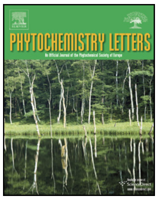
Phytochemistry Letters 10 (2014) 313–317

Contents lists available at ScienceDirect

Phytochemistry Letters

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



New labdane diterpenoids from *Leonurus japonicus* and their anti-inflammatory activity

Jing Qin [a,b], Hong-Mei Li [b], Ling-huan Gao [b], Dan Liu [b], Rong-Tao Li [b,*], Kuo-Hsiung Lee [c,d,**]

[a] *Faculty of Environmental Science and Engineering, Kunming University of Science and Technology, Kunming, PR China*
[b] *Faculty of Life Science and Technology, Kunming University of Science and Technology, Kunming 650500, Yunnan, PR China*
[c] *School of Pharmacy, University of North Carolina at Chapel Hill, NC 27599, USA*
[d] *Chinese Medicine Research and Development Center, China Medical University and Hospital, Taichung, Taiwan*

ARTICLE INFO

Article history:
Received 28 August 2014
Received in revised form 22 October 2014
Accepted 25 October 2014
Available online 6 November 2014

Keywords:
Leonurus japonicus
Labdane diterpenoids
Anti-inflammatory activity

ABSTRACT

Two new, 16-oxo-leoheteronone A (**1**) and 15-methoxyleoheteronin B (**2**), and four known, 8,9-secohispanolone (**3**), galeopsin (**4**), hispanone (**5**), and leoheteronin B (**6**), labdane diterpenoids were isolated from the aerial parts of *Leonurus japonicus*. Compound **3** was isolated for the first time as a naturally occurring compound. Structures of the two compounds were determined on the basis of extensive spectroscopic techniques, including 1D, 2D NMR, and HREIMS. In addition, compounds **1**–**3**, **5** and **6** were examined for inhibition of superoxide anion generation and elastase release, and the results suggested that compound **5** possesses anti-inflammatory activity.

1. Introduction

Leonurus japonicus Houtt (Labiatae), also known as Chinese motherwort, is an herbaceous flowering plant widely distributed in Asia, especially in China (Tasdemir et al., 1995). The aerial parts of this plant have been used as a famous traditional Chinese medicine (Yimucao) for thousands of years to treat menoxenia, dysmenorrhea, amenorrhea, lochia, body edema, oliguresis, sores, ulcerations, and other disorders in women (Cai, 2005). Modern pharmacological studies indicated that *L. japonicus* exhibits numerous pharmacological activities, such as cardioprotective, anti-oxidative, anti-cancer, analgesic, anti-inflammatory, neuroprotective, and antibacterial actions (Calis et al., 1992; Tasdemir et al., 1995). Prior phytochemical investigation found alkaloids (Calis et al., 1992; Chen et al., 2000; Tasdemir et al., 1995; Yeung et al., 1977), diterpenes (Boalino et al., 2004; Savona et al., 1982), flavones (Calis et al., 1992; Tasdemir et al., 1995), spirocyclic nortriterpenoids, phenylethanoid glycosides, and sesquiterpene glycosides as the main components of this plant (Shang et al., 2014). Recent studies have shown that some labdane diterpenes isolated from this plant exert anti-inflammatory (Traves et al., 2007), neuroprotective (Xu et al., 2011), antispasmodic, cytotoxic and trypanocidal (Scio et al., 2003) activities.

As part of our search for structurally interesting and potential anti-inflammatory terpenoids from the genus *Leonurus*, we carried out a phytochemical investigation on an EtOAc extract of the aerial parts of *L. japonicas*. We isolated, six labdane diterpenoids, including two new, 16-oxo-leoheteronone A (**1**) and 15-methoxyleoheteronin B (**2**), one new natural, 8,9-secohispanolone (**3**) (Rodriguez and Savona, 1980), and three known compounds, galeopsin (**4**) (Tasdemir et al., 1995), hispanone (**5**) (Gray et al., 2003), and leoheteronin B (**6**) (Giang et al., 2005a). The anti-inflammatory activities of the isolates were evaluated by assays for regulation of superoxide anion generation and elastase release (Yang et al., 2013; Yu et al., 2011). All compounds were also tested *in vitro* for anti-HIV activity. Herein, we report the isolation, structural elucidation, and results from anti-inflammatory and anti-HIV assays on these compounds.

