SHORT REPORT

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A New Oleanane Type Saponin from the Aerial Parts of *Elaeocarpus hainanensis*

Nguyen Huu Tung[1,2], Pham Quang Duong[3], Dinh Thi Phuong Anh[4], Nguyen Thi Thuy My[4], Le Dang Quang[5,6] and Vu Dinh Hoang[4,*]

[1]*Faculty of Pharmacy, Phenikaa University, Hanoi 100000, Vietnam*
[2] *Phenikaa Research and Technology Institute (PRATI), A&A Green Phoenix Group, 167 Hoang Ngan, Hanoi 10000, Vietnam*
[3]*Center For High Technology Development, Vietnam Academy of Science and Technology (VAST), Hanoi 100000, Vietnam*
[4] *School of Chemical Engineering, Hanoi University of Science and Technology (HUST), Hanoi 100000, Vietnam*
[5] *R&D Center of Bioactive Compounds, Vietnam Institute of Industrial Chemistry, Hanoi 10000, Vietnam*
[6] *Institute for Tropical Technology, Vietnam Academy of Science and Technology (VAST), Hanoi 100000, Vietnam*

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Abstract: *Elaeocarpus hainanensis* has been used in the Oriental medicine but there are very few studies on its phytochemical profile. Our ongoing research on chemical constituents from *Elaeocarpus* plants in Vietnam led to the isolation of a new oleanane-type saponin from the aerial part of *E. hainanensis*. Its structure was identified as 1*α*-hydroxy-olean-11-oxo-12-en-3-*O*-*β*-L-arabinopyranoside (**1**) on the basis of extensive chemical and spectroscopic analyses including NMR and MS spectra. The occurence of the unique oleanane-type saponins in *E. hainanensis* contributed to impact phytochemical profile associated with chemotaxanomy meaning of the title plant.

Keywords: *Elaeocarpus hainanensis*; Elaeocarpaceae; oleanane type saponin; 1*α*-hydroxy-olean-11-oxo-12-en-3-*O*-*β*-L-arabinopyranoside.

1. Plant Source

Elaeocarpus hainanensis Oliv. (Elaeocarpaceae) is distributed in subtropical areas of Vietnam and China as small evergreen tree [1,2] and has been used in the Oriental medicine but its phytochemical profile and biological activity are largely unknown. We have investigated on chemical constituents of *E. hainanensis* in Vietnam and herein, as part of ongoing study, report the new finding of triterpenoid saponin [3].

* Corresponding author: E-mail: hoang.vudinh@hust.edu.vn (V. D. Hoang), Phone +84-9146-61299

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The sample of *E. hainanensis* (aerial parts) was collected in Ha Tinh province, Vietnam and authenticated by Dr. Do Ngoc Dai in the Nghe An University of Economics. A voucher specimen (No.2-MS.104.01-2014.34) has been stored in the corresponding author's laboratory, Hanoi University of Science and Technology (HUST).

2. Previous Studies

The phytochemical profile of *Elaeocarpus* species has been reported and includes various cucurbitane-type triterpenoids, indolizidine-type alkaloids, flavonoids, tannins, glycosides, and phenolic compounds with cytotoxicity [4-6], anti-bacteria, anti-inflammation [7], hepatoprotection [8], analgesic with affinity for the human *δ*-opioid receptor [9]. In case of *E. hainanensis*, to date, there are only several cytotoxic cucurbitane-triterpenoids reported and, very recently, it is noteworthy that two novel triterpene glycosides were isolated for the first time [3], which supporting that the title plant has potential source of new natural compounds.

3. Present Study

Our ongoing study on triterpene saponin of the title sample led to the isolation of one novel triterpenoid glycoside, 1*α*-hydroxy-olean-11-oxo-12-en-3-*O*-*β*-L-arabinopyranoside (**1**). This paper deals with the isolation and structural identification of the newly isolated glycoside.

