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Two new oleanane-type triterpene saponins from the leaves of *Schefflera sessiliflora* De P. V.

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ABSTRACT

From the leaves of *Schefflera sessiliflora* De P. V., two new oleanane-type triterpene saponins, named scheffleraside A (**1**), scheffleraside B (**2**); together with two known saponins, chikusetsusaponin IVa (**3**), 3-*O*-[α-L-rhamnopyranosyl-(1 → 3)]-β-D-glucuronopyranosyl hederagenin (**4**) were isolated by various chromatography methods. Its chemical structure was elucidated by IR, UV, HR-ESI-MS, NMR 1D and 2D experiments and comparison of their NMR data with previous reported data.

1. Introduction

Schefflera is a large genus of the Araliaceae family with over 650 species (Hebbar and Nalini, 2014), which 56 species are found in Vietnam (La et al., 2013). Some species in the *Schefflera* genus, such as *Schefflera actinophylla*, *Schefflera arboricola*, *Schefflera heptaphylla*, *Schefflera impressa*, *Schefflera leucantha*, and *Schefflera-akwangsiensis* have been chemically investigated, which led to the isolation of triterpenoid (Wanas et al., 2010; Zhao et al., 2010; Wu et al., 2013; Srivastava, 1992; Pancharoen et al., 1994; Wang et al., 2014), sterol (Hansen and Boll, 1986), polyacetylene (Hansen and Boll, 1986), phenolic (Li et al., 2005), antraquinone (Srivastava, 1992), trisaccharide (Tran et al., 1991). *Schefflera sessiliflora* De P. V. (Araliaceae) is a new species in Viet Nam, it were discovered and indentified in 2004 (Nguyen et al., 2004). Pharmacological of extracts from this species possessed antistress, enhanced physical strength (Nguyen et al., 2004; Huynh et al., 2005); antioxidant activities in DPPH and MDA test (Vo et al., 2008); the androgenic effects (Tran et al., 2012). However, only two sapogenins: oleanolic acid and hederagenin from the hydrolyzed product of total saponins were isolated (Vo et al., 2003, 2004). This paper reports the isolation and structure elucidation of two new triterpene saponins (**1**, **2**) and 2 known saponins (**3**, **4**) from *S. sessiliflora* De P. V. growing in Vietnam.

2. Results and discussion

The 75% MeOH extract from the dried leaves of *S. sessiliflora* De P. V. was subjected to column chromatography over silica gel normal-phase and reversed-phase RP-18 to give two new triterpene saponins named scheffleraside A (3-*O*-[α-L-rhamnopyranosyl-(1→ 3)]-β-D-glucuronopyranosyl oleanolic acid 28-*O*-[α-L-rhamnopyranosyl-(1 → 4)]-β-D-glucopyranosyl ester) (**1**) and scheffleraside B (3-*O*-[α-L-rhamnopyranosyl-(1 → 3)]-β-D-(6′-*O*-methyl)glucuronopyranosyl oleanolic acid 28-*O*-[α-L-rhamnopyranosyl-(1 → 4)]-β-D-glucopyranosyl ester) (**2**), and two known saponins: 3-*O*-β-D-glucuronopyranosyl oleanolic acid 28-*O*-β-D-glucopyranosyl ester (chikusetsusaponin IVa) (**4**) (Mizui et al., 1990), 3-*O*-[α-L-rhamnopyranosyl-(1 → 3)]-β-D-glucuronopyranosyl hederagenin (**5**) (El and Morta, 1998).

Compound (**1**) was obtained as a white amorphous powder. The molecular formula was established as $C_{54}H_{86}O_{22}$ by HR-ESI-MS data ($[M-H]^-$ *m/z:* 1085.5521, calcd. 1085.5527 and $[M+Na]^+$ *m/z* 1109.5533, calcd. 1109.5503). The IR spectrum of **1** showed absorptions of hydroxyl (3418 cm^{-1}) and carbonyl (1726 cm^{-1}) groups. The ^{13}C NMR and DEPT spectrum (Table 1), showed **1** has fifty four carbons including: one carbonyl carbon, two olefinic

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Table 1
^{1}H (500 Hz) and ^{13}C (125 Hz) NMR spectral data for compounds **1** and **2** in pyridine-d_5.

