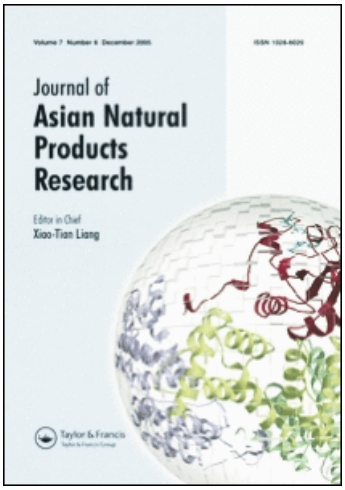

Journal of Asian Natural Products Research
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713454007

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To cite this Article Li, Xiang-Ri , Li, Zhi-Meng and Lin, Rui-Chao(2009) 'Two new triterpenes from *Lysimachia foenum-graecum*', Journal of Asian Natural Products Research, 11: 6, 529 — 533
To link to this Article: DOI: 10.1080/10286020902930884
URL: http://dx.doi.org/10.1080/10286020902930884

Journal of Asian Natural Products Research
Vol. 11, No. 6, June 2009, 529–533

Two new triterpenes from *Lysimachia foenum-graecum*

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(*Received 22 December 2008; final version received 23 March 2009*)

Two new oleanane-type triterpene saponins, lysimachiagenoside C (**1**) and lysimachiagenoside D (**2**), were isolated from the aerial parts of *Lysimachia foenum-graecum* Hance. The structures were elucidated on the basis of 1D and 2D NMR techniques, including $^{1}H-^{1}H$ COSY, HMQC, HMBC, TOCSY, and ROESY experiments as well as chemical methods.

Keywords: *Lysimachia foenum-graecum* Hance; triterpene saponin; lysimachiagenoside C; lysimachiagenoside D

1. Introduction

Lysimachia foenum-graecum Hance (Primulaceae), distributed mainly in Guangxi and Yunnan Provinces of China, has been commonly used as a perfume plant and pest repellent. In Chinese folk medicine, the plant has also been used for the treatment of cold and headache [1]. We have reported the isolation of lysimachiagenoside A and 21-*O*-angeloylbarringtogenol C from the aerial parts of *L. foenum-graecum*. In our recent study, lysimachiagenosides C and D, two new oleanane-type triterpenoid saponins were isolated. The structures were elucidated by 1D and 2D NMR techniques. In this paper, we describe the isolation and structural elucidation of lysimachiagenosides C and D.

2. Results and discussion

Compound **1** was obtained as a white powder. The MALDI-TOF-MS of **1** showed a pseudo-molecular ion $[M+Na]^+$ at *m/z* 1443, compatible with the molecular formula $C_{68}H_{108}O_{31}$, which was further verified by HR-FAB-MS at *m/z* 1443.6719 $[M+Na]^+$. Briefly, analysis of the NMR spectral data indicated that **1** was a saponin consisting of a triterpene aglycone and five monosaccharides. The ^{13}C NMR spectrum of **1** showed 68 carbon signals, from which 29 were assigned to five monosaccharide units, 30 to triterpene aglycone moiety, 5 to one angeloyl moiety, and the remaining 4 to two acetoxy moieties. The seven tertiary methyl groups and one tri-substituted olefinic proton (δ_H 5.41, s) in the ^{1}H NMR spectrum, together with the corresponding ^{13}C NMR signals, suggested an olean-12-ene skeleton. The downfield shift at δ_C 64.6 (C-28) indicated that the C-28 position of the aglycone was substituted by an angeloyl, which was confirmed by long-range correlations between the proton signals at δ_H 4.76, 5.08 (H_2-28, s) and the ester carbonyl