2. Results and discussion

A 75% acetone extract of *L. japonicas* was suspended in water and partitioned successively with EtOAc. The EtOAc extract (240 g) was subjected to Toyopearl HW-40 column chromatography (CC) on silica gel, Diaion CC, and HPLC, affording two new, 16-oxo-leoheteronone A (**1**) and 15-methoxyleoheteronin B (**2**), one new

---

[*] Corresponding author. Tel.: +86 871 65920569; fax: +86 871 65920570.
[**] Corresponding author at: School of Pharmacy, University of North Carolina at Chapel Hill, NC 27599, USA. Tel.: +919 962 0066; fax: +919 966 3893.
E-mail addresses: rongtaolikm@163.com (R.-T. Li), khlee@unc.edu (K.-H. Lee).

http://dx.doi.org/10.1016/j.phytol.2014.10.027
1874-3900/

natural, 8,9-secohispanolone (**3**) (Rodriguez and Savona, 1980), and three known, galeopsin (**4**) (Tasdemir et al., 1995), hispanone (**5**) (Gray et al., 2003), and leoheteronin B (**6**) (Giang et al., 2005a) labdane diterpenes.

16-Oxo-leoheteronone A (**1**), $[\alpha]_D^{24.3}$ −75.83 (c = 0.20, MeOH), was isolated as a white amorphous powder. Its molecular formula was determined as $C_{23}H_{34}O_7$ on the basis of HRESIMS (m/z 445.2207, $[M+Na]^+$), indicating seven degrees of unsaturation. The IR spectrum of **1** showed absorption bands for carbonyl groups (1792, 1739, and 1724 cm^{-1}) (Tasdemir et al., 1995). The ^1H and ^{13}C NMR (Table 1) data of **1** showed the presence of one methoxy group (δ_H 3.32, δ_C 57.4), one acetyl moiety (δ_H 2.08; δ_C 21.7, 170.1), and 20 additional carbon resonances, attributed to four tertiary methyl groups, seven methylenes, two methines (with one oxygenated), and seven quaternary carbons (with three oxygenated carbons, a keto carbonyl carbon at δ_C 206.4, and an ester carbonyl at δ_C 176.8). The above data, along with the characteristic signals of spiro carbons at δ_C 86.7 (s, C-13) and 100.2 (s, C-9) as well as the oxygenated methine at δ_C 103.0 (d, C-15), suggested that **1** was a labdane-type diterpenoid possessing one spiro-tetrahydrofuran ring and one five membered lactone ring. The structure of **1** was identical to that of leoheteronone A. (Giang et al., 2005b), except that C-16 was assigned as a carbonyl carbon [**1** (δ_C 176.8), leoheteronone A (δ_C 74.4; δ_H 3.82d, 3.49d)], as deduced from HMBC correlations from H_2-12 (δ_H 2.27, 2.37), H_2-14 (δ_H, 2.27, 2.55) and H-15 (δ_H 5.38) to the carbonyl group (δ_C 176.8, C-16). Detailed 2D analysis established the planar structure of **1** as shown in Fig. 1. The relative configuration of **1** was assigned from a ROESY experiment (Fig. 1). Correlations of H-5/Me-18, Me-19/Me-20, Me-20/OAc, Me-20/H_2-11, and H-15/H-12 suggested that H-5, Me-18, Me-17, H-15 had the same orientation (α), while Me-19, Me-20, and C-11 had the opposite orientation (β). According to these data, compound **1** was determined to be 8β-acetoxy-15α-methoxy-7,16-dioxo-9,13-epoxylabda-labdan-16,15-olide and named 16-oxo-leoheteronone A.

Compound **2**, $[\alpha]_D^{16.7}$ +12.81 (c = 0.14, MeOH), white amorphous powder, has the molecular formula $C_{21}H_{30}O_4$ as determined by HRESIMS (m/z 369.2044, $[M+Na]^+$) combined with ^1H and ^{13}C NMR data. IR absorptions were consistent with the presence of an α,β-unsaturated-γ-lactone unit (1770 cm^{-1}) and an α,β-unsaturated ketone (1661 cm^{-1}) group (Boalino et al., 2004), which was further supported by the UV spectrum of **2** [λ_{max} 249 nm (log ε 4.03)] (Boalino et al., 2004). Comparison of the 1D- (Table 1) and 2D-NMR spectra of **2** with those of leoheteronin B (**6**) suggested analogous structures (Giang et al., 2005a). The only difference was that **2** has a methoxy group connected at C-15 as deduced from the HMBC correlations of H-14 (δ_H 7.59 br.*s*) and OMe (δ_H 3.53s) with C-15 (δ_C103.9). ROESY (Fig. 2) correlations of H-5/Me-18 and Me-19/Me-20 indicated α-orientations for H-5 and Me-18 and β-orientations for Me-19 and Me-20. Therefore, compound **2** was established as 15-methoxy-7,16-dioxo-8,13-diene-labdan-16,15-olide and named 15-methoxyleoheteronin B.

