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Triterpenes from *Vitex limonifolia*

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Abstract

Using combined chromatographic methods, four triterpenoids, 2*α*,3*α*-dihydroxyurs-12-en-28-oic acid (**1**), euscaphic acid (**2**), 2*α*,3*α*-dihydroxy-19-oxo-18,19-*seco*-urs-11,13(18)-dien-28-oic acid (**3**), and maslinic acid (**4**) were isolated from the methanol extract of the leaves of *Vitex limonifolia*. Their structures were elucidated by 1D-, 2D-NMR spectra as well as by comparison with those reported in the literature. Compound **3** was reported from *Vitex* genus for the first time.

Keywords. *Vitex limonifolia*, triterpenoid, ursane, oleanane.

1. INTRODUCTION

Vitex is the genus of shrubs and trees and mainly distributed in tropics and subtropics [1]. Since ancient times, civilization used *Vitex* plants for treating many diseases such as malaria, herpes, itches, dermatitis or controlling menstruation [1]. Phytochemical study of the genus *Vitex* revealed the presence of flavonoids, terpenoids, ecdysteroids, iridoid glucosides, etc. [2]. However, phytochemistry study of this plant has not been studied yet. This paper reported the isolation and structure elucidation of four triterpenes from the methanol extract of the leaves of *V. limonifolia*.

2. MATERIAL AND METHODS

2.1. Plant materials

The leaves of *Vitex limonifolia* Wall. ex C.B.Clarke were collected in Bachma National Park, Thua Thien Hue, Vietnam in September 2015, and identified by one of the authors, Prof. Dr. Ninh Khac Ban. A voucher specimen was deposited at the Herbarium Institute of Marine Biochemistry, VAST.

2.2. General experimental procedures

Optical rotations were determined on a Jasco DIP-370 automatic polarimeter. The NMR spectra were recorded using a Bruker DRX 500 spectrometer (^{1}H-NMR, 500 MHz; ^{13}C-NMR, 125 MHz). Column chromatography was performed using silica-gel (Kieselgel 60, 230-400 mesh, Merck) or RP-18 resins (30-50 μm, Fujisilisa Chemical Ltd.), and thin layer chromatography (TLC) was performed using a precoated silica gel 60 F_{254} (0.25 mm, Merck) and RP-18 F_{254S} plates (0.25 mm, Merck).

2.3. Extraction and isolation

The dried leaves of *V. limonifolia* (4.2 kg) were extracted with hot MeOH three times (3 × 5 L) under reflux for 12 h to yield 350 g extract after evaporation of the solvent. This extract was suspended in H_2O and successively partitioned with CH_2Cl_2 and EtOAc to obtain the CH_2Cl_2 (VIL1, 130.0 g), EtOAc (VIL2, 27.0 g), and H_2O (VIL3, 190.0 g) extracts after removal of the solvents *in vacuo*. The VIL1 fraction was chromatographed on a silica gel column eluting with a gradient of *n*-hexane:acetone (100:0 → 0:1, v/v) to give six fractions, VIL1A – VIL1F.

The VIL1C fraction was chromatographed on an RP-18 column eluting with MeOH:water (5:1, v/v) to give three fractions, VIL1C1-VIL1C3. VIL1C3 was chromatographed on a silica gel column eluting with *n*-hexane:EtOAc (1.4:1, v/v) to yield **1** (16.0 mg).

The VIL1D fraction was chromatographed on an RP-18 column eluting with MeOH:water (4:1, v/v) to give five fractions, VIL1D1-VIL1D5. VIL1D2 was chromatographed on an RP-18 column eluting