The air-dried *E. hainanensis* sample was first extracted with methanol and then solvent partition procedure to obtain the *n*-BuOH portion found to contain triterpenoid saponin, which was followed by various column chromatography to yield the novel triterpene saponin (for the detailed separation, see Supporting information, S1-S2), whose structure, 1*α*-hydroxy-olean-11-oxo-12-en-3-*O*-*β*-L-arabinopyranoside (**1**) (Figure 1), was elucidated on the basis of extensive chemical and spectroscopic data (NMR and MS spectra).

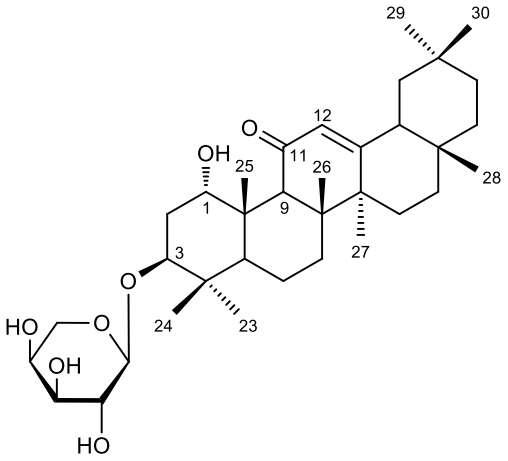

Figure 1. Chemical structure of the novel triterpene saponin (**1**) from *E. hainanensis*

*1α-Hydroxy-olean-11-oxo-12-en-3-O- β-L-arabinopyranoside (**1**):* white amorphous powder; $[\alpha]_D^{25}$ = +17 (*c* 0.20, CH_3OH); HR-ESI-MS: *m/z* 589.4132 $[M + H]^+$ (calcd for $C_{35}H_{57}O_7$, 589.4104); ^1H NMR (500 MHz, CD_3OD) and ^{13}C NMR (125 MHz, CD_3OD): see Table 1 and supporting information.

The new compound **1** was obtained as an amorphous powder. Its HRESIMS showed an $[M + H]^+$ adduct ion peak at *m/z* 589.4132 (calcd for $C_{35}H_{57}O_7$, 589.4104), corresponding to a molecular formula of $C_{35}H_{58}O_6$. The acid hydrolysis of **1** by HCl 1M gave L-arabinose, which was then confirmed in a gas chromatography procedure (Supporting information, S3). From the ^1H- and ^{13}C-NMR spectra (Table 1), chemical structure of **1** was proved to possess a *β*-L-arabinopyranoside and an oleanane-type aglycone. Comprehensive analysis of its NMR data indicated that the aglycone structure of **1** contains two oxygenated carbons, one ketone and one double bond in fashion of *α,β*-unsaturated ketone group [10-13].

Table 1. ^{1}H and ^{13}C NMR data in CD_3OD for the new compound **1** and the reference compound (1*α*-hydroxy-olean-12-en-3-*O*-*β*-L-arabinopyranose) [3]