Aglycone	**1**		**2**		Sugar	**1**		**2**	
	δ_H	δ_C	δ_H	δ_C		δ_H	δ_C	δ_H	δ_C
1	0.77, 1.30	38.6	0.78, 1.28	38.6	GlcA				
2	1.74, 2.24	26.4	1.72, 2.04	26.4	1′	4.78 d (6.5)	106.8	4.86 d (7.5)	107.0
3	3.26 br d (7.5)	89.1	3.26 dd (4.0, 11.5)	89.3	2′	4.00	75.9	4.03	75.7
4	–	39.4	–	39.5	3′	4.37	82.1	4.39	82.0
5	0.69	55.6	0.72	55.7	4′	4.28	72.0	4.39	71.4
6	1.21, 1.40	18.4	1.20, 1.41	18.4	5′	4.37	76.9	4.48	77.1
7	1.30, 1.40	33.1	1.28, 1.41	33.1	6′		[a]		170.7
8	–	39.8	–	39.9	6′-OMe			3.76 s	52.1
9	1.53	47.9	1.56	48.0	Rha I				
10	–	36.8	–	36.9	1″	6.28 br s	102.5	6.28 br s	102.9
11	1.81	23.7	1.83	23.7	2″	4.71 br s	72.5	4.73 d (1.5)	72.5
12	5.38 br s	122.9	5.38 br s	122.9	3″	4.56 d (8.0)	72.6	4.54	72.7
13	–	144.1	–	144.1	4″	4.28	74.1	4.32	74.1
14	–	42.0	–	42.1	5″	5.06	69.6	5.06	69.8
15	1.14, 1.30	28.2	1.14, 1.26	28.2	6″	1.68 d (5.0)	18.6	1.68 d (6.5)	18.6
16	1.89, 2.05	23.3	1.90, 2.04	23.3	Glc-28				
17	–	47.0	–	47.0	1‴	6.18 d (8.0)	95.3	6.20 d (8.0)	95.4
18	3.14 br d (10.0)	41.7	3.16 br d (13.0)	41.7	2‴	4.10	74.2	4.12	74.2
19	1.21, 1.74	46.1	1.20, 1.72	46.1	3‴	4.18	77.0	4.20	77.1
20	–	30.7	–	30.7	4‴	4.47	77.8	4.52	77.9
21	1.03, 1.30	34.0	1.04, 1.28	34.0	5‴	3.76 d (9.5)	77.8	3.78 d (9.5)	77.9
22	1.68, 1.74	32.5	1.72, 1.72	32.5	6‴	4.04, 4.18	60.9	4.05, 4.20	61.0
23	1.21 s	28.1	1.21 s	28.0	Rha II				
24	0.92 s	16.9	0.91 s	16.8	1⁗	5.85 br s	102.6	5.87 br s	102.7
25	0.77 s	15.4	0.78 s	15.5	2⁗	4.63 br s	72.5	4.64 d (1.5)	72.5
26	1.03 s	17.4	1.04 s	17.4	3⁗	4.47	72.7	4.52	72.7
27	1.24 s	26.0	1.24 s	26.1	4⁗	4.28	73.8	4.32	73.9
28	–	176.4	–	176.4	5⁗	4.86	70.3	4.90	70.4
29	0.89 s	33.1	0.90 s	33.1	6⁗	1.65 d (6.0)	18.4	1.66 d (6.5)	18.5
30	0.86 s	23.6	0.87 s	23.6					

[a] Not determined.

