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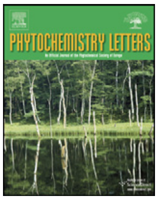

Triterpenoid saponins from *Sesbania vesicaria*

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ABSTRACT

Nine oleanane saponins including three new and six known were isolated from the seeds of *Sesbania vesicaria*. The new saponins were established as 3-*O*-[α-L-rhamnopyranosyl-(1 → 3)]-β-D-glucuronopyranosyl-3β,29-dihydroxy-olean-12-en-28-oic acid, 3-*O*-α-L-rhamnopyranosyl-28-*O*-β-D-glucopyransoyl-3β-hydroxy-olean-12-en-23-al-28-oate, and 3-*O*-α-L-rhamnopyranosyl-28-*O*-β-D-glucopyransoyl-3β,23-dihydroxy-olean-12-en-28-oate. All isolated saponins were assayed for their DNA topoisomerase I inhibition ability and cytotoxicity against A549 human lung adenocarcinoma epithelial cells with no positive activity detected ($IC_{50} > 312$ μM and $GI_{50} > 25$ μM, respectively).

1. Introduction

The genus *Sesbania* Scop. of the family Fabaceae consists of about 60 species widely distributed in tropical and subtropical regions of the world (Joshi-Saha and Gopalakrishna, 2007; Williams, 1983). In Asia and Africa, several *Sesbania* species are extensively utilized as forage and manure in their agroforestry systems (Gutteridge, 1998; Onim and Dzowela, 1988; Rinaudo et al., 1983). Some folk medicinal uses such as astringent, antihelminthic, contraceptive and arborifacient, have also been reported in the area (Onim and Dzowela, 1988). In the United States, *Sesbania vesicaria* (Jacq.) Elliott, *Sesbania punicea* (Cav.) Benth., *Sesbania drummondii* (Rydb.) Cory, and *Sesbania herbacea* (Mill.) McVaugh are widely distributed in the Southern Atlantic and Gulf Coastal plains as weeds and have long been known to be toxic to livestock and birds. Sesbanimides were identified as the cytotoxic compounds in *S. vesicaria*, *S. punicea*, and *S. drummondii* (Powell, 2009; Powell et al., 1990). In addition, triterpenoid saponins (Dorsaz et al., 1988; Haraguchi et al., 2000), flavonoids (Messens et al., 1989; Saxena and Mishra, 1999), fatty acids, sterols (Kohli, 1988; Miralles et al., 1992), and inositol (Misra and Siddiqi, 2005) have been isolated from various *Sesbania* species. In the course of discovering triterpenoid saponins from plants as DNA topoisomerase I (Topo I) inhibitors (Wang et al., 2010; Yuan et al., 2012; Zhang and Li, 2007), we conducted phytochemical investigations on saponins of *S. vesicaria*. Herein we report the isolation procedure and structural elucidation of these saponins.

2. Results and discussion

Three new oleanane saponins (**1**–**3**) together with six known saponins (**4**–**9**) were isolated from the *n*-BuOH soluble fraction of *S. vesicaria* (Fig. 1). The known saponins were identified as: 3-*O*-β-D-glucuronopyranosyl-28-*O*-β-D-glucopyransoyl-3β-hydroxy-olean-12-en-23-oic acid-28-oate (**4**) (Borel et al., 1987), 3-*O*-β-D-glucuronopyranosyl-28-*O*-β-D-glucopyransoyl-3β-hydroxy-olean-12-en-28-oate (**5**) (Borel et al., 1987; Dawidar et al., 1980), 3-*O*-[α-L-rhamnopyranosyl-(1 → 3)]-β-D-glucuronopyranosyl-28-*O*-β-D-glucopyransoyl-3β-hydroxy-olean-12-en-28-oate (**6**) (Dawidar et al., 1980; Dorsaz et al., 1988), 3-*O*-[α-L-rhamnopyranosyl-(1 → 3)]-(6-*O*-methyl)-β-D-glucuronopyranosyl-28-*O*-β-D-glucopyransoyl-3β-hydroxy-olean-12-en-28-oate (**7**) (Imai et al., 2006), 3-*O*-[β-D-glucopyranosyl-(1 → 2)]-[α-L-rhamnopyranosyl-(1 → 3)]-β-D-glucuronopyranosyl-28-*O*-β-D-glucopyransoyl-3β-hydroxy-olean-12-en-28-oate (**8**) (Dawidar et al., 1980; Dorsaz et al., 1988), 3-*O*-[β-D-xylopyranosyl-(1 → 2)]-[α-L-rhamnopyranosyl-(1 → 3)]-β-D-glucuronopyranosyl-28-*O*-β-D-glucopyransoyl-3β-hydroxy-olean-12-en-28-oate (**9**) (Dawidar et al., 1980; Dorsaz et al., 1988). The determination of the structures of the three new saponins (**1**–**3**) was achieved by spectroscopic and chemical analysis as described below.