Carbon	The new compound **1**			The reference compound (1*α*-hydroxy-olean-12-en-3-*O*-*β*-L-arabinopyranose)	
	δ_C	DEPT	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)
1	73.6	CH	4.61 br s	72.7	3.56 br s
2	33.7	CH_2	2.05 m	33.8	2.03 m
3	85.1	CH	3.64 dd(11.5,5.5)	85.3	3.67 dd(10.5, 6.5)
4	40.5	C		39.8	
5	48.5	CH	1.23 m	49.0	1.23 m
6	27.6	CH_2	2.18 dd(10.5,5.0), 1.91 m	27.7	2.02 dd(14.0, 2.5), 0.80 m
7	27.4	CH	1.23 m, 1.01 m	27.0	1.80 m, 0.99 m
8	46.5	C		42.9	
9	54.6	CH	3.39 s	38.7	2.40 t(8.5)
10	42.3	C		41.3	
11	203.5	C		23.7	1.91 br d(7.0)
12	128.7	CH	5.55 s	122.8	5.19 s
13	173.8	C		145.7	
14	45.2	C		40.4	
15	33.5	CH_2	1.68 m, 1.48 m	33.0	1.54 m, 1.30 m
16	18.2	CH_2	1.65 m, 1.54 m	18.8	1.59 m, 1.45 m
17	31.9	C		33.2	
18	49.0	CH	2.22 dd(14.0,4.0), 1.90 m	48.2	1.97 m
19	46.4	CH	1.80 m, 1.07 m	47.7	1.71 br t(13.5), 0.99 m
20	32.0	C		31.6	
21	37.6	CH_2	1.51 m, 1.34 m	37.9	1.45 m, 1.21 m
22	35.5	CH_2	1.46 m, 1.19 m	35.5	1.37 m, 1.12 m
23	16.5	CH_3	0.89 s	16.6	0.88 s
24	28.3	CH_3	1.10 s	28.3	1.08 s
25	18.2	CH_3	1.17 s	16.8	0.98 s
26	19.5	CH_3	1.18 s	17.4	1.01 s
27	24.0	CH_3	1.44 s	26.4	1.20 s
28	29.3	CH_3	0.92 s	28.8	0.85 s
29	24.0	CH_3	0.96 s	24.0	0.87 s
30	33.5	CH_3	0.95 s	33.7	0.87 s
1′	107.2	CH	4.31 d(7.0)	106.4	4.34 d(6.0)
2′	74.4	CH	3.59 t(7.0)	72.2	3.61 br t(7.5)
3′	72.9	CH	3.52 m	73.7	3.54 m
4′	69.5	CH	3.82 m	68.6	3.84 m
5′	66.4	CH_2	3.86 dd(12.5,3.5), 3.52 m	65.5	3.84 overlapped, 3.51 m