carbons, four anomeric carbons, seventeen oxygenated methine carbons, one oxygenated methylene carbon, six quaternary carbons, three methine carbons, ten methylene carbons, and nine methyl carbons. The presence of seven tertiary methyl groups, two olefinic carbons at δ_C 144.0 (C-13) and 122.8 (C-12), and one carbonyl carbon 176.4 (C-28) which indicated an olean-12-en-28-oic acid as an aglycone (Tran et al., 1991). Thus **1** was an oleanane-type triterpenoid saponin bearing four sugar (six carbons) units. The ^{1}H NMR data (Table 1) also indicated aglycone of **1** was oleanolic acid with: one olefinic proton at δ_H 5.38 (*br s*, H-12); one oxygenated methine proton at δ_H 3.26 (*br d*, 7.5, H-3); one methine proton at δ_H 3.14 (*br d*, 10.0, H-18) and seven tertiary methyl groups at δ_H 0.76–1.24 (Maeda et al., 1994). Moreover, four anomeric protons at δ_H 4.78 (*d*, 6.5, H-1′); 6.18 (*d*, 8.0, H-1‴); 6.28 (*br s*, H-1″); 5.85 (*br s* H-1⁗) to four anomeric carbons at δ_C 106.8 (C-1′), 95.3 (C-1‴), 102.5 (C-1″), 102.6 (C-1⁗) were assigned to β-D-glucuronic (GlcA), β-D-glucose (Glc) and two α-L-rhamnose (Rha) units, respectively. It was completely appropriated with HR-ESI-MS data, a peak $[M+2Na-H]^+$ *m/z* 1131.5492 was indicated the presence of a sugar acid (Madl et al., 2006). The COSY, HSQC and NOESY spectrum, allowed analysis of their spin systems and assigned of their proton resonances to determine clearly every sugar unit. Beside, through acid hydrolysis followed by co-TLC in comparison with standard sugars the identification of the sugars was determined to be D for Glc, GlcA and L for Rha (see Section 3). The HMBC spectrum (Fig. 1), showed correlations between anomeric proton at δ_H 4.78 (*d*, 6.5, H-1′) of GlcA and carbons at δ_C 89.1 (C-3) of aglycone; between anomeric proton at δ_H 6.28 (*br s*, H-1″) of Rha I unit and carbons at δ_C 82.1 (C-3′) of GlcA; between oxygenated methine proton at δ_H 4.37 (*m*, H-3′) and carbon acetal at δ_C 102.5 (C-1″). On the other hands, anomeric proton at δ_H 6.18 (*d*, 8.0, H-1‴) of Glc correlated with carbonyl carbon at δ_C 176.4 (C-28); anomeric proton at δ_H 5.85 (*br s*, H-1⁗) of Rha II correlated with carbon at δ_C 77.8 (C-4‴) of Glc; between oxygenated methine proton at δ_H 4.47 (*m*, H-4‴) and carbon acetal at δ_C 102.6 (C-1⁗). These connectivities were also confirmed by correlations observed in the NOESY spectrum between H-3 (δ_H 3.26) of aglycone and H-1′ (δ_H 4.78) of GlcA; between H-1″ (δ_H 6.28) of RhaI and H-3′ (δ_H 4.37) of GlcA. Based on data of HR-ESI-MS, 1D, 2D-NMR and compared with previous published data (Tapondjou et al., 2006; Wang et al., 2014), the structure of **1** was identified as 3-*O*-[α-L-rhamnopyranosyl-(1 → 3)]-β-D-glucuronopyranosyl oleanolic acid 28-*O*-[α-L-rhamnopyranosyl-(1 → 4)]-β-D-glucopyranosyl ester, and named scheffleraside A.

Compound (**2**) was obtained as a white amorphous powder. The molecular formula was established as $C_{55}H_{88}O_{22}$ by HR-ESI-MS data ($[M+Na]^+$ *m/z* 1123.5633, calcd. 1123.5659). The ^{1}H and ^{13}C NMR data (Table 1) demonstrated that **2** has the same aglycone and sugar chains as **1**, except for presence of an oxygenated methyl group [δ_H 3.76 (*s*); δ_C 52.1]. Further, the HMBC spectrum (Fig. 1) showed correlation between this oxygenated methyl at δ_H 3.76 (*s*) and carbon carbonyl of glucuronic unit at δ_C 170.7. Based on data of HR-ESI-MS, 1D, 2D-NMR and compared with previous published data (Tapondjou et al., 2006; Wang et al., 2014), the structure of **2** was identified as 3-*O*-[α-L-rhamnopyranosyl-(1 → 3)]-β-D-(6′-*O*-methyl)glucuronopyranosyl oleanolic acid 28-*O*-[α-L-rhamnopyranosyl-(1 → 4)]-β-D-glucopyranosyl ester, and named scheffleraside B.