The molecular formula of **1** was determined to be $C_{42}H_{66}O_{14}$ from its HRESIMS data ($[M-H]^-$ *m*/*z*, 793.4379) and ^{13}C NMR spectrum (Table 1). The NMR data displayed molecular characteristic of a 30 carbon tritepenoid aglycone and two six-carbon saccharide moieties. The six tertiary methyl signals at δ_H 0.75, 0.94, 1.00, 1.23, 1.25, and 1.34 and Δ^{12} broad triplet-like olefinic signal at δ_H 5.50 revealed the olean-12-ene aglycone. The methyl singlets of δ_H 1.25, 0.94, 0.75 and 1.34 can be assigned as H-23, H-24, H-25

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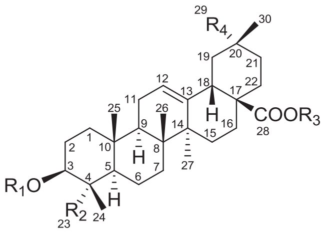

1 R_1=α-L-Rha-(1→3)-β-D-GlcA, R_2=CH_3, R_3=H, R_4=CH_2OH
2 R_1=α-L-Rha, R_2=CHO, R_3=β-D-Glc, R_4=CH_3
3 R_1=α-L-Rha, R_2=CH_2OH, R_3=β-D-Glc, R_4=CH_3
4 R_1=β-D-GlcA, R_2=COOH, R_3=β-D-Glc, R_4=CH_3
5 R_1=β-D-GlcA, R_2=CH_3, R_3=β-D-Glc, R_4=CH_3
6 R_1=α-L-Rha-(1→3)-β-D-GlcA, R_2=CH_3, R_3=β-D-Glc, R_4=CH_3
7 R_1=α-L-Rha-(1→3)-6-OMe-β-D-GlcA, R_2=CH_3, R_3=β-D-Glc, R_4=CH_3
8 R_1=β-D-Glc-(1→2)-[α-L-Rha-(1→3)]-β-D-GlcA, R_2=CH_3, R_3=β-D-Glc, R_4=CH_3
9 R_1=β-D-Xyl-(1→2)-[α-L-Rha-(1→3)]-β-D-GlcA, R_2=CH_3, R_3=β-D-Glc, R_4=CH_3

Fig. 1. The structures of triterpenoid saponins **1–9** from *S. vesicaria.*

and H-27 from HMBC correlations with C-3, C-5 and C-13. Further analysis of HMBC and HSQC correlations and ^{13}C NMR data led to the assignment of H-26. The HMBC correlations of the methyl singlet at δ_H 1.23 and δ_C 20.1 with a hydroxymethyl singlet at δ_H 3.62 and δ_C 74.2 revealed the hydroxylated C-29 or C-30. The hydroxylation was determined to be at C-29 by the NOESY experiment and ^{13}C NMR shifts of C-29 and C-30 (Ikuta and Itokawa, 1989; Plasman et al., 2000). After extensive 2D-NMR studies and spectral data comparison, the tritepenoid aglycone was determined to be 3β,29-dihydroxy-olean-12-en-28-oic acid. The two saccharide moieties had two anomeric protons resonating at δ_H 4.93 and 6.36 and their correlating resonances occupied at δ_C 107.4 and 103.3, respectively. By chemical degradation and HPLC analysis, the two monosaccharides of **1** were determined as D-glucuronic acid and L-rhamnose. The signals of the D-glucuronic acid and L-rhamnose were assigned by means of HSQC and DQF-COSY experiments (Table 1). The coupling constants of the protons on the six-membered sugar ring were consistent with the identification of the sugars as glucuronic acid and rhamnose. Both monosaccharides were in the pyranose form as determined from their ^{13}C NMR data. The anomeric configuration in the glucuronopyranosyl was defined as β according to its $^3J_{H1',\,H2'}$ (7.9 Hz) and the rhamnopyranosyl was defined as α according to its $^1J_{C1'',\,H1''}$ (171.1 Hz) (Jia et al., 1998). The following HMBC correlations established the linkages of the glucuronopyranosyl to C-3 and rhamnopyranosyl to C-3′: H-1′ of GlcA (δ_H 4.93) with C-3 of the aglycone (δ_C 89.5), H-1″ of Rha (δ_H 6.36) with C-3′ of GlcA (δ_C 82.6). The linkages were also confirmed by the NOESY experiment. Therefore, the structure of **1** was determined to be 3-*O*-[α-L-rhamnopyranosyl-(1 → 3)]-β-D-glucuronopyranosyl-3β,29-dihydroxy-olean-12-en-28-oic acid.