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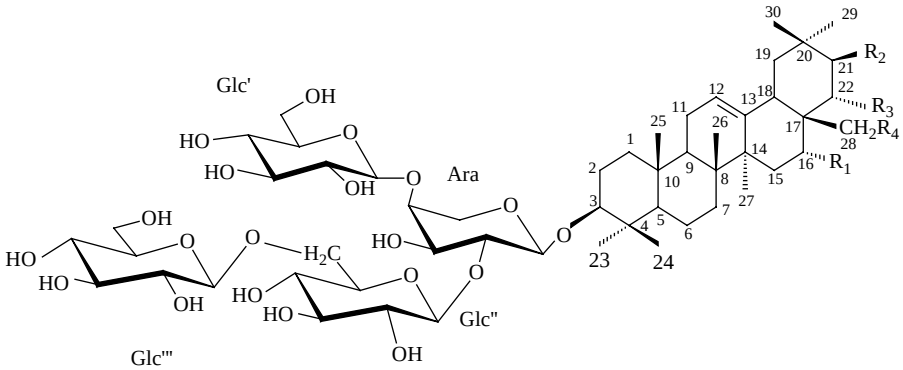
ISSN 1028-6020 print/ISSN 1477-2213 online

DOI: 10.1080/10286020902930884
http://www.informaworld.com

signal at δ_C 167.8 and between the angeloyl methyl proton signal at δ_H 1.96 (H-4′, 3H, s) and δ_H 2.07 (H-5′, 3H, s) and the carbon signals at δ_C 167.8 (—CO—) in the HMBC spectrum. Detailed comparison of the ^{13}C and ^{1}H NMR spectral data of **1** with those reported in the literature, suggested that the aglycone of **1** was [3β,16α,22α]-olean-12-ene-3,22-diol 16-*O*-acetyl-28-(2-methyl-2-butenoate) (16-*O*-acetyl-21-dehydroxy-28-*O*-angeloyl-barringtogenol C) [2–4]. Five monosaccharide units were determined from the TOCSY spectrum with the aid of COSY, HMQC, and HMBC spectra. Starting from the anomeric proton signal at δ_H 5.08 (1H, brs), six correlated carbon signals were observed in the TOCSY spectrum and determined in sequence to be at δ_C 106.4 (C-1), 72.8 (C-2), 75.1 (C-3), 71.2 (C-4), 78.1 (C-5), and 65.5 (C-6), which suggested a glucosyl group. The downfield shift at δ_C 65.5 (C-6) indicated that the C-6 position of the glucosyl group was substituted by an acetoxy, which was confirmed by long-range correlations between the proton signal at δ_H 4.56, 4.22 (H_2-6, s) and the ester carbonyl signal at δ_C 170.5 and between the acetyl methyl proton signal at δ_H 2.09 (3H, s) and the carbon signals at δ_C 65.5 (C-6) and 170.5 (—CO—) in the HMBC spectrum. Similarly, the other three glycosyl groups were identified from analysis of the TOCSY spectra (Table 1). The remaining five carbon signals suggested the presence of a pentosyl group whose anomeric proton at δ_H 4.80 (1H, brs) was only correlated to four carbon signals at δ_C 104.6 (C-1), 81.1 (C-2), 72.8 (C-3), and 77.2 (C-4) in TOCSY spectrum, implying an arabinosyl group. The oxygen-bearing methylene at δ_C 64.2 was assigned to be at the C-5 position of arabinosyl group based



	R_1	R_2	R_3	R_4
1	OAc	H	Glc''''	angelate
2	OAc	Glc''''	H	angelate

Figure 1. Structures of compounds **1** and **2**.

Table 1. ^{13}C NMR spectral data of compounds **1** and **2** (500 MHz, pyridine-d_5).

