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Heptaelliptoic Acid A, A New Betulinic Acid Saponin from the Leaves of *Heptapleurum ellipticum*

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Abstract: One new 3-*O*-glycoside of betulinic acid, named heptaelliptoic acid A (**1**), together with one known betulinic acid analogue (**2**), and four other compounds (**3**–**6**) were separated by combinatively chromatographic techniques. For the first time, all of the purified compounds (**1**–**6**) were reported from the *H. ellipticum* species. Their structures were obviously elucidated basing exhaustive and pervasive UV-VIS, FT-IR, HR-MS-ESI, and NMR experiment data. Compounds **2**-**4** were significantly displayed the *in vitro* α-glucosidase inhibition (IC_{50} values of 11.53, 28.75, and 10.90 μM, respectively) better than the acarbose positive drug (IC_{50} value of 214.50 μM).

Keywords: Araliaceae; *Heptapleurum ellipticum*; heptaelliptoic acid A; α-glucosidase inhibition; betulinic acid saponin.

1. Plant Source

Heptapleurum ellipticum (synonym: *Schefflera elliptica*) distributing fifty-six species in Vietnam is used as a traditional medicine [1-3]. The leaves of *H. ellipticum* collected at An Giang

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province, were scientifically characterization by Assoc. Prof. Dr. Van Son Dang, ITB. The herbal specimen in Kien Giang University was made with coded KGU/NGHIA-SE0523.

2. Previous Studies

In our published studies on the phytochemistry of this species, five triterpenoids, three flavonoids, and two cerebrosides were reported [3-4].

3. Present Study

The leaves of *H. ellipticum* (10 kg) were dried and extracted at room temperature in ethanol 96^o to provide the total extract (SEEt). This extract (960 g) was respectively subjected to a liquid-liquid fractionation with *n*-hexane, and EtOAc solvents to deliver SEH (220 g) and SEE (150 g) extracts, along with an aqueous layer. The aqueous portion (700 g) was eluted by Diaion HP-20 column with H_2O:MeOH (0:100→100:0, *v*/*v*), and then gave five major fractions (I-V), respectively. Fraction IV (70 g) was subjected to silica gel column chromatography (CC) with mobile phase (EtOAc:MeOH) gradient (0→100%), and gave six sub-fractions (IV.1-IV.6). Fraction IV.1 (12 g) was chromatographed on silica gel with solvent system $CHCl_3$:MeOH:H_2O (90:10:0.1→80:20:0.2, *v*/*v*/*v*), and then separated by RP-18 using gradient mixtures of MeOH:H_2O (4:1, v/v) to give **1** (15 mg), **2** (20 mg). The SEE extract was objected to a normal-phase CC via a mixture of solvents containing *n*-hexane, EtOAc, and MeOH (the ratio of 25:75:0 to 0:80:20, *v*/*v*/*v*) to give six fractions, SEE.I–SEE.VI. The SEE.II fraction (27.0 g) was chromatographed on a silica gel column with the solvent system of *n*-hexane:EtOAc (80:20→30:70, *v*/*v*) to yield six sub-fractions, SEE.II.1–SEE.II.6. The SEE.II.1 sub-fraction (4.0 g) was separated on a normal-phase CC using $CHCl_3$:MeOH (99:1, *v*/*v*), and further on a reversed phase-C_{18} CC using gradient mixtures of MeOH:H_2O (70:30, v/v) to obtain **3** (15.0 mg) and **4** (10 mg). Likewise, **5** (10 mg) and **6** (9 mg) were respectively yielded from sub-fractions SEE.II.6 (6.5 g) and SEE.II.2 (3.5 g).

*Heptaelliptoic acid A (**1**)*: Amorphous powder (MeOH); $[\alpha]_D^{25}$ -1.0 (c 0.1, MeOH). UV (MeOH, λ_{max} nm): 203. IR (KBr, υ_{max}, cm^{-1}): 3416, 2942, 2871, 1692, 1616, 1077, 1044. HR-ESI-MS *m/z* 773.4464 $[M+Na]^+$ (calcd for $C_{41}H_{66}O_{12}Na$, 773.4452). ^1H NMR (600 MHz, methanol-d_4, *J*/Hz), and ^{13}C NMR (125 MHz, methanol-d_4): see in Table 1.

