Fitoterapia 127 (2018) 123–128

Contents lists available at ScienceDirect

Fitoterapia

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

Megastigmane glycosides from *Urena lobata*

Cong Su[1], Bowen Qi[1], Juan Wang, Ning Ding, Yun Wu, Xiao-Ping Shi, Zhi-Xiang Zhu, Xiao Liu, Xiao-Hui Wang, Jiao Zheng, Peng-Fei Tu, She-Po Shi*

Modern Research Center for Traditional Chinese Medicine, School of Chinese Materia Medica, Beijing University of Chinese Medicine, Beijing 100029, People's Republic of China

ARTICLE INFO

Keywords:
Urena lobata L.
Megastigmane glycosides
Urenalobasides A–E
Anti-inflammatory
Nitric oxide production

ABSTRACT

Five new megastigmane glycosides, urenalobasides A–E (**1–5**), together with 11 known ones (**6–16**) were isolated from *Urena lobata*. Their structures were determined by extensive spectroscopic and spectrometric data (1D and 2D NMR, IR, and HRESIMS) and calculated electronic circular dichroism method. Compounds **1** and **2** are two unusual megastigmanes structurally containing a 6/5 fused ring system. Compound **3** exhibits inhibition of nitric oxide production in lipopolysaccharide (LPS)-stimulated RAW264.7 macrophage cells with IC_{50} value of 53.7 ± 1.0 μM (positive control, dexamethasone, IC_{50} = 16.6 ± 0.8 μM).

1. Introduction

Urena lobata, belonging to the family Malvaceae, is widely distributed in Asia, South America, and Africa [1]. In south of China, the aerial part of *U. lobata* is commonly used as anti-inflammatory, antipyretic, antalgesic, and antibacterial agents for the treatment of fever, rheumatism, and diarrhea. In addition, decoctions and preparations containing *U. lobata* are also popularly used to treat gynopathies such as pathological leucorrhea and gonorrhea [2]. Previous investigations led to the isolation and structural elucidation of flavonoids, coumarins, and lignans from *U. lobata* [1,3–6]. However, as a widely used herbal medicine, the bioactive constituents of *U. lobata* remain largely unknown. Therefore, further investigations on the bioactive constituents of *U. lobata* are quite essential. In our previous reports, 24 flavonoids were identified from *U. lobata* [7,8]. As an ongoing study, five new megastigmane glycosides, urenalobasides A–E (**1–5**), together with 11 known ones (**6–16**) were obtained (Fig. 1). Herein, the isolation and structural elucidation of the new compounds as well as their inhibitory effects on nitric oxide (NO) production in LPS-stimulated RAW264.7 macrophage cells are described.

2. Experimental

2.1. General experimental procedures

Optical rotations were obtained on a Rudolph Autopol IV automatic polarimeter (NJ, USA). IR spectra were recorded on a Thermo Nicolet Nexus 470 FT-IR spectrophotometer (MA, USA) with KBr pellets. UV spectra were obtained using a Shimadzu UV-2450 spectrophotometer (Tokyo, Japan). NMR spectra were recorded on a Varian INOVA-500 spectrometer (CA, USA) operating at 500 MHz for ^{1}H NMR and 125 MHz for ^{13}C NMR. HRESIMS was recorded on an LCMS-IT-TOF system, fitted with a Prominence UFLC system and an ESI interface (Shimadzu, Kyoto, Japan). Silica gel (200–300 mesh, Qingdao Marine Chemical Inc., Qingdao, China), LiChroprep RP-C_{18} gel (40–63 μm, Merck, Germany), D101 macroporous adsorption resin (Qingdao Marine Chemical Inc., Qingdao, China) and Sephadex LH-20 (Pharmacia) were used for open column chromatography (CC). HPLC was performed on a Shimadzu LC-20AT pump system (Shimadzu Corporation, Tokyo, Japan), equipped with a SPD-M20A photodiode array detector monitoring at 254 nm. A semi-preparative HPLC column (YMC-Pack C_{18}, 250 × 10 mm, 5 μm) was utilized for compounds separation and purification. TLC was performed using GF_{254} plates (Qingdao Marine Chemical Inc., Qingdao, China).

2.2. Plant material

Urena lobata L. was collected in Guangxi province, China, in September 2013. The plant material was authenticated by one of the authors (S.-P. Shi), and a voucher specimen (DTH2013029) is deposited at the Modern Research Center for Traditional Chinese Medicine, Beijing University of Chinese Medicine, Beijing, China.