Assignments were confirmed by DEPT, COSY, HSQC, HMBC, and NOESY spectra

The configuration of the anomeric proton at δ_H 4.31 in L-arabinose unit was proposed to be β on the basis of the relative coupling constant value (J = 7.0 Hz) in the ^{1}H-NMR spectrum of **1** [14]. Moreover, starting from the anomeric proton and aids by COSY and HSQC spectra, the L-arabinopyranoside moiety was clarified by the array of proton signals at δ_H 4.31 (1H, d, J = 7.0 Hz, H-1′), 3.59 (1H, t, J = 7.0 Hz, H-2′), 3.52 (1H, m, H-3′), 3.82 (1H, m, H-4′), 3.86 (1H, dd, J = 12.5, 3.5 H-5′a), 3.52 (1H, m, H-5′b) and the respective carbons at δ_C 107.2, 74.4, 72.9, 69.5 and 66.4 in the ^{13}C NMR and DEPT spectra, respectively [14]. The ^{1}H-NMR spectrum of **1** also revealed the proton signals attributable to the oleanane-type triterpene moiety having an olefinic proton at δ_H 5.55 (1H, br s, H-12) and eight tertiary methyl protons at δ_H 0.89, 1.10, 1.17, 1.18, 1.44, 0.92, 0.96, 0.95 (3H each, all s, CH_3-23, 24, 25, 26, 27, 28, 29, 30) that correlated in HSQC experiments with the carbon signals at δ_C 16.6, 28.3, 18.2, 19.5, 24.0, 29.3, 24.0 and 33.5, respectively. Extracting the array of the five carbons of the arabinose unit, the ^{13}C and DEPT-NMR spectra also showed 30 carbon signals of the oleanane-type triterpene skeleton including two oxygenated methines at δ_C 73.6 (C-1) and 85.1 (C-3), one carbonyl carbon at δ_C 203.5 (C-11), two olefinic carbons [δ_C 128.7 (C-12) and 173.8 (C-13)], eight methylene carbons (δ_C 33.7 (C-2), 27.6 (C-6), 27.4 (C-7), 33.5 (C-15), 18.2 (C-16), 46.4 (C-19), 37.6 (C-21) and 32.0 (C-22)), three methine carbons [δ_C 48.5 (C-5), 54.6 (C-9) and 49.0 (C-18)], and six quaternary carbons [δ_C 40.5 (C-4), 46.5 (C-8), 42.3 (C-10), 45.2 (C-14), 32.0 (C-17) and 32.0 (C-20)]. The downed-field chemical shifts of the two olefinic carbons [δ_C 128.7 (C-12) and 173.8 (C-13)] in comparison to those of olenane-12-en triterpenes and the appearance of the carbonyl carbon at δ_C 203.5 proposed an α,β-unsaturated ketone group, which is featured for hydroxyglycyrrhetic acid analogs wellknown in licorice (*Glycyrrhiza* spp.) [10-12]. Therefore, the aglycone part of **1** was suggested to have the 1α, 3β-dihydroxy-olean-11-oxo-12-ene skeleton [10-13]. The agreement with the literature data [10-13], especially the reference compound 1α-hydroxy-olean-12-en-3-*O*-β-L-arabinopyranose [3], and extensive analyses of the HMBC and COSY spectra led to confirm the positions of α,β-unsaturated ketone group and two hydroxyl groups at δ_C 73.6 (C-1) and 85.1 (C-3) (Figure 2A). The HMBC spectrum of **1** also disclosed the crosspeak between H-1′ (δ_H 4.31) and C-3 (δ_C 85.1), indicating the linkage at C-3 of the arabinose unit (Figure 2A). The stereochemistry including absolute configuration of **1** was confirmed by the NOESY NMR procedure (Figure 2B), in which the correlations of H-1′/H-3, H-1/H-24, and H-1/H-25 were observed. Hence, the chemical structure of **1**, a new natural compound as confirmed by on air SciFinder database (Supporting information, S11), was unambiguously deduced as 1α-hydroxy-olean-11-oxo-12-en-3-*O*-β-L-arabinopyranoside (Figure 1).

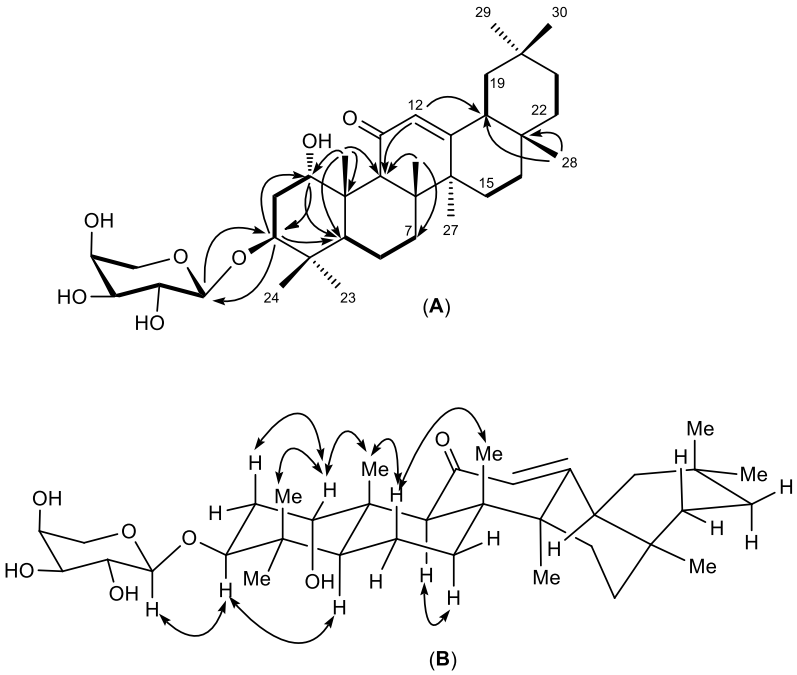

Figure 2. Important HMBC (arrow) and H-H COSY (bold line) crosspeaks (A) and stereochemistry with selected NOESY correlations of **1** (B)