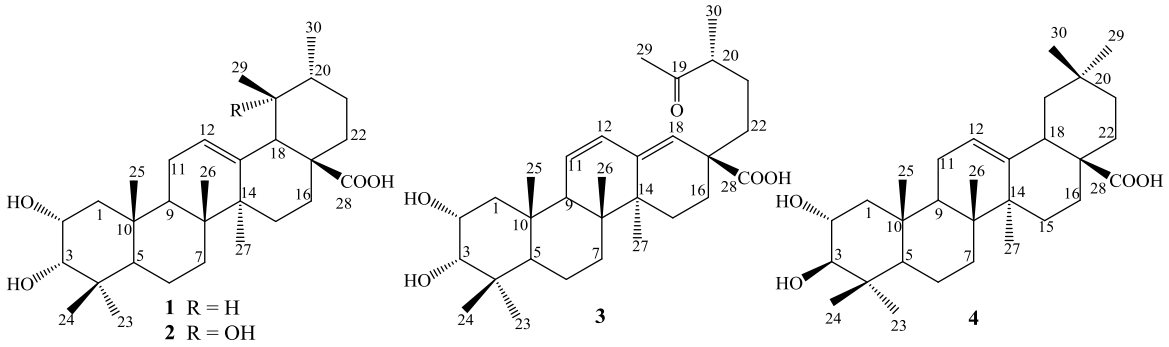

Figure 1: The chemical structures of compounds **1**−**4**

with acetone:water (1.8:1, v/v) to give three smaller fractions, VIL1D2A-VIL1D2C. Compounds **2** (18.0 mg) and **3** (4.5 mg) were obtained from VIL1D2A fraction using silica gel column (*n*-hexane:EtOAc, 1.4:1, v/v as eluent solvent). Compound **4** (7.0 mg) was obtained from VIL1D2C using silica gel column with solvent of *n*-hexane:EtOAc (1.4:1, v/v).

2*α*,3*α*-Dihydroxyurs-12-en-28-oic acid (1): white amorphous powder; $[\alpha]_D^{25}$: +30.0 (*c* 0.1, MeOH); ESIMS *m/z* 473 $[M+H]^+$, $C_{30}H_{48}O_4$; MW: 472; ^{1}H- and ^{13}C-NMR (DMSO-d_6), see table 1.

Euscaphic acid (2): white amorphous powder; $[\alpha]_D^{25}$: +20.0 (*c* 0.1, MeOH); ESIMS *m/z* 489 $[M+H]^+$, $C_{30}H_{48}O_5$; MW: 488; ^{1}H- and ^{13}C-NMR (DMSO-d_6), see table 1.

2*α*,3*α*-Dihydroxy-19-oxo-18,19-*seco*-urs-11,13(18)-dien-28-oic acid (3): white amorphous powder; $[\alpha]_D^{25}$: –40.0 (*c* 0.1, MeOH); ESIMS *m/z* 487 $[M+H]^+$, $C_{30}H_{46}O_5$; MW: 486; ^{1}H- and ^{13}C-NMR (CD_3OD), see table 1.

Maslinic acid (4): white amorphous powder; $[\alpha]_D^{25}$: –64.1 (*c* 0.1, MeOH); ESIMS *m/z* 473 $[M+H]^+$, $C_{30}H_{48}O_4$; MW: 472; ^{1}H- and ^{13}C-NMR (CD_3OD), see table 1.

3. RESULTS AND DISCUSSION

Compound **1** was obtained as a white powder. The ^{1}H-NMR spectrum of **1** showed the signals of one olefinic proton at δ_H 5.14 (1H, t, J = 3.0 Hz) and seven methyl groups at δ_H 0.70 (3H, s), 0.78 (3H, s), 0.82 (3H, d, J = 6.5 Hz), 0.88 (3H, s), 0.89 (3H, s), 0.91 (3H, d, J = 7.0 Hz), 1.04 (3H, s), two oximethine protons at δ_H 3.15 (br s) and 3.77 (br d, J = 11.0 Hz). The ^{13}C-NMR and DEPT spectra of **1** displayed the signals of thirty carbons, including seven methyls, eight methylenes, eight methines, and seven quaternary carbons in which two olefinic carbons at δ_C 124.5 and 138.2. Analysis the ^{1}H- and ^{13}C-NMR spectra of **1** suggested the presence of an ursan-12-ene-*type* triterpene skeleton [3]. In addition, the NMR data of **1** was found to be similar to those of 2*α*,3*α*-dihydroxyurs-12-en-28-oic acid methyl ester [4] except for the disappearance of methyl ester group. The HMBC correlations between H-23 (δ_H 0.89)/H-24 (δ_H 0.78) and C-3 (δ_C 77.8)/C-4 (δ_C 38.0)/C-5 (δ_C 47.6) indicated the position of the hydroxyl group at C-3. The HMBC correlations between H-1 (δ_H 1.13 and 1.41)/H-3 (δ_H 3.15) and C-2 (δ_C 64.7) indicated the hydroxyl group was located at C-2. Beside, the large coupling constant of H-1 and H-2, J = 11.0 Hz [H-2: δ_H 3.77 (br d, J = 11.0 Hz)] and small coupling constant of H-2 and H-3 (J~0 Hz) [H-3: δ_H 3.15 (br s)] suggested the configurations of two hydroxyl groups at C-2/C-3 as *equatorial* and *axial*, respectively. The HMBC correlations between H-27 (δ_H 1.04) and C-8 (δ_C 39.1)/C-13 (δ_C 138.2)/C-14 (δ_C 41.7)/C-15 (δ_C 28.2); between H-12 (δ_H 5.14) and C-9 (δ_C 46.8)/C-14 (δ_C 41.7)/C-18 (δ_C 52.4) proved the position of the double bond at C-12/C-13. The ESIMS of **1** exhibited an ion peak at *m/z* 473 $[M+H]^+$, corresponding to the formula of $C_{30}H_{48}O_4$. Consequently, compound **1** was identified as 2*α*,3*α*-dihydroxyurs-12-en-28-oic acid and previously reported from *V. altissima* [5] and *V. negundo* [6].