Compound (**1**) was supplied as an amorphous powder. Its formular was determined as $C_{41}H_{66}O_{12}$ basing on the HR-ESI-MS data *m/z* 773.4464 $[M+Na]^+$ (calcd for $C_{41}H_{66}O_{12}Na^+$, 773.4452). The FT-IR spectroscopy of **1** clearly exhibited the specific absorptions of the hydroxyl (3416 cm^{-1}), carboxyl (1692 cm^{-1}), and C-O stretch (1044 cm^{-1}) functional groups. The ^{13}C & ^1H NMR, and integrating HSQC spectrum of **1** (Table 1) certificated forty-one carbons comprising one carboxylic carbon at δ_C 181.7 (C-28), one olefinic carbon at δ_C 152.4 (C-20), one exocyclic methylene carbon at δ_C 109.9 (C-29)/δ_H 4.70 (1H, d, *J* = 1.8 Hz, H-29a), 4.57 (1H, d, *J* = 1.2 Hz, H-29b), two anomeric carbons with one of them at δ_C 105.3 (C-1′)/δ_H 4.42 (1H, d, *J* = 7.8 Hz, H-1′), eight oxygenated methine carbons with five of them at δ_C 91.0 (C-3)/δ_H 3.12 (1H, dd, *J* = 10.2, 4.2 Hz, H-3), 83.3 (C-2′), 78.3 (C-3′), 71.5 (C-4′), 77.5 (C-5′), two oxygenated methylene carbons with one of them at δ_C 62.7 (C-6′), five quaternary carbons, ten methylene carbons, five methine carbons, and six methyl carbons, were disclosed a betulinic acid skeleton bearing one 3-*O*-*β*-D-glucopyranosyl (Glc) unit likely those of 28-*O*-*β*-D-glucopyranosylbetulinic acid 3-*O*-*β*-D-glucopyranoside [5]. However, the presence of one anomeric carbons at δ_C 106.3 (C-1″)/δ_H 4.51 (1H, d, *J* = 7.2 Hz, H-1″), three oxygenated methine carbons at δ_C 76.3 (C-2″), 77.8 (C-3″), 71.2 (C-4″), one oxygenated methylene carbon at δ_C 67.2 (C-5″)/δ_H 3.80 (1H, dd, *J* = 11.4, 5.4 Hz, H-5″a), 3.13 (1H, dd, *J* =10.8, 6.6 Hz, H-5″b), and the multiplicities and large coupling constants of proton H-4″ at δ_H 3.45 (1H, ddd, *J* = 10.2, 8.4, 5.4 Hz) were clearly distinguished one *O*-*β*-D-xylopyranosyl (Xyl) unit, by comparising with those of a Xyl

moiety in 2*α*-hydroxy-3*β*-[(*O*-*β*-D-xylopyranosyl-(1→2)-*β*-D-glucopyranosyl)oxy]lup-20(29)-en-28-oic acid *α*-L rhamnopyranosyl ester (Table 1) [6], and those of Arabinopyranosyl (Ara) unit [δ_C 107.1 (C-1′′), 72.8 (C-2′′), 74.3 (C-3′′), 69.5 (C-4′′)/δ_H 3.82 (1H, dd, *J* = 3.5, 3.0 Hz), 66.3 (C-5′′)] in chenoalbumoside A [7]. Furthermore, the Rf comparison between the aqueous layer from the hydrolysis of compound **1** and the D-glucose, D-xylose authentic standards by TLC.