* Corresponding author.
E-mail address: shishepo@163.com (S.-P. Shi).
[1] These two authors contributed equally to this work.

https://doi.org/10.1016/j.fitote.2018.02.017
Received 25 November 2017; Received in revised form 5 February 2018; Accepted 10 February 2018
Available online 12 February 2018
0367-326X/

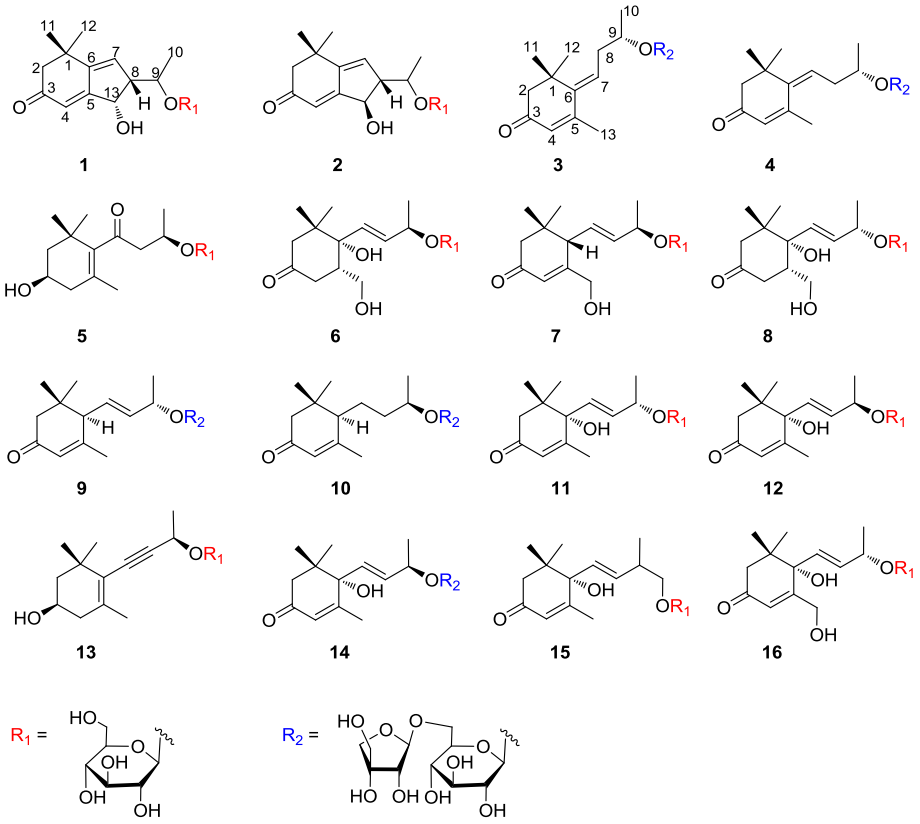

Fig. 1. Structures of compounds **1–16.**

Table 1
^{13}C NMR (125 MHz) data of compounds **1–5** (δ in ppm, in CD_3OD).

Position	1[a]	2[a]	3[a]	4[a]	5[a]
	δ_C, type	δ_C, type	δ_C, type	δ_C, type	δ_C, type
1	35.2,C	35.2, C	39.2, C	41.9, C	37.1, C
2	52.4,CH_2	52.3, CH_2	54.6, CH_2	53.7, CH_2	49.0, CH_2
3	202.3, C	202.3, C	202.3, C	201.9, C	65.0, CH
4	118.0, CH	118.5, CH	125.3, CH	128.8, CH	41.7, CH_2
5	171.5, C	171.1, C	159.6, C	160.0, C	143.7, C
6	148.9, C	149.5, C	142.6, C	144.4, C	129.2, C
7	136.1, CH	137.0, CH	135.4, CH	130.2, CH	211.7, C
8	60.5, CH	60.9, CH	38.5, CH_2	38.7, CH_2	54.4, CH_2
9	75.4, CH	75.9, CH	74.9, CH	74.9, CH	71.7, CH
10	18.1, CH_3	17.6, CH_3	20.2, CH_3	20.1, CH_3	20.2, CH_3
11	27.7, CH_3	27.8, CH_3	29.1, CH_3	28.3, CH_3	29.4, CH_3
12	28.4, CH_3	28.7, CH_3	29.2, CH_3	28.4, CH_3	29.9, CH_3
13	75.1, CH	75.5, CH	22.9, CH_3	25.1, CH_3	20.7, CH[3]
Glu-1	102.2, CH	101.9, CH	102.5, CH	102.5, CH	102.5, CH
Glu-2	75.0, CH	75.0, CH	75.9, CH	75.9, CH	75.1, CH
Glu-3	78.1, CH	77.9, CH	77.0, CH	77.0, CH	77.8, CH
Glu-4	71.8, CH	71.8, CH	71.8, CH	71.9, CH	71.6, CH
Glu-5	77.9, CH	78.1, CH	78.1, CH	78.1, CH	78.1, CH
Glu-6	63.0, CH_2	62.9, CH_2	69.1, CH_2	69.1, CH_2	62.9, CH_2
Api-1			111.0, CH	111.0, CH	
Api-2			78.0, CH	78.0, CH	
Api-3			80.4, C	80.5, C	
Api-4			75.0, CH_2	75.1, CH_2	
Api-5			65.4, CH_2	65.5, CH_2	