Analysis the NMR spectra of **2** exhibited the structure was similar to those of **1** except for the additional hydroxyl group at C-19. This was confirmed by the HMBC correlations between H-29 (δ_H 1.08) and C-18 (δ_C 53.2)/C-19 (δ_C 71.6)/C-20 (δ_C 41.4); between H-30 (δ_H 0.84) and C-19 (δ_C 71.6)/C-20 (δ_C 41.4)/C-21 (δ_C 25.9). The molecular formular of **2** was futher confirmed by the exhibition of an ESIMS ion peak at *m/z* 489 $[M+H]^+$, corresponding to the formula of $C_{30}H_{48}O_5$. Thus, the structure of **2** was determined to be euscaphic acid [3]. This compound was already isolated from *V. altissima* [5], *V. negundo* [6].

Table 1: The ^{1}H- and ^{13}C-NMR data for compounds **1-4**

C	**1**			**2**			**3**			**4**		
	δ_C[#]	δ_C[a,c]	δ_H[a,d] (*J* in Hz)	δ_C[¥]	δ_C[a,c]	δ_H[a,d] (*J* in Hz)	δ_C[$]	δ_C[b,c]	δ_H[b,d] (*J* in Hz)	δ_C[£]	δ_C[b,c]	δ_H[b,d] (*J* in Hz)
1	42.2	41.7	1.13 (m) 1.41 (m)	42.6	41.6	1.15 (m) 1.39 (m)	43.0	42.3	1.32 (m) 1.85 (m)	46.8	48.2	0.92 (m) 1.95 (m)
2	66.7	64.7	3.77 (br d, 11.0)	66.2	64.7	3.77 (br d,11.0)	66.3	67.0	4.00 (dt, 4.0, 11.0)	68.8	69.5	3.64 (dt, 4.5, 10.0)
3	79.2	77.8	3.15 (br s)	79.3	77.9	3.15 (d, 1.0)	79.8	80.1	3.37 (br s)	83.8	84.4	2.93 (d, 10.0)
4	39.3	38.0	-	38.9	38.0	-	39.3	39.5	-	39.1	40.6	-
5	48.3	47.6	1.11 (m)	48.8	47.6	1.15 (dd, 4.0, 13.5)	48.7	49.0	1.36 (m)	55.3	56.7	0.86 (m)
6	18.2	17.6	1.27 (m) 1.35 (m)	18.8	17.7	1.28 (m) 1.35 (m)	18.6	19.0	1.45 (m) 1.54 (m)	18.3	19.6	1.45 (m) 1.58 (m)
7	33.0	32.6	1.25 (m) 1.42 (m)	33.7	32.6	1.21 (m) 1.43 (m)	32.9	33.2	1.30 (m) 1.42 (m)	32.6	33.8	1.52 (m) 1.57 (m)
8	40.0	39.1	-	40.8	39.5	-	41.6	41.9	-	39.1	40.5	-
9	47.6	46.8	1.54 (m)	47.8	46.5	1.66 (m)	55.0	55.3	2.19 (br s)	47.5	49.0	1.65 (m)
10	38.4	37.8	-	38.8	37.8	-	38.8	39.1	-	38.3	39.3	-
11	23.4	22.9	1.85 (m)	24.3	23.1	1.89 (m)	128.0	128.2	5.71 (dd, 1.5, 10.5)	23.5	24.6	1.92 (m) 1.96 (m)