C no.	**1**	**2**	C no.	**1**	**2**
1	38.7	38.7	Ara-1	104.6	104.5
2	26.4	26.9	2	81.1	80.9
3	88.6	88.6	3	72.8	72.7
4	39.4	39.4	4	77.2	77.1
5	55.6	55.6	5	64.2	64.2
6	18.2	18.2	Glc′-1	105.6	105.8
7	33.0	32.9	2	75.4	75.0
8	39.9	39.8	3	78.1	78.1
9	46.9	46.8	4	71.4	71.2
10	36.7	36.9	5	78.0	78.0
11	23.8	23.5	6	62.4	62.5
12	123.8	124.1	Glc″-1	105.8	105.5
13	141.3	141.7	2	75.4	75.3
14	41.2	41.2	3	78.3	78.3
15	31.0	30.6	4	71.3	71.2
16	71.7	73.7	5	78.2	78.2
17	43.7	42.7	6	69.9	69.9
18	40.9	40.8	Glc‴-1	105.9	105.7
19	46.8	46.8	2	75.5	75.3
20	31.5	32.0	3	78.4	78.4
21	44.5	78.3	4	71.6	71.6
22	74.2	29.9	5	78.3	78.2
23	28.0	28.0	6	62.5	62.6
24	16.7	16.6	Glc⁗-1	106.4	100.7
25	16.1	16.0	2	72.8	72.7
26	16.8	16.7	3	75.1	75.1
27	26.9	26.7	4	71.2	71.2
28	64.6	64.7	5	78.1	78.4
29	33.5	33.1	6	65.5	65.2
30	24.9	20.2	1	170.5	170.8
1′	167.8	167.9	2	20.8	20.8
2′	128.3	128.3			
3′	138.2	137.9			
4′	15.7	15.7			
5′	20.9	20.8			
1″	169.6	169.7			
2″	21.9	21.7			

on ^{1}H–^{1}H COSY and HMBC experiments. The above inferences for the monosaccharide unit were further confirmed by TLC analysis of the acid hydrolysate of compound **1**. The glycosidic position of the aglycone was determined to be at the C-3 position on the basis of HMBC correlation between the anomeric proton of arabinosyl group at δ_H 4.79 (1H, brs) with C-3. The connectivity among the monosaccharide units was established with the following HMBC correlations: H-1 (δ_H 5.08) of inner glucosyl group with C-2 (δ_C 81.1) of arabinosyl group; H-1 (δ_H 5.10) of outer glucosyl group with C-4 (δ_C 77.2) of arabinosyl group; and H-1 (δ_H 5.09) of the outest glucosyl group with C-6 (δ_C 69.9) of inner glucosyl group. Another glycosidic position of the aglycone was determined to be at the C-22 position on the basis of HMBC correlation between the anomeric proton of glucosyl group at δ_H 4.93 (1H, d, 7.8) with C-22 (δ_C 74.2) of the aglycone. The anomeric configurations of four glucosyl groups were determined to be β-oriented from the coupling constants of the anomeric protons. Similarly, the

Table 2. ^{1}H NMR spectral data for the sugar moiety of compounds **1** and **2** (125 MHz, pyridine-d_5).

H no.	**1**	**2**
Ara-1	4.79 (brs)	4.79 (brs)
2	4.56	4.48
3	4.69	4.64
4	4.29	4.43
5	4.47, 3.84	4.47, 3.80
Glc′-1	5.10 (1H, 7.6)	5.10 (1H, 7.6)
2	3.97	3.96
3	4.05	4.08
4	4.34	4.26
5	3.74	3.70
6	4.51, 4.40	4.45, 4.35
Glc″-1	5.08 (1H, 7.8)	5.05 (1H, 7.8)
2	4.05	4.03
3	3.96	3.85
4	4.03	4.01
5	4.16	4.14
6	4.78, 4.31	4.70, 4.25
Glc‴-1	5.09 (1H, 7.8)	5.08 (1H, 7.8)
2	4.08	4.04
3	4.15	4.13
4	4.31	4.25
5	3.76	3.70
6	4.51, 4.40	4.45, 4.35
Glc⁗-1	4.93 (1H, 7.8)	4.86 (1H, 7.8)
2	3.97	3.92
3	4.19	4.20
4	4.30	4.25
5	3.90	3.85
6	4.56, 4.22	4.83, 3.94
CO_2CH_3	2.09 (3H, s)	2.05 (3H, s)

anomeric protons of arabinosyl groups were found to be in the α-orientation (Table 2). Thus, the complete structure of **1** was elucidated as 3-*O*-β-glucopyranosyl (1 → 4)-β-glucopyranosyl(1 → 2)[β-glucopyranosyl(1 → 4)]-α-arabinopyranosyl-16-*O*-acetyl-21-dehydroxy-28-*O*-angeloylbarringtogenol C 22-*O*-β-6-acetyl-glucopyranoside, named lysimachigenoside C (Figure 1).

