Phytochemistry xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Phytochemistry

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

Oleanane-*type* saponins from *Glochidion glomerulatum* and their cytotoxic activities

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ARTICLE INFO

Article history:
Received 21 November 2014
Received in revised form 1 April 2015
Accepted 6 May 2015
Available online xxxx

Keywords:
Glochidion glomerulatum
Euphorbiaceae
Oleanane-*type* saponins
Cytotoxic activity

ABSTRACT

Eight oleanane-*type* saponins, glomerulosides A–H, were isolated from leaves of *Glochidion glomerulatum*. All isolated compounds were evaluated for cytotoxic activity on four human cancer cell lines, A-549, HT-29, OVCAR, and MCF-7. Glomerulosides C and E, which contain a benzoyloxy group at C-22, showed significant cytotoxic activities against the A-549, HT-29, and OVCAR cancer cell lines with IC_{50} values ranging from 5.9 to 9.8 μM. Glomeruloside A showed cytotoxicity on HT-29 and OVCAR cell lines with IC_{50} values of 7.3 and 6.6 μM, respectively. Moreover, glomeruloside B exhibited significant activity on A-549 and HT-29 cancer cell lines with IC_{50} values of 9.7 and 7.5 μM. In contrast, glomerulosides F–H, lacking a benzoyloxy group, showed only moderate cytotoxic activity.

1. Introduction

Cancer remains the second most common cause of death in the United States and other countries in the world. In 2014, there will be estimated 1,665,540 new cancer cases and 585,720 deaths in the United States (Cancer Facts and Figures, 2014). Accordingly, there have been continuing efforts to develop new and effective anticancer agents. However, the frequency and severity of side-effects which are characteristic of some anticancer drugs have not been yet completely understood. Natural products have attracted interest as potential sources of novel drugs with a wide range of biological and pharmacological activities, including cytotoxic activity (Vickers, 2002). As a result, more than 60% of currently available anticancer drugs are natural compounds or are related to them (Newman and Cragg, 2007). Therefore, in the present study, a search for new and effective cytotoxic compounds from natural products has been conducted.

Glochidion is a large genus of the Euphorbiaceae family, comprising more than 250 species in the world. *Glochidion glomerulatum* (Miq.) Boerl. is a shrub or small tree distributed throughout Southeast Asia. The leaves of *G. glomerulatum* have been used in folk medicine to treat dysentery (Chi, 2012). A number of phytochemical studies of genus *Glochidion* has reported the isolation of oleanane-*type* saponins (Kiem et al., 2009) and flavonoids (Yin et al., 2010). In addition, oleanane saponins displayed cytotoxic activities (Nhiem et al., 2012). However, chemical components of *G. glomerulatum* have not yet been studied. As a part of an ongoing investigation on new cytotoxic compounds from Vietnamese plants (Ky et al., 2010; Nhiem et al., 2012), the methanol extract from *G. glomerulatum* leaves was found to have cytotoxic activity, and its phytochemical investigation led to isolation of eight new oleanane-*type* saponins. Herein, reported are the isolation, structural elucidation of oleanane-*type* saponins, and their cytotoxic activity against four human cancer cell lines, A-549, HT-29, OVCAR, and MCF-7.

2. Results and discussion

The methanol extract of the *G. glomerulatum* leaves was suspended in water and then partitioned with $CHCl_3$ and EtOAc to obtain three layers. The aqueous layer was subjected to Diaion HP-20P column chromatography to remove sugar and phenolics. The crude saponin fraction was separated using a combination of silica gel and RP-18 column chromatographic steps. The obtained fractions were further purified by HPLC to afford eight new

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http://dx.doi.org/10.1016/j.phytochem.2015.05.001
0031-9422/

triterpene saponins (Fig. 1). Their structures were elucidated by extensive spectroscopic methods including 1D and 2D NMR experiments, as well as by HRESIMS analysis.