[a] Assignments were carried out based on HSQC and HMBC experiments.

2.3. Extraction and isolation

The air-dried *U. lobata* (13 kg) was refluxed with 95% EtOH for three times (3 × 180 L, each for 1 h). After removal of solvent under reduced pressure, the residue (1.35 kg) was suspended in water (6 L), and partitioned with petroleum ether (2 × 6 L), EtOAc (5 × 6 L), and *n*-BuOH (3 × 6 L), successively. The *n*-BuOH extract (128 g) was subjected to D101 macroporous adsorption resin chromatography and eluted with H_2O–EtOH (100:0, 90:10, 50:50, 20:80, 0:100) to yield five fractions (Fr.1–5). Fr. 2 (20 g) and Fr. 3 (40 g) were combined and subjected to silica gel chromatography and eluted with a gradient of EtOAc-MeOH-H_2O from 30:2:1 to 5:2:1 to give five subfractions (Subfr. A – E). Subfr. C (6.3 g) and subfr. D (7.7 g) were combined and chromatographed on Sephadex LH-20 column eluted with MeOH to give seven subfractions (Fr. C1 – C7). Fr. C4 (4.0 g) was subjected to silica gel column eluted with a stepwise gradient of petroleum CH_2Cl_2–MeOH (20:1 → 0:1) to afford six fractions (Fr. C4a – C4f). Fr. C4e (1.5 g) was further separated using ODS C_{18} column chromatography and eluted with MeOH–H_2O (20:1, 15:1, 10:1, v/v) to obtain four fractions (Subfr. C4e1 – C4e4). Subfr. C4e1 was repeatedly separated and purified by semi-preparative HPLC (8% aqueous MeCN) to give compounds **1** (6.0 mg, t_R 45.0 min), **2** (1.4 mg, t_R 63.0 min), **5** (1.8 mg, t_R 52.5 min), **6** (2.0 mg, t_R 57.0 min), **7** (1.4 mg, t_R 23.0 min), and **8** (1.5 mg, t_R 48.5 min). Subfr. C4e2 was separated by semi-preparative HPLC (15% aqueous MeOH) to give compounds **3** (13.0 mg, t_R 32.0 min), **4** (12.4 mg, t_R 35.0 min), **9** (11.8 mg, t_R 42.5 min), and **10** (10.0 mg, t_R 45.5 min). Subfr. C4e3 was subjected to semi-preparative HPLC (12% aqueous MeCN) to yield compounds **11** (3.0 mg, t_R 35.0 min), **12** (2.4 mg, t_R 43.0 min), and **13** (1.8 mg, t_R 52.5 min). Subfr. C4e4 was separated by semi-preparative HPLC (30% aqueous MeOH) to afford compounds **14** (23.0 mg, t_R 55.0 min), **15** (2.4 mg, t_R 53.0 min), and **16** (5.8 mg, t_R 62.5 min).

Urenalobaside A (**1**): Pale yellow gum, $[\alpha]_D^{25}$: −55.4 (*c* 0.05, MeOH); UV (MeOH) λ_{max} (log ε): 206 (3.09), 289 (3.55); IR (KBr) ν_{max}: 3389, 2940, 1655, 1653, 1082 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; negative-ion HRESIMS: *m/z* 419.1469 $[M + Cl]^-$ (calcd for $C_{19}H_{28}O_8Cl$, 419.1478).

Urenalobaside B (**2**): Pale yellow gum, $[\alpha]_D^{25}$: −14.1 (*c* 0.05, MeOH); UV (MeOH) λ_{max} (log ε): 206 (3.08), 289 (3.55); IR (KBr) ν_{max}: