Fitoterapia 95 (2014) 121–126

Contents lists available at ScienceDirect

Fitoterapia

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

Alkaloids from *Lycoris aurea* and their cytotoxicities against the head and neck squamous cell carcinoma

Jin-Hui Song [a,1], Li Zhang [b,*,1], Yan Song [c,**]

[a] Department of Otolaryngology, Shenyang 463 Hospital, Xiao He Yan Road 46, Shenyang, Liaoning 110042 China
[b] Department of Oral and Maxillofacial Surgery, General Hospital of Shenyang Military Area Command, No.83, Wenhua Road, Shenhe District, Shenyang, Liaoning 110840 China
[c] Department of Pharmacy, 455 Hospital of People's Liberation Army, West Huaihai Road 338, Shanghai 200052 China

ARTICLE INFO

Article history:
Received 15 February 2014
Accepted in revised form 27 February 2014
Available online 14 March 2014

Keywords:
Lycoris aurea
Amaryllidaceae
Alkaloids
Cytotoxicity
Anti-inflammatory

ABSTRACT

Phytochemical investigation of the 80% EtOH extract of the bulbs of *Lycoris aurea* led to the isolation of six new alkaloids, 2-demethyl-isocorydione (1), 8-demethyl-dehydrocrebanine (2), 1-hydroxy-anhydrolycorin-7-one (3), (+)-1,2-dihydroxy-anhydrolycorine *N*-oxide (4), 5,6-dihydro-5-methyl-2-hydroxyphenanthridine (5), and (+)-8-hydroxy-homolycorine-α-*N*-oxide (6), and together with two known compounds, isocorydione (7) and anhydrolycorin-7-one (8). Structural elucidation of all the compounds was performed by spectral methods such as 1D and 2D (^{1}H–^{1}H COSY, HMQC, and HMBC) NMR spectroscopy, in addition to high resolution mass spectrometry. All the alkaloids were *in vitro* evaluated for their cytotoxic activities against seven tumor cell lines of the head and neck squamous cell carcinoma and anti-inflammatory activities. Compounds 1, 2, 6, and 7 exhibited significant cytotoxicities against all the tested cell lines. Moreover, alkaloids 1, 2, and 7 possessed selective inhibition of Cox-2 (>85%).

1. Introduction

The genus *Lycoris* (Amaryllidaceae) comprises more than 20 species and mainly distributes in the temperate woodlands of eastern Asia, particularly in China and Japan [1,2]. Plants of the Amaryllidaceae family are well-known not only for their ornamental value but also for the use as herbal remedies [3–6]. The major chemical constituents of this family Amaryllidaceae are alkaloids, which are known to have various chemical structures and a wide range of biological activities. The alkaloids affect the central nervous system and have acetylcholinesterase-inhibitory, analgesic, anti-inflammatory, antiviral, antimalarial, antitumor, or anti-neoplastic activity [7–14]. Galanthamine is a long-acting, selective, reversible and competitive acetylcholine esterase inhibitor that has been approved for use in the European Union and the United States for the treatment of Alzheimer's disease (AD). Lycorine is an antiviral agent and a powerful inhibitor of cell division and growth in higher plants [15,16]. *Lycoris aurea* is endemic to the southwest district of Hubei Province, China. Previous investigation in *L. aurea* had reported a new alkaloid, 3-*O*-ethyltazettinol [3]. Present studies on the chemical constituents of the EtOH extract of the bulbs of *L. aurea* resulted in the isolation of six new alkaloids, 2-demethyl-isocorydione (1), 8-demethyl-dehydrocrebanine (2), 1-hydroxy-anhydrolycorin-7-one (3), (+)-1,2-dihydroxy-anhydrolycorine *N*-oxide (4), 5,6-dihydro-5-methyl-2-hydroxy-phenanthridine (5), and (+)-8-hydroxy-homolycorine-α-*N*-oxide (6), as well as two known alkaloids, isocorydione (7) and anhydrolycorin-7-one (8) (Fig. 1). This paper describes the isolation and structure elucidation of the new compounds on the basis of based on their chromatographic properties, chemical and physicochemical methods. Furthermore, all the isolated alkaloids were *in vitro* evaluated for their cytotoxic potential and anti-inflammatory activities.