Compound **2** was assigned a molecular formula of $C_{42}H_{66}O_{13}$, as deduced from its HRESIMS data ([M−H]$^-$ *m*/*z*, 777.4420). The tritepenoid aglycone of **2** had six tertiary methyl singlets (δ_H 0.82, 0.88, 0.91, 1.04, 1.24 and 1.26). Among them, only one singlet at δ_H 1.26 was correlated with the typical C-3 signal at δ_C 82.6 in the HMBC experiment, which implied possible modifications at C-23 or C-24. The singlet at δ_H 1.26 also showed long range correlation with a aldehyde carbonyl signal at δ_C 207.2 (δ_H at 9.68). The evidence of the upfield chemical shift of C-24 (up to δ_C 10.5) revealed the aldehyde group at C-23, which was confirmed by the NOESY experiment (Murakami et al., 2001). The aglycone was deduced to be 3β-hydroxy-olean-12-en-23-al-28-oate. The chemical shifts of C-3 at δ_C 82.6 and C-28 at δ_C 176.8 indicated that **2** was a bisdesmosdic glycoside. Acid hydrolysis of **2** liberated L-rhamnose and D-glucose. With the aid of DQF-COSY and HSQC spectra, the signals of the two monosaccharides were assigned. The characteristics of the coupling constants of ^{1}H NMR and the shifts of ^{13}C NMR of **2** supported that the two monosaccharides were L-rhamnopyranosyl and D-glucopyranosyl. The HMBC correlation of H-1′ (δ_H 6.29, *brs*) of L-rhamnopyranosyl with C-3 (δ_C 82.6) of the aglycone and H-1″ (δ_H 6.31, *d*, 8.3 Hz) of D-glucopyranosyl with C-28 (δ_C 176.8) indicated the linkage of L-rhamnopyranosyl to C-3 and D-glucopyranosyl to C-28, respectively. The anomeric configurations of the glucopyranosyl and rhamnopyranosyl were determined by the coupling constants of $^3J_{H1'',\,H2''}$ (8.3 Hz) and $^1J_{C1',\,H1'}$ (171.0 Hz) and NOESY. Based upon the above evidence, the structure of **2** was established as 3-*O*-α-L-rhamnopyranosyl-28-*O*-β-D-glucopyransoyl-3β-hydroxy-olean-12-en-23-al-28-oate.

Compound **3** had a molecular formula of $C_{42}H_{68}O_{13}$ as deduced from its negative HRESIMS (*m*/*z* 779.4585 [M−H]$^-$) and ^{13}C NMR spectrum (Table 1). Detailed NMR analysis concluded that the structure of compound **3** was very close to that of **2** and they only differed in the substitute at C-23. In saponin **2**, the signals at δ_C 207.2 (CH) and δ_H 9.68 (1H, *s*) for the aldehyde group were replaced by signals at δ_C 64.4 (CH_2) and δ_H 4.34 (1H, d, *J* = 9.7 Hz), 3.69 (1H, d, *J* = 9.7 Hz) for CH_2OH in saponin **3**. Accordingly, the structure of **3** was determined to be 3-*O*-α-L-rhamnopyranosyl-28-*O*-β-D-glucopyransoyl-3β,23-dihydroxy-olean-12-en-28-oate.

Compounds **1–9** were evaluated for their DNA topoisomerase I (Topo I) inhibition activity and cytotoxicity against A549 human lung adenocarcinoma epithelial cells. None of them showed positive activity in our assays (Topo I inhibition $IC_{50} > 312$ μM and cytotoxicity $GI_{50} > 25$ μM).

3. Experimental

3.1. General experimental procedures

NMR experiments were performed using a JEOL ECS 400 spectrometer, with spectroscopic data referenced to the solvent used. HR-mass spectra were acquired using a MDS Sciex API QStar Pulsar mass spectrometer. Optical rotation values were measured on a JASCO P-1010 polarimeter. Octadecyl-functionalized silica gel and Diaion® HP-20 (ODS, Aldrich) was used for open column chromatography. HPLC analysis was performed on an Agilent 1260 HPLC system using Agilent ODS columns (Column A: Zorbax SB-C18, 4.6 × 250 mm, 5 μm). Preparative HPLC was performed with a Lab-Alliance Series III Isocratic HPLC System using an Alltima C18 column (Column B: 250 × 22 mm, 10 μm, Grace).

3.2. Plant material

Seeds of *S. vesicaria* were collected in Kilgore, TX. The plant sample was identified by Dr. Shiyou Li and the voucher specimen (TX-Kilgore-#259-20071009-#001-Sd) was deposited at the National Center for Pharmaceutical Crops at Stephen F. Austin State University, USA.

3.3. Extraction and isolation

The seeds of *S. vesicaria* (1.2 kg dry weight) were ground to a coarse powder and percolated with 95% ethanol using a 75 mm × 600 mm glass column. After evaporated under reduced pressure, the extracts gave a total of 130 g residue. The residue was suspended in 500 mL water and then partitioned successively with *n*-hexane, ethyl acetate and *n*-butanol. The *n*-butanol soluble fraction (20 g) was loaded on a Diaion® HP-20 absorbent resin