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Two new oleanane-type saponins from *Elaeocarpus hainanensis* Oliv. growing in Vietnam

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ABSTRACT

Two new oleanane-type saponins, 1α-hydroxy-olean-12-en-3-*O*-β-D-xylopyranoside (**1**) and 1α-hydroxy-olean-12-en-3-*O*-α-L-arabinopyranoside (**2**), along with six known compounds (**3**-**8**), were isolated from the leaves and twigs of *Elaeocarpus hainanensis* Oliv. Their structures were determined based on chemical and spectroscopic analyses. This is the first report on oleanane-type triterpenoids in the genus *Elaeocarpus*.

1. Introduction

Elaeocarpus is a genus of evergreen or semi-evergreen trees and shrubs with about 350 species, approximately 40 of which are found in Vietnam. Among them, *E. hainanensis* is distributed in Vietnam and China (Tang and Phengklai, 2007) and has been used in the oriental medicine to treat certain diseases (Institutum Botanicum Academiae Sinicae, 1994). Previous phytochemical studies on *Elaeocarpus* species have resulted in the isolation of various cucurbitane-type triterpenoids, indolizidine alkaloids, tannins, flavonoids and other phenolic compounds with cytotoxic (Ito et al., 2009; Kinghorn et al., 1999; Meng et al., 2008), anti-bacterial and anti-inflammatory (Singh et al., 2000), hepatoprotective (Bhattacharya et al., 1975) and analgesic (Carroll et al., 2005) activities. In our search for new bioactive compounds from *Elaeocarpus* plants in Vietnam, we performed phytochemical investigation on *E. hainanensis*. Herein we report on the isolation and structural determination of two new oleanane-type saponins and six known compounds from the leaves and twigs of *E. hainanensis* Oliv.

2. Results and discussion

The methanol extract of the leaves and twigs of *E. hainanensis* was suspended in water and then partitioned successively with *n*-hexane, dichloromethane (DCM)and *n*-BuOH. The DCM and *n*-BuOH residues were further fractionated and purified by various column chromatography using silica gel, Sephadex LH-20 and reversed-phase C_{18} to afford two new oleanane-type saponins (1α-hydroxy-olean-12-en-3-*O*-β-D-xylopyranoside (**1**) and 1α-hydroxy-olean-12-en-3-*O*-β-L-arabinopyranoside (**2**) (Fig. 1), and six known compounds including one diterpene, blumenol A (**3**) (Gilda et al., 2009) and five cucurbitacin-type triterpenes, cucurbitacin D (**4**), cucurbitacin F (**5**) (Kim et al., 1997), cucurbitacin I (**6**) (Seger et al., 2005), 3-epi-isocucurbitacin D (**7**) (Halaweish, 1993) and cucurbitacin H (**8**) (Fujita et al., 1995)

Compound **1** was obtained as a white amorphous powder. The molecular formula of **1** was established to be $C_{35}H_{58}O_6$ from its HRESIMS at *m/z* 619.4212 ($[M + HCOO]^-$, calcd for $C_{36}H_{59}O_8^-$, 619.4215 and ^{13}C-NMR spectrum (Table 1). The acid hydrolysis of **1** liberated D-xylose as confirmed in a gas chromatography (GC) experiment. From the ^{1}H- and ^{13}C-NMR spectra (Table 1), **1** was proposed to consist of a β-D-xylopyranosyl unit and an aglycone with two oxygenated carbons and one double bond. The anomeric configuration of the sugar was confirmed to be β on the basis of the large coupling constant (J = 7.5 Hz) of the anomeric proton at δ_H 4.31 in the ^{1}H-NMR spectrum. In addition, the D-xylopyranosyl moiety was determined based on HSQC correlations of the signals at δ_H 4.31 (1H, d, J = 7.5 Hz, H-1′), 3.23 (1H, overlapped, H-2′), 3.33 (1H, m, H-3′), 3.49 (1H, dd, J = 15.0, 9.0 Hz, H-4′), 3.85 (1H, dd, J = 11.5, 5.0 H-5a′), 3.23 (1H, overlapped, H-5b′) in the ^{1}H-NMR spectrum and signals at δ_C 107.0, 74.9, 77.5, 70.8 and 66.3 in the ^{13}C and DEPT-NMR spectra, respectively (Agrawal, 1992). Furthermore, the ^{1}H-NMR spectrum showed the signals

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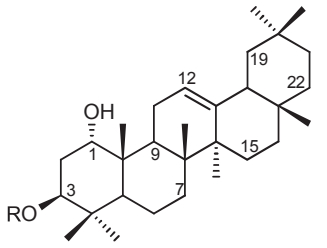

1 R = -β-D-xylopyranossyl
2 R = -α-L-arabinopyranosyl

Fig. 1. Chemical structures of new compounds isolated from *E. hainanensis*.