Compound **1** was obtained as a white amorphous powder and its molecular formula was determined as $C_{48}H_{72}O_{15}$ by HRESIMS ion at *m/z* 923.4563 $[M+Cl]^-$ (calcd for $C_{48}H_{72}O_{15}Cl$, 923.4560). The ^1H NMR spectrum showed signals for the protons of six methyl groups at δ_H 0.74, 0.94, 1.03, 1.04, 1.07, and 1.32 (each, 3H, s), one olefinic proton at δ_H 5.36 (1H, br s), which indicated an oleanane aglycone. In addition to these, protons of a benzoyloxy unit were observed at δ_H 7.49 (2H, t, *J* = 7.6 Hz), 7.60 (1H, t, *J* = 7.6 Hz), and 8.05 (2H, d, *J* = 7.6 Hz). Two anomeric protons at δ_H 4.35 (1H, d, *J* = 7.6 Hz) and 4.54 (1H, d, *J* = 7.6 Hz) showed the presence of two sugar moieties. The ^{13}C NMR and DEPT spectra showed the presence of 48 carbons, including 1 carbonyl, 8 quaternary, 21 methine, 12 methylene, and 6 methyl carbons (Table 1). The NMR spectroscopic data of **1** were similar to those of glochierioside A (Kiem et al., 2009), except for addition of a hydroxyl group at C-23. The HMBC correlations (Fig. 2) between H-3 (δ_H 3.63) and C-4 (δ_C 43.9)/C-5 (δ_C 48.1)/C-23 (δ_C 65.0)/C-24 (δ_C 13.1) as well as between H-23 (δ_H 3.32 and 3.65)/H-24 (δ_H 0.74) and C-3 (δ_C 83.3)/C-4 (δ_C 43.9)/C-5 (δ_C 48.1) suggested the locations of the hydroxyl groups at C-3 and C-23, respectively. In addition, the HMBC correlations from H-18 (δ_H 2.46) to C-16 (δ_C 69.5)/C-17 (δ_C 44.8)/C-22 (δ_C 72.1)/C-28 (δ_C 64.7) confirmed the locations of oxygenated groups at C-16, C-22, and C-28. This was also confirmed by the COSY correlations of H-15 (δ_H 1.52 and 1.98)/H-16 (δ_H 4.32); H-21 (δ_H 1.78)/H-22 (δ_H 5.91). The location of a benzoyl group at C-22 was assigned based on the HMBC cross-peak from H-22 (δ_H 5.91) to C-7′ (δ_C 167.2). The α-orientations of H-3 and the hydroxyl methylene group at C-4 were determined by observation of ROESY correlations of H-3 (δ_H 3.63)/H-5 (δ_H 1.66)/H-23 (δ_H 3.32 and 3.65); H-24 (δ_H 0.74)/H-25 (δ_H 1.03). Furthermore, the α-orientations of both H-16 and H-22 were also confirmed by ROESY correlations between H-16 (δ_H 4.32) and H-27 (δ_H 1.32), and H-16 (δ_H 4.32) and H-22 (δ_H 5.91) (Fig. 2). Acid hydrolysis of **1** afforded L-arabinose and D-glucose as sugar components (identified as TMS derivatives by GC). In addition, the HMBC correlation between glc H-1‴ (δ_H 4.54) and ara C-3″ (δ_C 84.2); ara H-1″ (δ_C 4.35) and C-3 (δ_C 83.3) were observed. These results indicated the sugar linkages of **1** to be *O*-β-D-glucopyranosyl-(1 → 3)-α-L-arabinopyranoside, with the location of the sugar moiety being at C-3 of aglycone. This was also in agreement with the ^{13}C NMR data reported for glochierioside A from another member of the Glochidion genus, *G. eriocarpum* (Kiem et al., 2009). Thus, the structure of **1** was elucidated to be 22β-benzoyloxy-3β,16β,23,28-tetrahydroxyolean-12-ene 3-*O*-β-D-glucopyranosyl-(1 → 3)-α-L-arabinopyranoside, a new compound and named glomeruloside A.

The molecular formula of **2** was determined as $C_{47}H_{78}O_{16}$ by the HRESIMS ion at *m/z* 933.4996 $[M+Cl]^-$ (calcd for $C_{47}H_{78}O_{16}Cl$, 933.4978). The ^1H NMR and ^{13}C NMR spectra exhibited an oleanane skeleton and three sugar units (Table 2). In addition, the NMR data of **2** were similar to those of glomeruloside A (**1**), except for the addition of a sugar unit at glc C-4‴ and the absence of functional groups at C-16 and C-22. The aglycone was recognized to be 3β,23,28-trihydroxyolean-12-ene (Gohar et al., 2012). Acid hydrolysis of **2** confirmed the presence of L-arabinose, D-glucose, and L-rhamnose as sugar components (identified as TMS derivatives by GC). Furthermore, the ^1H and ^{13}C NMR data showed the sugar units to be α-L-arabinopyranose, β-D-glucopyranose, and α-L-rhamnopyranose. The HMBC correlations (Fig. 2) between rha H-1⁗ (δ_H 4.86) and glc C-4‴ (δ_C 79.1); glc H-1‴ (δ_H 4.55) and ara C-3″ (δ_C 84.4); ara H-1″ (δ_H 4.34) and aglycone C-3 (δ_C 83.5) confirmed the sugar linkages to be 3-*O*-α-L-rhamnopyranosyl (1 → 4)-β-D-glucopyranosyl (1 → 3)-α-L-arabinopyranoside. To the best of our knowledge, the trisaccharide sequence of oleanane saponin was reported for the first time. Consequently, the structure of **2** was determined to be 3β,23,28-trihydroxyolean-12-ene 3-*O*-α-L-rhamnopyranosyl (1 → 4)-β-D-glucopyranosyl (1 → 3)-α-L-arabinopyranoside, a new compound named glomeruloside B.