* Corresponding author. Tel./fax: +86 24 28851331.
** Corresponding author.
E-mail addresses: lzhangsy@sohu.com (L. Zhang), songyan455@gmail.com (Y. Song).
[1] These two authors contributed equally to this work.

http://dx.doi.org/10.1016/j.fitote.2014.03.006
0367-326X/

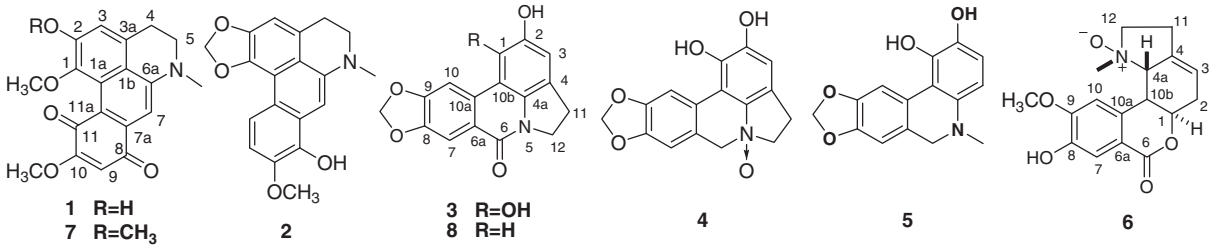

Fig. 1. Structures of compounds 1–8.

2. Experimental

2.1. General

Optical rotations were measured on a Perkin-Elmer 341 polarimeter (Na filter, $\gamma = 589$ nm). UV spectra were obtained on a Shimadzu UV-2550 spectrophotometer, whereas IR spectra were recorded on a Perkin-Elmer 577 spectrometer with KBr disks. NMR spectra were measured on a Bruker AM-600 spectrometer. EIMS and HREIMS (70 eV) were carried out on a Finnigan MAT 95 mass spectrometer. All solvents used were of analytical grade (Shanghai Chemical Reagents Company Ltd, Shanghai, China). Silica gel (200–300 mesh), silica gel H (Qingdao Haiyang Chemical Co. Ltd, Qingdao,China), C18 reversed-phase silica gel (150–200 mesh, Merck, New York, America), and MCI gel (CHP20P, 75–150 mm, Mitsubishi Chemical Industries Ltd, Tokyo, Japan) were used for column chromatography. HPLC separation was performed on an instrument consisting of a Waters 600 controller, a Waters 600 pump, and a Waters 2487 dual λ absorbance detector, with a Prevail (250 × 10 mm i.d.) preparative column packed with C18 (5 μm). Precoated thin-layer chromatography (TLC) plates with silica gel GF_{254} (Qingdao Haiyang Chemical Co. Ltd, Qingdao, China) were used for TLC.

Table 1
^{1}H NMR data of compounds 1 and 2 in $CDCl_3$ (δ in ppm and J in Hz).

No.	1		2	
	δ ^{1}H (Hz)	δ ^{13}C	δ ^{1}H (Hz)	δ ^{13}C
1	–	143.9, s	–	141.8, s
1a	–	127.4, s	–	126.3, s
1b	–	120.9, s	–	117.4, s
2	–	150.5, s	–	145.5, s
3	6.92 (*s*)	113.5, d	6.89 (*s*)	107.1, d
3a	–	128.0, s	–	128.9, s
4	3.10 (*t*, 6.5)	29.0, t	3.15 (*t*, 6.2)	29.5, t
5	3.45 (*t*, 6.5)	50.1, t	3.36 (*t*, 6.2)	50.6, t
6a	–	150.0, s	–	150.3, s
7	6.94 (*s*)	98.1, d	6.86 (*s*)	101.6, d
7a	–	136.2, s	–	134.2, s
8	–	186.5, s	–	141.6, s
9	5.90 (*s*)	105.0, d	–	148.0, s
10	–	163.7, s	7.07 (*d*, 8.8)	101.0, d
11	–	178.2, s	8.63 (*d*, 8.8)	123.7, d
11a	–	117.5, s	–	118.1, s
N-CH$_3$	3.16 (*s*)	39.9, q	3.21 (*s*)	41.2, q
OCH_2O	–	–	6.19 (*s*)	101.2, t
1-OCH_3	3.92 (*s*)	60.6, q	–	–
9-OCH_3	–	–	3.99 (*s*)	56.3, q
10-OCH_3	3.87 (*s*)	56.2, q	–	–