Table 1
The ^{1}H- and ^{13}C-NMR spectral data of compounds **1** and **2**.

Position	**1** (CD_3OD)		**2** (CD_3OD + $CDCl_3$)	
	δ_H mult. (*J* in Hz)	δ_C	δ_H mult. (*J* in Hz)	δ_C
1	3.55 (1H, br s)	72.8	3.56 (1H, br s)	72.7
2	1.96 (2H, m)	34.0	2.03 (2H, m)	33.8
3	3.67 (1H, dd, *J* = 11.0, 5.0 Hz)	85.5	3.67 (1H, dd, *J* = 10.5, 6.5 Hz)	85.3
4	–	39.9	–	39.8
5	1.23 (1H, overlapped)	49.0	1.23 (1H, m)	49.0
6	2.02 (1H, dd, *J* = 14.0, 2.5 Hz) 0.80 (1H, overlapped)	27.8	2.02 (1H, dd, *J* = 14.0, 2.5 Hz) 0.80 (1H, overlapped)	27.7
7	1.80 (1H, br t, *J* = 13.5 Hz) 0.99 (1H, overlapped)	27.1	1.80 (1H, m) 0.99 (1H, m)	27.0
8	–	43.0	–	42.9
9	2.40 (1H, t, *J* = 8.5 Hz)	38.8	2.40 (1H, t, *J* = 8.5 Hz)	38.7
10	–	41.4	–	41.3
11	1.91 (2H, br d, *J* = 6.0 Hz)	23.8	1.91 (2H, br d, *J* = 7.0 Hz)	23.7
12	5.19 (1H, br s)	122.9	5.19 (1H, s)	122.8
13	–	145.9	–	145.7
14	–	40.5	–	40.4
15	1.53 (1H, m) 1.29 (1H, m)	33.1	1.54 (1H, m) 1.30 (1H, m)	33.0
16	1.59 (1H, m) 1.47 (1H, m)	18.9	1.59 (1H, m) 1.45 (1H, m)	18.8
17	–	33.3		33.2
18	2.00 (1H, dd, *J* = 14.0, 2.5 Hz)	48.3	1.97 (1H, m)	48.2
19	1.72 (1H, br t, *J* = 14.0) 1.00 (1H, overlapped)	47.8	1.71 (1H, br t, *J* = 13.5 Hz) 0.99 (1H, overlapped)	47.7
20	–	31.7	–	31.6
21	1.44 (1H, m) 1.22 (1H, m)	38.0	1.45 (1H, m) 1.21(1H, m)	37.9
22	1.36 (1H, m) 1.09 (1H, m)	35.6	1.37 (1H, m) 1.12 (1H, m)	35.5
23	0.88 (3H, s)	16.7	0.88 (3H, s)	16.6
24	1.08 (3H, s)	28.3	1.08 (3H, s)	28.3
25	0.97 (3H, s)	16.8	0.98 (3H, s)	16.8
26	1.00 (3H, s)	17.5	1.01 (3H, s)	17.4
27	1.19 (3H, s)	26.5	1.20 (3H, s)	26.4
28	0.85 (3H, s)	28.9	0.85 (3H, s)	28.8
29	0.87 (3H, s)	24.1	0.87 (3H, s)	24.0
30	0.87 (3H, s)	33.8	0.87 (3H, s)	33.7
1′	4.31 (1H, d, *J* = 7.5 Hz)	107.0	4.34 (1H, d, *J* = 6.0 Hz)	106.4
2′	3.23 (1H, overlapped)	74.9	3.61 (1H, br t, *J* = 7.5 Hz)	72.2
3′	3.33 (1H, m)	77.5	3.54 (1H, overlapped)	73.7
4′	3.49 (1H, dd, *J* = 15.0, 9.0 Hz)	70.8	3.84(1H, overlapped)	68.6
5′	3.85 (1H, dd, *J* = 11.5, 5.0 Hz) 3.23 (1H, overlapped)	66.3	3.84 (1H, overlapped) 3.51 (1H, overlapped)	65.5

assignable to the sapogenol moiety including an olefinic proton at δ_H 5.19 (1H, br s, H-12) and protons of eight tertiary methyls at δ_H 0.88, 1.08, 0.97, 1.00, 1.19, 0.85, 0.87 and 0.87 (3H each, all s, CH_3-23, 24,