Compound **2**, a white powder, displayed a quasi-molecular ion peak at *m/z* 1443.6716 $[M+Na]^+$ in HR-FAB-MS, which is consistent with the molecular formula $C_{68}H_{108}O_{31}$. The comparison of NMR spectral data (see Tables 1 and 2) between **1** and **2** revealed great similarity. The only difference was that the C-21 position of **2** was substituted by a glucose based on the TOCSY and HMBC spectra, not C-22 position like compound **1**. In the TOCSY spectrum, an anomeric proton at δ_H 4.84 was correlated with six carbons at δ_C 100.7 (C-1), 75.1 (C-2), 72.7 (C-3), 71.2 (C-4), 78.4 (C-5), and 65.2 (C-6) indicating a glucose unit. The downfield shift at δ_C 65.2 (C-6) indicated that the C-6 position of the glucosyl group was substituted by an acetoxy, which was confirmed by long-range correlations between the proton signal at δ_H 4.83, 3.94 (H_2-6, s) and the ester carbonyl signal at δ_C 170.8 and between the acetyl methyl proton signal at δ_H 2.05 (3H, s) and the carbon signals at δ_C 65.2 (C-6) and 170.8 (—CO—) in the HMBC spectrum. The glucose was attached to C-21 of the aglycone based on the HMBC correlations between the anomeric protons at δ_H 4.86 and C-21. The configuration of the anomeric proton of glucose was established to be β, based on the coupling constant of the anomeric proton. Thus, the structure of **2** was established as 3-*O*-β-glucopyranosyl(1 → 4)-β-glucopyranosyl (1 → 2)-[β-glucopyranosyl(1 → 4)]-α-arabinopyranosyl-16-*O*-acetyl-22-dehydroxy-28-*O*-angeloylbarringtogenol C 21-*O*-β-6-acetyl-glucopyranoside, named lysimachigenoside D (Figure 1).

3. Experimental

3.1 General experimental procedures

Optical rotations were determined with a Perkin-Elmer model 241 polarimeter. UV spectra were taken on a Perkin-Elmer-554 spectrometer. IR spectra were run on a Nicolet Impact 400 grating infrared spectrophotometer. 1D and 2D NMR spectra were recorded with a Bruker Avance 500 spectrometer. Chemical shifts (δ) are given with TMS as an internal standard. MS and HR-MS were obtained using ESQUIRE-LC or APEX II. FT-ICR-MS instruments.

Silica gel precoated plates (Qingdao Ocean Chemical Co., Qingdao, China) were used in TLC. Detection was carried out by spraying with 10% H_2SO_4 solution followed by heating.

3.2 Plant material

The aerial parts of *L. foenum-graecum* were collected in Kunming City, Yunnan Province of China, in June 2001, and were identified by Prof. Zhang Ji. A voucher specimen (No. 0108127) is deposited in the Institute of Chinese Materia Medica, National Institute for the Control of Pharmaceutical and Biological Products.