To the best of our knowledge, oleanane triterpenoids and saponins are considered most abundant among the triterpenoid skeletons in the plant kingdom due to variety of oxidation degree, especially hydroxylation at certain positions, but the hydroxylation at C-1 is noteworthy that being not common [13]. It is then the saponins having a monosaccharide unit such as *O*-*β*-L-arabinopyranoside attached at C-3 of the aglycone of 1*α*,3*β*-dihydroxy-olean-12-en are very rare with only several analogs discovered from natural sources.

4. Chemotaxonomic Significance and Conclusions

Together with the previous report [3], in our systematic phytochemical investigation on the title plant growing in Vietnam, three oleanane-type triterpenoid glycosides, five cucurbitane-type triterpenoids and one sesquiterpenoid were isolated (Supporting information, Figure S9), which contributes noteworthily to phytochemical database of *E. hainanensis* with sixteen compounds totally up to date. Of them, cucurbitane triterpenoids are dominated with twelve compounds (Supporting information, S4).

The genus *Elaeocarpus* is relatively big in the family Elaeocarpaceae with more than 300 species and as in the phytochemical database to date, there are more than eighty compounds indentified and including mainly alkaloids (indole, indolizidine and pyrrolidine derivatives) with more than thirty compounds [15]. Besides and in consistent with our investigation, it become more evident that the triterpenoid component is also principal in the phytochemical profile of *Elaeocarpus* species with twenty-seven triterpenoids characterized, in which various bioactive cucurbitane-triterpenes (twenty-three compounds) reported from from *E. chinensis*, *E. hainanensis*, *E. dolichostylus* and *E. floribundus* [5,6,16,17], but the oleanane triterpenes had not been characterized from the genus *Elaeocarpus* up to our investigation on the title plant [3] (Supporting information, S4). The oleanane triterpenoids, which have been found only in the *E. hainanensis*, might be applicable as chemotaxanomic marker for this plant.

Triterpenoid including saponin, one of major class of natural products, are widely distributed in nature, occurring primarily in the plant kingdom and be classified according to their own triterpene skeletons. The biosynthesis of the triterpene saponins was well documented to speculate their classification by the C_{30} carbon skeletons and resulted in impact meaning of chemotaxonomy. Of the ten sapogenin skeletons (dammarane, tirucallane, lupane, hopane, oleanane, taraxasterane, ursane, cucurbitane, cycloartane, lanostane), it can be seen that oleanane-type saponin is the most abundant skeleton, while hopane- and tirucallane-type saponins are considered least naturally occuring [18]. Then, our findings revealed the different and various pathways in the triterpene biosynthesis with cucurbitane, oleanane and tirucallanane skeletons in the *Elaeocarpus* species up to date.

In addition, the literature revealed that mostly less polar secondary metabolites have been reported from *Elaeocarpus* species, so phytochemical analysis should be carried out on the respective polar portion in advance. Taken together, it is suggested that *Elaeocarpus* spp. are potential sources for novel natural products and our studies propose variation in the biosynthesis of the triterpenoid in *E. hainanensis* and significantly contribute to chemotaxonomic meaning of *E. hannanensis* and the *Elaeocarpus* species.

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Supporting Information

Supporting Information accompanies this paper on http://www.acgpubs.org/journal/records-of-natural-products

ORCID

Nguyen Huu Tung: 0000-0001-6005-9280
Pham Quang Duong: 0000-0002-3036-8581
Dinh Thi Phuong Anh: 0000-0002-3838-7568
Nguyen Thi Thuy My: 0000-0002-7769-2741
Le Dang Quang: 0000-0001-7203-8081
Vu Dinh Hoang: 0000-0002-2112-6732

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