Some species in the *Schefflera* genus, such as *S. actinophylla* (Wanas et al., 2010), *S. arboricola* (Zhao et al., 2010), *S. impressa* (Srivastava, 1992), *S. leucantha* (Pancharoen et al., 1994) are reported as a rich source of saponins which possessed lupane-type and/or ursane-type aglycones. Whereas, the genins of *S. heptaphylla* (Maeda et al., 1994), *S. kwangsiensis* (Wang et al., 2014) as well as *S. sessiliflora* were oleanolic acid and hederagenin. However, all compounds were isolated for the first time from the genus *Schefflera*.

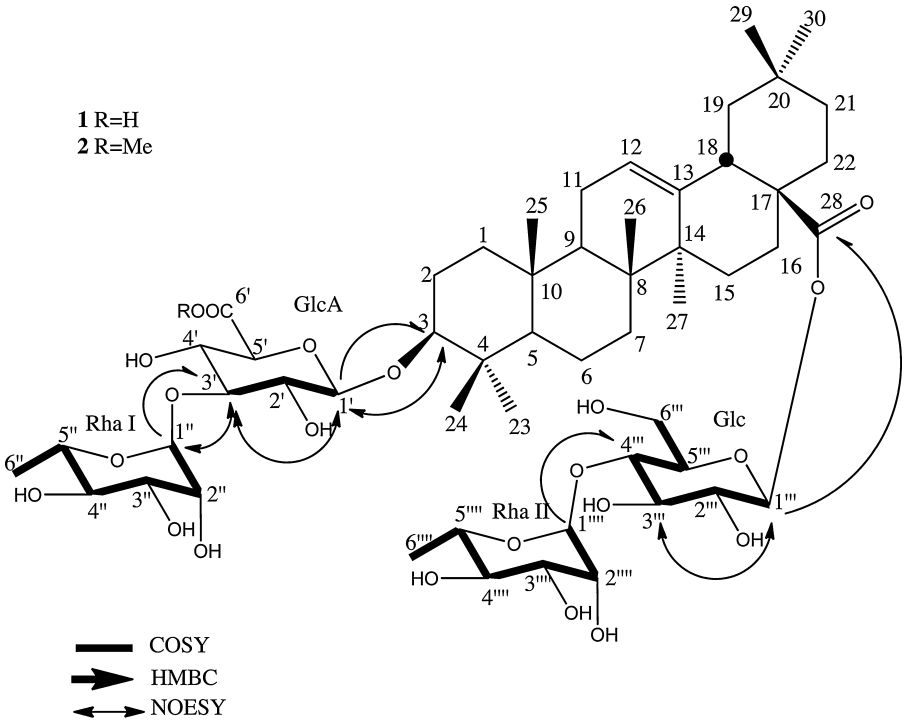

Fig. 1. Chemical structures and selected HMBC, COSY and NOESY correlations of **1** and **2**.

3. Experimental

3.1. General experimental procedures

The high resolution electrospray ionization mass spectroscopy (HR-ESI-MS) was recorded on a Bruker MicrOTOF-QII spectrometer. The ^{1}H NMR (500 MHz), ^{13}C NMR (125 MHz), DEPT, COSY, HSQC and HMBC spectra were recorded on a Bruker AM500 FT-NMR spectrometer using tetramethylsilane (TMS) as internal standard. Column chromatography was carried out using Merck Silica gel normal-phase (230–240 mesh) and reversed-phase C_{18} (Merck). Analytical TLC was carried out in silica gel plates (Merck DC-Alufolien 60 F_{254}). Compounds were visualized by spraying with aqueous 10% H_2SO_4 and heating for 3–5 min.