The HRESIMS of **3** corresponded to the molecular formula of $C_{54}H_{82}O_{19}$ (calcd for $C_{54}H_{82}O_{19}Cl$, 1069.5139). The NMR spectroscopic data of **3** was similar to glochierioside A (Kiem et al., 2009) except for an additional sugar unit (Table 1). The trisaccharide proved to be *O*-β-D-glucopyranosyl (1 → 2)-[β-D-glucopyranosyl (1 → 3)]-α-L-arabinopyranoside by HMBC and COSY experiments and the location of this sugar moiety was determined to be C-3 by the HMBC correlation between ara H-1″ (δ_H 4.41) and

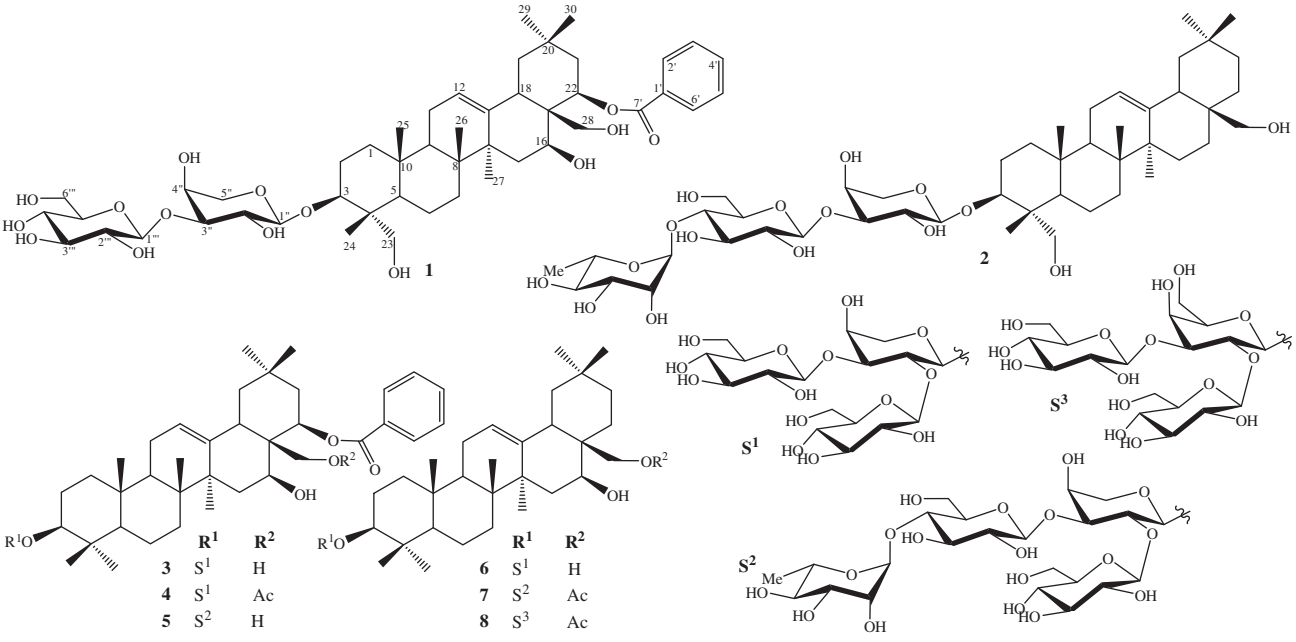

Fig. 1. Chemical structures of compounds of **1–8**.

Please cite this article in press as: Thu, V.K., et al. Oleanane-*type* saponins from *Glochidion glomerulatum* and their cytotoxic activities. Phytochemistry (2015), http://dx.doi.org/10.1016/j.phytochem.2015.05.001