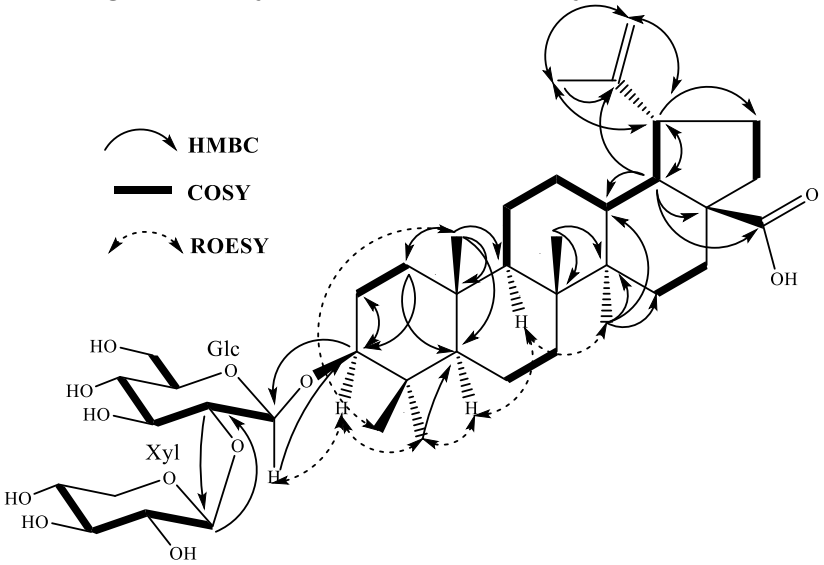

Figure 1. Selected COSY, HMBC, and ROESY correlations of **1**

Moreover, the HMBC spectrum of **1** (Figure 1) displayed two anomeric protons at δ_H 4.42 (1H, d, *J* = 7.8 Hz, H-1′) and 4.51 (1H, d, *J* = 7.2 Hz, H-1′′) respectively correlated with two oxymethine carbons at δ_C 91.0 (C-3) and 83.3 (C-2′), which were plainly recognized the glycoside chain of **1** was 3-*O*-*β*-D-Xyl-(1→2)-*β*-D-Glc, instead of the 3-*O*-*α*-L-Ara-(1→2)-*β*-D-Glc sugar chain in coccinioside-K [8]. On the other hand, the *α*-oriented methyl protons δ_H 1.05 (3H, s, H-23), and methine proton δ_H 0.73 (1H, d, *J* = 10.8 Hz, H-5) correlated with oxymethine proton δ_H 3.12 (1H, dd, *J* = 10.2, 4.2 Hz, H-3) in the ROESY spectroscopy of **1** (Figure 1), together with the doublet of doublet splitting and large-small coupling constants (*J* = 10.2, 4.2 Hz) of proton (H-3), which were significantly affirmed a hydroxyl moiety at carbon C-3 was 3*β* orientation. Based on data of HRMS-ESI, 1D & 2D-NMR, and comparison to the published spectral data [5-6], the structure of **1** was designated as 3-*O*-*β*-D-xylopyranosyl-(1→2)-*β*-D-glucopyranosylbetulinic acid, and trivially named heptaelliptoic acid A.

The HRMS-ESI, NMR data of isolated compounds were consistent with those in the published papers for 3-*O*-*β*-D-xylopyranosyl-(1→2)-*β*-D-glucuronopyranosylbetulinic acid (**2**) [9], and four known compounds, oleanolic acid (**3**) [10], ursolic acid (**4**) [11], 3-*O*-*β*-D-glucopyranosylstigmasterol (**5**) [12], and 3'-*O*-methylluteolin (**6**) [13] (Figure 2).

*Hydrolysis of glycoside***:** The acid hydrolysis of new compound **1** was detailed reported papers in the literature [3,14].

α-Glucosidase Inhibition Assay: The *in vitro α*-glucosidase inhibitory activities of all isolated compounds (**1**-**6**) were examined as our published method [3]. As the results, the isolated compounds **2**-**4** showed meaningfully better *α*-glucosidase inhibition (IC_{50} values of 11.53, 28.75, and 10.90 μM, respectively) than the acarbose control (IC_{50} values of 214.50 μM), whereas compounds **1, 5** and **6** did not exhibit activity (Table S1). Additionally, these results were completely consistent with the *α*-glucosidase inhibitions of those compounds that were previously evaluated [15, 16].