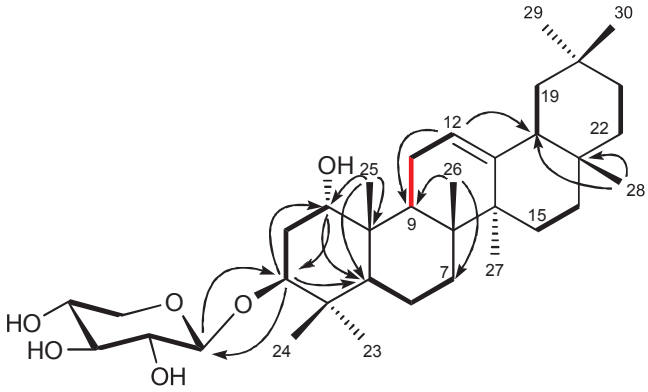

Fig. 2. Key HMBC (arrows) and H-H COSY (bold lines) correlations of **1**.

25, 26, 27, 28, 29, 30) that correlated in HSQC experiments with the carbon signals at δ_C 16.7, 28.3, 16.8, 17.5, 26.5, 28.9, 24.1 and 33.8, respectively. Apart from an array of the five carbons of the xylose unit, the ^{13}C and DEPT-NMR spectra also exhibited 30 carbon signals of aglycone part including two oxygenated methine carbons at δ_C 72.8 (C-1) and 85.5 (C-3), two olefinic carbons (δ_C 122.9 (C-12) and 145.9 (C-13)), nine methylene carbons (δ_C 34.0 (C-2), 27.8 (C-6), 27.1 (C-7), 23.8 (C-11), 33.1 (C-15), 18.9 (C-16), 47.8 (C-19), 38.0 (C-21) and 35.6 (C-22)), three methine carbons (δ_C 49.0 (C-5), 38.8 (C-9) and 48.3 (C-18)) and six quaternary carbons (δ_C 39.9 (C-4), 43.0 (C-8), 41.4 (C-10), 40.5 (C-14), 33.3 (C-17) and 31.7 (C-20)). Therefore, the aglycone part of **1** was suggested to have a 1α, 3β-dihydroxy-olean-12-ene skeleton (Mahato and Kundu, 1994). The consistency with the literature of similar triterpenes (Topcu, 2006) and extensive analyses of the HMBC and COSY spectra confirmed the substituted positions of the double bond at C-12/C-13 and two hydroxyl groups at δ_C 72.8 (C-1) and 85.5 (C-3) (Fig. 2). The HMBC spectrum also indicated the presence of the anomeric proton signal at δ_H 4.31 (H-1′) in correlation with the oxygenated carbon signal at δ_C 85.5 (C-3), suggesting that the xylopyranose moiety is directly linked to the aglycone at C-3 (Fig. 2). The stereochemistry of **1** was further established from the NOESY spectrum (Fig. 3), in which the NOE cross-peaks of H-1′/H-3, H-1/H_3-24, and H-1/H-26 were observed. Consequently, based on the above evidence, the structure of **1** was unambiguously identified as 1α-hydroxy-olean-12-en-3-*O*-β-D-xylopyranoside.

Compound **2** was also isolated as a white amorphous powder and had a molecular formula of $C_{35}H_{58}O_6$ as deduced from its negative HRESIMS (*m/z* 619.4207 ([M + HCOO]$^-$, calcd for $C_{36}H_{59}O_8^-$, 619.4215) and ^{13}C-NMR spectrum (Table 1). The structure of **2**, consisting of the aglycone and sugar moieties, was elucidated based on the extensive analysis of 1D and 2D NMR spectra in respect to **1** (Figs. 2 and 3). Detailed comparison of the ^{1}H and ^{13}C-NMR spectral data of **2** with those of **1** revealed that they are similar except for the monosaccharide substituent on C-3. The D-xylopyranose unit in **1** was replaced with an L-arabinopyranose unit in **2** (Table 1). The L-arabinopyranosyl moiety was determined based on HSQC correlations of the signals at δ_H 4.34 (1H, d, *J* = 6.0 Hz, H-1′), 3.61 (1H, br t, *J* = 7.5 H-2′), 3.54 (1H, overlapped, H-3′), 3.84 (2H, overlapped, H-4′, 5a′) and 3.51 (1H, overlapped, H-5b′) in the ^{1}H-NMR spectrum and signals at δ_C 106.4, 72.2, 73.7, 68.6 and 65.5 in the ^{13}C and DEPT-NMR spectra, respectively (Agrawal, 1992). Additionally, the acid hydrolysis of **2** liberated L-arabinose, which was identified by GC analysis of the trimethylsilyl derivative of the sugar compared with that of the authentic sample. The HMBC correlation from δ_H 4.34 (1H, d, *J* = 6.0 Hz, H-1′) of L-arabinopyranose to δ_C 85.3 (C-3) in the aglycone moiety of **2** further confirmed the linkage position of the sugar. Based on the above evidence, the structure of **2** was determined as 1α-hydroxy-olean-12-en-3-*O*-α-L-arabinopyranoside, and is another new oleanane-type glycoside from the title plant.

