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Cytotoxic triterpenoid saponins from *Lysimachia foenum-graecum*

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ABSTRACT

Eleven oleanane-type triterpenoid saponins, foegraecumosides A–K, and eight known ones, were isolated from the aerial parts of *Lysimachia foenum-graecum*. Their structures were elucidated by spectroscopic data analyses and chemical methods. All isolated saponins were evaluated for their cytotoxicity against four human cancer cell lines (NCI-H460, MGC-803, HepG2, and T24). Seven saponins containing the aglycone cyclamiretin A exhibited moderate cytotoxicity against all tested human cancer cell lines, with IC_{50} values of 9.3–24.5 μM. Simultaneously, the cytotoxic activities of foegraecumosides A and B, lysichriside A, ardisiacrispins A and B, cyclaminorin, and 3-*O*-α-L-rhamnopyranosyl-(1 → 2)-β-D-glucopyranosyl-(1 → 4)-α-L-arabinopyranosyl-cyclamiretin A were tested on drug-resistant lung cancer cell lines (A549 and A549/CDDP, respectively). Ardisiacrispin B displayed moderate cytotoxicity against A549/CDDP, with an IC_{50} value of 8.7 μM and a resistant factor (RF) of 0.9.

1. Introduction

The genus *Lysimachia* of the family Primulaceae, comprising about 180 species, is widely distributed throughout the world. However, data from recent phylogenetic analyses suggested its relocation to the family Myrsinaceae (Podolak et al., 2013). *L. foenum-graecum* is one of the best-known plants from this genus, distributed mainly in the Guangxi and Yunnan Provinces of China. The aerial parts of this plant have been used not only as a perfumery plant and an insect repellent, but also for the treatment of colds and headaches in traditional Chinese medicine (The Health Administration of Beijing, 1998). Previous phytochemical investigation of this species led to isolation of several triterpenoid saponins and flavonoids (Shen et al., 2005; Li et al., 2007, 2009a,b; 2010). In a continuing search for bioactive constituents from medicinal plants in the Guangxi Zhuang Autonomous Region, the aerial parts of *L. foenum-graecum* were investigated and eleven new oleanane-type triterpenoid saponins, named foegraecumosides A–K (**1**–**11**) (Fig. 1) were isolated, together with eight known ones (**12**–**19**) (Fig. 1). The isolated saponins were evaluated for their cytotoxic activities against a panel of drug-sensitive and drug-resistant human cancer cell lines. Herein, described are the isolation, structural elucidation, and biological assays of these compounds.

2. Results and discussion

The aerial parts of *L. foenum-graecum* were collected from the Jinxiu County of Guangxi Province and extracted with 95% aqueous EtOH. The EtOH extract was suspended in H_2O and partitioned successively with EtOAc and *n*-BuOH. The *n*-BuOH-soluble extract was subjected to macroporous resin column chromatography to give a crude saponin fraction, which showed cytotoxicity against three human cancer cell lines (HepG2, MGC-803, and T24) with IC_{50} values of 2.6–7.1 μg/mL. The crude saponin fraction was subjected to further column chromatography and purified by preparative HPLC, to afford eleven new (**1**–**11**) and eight known (**12**–**19**)