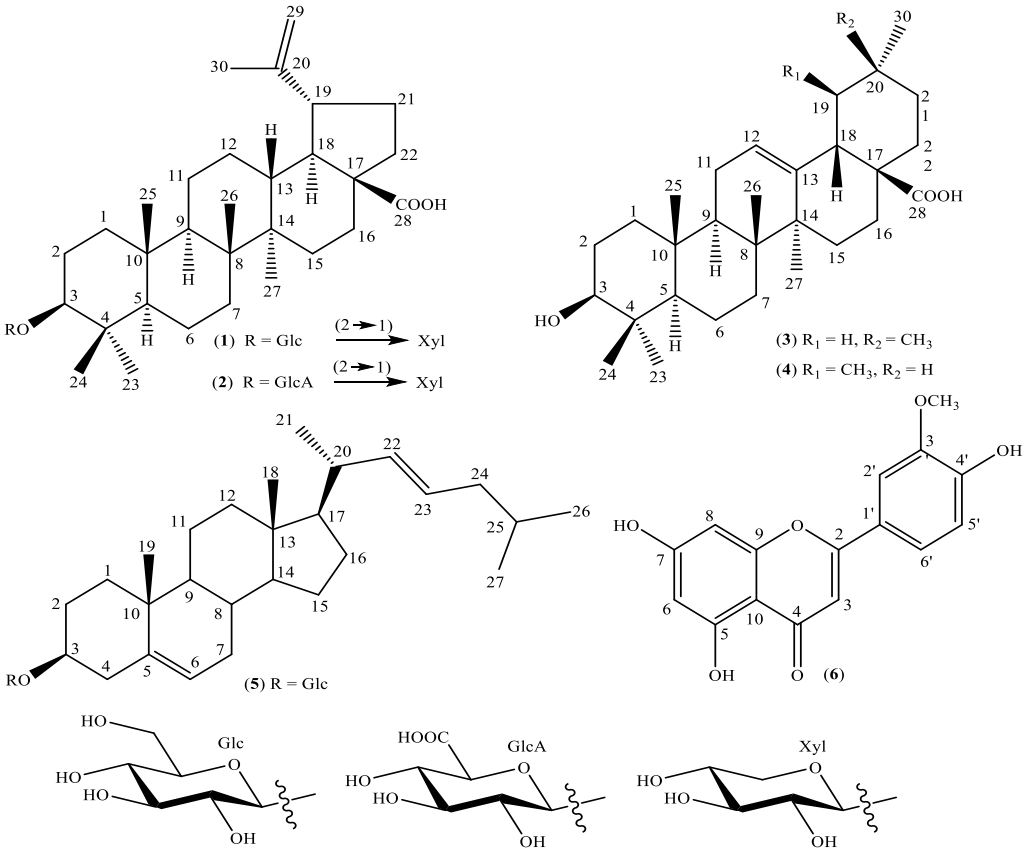

Figure 2. Chemical structures of **1**-**6**

Table 1. NMR spectral data for compound **1**in methanol-d_4 (δ in ppm, J in Hz)

No.	**1**	
	δ_H	δ
1	1.69 (1H, m) 0.92 (1H, m)	40.1
2	1.94 (1H, m) 1.70 (1H, m)	27.3
3	3.12 (1H, dd, J = 10.2, 4.2)	91.0
4	-	40.4
5	0.71 (1H, d, J =10.8)	57.3
6	1.55 1H, m) 1.42 (1H, m)	19.3
7	1.42 (1H, m)	35.7
8	-	42.0
9	1.30 (1H, m)	52.1
10	-	38.1
11	1.41 (1H, m) 1.26 (1H, m)	22.2
12	1.05 (1H, m) 1.71 (1H, m)	27.0
13	2.39 (1H, td, J = 12.0, 0.6)	39.6
14	-	43.6
15	1.15 (1H, m) 1.56 (1H, m)	31.0
16	2.23 (1H, d, J =12.0)	33.8

	1.39 (1H, m)	
17	-	57.3
18	1.61 (1H, m)	50.7
19	3.07 (1H, td, *J* = 12.0, 3.0)	48.5
20	-	152.4
21	1.92 (1H, m) 1.34 (1H, m)	31.9
22	1.92 (1H, m) 1.40 (1H, m)	38.5
23	1.03 (3H, s)	28.2
24	0.81 (3H, s)	16.4
25	0.86 (3H, s)	16.8
26	0.98 (3H, s)	16.8
27	0.99 (3H, s)	15.1
28	-	181.7
29	4.70 (1H, d, *J* = 1.8) 4.57 (1H, d, *J* = 1.2)	109.9
30	1.69 (3H, s)	19.6
3-*O*-Glc		
1′	4.42 (1H, d, *J* = 7.8)	105.3
2′	3.42 (1H, dd, *J* = 9.0, 7.8)	83.3
3′	3.53 (1H, m)	78.3
4′	3.32 (1H, m)	71.5
5′	3.22 (1H, m)	77.5
6′	3.84 (1H, m) 3.67 (1H, dd, *J* = 12.0, 5.4)	62.7
Xyl		
1″	4.51 (1H, d, *J* = 7.2)	106.3
2″	3.21 (1H, m)	76.3
3″	3.34 (1H, m)	77.8
4″	3.45 (1H, ddd, *J* = 10.2, 8.4, 5.4)	71.2
5″	3.80 (1H, dd, *J* = 11.4, 5.4) 3.13 (1H, dd, *J* = 10.8, 6.0)	67.2

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Supporting Information

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A new betulinic acid saponin from *Heptapleurum ellipticum*

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