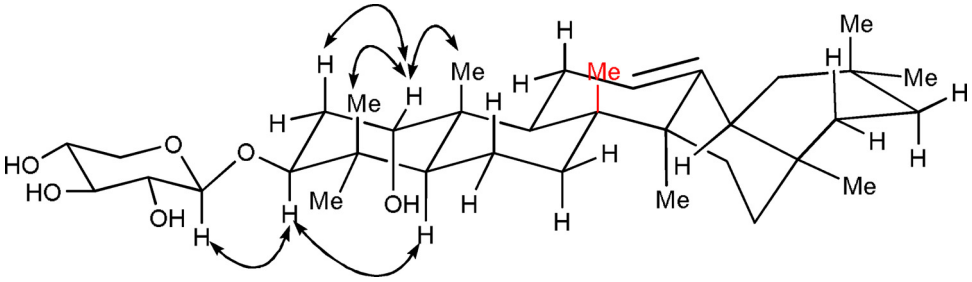

Fig. 3. Selected NOESY correlations of compound **1**.

In the review of the phytochemical profile of *Elaeocarpus* genus, several cucurbitane-type triterpenes have been reported from *E. chinensis*, *E. hainanensis*, etc. (Kinghorn et al., 1999; Meng et al., 2008), but the oleanane-type triterpenes have not been identified from *Elaeocarpus* sp. to date. These results propose a variation in the biosynthesis of the triterpenoids in *E. hainanensis* and make a significant contribution to the chemotaxonomic classification of the *Elaeocarpus* species.

3. Experimental

3.1. General experimental procedures

The silica gel 60 Å grade (40–63 μm) for column chromatography (CC) was purchased from Merck (Darmstadt, Germany). Sephadex LH-20 (25–100 μm) were purchased from Sigma-Aldrich (St Louis, MO). Thin-layer chromatography (TLC) plates (silica gel 60 F_{254}, 0.2 mm) were obtained from Merck (Germany). Chemical spots on TLC plates after development were detected using 10% H_2SO_4 in methanol. All solvents were distilled and purified before use. The HR-MS spectra were measured in the ESI mode on Agilent 6530 Accurate-Mass Q-TOF LC/MS spectrometer (Agilent Technologies, USA). The ^{1}H- and ^{13}C NMR spectra were recorded on a Bruker AM500 FT-NMR spectrometer (Bruker Spin, Germany), resonating at 500 MHz and 125 MHz respectively. Chemical shifts (δ) were expressed in ppm values with reference to tetramethylsilane and coupling constants (J) were given in Hz. Gas chromatography-GC (Shimadzu GC-2010 plus QP2020, Shimadzu Corp., Japan) using a Shimadzu SH-Rxi-5 Sil capillary column (0.25 mm ID × 30 mm) [column temperature 210 °C; detector temperature 300 °C; injector temperature 270 °C; He gas flow rate 30 mL/min (splitting ratio: 1/30)] was used for sugar determination.

3.2. Plant material

Elaeocarpus hainanensis Oliv. sample was collected in Ha Tinh province, Vietnam and identified by Dr. Do Ngoc Dai, Department of Forestry, Nghe An University of Economics. A voucher specimen (No.2-MS.104.01–2014.34) has been deposited in the School of Chemical Engineering, Hanoi University of Science and Technology.