Compound **3**, $[\alpha]_D^{22.9}$ −15.87 (c = 0.20, MeOH), white amorphous powder, has the molecular formula $C_{20}H_{30}O_3$ as determined by HRESIMS (341.2094, $[M+Na]^+$). Its ^1H and ^{13}C NMR (Table 1) spectroscopie data indicated the presence of four methyls, seven methylenes, four methines (including three unsaturated), and five quaternary carbons [including one unsaturated, as well as two carbonyl groups at δ_C 210.7 (s, C-7) and 215.4 (s, C-9)]. The ^{13}C NMR and HSQC spectroscopic data (δ_C: 125.6 (s, C-13), 112.3 (d, C-14), 143.8 (d, C-15), 140.1 (d, C-16); δ_H: 6.47 (br.*s*, H-14), 7.58 (br.*s*, H-15), 7.54 (s, H-16)) further confirmed the presence of a furan ring. All data were those reported for a previously synthesized *diketone* compound (Rodriguez and Savona, 1980). Thus, compound **3** was isolated for the first time as natural product, identified as 8,9-*seco*-15,16-epoxylabda-13(16),14-diene-7,9-dione, and named 8,9-secohispanolone.

Table 1
^1H (600 MHz) and ^{13}C NMR (150 MHz) data for **1**–**3** in C_5D_5N-d_5 (δ in ppm and J in Hz).

No.	**1**		**2**		**3**	
	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1a	33.5 (t)	1.37 (br.d, 12.8)	36.2 (t)	1.81 (br.d, 12.3)	36.3 (t)	1.33 (m)
1b		2.37 (m)		1.21 (m)		2.24 (m)
2a	18.8 (t)	1.49 (m)	19.2 (t)	1.51 (m)	19.1 (t)	1.41 (m)
2b		1.57 (m)		1.39 (m)		1.47 (m)
3a	41.8 (t)	1.16 (m)	41.5 (t)	1.33 (m)	41.3 (t)	1.33 (m)
3b		1.22 (m)		1.07 (m)		1.35 (m)
4	34.9 (s)		33.5 (s)		34.6 (s)	
5	50.6 (d)	1.95 (dd, 14.2, 2.4)	51.0 (d)	1.57 (dd, 12.0, 3.0)	43.4 (d)	1.67 (td, 12.5, 3.9)
6a	37.2 (t)	2.79 (dd, 14.2, 11.8)	36.1 (t)	2.41 (m)	41.4 (t)	2.39 (m)
6b		2.54 (dd, 11.8, 2.4)		2.57 (dd, 17.2, 3.4)		2.24 (dd, 17.8, 5.1)
7	206.4 (s)		199.4 (s)		210.7 (s)	
8	88.3 (s)		131.3 (s)		38.4 (t)	2.42 (m)
9	100.2 (s)		165.7 (s)		215.4 (s)	
10	44.8 (s)		41.8 (s)		52.9 (s)	
11a	29.4 (t)	2.36 (overlap)	27.8 (t)	2.41 (m)	35.8 (t)	2.69 (m)
11b				2.49 (m)		
12a	37.4 (t)	2.27 (m)	25.4 (t)	2.42 (m)	20.2 (t)	2.81 (m)
12b		2.37 (m)		2.50 (m)		
13	86.7 (s)		138.2 (s)		125.6 (s)	
14a	44.1 (t)	2.55 (dd, 13.5, 5.6)	144.1 (d)	7.59 (br.s)	112.3 (d)	6.47 (br.s)
14b		2.27 (m)				
15	103.0 (d)	5.38 (dd, 5.6, 2.6)	103.9 (d)	6.05 (br.s)	143.8 (d)	7.58 (br.s)
16	176.8 (s)		172.1 (s)		140.1 (d)	7.54 (s)
17	16.8 (q)	1.60 (s)	12.1 (q)	1.95 (s)	8.6 (q)	1.03 (t, 7.3)
18	33.2 (q)	0.73 (s)	21.7 (q)	0.79 (s)	33.3 (q)	0.91 (s)
19	21.9 (q)	0.72 (s)	33.0 (q)	0.78 (s)	23.3 (q)	0.90 (s)
20	19.3 (q)	1.25 (s)	18.2 (q)	0.95 (s)	18.0 (q)	1.15 (s)
OCH_3	57.4 (q)	3.32 (s)	57.3 (q)	3.53 (s)		
$OCOCH_3$	170.1 (s)					
$OCOCH_3$	21.7 (q)	2.08 (s)				