3.3 Extraction and isolation

The aerial parts of *L. foenum-graecum* (3.0 kg) were extracted with 70% EtOH (2× 52 l) under reflux. The combined filtrate was partitioned into petroleum ether, CH_2Cl_2, and remaining ethanol fractions. The remaining ethanol fractions (160 g) were absorbed on a Diaion SP825 column, and then sequentially eluted with H_2O and EtOH. The fraction eluted with 50% EtOH (25 g) was subjected to silica gel column chromatography (Ø 7 × 100 cm) using $CHCl_3$–MeOH gradient system to yield fractions I–VIII. Fraction II (1250 mg) was purified by repeated silica gel column chromatography using $CHCl_3$–MeOH gradient system and a reversed-phase column (Rp18, Ø 3.5 × 60 cm) using 60% MeOH as eluent to give compounds **1** (7 mg) and **2** (6 mg).

3.3.1 Lysimachigenoside C (**1**)

White powder; $[\alpha]_D^{24}$ −3.8 ($c = 0.14$, MeOH); UV (MeOH) λ_{max} (log ε): 211 (4.34) nm; IR (KBr) ν_{max} (cm^{-1}): 3434 (OH), 1243 (C═C); ^{1}H NMR (500 MHz, pyridine-d_5): δ 1.17 (3H, s, Me-23), 1.00 (3H, s, Me-24), 0.82 (3H, s, Me-25), 0.87 (3H, s, Me-26), 1.05 (3H, s, Me-29), 1.20 (3H, s, Me-30), 3.07 (1H, dd-like, H-3), 0.62 (1H, d, $J = 10.3$ Hz, H-5), 1.61 (1H, m, H-9), 5.41 (1H, brs, H-12), 4.74 (1H, brs, H-16), 3.11 (1H, dd-like, H-18), 2.22 (1H, overlap, H-21a), 2.09 (1H, overlap, H-21b), 4.51 (1H, d, $J = 10.1$ Hz, H-22), 4.76 (1H, overlap, H-28a), 5.08 (1H, overlap, H-28b), 5.99 (1H, d, $J = 7.0$ Hz, H-3′), 1.96 (3H, d, $J = 7.0$ Hz, H-5′), 2.07 (3H, s, H-4′), 2.22 (3H, s, $COCH_3$); ^{1}H and ^{13}C NMR data, see Tables 1 and 2; MALDI-TOF-MS: *m/z* 1443 $[M+Na]^+$; HR-FAB-MS: *m/z* 1443.6719 $[M+Na]^+$ (calcd for $C_{68}H_{108}O_{31}Na$, 1443.6772).

3.3.2 Lysimachigenoside D (**2**)

White powder; $[\alpha]_D^{24}$ −10.6 ($c = 0.14$, MeOH); UV (MeOH) λ_{max} (log ε): 208 (4.16) nm; IR (KBr) ν_{max} (cm^{-1}): 3430 (OH), 1715 (C═O), 1245 (C═C); ^{1}H NMR (500 MHz, pyridine-d_5): δ 1.18 (3H, s, Me-23), 1.00 (3H, s, Me-24), 0.82 (3H, s, Me-25), 0.98 (3H, s, Me-26), 1.08 (3H, s, Me-29), 1.36 (3H, s, Me-30), 3.06 (1H, dd-like, H-3), 0.62 (1H, d, $J = 10.0$ Hz, H-5), 1.56 (1H, m, H-9), 5.20 (1H, brs, H-12), 4.40 (1H, brs, H-16), 3.11 (1H, dd-like, H-18), 5.70 (1H, brs, H-21), 1.67 (1H, overlap, H-22a), 2.05 (1H, overlap, H-22b), 4.47 (1H, d, $J = 10.0$ Hz, H-28a), 3.80 (1H, d, $J = 10.0$ Hz, H-28b), 5.98 (1H, qq-like, H-3′), 1.93 (3H, d, $J = 7.0$ Hz, H-5′), 2.06 (3H, s, H-4′), 2.20 (3H, s, $COCH_3$); ^{1}H and ^{13}C NMR data, see Tables 1 and 2; MALDI-TOF-MS: *m/z* 1443 $[M+Na]^+$; HR-FAB-MS: *m/z* 1443.6716 $[M+Na]^+$ (calcd for $C_{68}H_{108}O_{31}Na$, 1443.6772).

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