3.3. Extraction and isolation

E. hainanensis sample (5.8 kg) was extracted with methanol (MeOH) at room temperature (3 × 20 L × 24 h) and concentrated *in vacuum* at 55 °C. The obtained residue (354.2 g) was suspended in water (1000 mL) and partitioned successively with *n*-hexane, dichloromethane (DCM), and *n*-BuOH (each 3 × 1000 mL) to give residues of *n*-hexane (23.8 g), DCM (80.4 g), and *n*-BuOH (28.70 g), respectively.

The DCM residue (60 g) was fractionated on a silica gel column (640 g silica gel) and eluting with a gradient of DCM-MeOH (1:0-0:1, v/v) to give seven fractions (F1-F7). Fraction F3 (10 g) was repeatedly chromatographed on a silica gel column (240 g silica gel) using mixtures of DCM-acetone to yield five fractions (F3.1-F3.5). Fraction F3.4 was purified by Sephadex LH-20 column chromatography with MeOH to furnish compound **3** (8 mg), compound **4** (29 mg) and compound **5** (3.1 mg). Fraction F4 (12 g) was subjected to CC (240 g silica gel) with a gradient of DCM-acetone to yield a mixture of compounds **4** and **6** (2:3), compound **7** (13.5 mg) and compound **8** (17 mg), respectively.

Next, the *n*-BuOH residue (25 g) was subjected to a silica gel column (Φ60 mm × 80 mm) with stepwise gradient of CH_2Cl_2-MeOH (5:1→1:1, v/v, 600 mL/fraction) to give 4 fractions (B1-B4). Fraction B1 (4.3 g) was then loaded onto a silica gel column (Φ 45 mm × 350 mm) with an eluent of $CHCl_3$-MeOH-H_2O (3:1:0.1, v/v/v, 1500 mL) to yield four sub-fractions (B2.1-B2.4). Sub-fraction B2.2 (920 mg) was further purified on a reversed-phase C_{18} column (Φ30 mm × 350 mm) eluting with MeOH-H_2O (1:1, v/v, 1400 mL) to furnish compound **1** (10.5 mg). Finally, compound **2** (12 mg) was obtained from sub-fraction B2.4 (1100 mg) by using RP C_{18} column chromatography(Φ30 mm × 350 mm) with the eluent of MeOH-H_2O (1:1, v/v, 1600 ml).

*1α-Hydroxy-olean-12-en-3-O-β-*D*-xylopyranoside* ***(1)****:* white amorphous powder; $C_{35}H_{58}O_6$; HR-ESI-MS $[M + HCOO]^-$ *m/z* 619.4212 (calcd for 619.4215); ^{1}H-NMR (CD_3OD, 500 MHz) and ^{13}C-NMR (CD_3OD, 125 MHz): See Table 1.

*1α-Hydroxy-olean-12-en-3-O-β-*L*-arabinopyranoside* ***(2)****:* white amorphous powder; $C_{35}H_{58}O_6$; HR-ESI-MS $[M + HCOO]^-$ *m/z* 619.4207 (calcd for 619.4215); ^{1}H-NMR (CD_3OD + $CDCl_3$, 500 MHz) and ^{13}C-NMR (CD_3OD + $CDCl_3$, 125 MHz): See Table 1.

Acid hydrolysis of 1 and 2: A solution of a compound (2.0 mg) in HCl 1.0 M (3.0 mL) was heated under reflux for 2 h. Then, the reaction mixture was concentrated *in vacuum* to dryness. The residue was extracted with $CHCl_3$ and H_2O (5 mL each, 3 times). Next, the sugar residue obtained by concentration of the water layer was dissolved in dry pyridine (0.1 mL). Then L-cysteine methyl ester hydrochloride in pyridine (0.06 M, 0.1 mL) was added to the solution. After heating the reaction mixture at 60 °C for 2 h, 0.1 mL of trimethylsilylimidazole was added. Heating at 60 °C was continued for a further 2 h, and the mixture was evaporated *in vacuum* to give a dried product, which was partitioned between *n*-hexane and H_2O. The *n*-hexane layer was analyzed using the GC procedure (General Procedures). The peaks of the hydrolysates of the respective glycosides were detected at t_R 8.21 min (D-xylose) for **1** and at t_R 4.50 min (L-arabinose) for **2**. The retention times for the authentic samples (Sigma) after being treated similarly were 8.21 min (D-xylose), 8.66 min (L-xylose), and 4.50 min (L-arabinose), respectively. Co-injection of the hydrolysates of the compounds with standard D-xylose in **1** and L-arabinose in **2** gave single peaks.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.phytol.2018.07.003.

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