2.2. Plant material

The bulbs of *L. aurea* were bought from Hubei Chinese Traditional Medicine Market, Wuhang, Hubei Province, China, in June 2012. A specimen (201206001A) was identified by one of the authors (Y. Song) and in the Herbarium of Shengyang Medicine College, Shengyang, China.

2.3. Extraction and isolation

The bulbs of *L. aurea* (10.0 kg) were cut into small pieces and were extracted with 80% EtOH (20 L × 3) at room temperature, each time lasted 24 h. After removal of EtOH under reduced pressure at 55 °C, the aqueous brownish syrup (1 L) was suspended in H_2O (l L) and then partitioned with chloroform (1 L × 3) to afford chloroform fraction (102.3 g). The chloroform fraction was further fractionated through a silica gel column using increasing volume of acetone in petroleum ether (100:1, 50:1, 30:1, 15:1, 10:1, 7:1, 5:1, 3:1, 1:1, *v/v*, each 3 L) as the eluents to give 12 fractions according to TLC analysis. Fraction 4 (5.8 g) was applied to an ODS MPLC column (100 g) and eluted with MeOH–H_2O (20:80, 30:70, 40:60, each 500 mL) to yield 3 subfractions (Fr. 4-1 and Fr. 4-3). Subfraction 4-1 (MeOH–H_2O, 350 mg) was purified by a preparative RP-HPLC (ODS column, 250 × 20 mm) using MeOH/H_2O (25:75) as mobile phase to obtain 4 (59 mg, 22.33 min) and 7 (69 mg, 23.90 min). Subfraction 4-2 (99 mg) was purified by a preparative RP-HPLC (ODS column, 250 × 20 mm) eluted with MeOH/H_2O (22:78) to get 5 (61 mg, 23.15 min) and 8 (57 mg, 24.63 min). Subfraction 4-3 (978 mg) was chromatographed by a Sephadex LH-20 column eluted with MeOH/H_2O (50:50), and purified by a preparative RP-HPLC (ODS column, 250 × 20 mm) using MeOH/H_2O (30:70) as mobile phase to yield 1 (88 mg, 22.75 min). Fraction 5 (1.3 g) was applied to an ODS column eluted with MeOH/H_2O (30:70, 40:60, 50:50, each 500 mL) to provide 4 Subfractions (Fr. 5-1 and Fr. 5-4); Subfraction 5-2 (MeOH–H_2O 20:80, 226 mg) was repeatedly chromatographed on silica gel (150 g, 60 × 2.8 cm, chloroform:methanol, 20:1 → 10:1) and then purified by a Sephadex LH-20 column eluted with MeOH/H_2O (50:50) to afford 2 (78 mg). Subfraction 5-3 (387 mg) was purified by a preparative RP-HPLC (ODS column, 250 × 20 mm) eluted with MeOH/H_2O (20:80) to get 3 (77 mg, 23.17 min) and 6 (75 mg, 24.65 min).

2-Demethyl-isocorydione (1): violet amorphous powder; $[\alpha]_D^{23.3} = +76.3$ ($c = 0.18$, MeOH); UV ($CDCl_3$) $\lambda_{max}(\log \varepsilon)$ 304 (4.07), 283 (3.77), 217 (4.14) nm; IR (KBr) ν_{max} 3030, 1711, 1654, 1453, 1253 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz) data see Table 1; EIMS m/z 339 $[M]^+$;