12	125.8	124.5	5.14 (t, 3.0)	128.7	126.8	5.17 (br s)	130.9	131.3	6.01 (dd, 3.0, 10.5)	122.0	123.5	5.27 (t, 3.5)
13	138.7	138.2	-	139.6	138.6	-	142.9	144.0	-	143.6	145.3	-
14	42.4	41.7	-	42.3	41.2	-	41.9	42.4	-	41.7	42.9	-
15	28.2	27.4	0.98 (m) 1.80 (m)	29.1	28.0	0.89 (m) 1.69 (m)	26.9	27.1	1.16 1.82	27.6	28.8	1.10 (m) 1.79 (m)
16	24.4	23.8	1.52 (m) 1.93 (m)	26.3	25.2	1.39 (m) 2.49 (m)	27.8	27.9	1.48 (m) 2.20 (m)	23.1	24.0	1.62 (m) 2.03 (m)
17	48.3	46.9	-	48.3	46.9	-	47.9	48.5	-	46.6	47.6	-
18	53.2	52.4	2.11 (d, 11.5)	54.4	53.2	2.37 (s)	129.2	128.4	5.41 (s)	41.3	42.7	2.87 (dd, 4.0, 14.0)
19	39.1	38.5	1.31 (m)	73.2	71.6	-	211.8	215.1	-	45.8	47.2	1.16 (m) 1.51 (m)
20	38.5	38.4	0.94 (m)	42.5	41.4	1.13 (m)	47.8	48.7	2.56 (m)	30.7	31.6	-
21	30.8	30.2	1.28 (m) 1.43 (m)	27.0	25.9	1.12 (m) 1.61 (m)	28.6	28.7	1.34 (m) 1.70 (m)	33.8	34.9	1.22 (m) 1.42 (m)
22	36.8	36.3	1.51 (m) 1.59 (m)	38.4	37.3	1.50 (m) 1.59 (m)	39.4	39.4	1.42 (m) 1.69 (m)	32.3	33.9	1.36 (m) 1.76 (m)
23	28.6	28.9	0.89 (s)	29.5	28.9	0.88 (s)	29.7	29.1	1.01 (s)	28.6	29.3	1.04 (s)
24	22.0	21.9	0.78 (s)	22.3	21.8	0.78 (s)	22.1	21.8	0.88 (s)	16.8	17.7	0.84 (s)
25	16.5	16.2	0.88 (s)	16.7	16.1	0.88 (s)	19.6	20.3	0.99 (s)	16.8	17.5	0.83 (s)
26	17.1	17.0	0.70 (s)	17.4	16.6	0.68 (s)	17.2	17.1	0.75 (s)	16.8	17.1	1.03 (s)
27	23.9	23.2	1.04 (s)	24.7	24.1	1.29 (s)	20.4	19.4	1.00 (s)	26.0	26.4	1.18 (s)
28	178.4	178.3	-	179.4	179.0	-	178.3	178.5	-	178.0	181.8	-
29	17.1	16.9	0.82 (d, 6.5)	27.2	26.4	1.08 (s)	28.4	28.3	2.16 (s)	33.1	33.6	0.93 (s)
30	21.2	21.1	0.91 (d, 7.0)	16.6	16.3	0.84 (d, 6.5)	16.7	16.5	1.10 (d, 7.0	23.5	24.0	0.96 (s)

[a]recorded in DMSO-d_6, [b]CD_3OD, [c]125MHz, [d]500MHz, [#]δ_C of methyl 2*α*,3*α*-dihydroxy-urs-12-en-28-oate [4], [¥]δ_C of euscaphic acid [3], [$]$\delta_C$ of 2*α*,3*α*-dihydroxy-19-oxo-18,19-*seco*-urs-11,13(18)-diene-28-oic acid [7], [£]δ_C of maslinic acid [8].