3.2. Plant material

The leaves of *S. sessiliflora* De P. V. were provided by Center Cultivation and Processing of Medicinal Plants Da Lat, No. 18 Hoang Van Thu Street, Da Lat City, Viet Nam and identified by Dr. Phan Van De – Department of Medicinal Resources, The Research Center of Ginseng and Medicinal Materials, Ho Chi Minh City, Viet Nam. A voucher specimen was deposited in Bioactive Compounds Laboratory, Institute of Chemical Technology, Vietnam Academy of Science and Technology, Viet Nam.

The leaves were collected, washed, dried, and powdered.

3.3. Extraction and isolation

Powder of leaves *S. sessiliflora* De P. V. (5 kg) were extracted with 96% EtOH (v/v), filtered residue, removed solvents under low pressure, obtained crude extract (890 g). Then, crude extract was added water and extracted with *n*-hexane, ethyl acetate, obtained extracts respectively. The aqueous portion was eluted by Diaion HP-20 column with: H_2O, 25% MeOH, 50% MeOH, 75% MeOH and 100% MeOH. Then distilled solvents, gave five major fractions (I–V), respectively. Fraction IV (80 g) was subjected by column chromatography with mobile phase (EtOAc–MeOH) gradient (0–100%) gave seven subfractions (IV.1–IV.7). Fraction IV.2 (15 g), was eluted with solvent system $CHCl_3$–MeOH–H_2O (85:15:0.1 → 75:25:0.3, v/v), then, separated by RP-18 using gradient mixtures of MeOH–H_2O gave **3** (15 mg) and **4** (8 mg). Similar, Fraction IV.3 (20 g) was eluted with solvent system $CHCl_3$–MeOH–H_2O (80:20:0.2 → 70:30:0.5, v/v), then, separated by RP-18 using gradient mixtures of MeOH–H_2O gave **1** (12 mg) and **2** (16 mg).

3.3.1. Scheffleraside A (*1*)

Amorphous powder (MeOH); $[\alpha]_D^{25}$ −17.0 (c 0.10, MeOH); IR (KBr) υ_{max}: 3418, 2927, 1726, 1615, 1075, 1030 cm^{-1}; UV (MeOH) λ_{max}: 206 and 220 nm; HR-ESI-MS *m/z* 1085.5521 $[M-H]^-$ (calcd for $C_{54}H_{85}O_{22}$, 1085.5527) and *m/z* 1109.5533 $[M+Na]^+$ (calcd for $C_{54}H_{86}O_{22}Na$, 1109.5503); ^{1}H and ^{13}C NMR data (pyridine-d_5), see Table 1.

3.3.2. Scheffleraside B (*2*)

Amorphous powder (MeOH); $[\alpha]_D^{25}$ −13.5 (c 0.10, MeOH); IR (KBr) υ_{max}: 3423, 2924, 1741, 1635, 1067, 1039 cm^{-1}; UV (MeOH) λ_{max}: 240 nm; HR-ESI-MS *m/z* 1123.5633 $[M+Na]^+$, calcd for $C_{58}H_{88}O_{22}Na$, 1123.5659; ^{1}H and ^{13}C NMR data (pyridine-d_5), see Table 1.

3.4. Acid hydrolysis

Each saponin (2 mg) was refluxed with 2 N aq. CH_3COOH (5 ml) for 2 h at 100 °C. After extraction with CH_3Cl (3× 5 ml), the aqueous layer was repeatedly evaporated to dryness with MeOH until neutral, and then analyzed by TLC over silica gel (MeCOEt–isoPrOH–Me_2CO–H_2O 20:10:7:6) by comparison with authentic samples (L-rhamnose *Rf* 0.65; D-glucose *Rf* 0.40; D-glucuronic acid *Rf* 0.05) (Haddad et al., 2013; Voutquenne-Nazabadioko et al., 2013).

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