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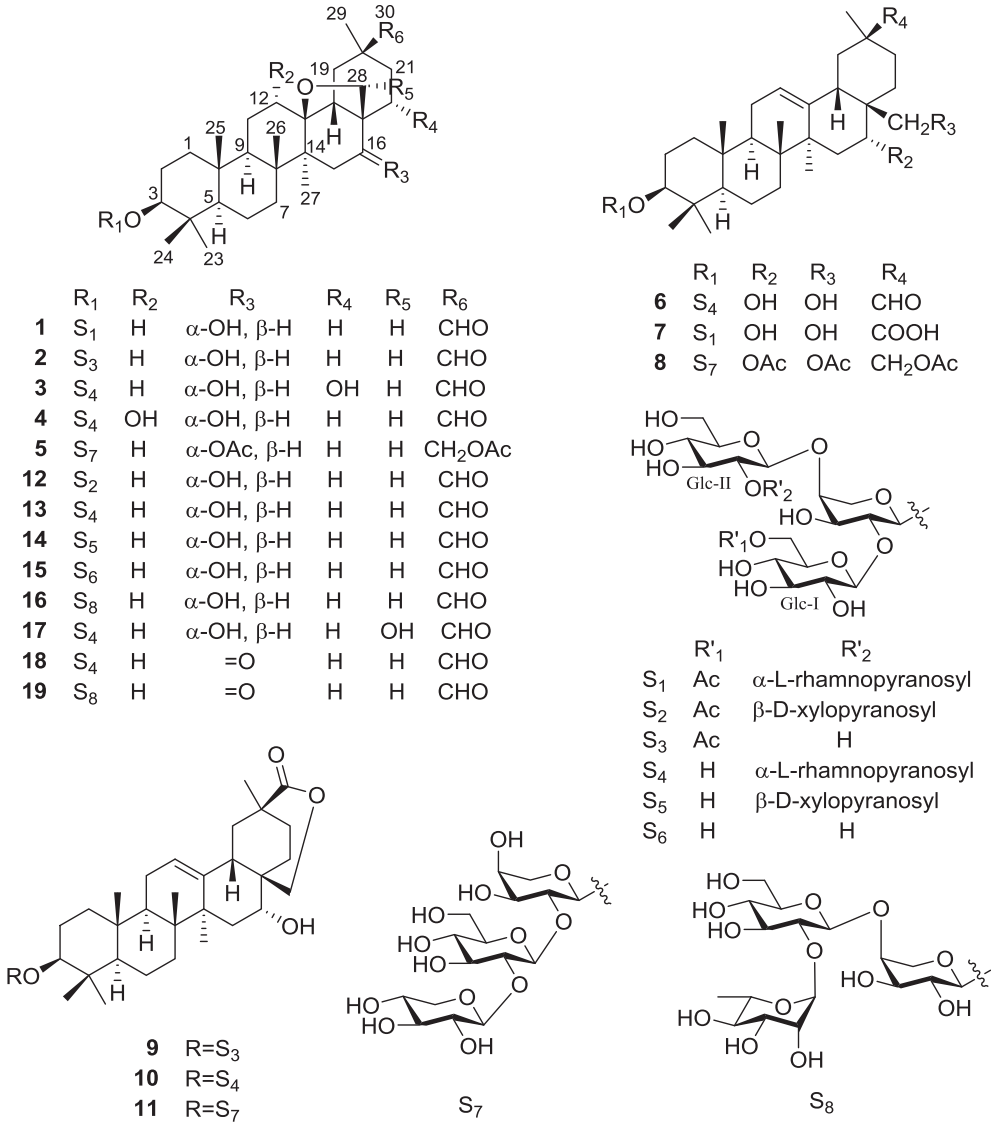

	R_1	R_2	R_3	R_4	R_5	R_6
1	S_1	H	α-OH, β-H	H	H	CHO
2	S_3	H	α-OH, β-H	H	H	CHO
3	S_4	H	α-OH, β-H	OH	H	CHO
4	S_4	OH	α-OH, β-H	H	H	CHO
5	S_7	H	α-OAc, β-H	H	H	CH_2OAc
12	S_2	H	α-OH, β-H	H	H	CHO
13	S_4	H	α-OH, β-H	H	H	CHO
14	S_5	H	α-OH, β-H	H	H	CHO
15	S_6	H	α-OH, β-H	H	H	CHO
16	S_8	H	α-OH, β-H	H	H	CHO
17	S_4	H	α-OH, β-H	H	OH	CHO
18	S_4	H	=O	H	H	CHO
19	S_8	H	=O	H	H	CHO

