1.74 s	25.8	1.77 s	25.8	1.73 s	25.8
1‴	3.34 d (6.8)	29.3	3.34 d (6.8)	29.4	6.35 d (9.6)	122.1
2‴	5.30 t (6.8)	121.7	5.30 t (6.8)	121.6	5.64 d (9.6)	131.0
3‴	–	134.3	–	134.6	–	77.2
4‴	1.73 s	17.9	1.75 s	17.9	1.44 s	28.3
5‴	1.74 s	25.8	1.77 s	25.8	1.44 s	28.3

[a] Data are based on DEPT, ^1H–^1H COSY, HMQC and HMBC experiments. ^1H NMR (600 MHz, *δ*, *J* in Hz in parentheses), ^{13}C NMR (150 MHz, *δ*).
[b] Spectra were recorded in $CDCl_3$.