Compund **3** was obtained as a white powder. The ^{1}H-NMR spectrum of **3** showed the signals of three olefinic protons at δ_H 5.41 (s), 5.71 (dd, *J* = 1.5, 10.5 Hz), and 6.01 (dd, *J* = 3.0, 10.5 Hz), two hydroxymethine protons at δ_H 3.37 (br s) and 4.00 (dt, *J* = 4.0, 11.0 Hz), and six methyl groups at δ_H 0.75 (s), 0.88 (s), 0.99 (d, *J* = 6.5 Hz), 1.00 (s), 1.01 (s), 1.10 (d, *J* = 7.0 Hz), and 2.16 (s). The ^{13}C-NMR and DEPT spectra of **3** displayed the signals of 30 carbons, including seven methyls at δ_C 16.5, 17.1, 19.4, 20.3, 21.8, 28.3, 29.1 and four olefinics at δ_C 128.2, 128.4, 131.3, 144.0 and one carbonyl carbon at δ_C 178.5. Analysis the ^{1}H- and ^{13}C-NMR spectra of **3** suggested **3** was very similar to those of 2*α*,3*α*-dihydroxy-19-oxo-18,19-*seco*-urs-11,13(18)-dien-28-oic acid [7]. The HMBC correlations between H-23 (δ_H 1.01)/H-24 (δ_H 0.88) and C-3 (δ_C 80.1); between H-3 (δ_H 3.37) and C-2 (δ_C 67.0) indicated the position of the hydroxyl group at C-3. The HMBC correlations indicated the hydroxyl group was located at C-2. The HMBC correlations from H-27 (δ_H 1.00) to C-8 (δ_C 41.9)/C-13 (δ_C 144.0)/C-14 (δ_C 42.4)/C-15 (δ_C 27.1); from H-12 (δ_H 6.01) to C-9 (δ_C 55.3)/C-11 (δ_C 128.2)/C-13 (δ_C 144.0)/C-18 (δ_C 128.4); and from H-18 (δ_H 5.41) to C-12 (δ_C 131.3)/C-14 (δ_C 42.4)/C-16 (δ_C 27.9)/C-17 (δ_C 48.5)/ C-22 (δ_C 39.4) proved the location of two the double bond at C-11/C-12 and C-13/C-18. The HMBC correlations between H-29 (δ_H 2.16) and C-19 (δ_C 215.1)/C-20 (δ_C 48.7); between H-30 (δ_H 1.10) and C-19 (δ_C 215.1)/C-20 (δ_C 47.8)/C-21 (δ_C 28.7) proved the oxo group at C-19. All NMR assignments of **3** were confirmed by detailed analyses of HSQC and HMBC spectra (table 1), which are in good agreement with those reported for 2*α*,3*α*-dihydroxy-19-oxo-18,19-*seco*-urs-11,13(18)-dien-28-oic acid in the literature [7], and further confirmed by the exhibition of an ESIMS ion peak at *m/z* 487 $[M+H]^+$, corresponding to the formula of $C_{30}H_{46}O_5$. Thus, compound **3** was identified as 2*α*,3*α*-dihydroxy-19-oxo-18,19-*seco*-urs-11,13(18)-dien-28-oic acid.

Analysis the NMR and mass spectra of **4** indicated that the structure of **4** was similar to those of maslinic acid [8]. The HMBC correlations between two methyl groups H-23 (δ_H 1.04)/H-24 (δ_H 0.84) and C-3 (δ_C 84.4); between H-3 (δ_H 2.93) and C-2 (δ_C 69.5) indicated two hydroxyl groups located at C-3 and C-2. The HMBC correlations between two methyl groups H-29 (δ_H 0.93)/H-30 (δ_H 0.96) and C-19 (δ_C 47.2)/C-20 (δ_C 31.6)/C-21 (δ_C 34.9) confirmed the location of two methyl groups at C-20. The HMBC correlations between H-27 (δ_H 1.18) and C-8 (δ_C 40.5)/C-14 (δ_C 42.9)/C-15 (δ_C 28.8) proved the double bond at C-12/C-13. Consequently, **4** was elucidated as maslinic acid [8] and previously reported from *V. altissima* [5], and *V. negundo* [9].

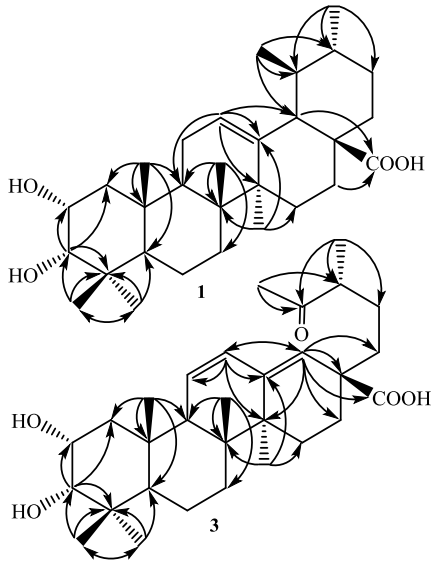

Figure 2: The key HMBC correlations of **1** and **3**

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