	R_1	R_2	R_3	R_4
6	S_4	OH	OH	CHO
7	S_1	OH	OH	COOH
8	S_7	OAc	OAc	CH_2OAc

	R'_1	R'_2
S_1	Ac	α-L-rhamnopyranosyl
S_2	Ac	β-D-xylopyranosyl
S_3	Ac	H
S_4	H	α-L-rhamnopyranosyl
S_5	H	β-D-xylopyranosyl
S_6	H	H

9 R=S_3
10 R=S_4
11 R=S_7

Fig. 1. Structures of compounds **1**–**19**.

oleanane-type triterpenoid saponins. All of the new compounds (**1**–**11**) were obtained as amorphous powders. Acid hydrolysis of each compound (**1**–**11**) and subsequent HPLC analysis of the sugars using an optical detector (Yoshikawa et al., 2003, 2007) allowed the characterisation of D-glucose, L-arabinose, and L-rhamnose for compounds **1**, **3**, **4**, **6**, **7**, and **10**, D-glucose and L-arabinose for compounds **2** and **9**, and D-glucose, L-arabinose, and D-xylose for compounds **5**, **8**, and **11**, respectively.

Foegraecumoside A (**1**) possessed the molecular formula of $C_{55}H_{88}O_{23}$ on the basis of its positive-ion HRESIMS (*m/z* 1139.5608 $[M + Na]^+$, calcd 1139.5609) and ^{13}C NMR data (Table 2). Its ^1H NMR spectrum (Table 1) showed six methyl singlets at δ_H 0.83, 0.99, 1.02, 1.21, 1.27, and 1.51, one oxygenated methylene at δ_H 3.14 and 3.52 (each 1H, d, $J = 7.5$ Hz), and one aldehyde proton at δ 9.60 (1H, s), which were unambiguously designated by HSQC experiment. Its ^{13}C NMR (Table 2) and DEPT spectra displayed six sp^3 carbon resonances at δ_C 16.38, 16.42, 18.5, 19.7, 24.1, and 28.0, an oxygenated methylene at δ_C 77.6, a quaternary carbon resonance at δ_C 86.4, and an aldehyde carbon at δ_C 207.5. The NOESY correlation of H-30 (δ_H 9.60)/H-18 (δ_H 1.35) confirmed the β-orientation of the aldehyde group. These data indicated that **1** was based on a 13,28-epoxy-oleanane skeleton. On the basis of its 2D NMR (^1H–^1H COSY, HSQC, and HMBC) analyses and comparison with literature data, the aglycone of **1** was identified as 3β,16α-dihydroxy-13β,28-epoxy-oleanan-30-al (cyclamiretin A) (Dong et al., 2011).

The ^1H NMR spectrum of **1** exhibited signals of four sugar anomeric protons at δ_H 4.87 (1H, d, $J = 5.0$ Hz), 5.12 (1H, d, $J = 8.0$ Hz), 5.38 (1H, d, $J = 7.5$ Hz), and 6.35 (1H, br s), which correlated to four anomeric carbons at δ_C 104.4, 103.5, 105.0, and 101.6, respectively, according to the HSQC spectrum. The extensive 2D NMR spectrocopic analyses together with results of acid hydrolysis of **1** allowed the characterisation of two β-D-glucopyranosyls (Glc), one α-L-rhamnopyranosyl (Rha), and one α-L-arabinopyranosyl (Ara), respectively. In the same way, these sugars were identified in compounds **3**, **4**, **6**, **7**, and **10**. The coupling constants also confirmed the β-glycosidic linkages for two glucopyranosyl units. The arabinopyranosyl unit was determined to be the α-anomer on the basis of: the $^3J_{H1,H2}$ value (5.0 Hz) and the correlations of Ara-H-1/Ara-H-3 and Ara-H-1/Ara-H-5 in the NOESY spectrum (Chang et al., 2007; Liang et al., 2011), as well as the ^1H non-splitting pattern (br s) and the large $J_{C1\text{-}H1}$ coupling constants (171 Hz) which confirmed the α-anomeric orientation of the

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