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Triterpenoid saponins from *Meryta lanceolata*

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Abstract

Four new oleanane-type saponins and a known one were isolated from the leaves and stems of *Meryta lanceolata*. The new saponins were characterised by spectroscopic means and chemical hydrolysis as 3-*O*-{β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl-(1→3)-[β-D-glucopyranosyl-(1→2)]-α-L-arabinopyranosyl}oleanolic acid 28-*O*-[α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl] ester, 3-*O*-{β-D- glucopyranosyl-(1→3)-β-D-glucopyranosyl-(1→3)-[β-D-glucopyranosyl-(1→2)]-α-L-arabinopyranosyl}oleanolic acid 28-*O*-[α-L-rhamnopyranosyl-(1→4)-β-D-6-*O*-acetyl glucopyranosyl-(1→6)-β-D-glucopyranosyl] ester, 3-*O*-[β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl-(1→3)-α-L-arabinopyranosyl]oleanolic acid 28-*O*-[α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl] ester and 3-*O*-[β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl-(1→3)-α-L-arabinopyranosyl]echinocystic acid 28-*O*-[α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl] ester. The NMR assignments were made by means of HOHAHA, ^{1}H–^{1}H COSY, HMQC, HMBC and NOE difference studies.

Keywords: Meryta lanceolata; Araliaceae; Triterpenoid saponin; Oleanolic acid; Echinocystic acid

1. Introduction

Meryta lanceolata hort (Araliaceae) is an ornamental tree cultivated in gardens in Egypt. There have been no phytochemical reports on this species. As a part of our continuing search for bioactive saponins from plants grown in Egypt (Miyase et al., 1996; Abdel-Khalik et al., 2000; Melek et al., 2000), we report here the isolation and structure elucidation of four new saponins **1**–**4** in addition to a known one **5**.

2. Results and discussion

The methanolic extract of *M. lanceolata* was concentrated and diluted with acetone to precipitate the crude saponin mixture. The mixture was dissolved in water and passed through a porous polymer gel (Mitsubishi HP-20) column and the adsorbed materials were eluted with methanol. The methanolic eluate was subjected to silica gel column chromatography and HPLC to give the saponins **1**–**5**. The known saponin 5 was identified as leonticin E (Chen et al., 1997). The NMR data of the new saponins **1**–**4** are shown in Tables 1 and 2.

Saponin **1** had a molecular formula $C_{71}H_{116}O_{36}$ determined from the quasi-molecular ion $[M+Na]^+$ peak at m/z 1567 in its FAB–mass spectrum and ^{13}C NMR data. Its spectral features suggested that **1** was oleanolic acid glycoside. The ^{13}C NMR spectrum showed 71 signals, of which 30 were assigned to the oleanolic acid moiety and 41 to the saccharide portion. The sugar portion of **1** contained in the ^{1}H NMR spectrum seven anomeric proton signals at δ 4.76 (*d*, $J=6.2$ Hz), 5.44 (*d*, $J=8.0$ Hz), 5.25 (*d*, $J=8.0$ Hz), 5.15 (*d*, $J=8.0$ Hz), 6.22 (*d*, $J=8.0$ Hz), 4.97 (*d*, $J=8.0$ Hz), 5.81 (*br s*) and one methyl doublets at δ 1.69 ($J=6.0$ Hz), suggesting the occurrence of one deoxyhexose unit. The sugar moieties were assigned mainly from the HOHAHA, ^{1}H–^{1}H COSY, HMQC and HMBC spectra which allowed the identification of one α-arabino-pyranose (Ara) unit with the anomeric proton signal at δ 4.76 and five β-glucopyranose (Glc) units with anomeric protons resonating at δ 5.44, 5.25, 5.15, 4.97, 6.22. The

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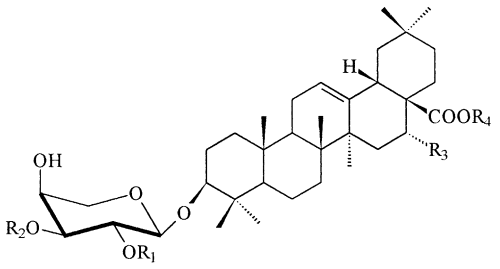

	R_1	R_2	R_3	R_4
1-	Glc	Glc^1 - ^{3}Glc -	H	-Glc^6 – $^1Glc^4$ – ^{1}Rha
2-	Glc	Glc^1 – ^{3}Glc -	H	$-Glc^6(-Ac^1)-Glc^4-Rha^1$
3-	H	Glc^1 – $^2Glc^1$ - ^{3}Glc -	H	-Glc^6 – $^1Glc^4$ – ^{1}Rha
4-	H	Glc^1 – $^2Glc^1$ - ^{3}Glc -	OH	-Glc^6 – $^1Glc^4$ – ^{1}Rha
5-	Glc	Glc	H	-Glc^6 – $^1Glc^4$ – ^{1}Rha

remaining sugar moiety with anomeric proton signal at δ 5.81 was ascribable to α-rhamnopyranose (Rha) unit. The nature of the sugar components was further identified by acid hydrolysis of **1** which yielded oleanolic acid and the sugars L-arabinose, D-glucose and L-rhamnose identified by GC analysis after being converted to their thiazolidine derivatives (Hara et al., 1986). Considering the δ values of the signals due to C-3 (δ 89.1) and C-28 (δ 176.5) in the ^{13}C NMR spectrum, saponin **1** was a 3,28 bisdesmoside. The structure of the sugar chain at C-3 was unambiguously defined by the HMBC correlations. A cross peak due to long-range correlation between C-3 signal (δ 89.1) of the aglycone and Ara H-1 signal (δ 4.76) indicated that this pentose was directly attached to the aglycone. Further correlations were observed between signals of Ara C-2 (δ 77.3) and Glc I H-1 (δ 5.44), Ara C-3 (δ 83.3) and Glc II H-1 (δ 5.25), Glc II C-3 (δ 88.0) and Glc III H-1 (δ 5.15). The structure of the oligosaccharide chain at C-28 was identified from the HMBC correlations between signals of Glc V C-4 (δ 78.5) and Rha H-1 (δ 5.81), Glc IV C-6 (δ 69.3) and Glc V H-1 (δ 4.97). A cross peak correlating signals due to Glc IV H-1 (δ 6.22) and aglycone C-28 (δ 176.5) provided definitive evidence of an ester linkage between the trisaccharide chain and the aglycone. The deduced branched nature of the tetrasaccharide chain connected at C-3 of the aglycone was further verified from the inter-residue NOEs observed in a NOE difference experiment. Thus, saponin **1** was defined as 3-*O*-{β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl-(1→3)-[β-D-glucopyranosyl-(1→2)]-α-L-arabinopyranosyl} oleanolic acid 28-*O*-[α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl] ester.

Saponin **2** possessed a molecular formula $C_{73}H_{118}O_{37}$, determined from the pseudo-molecular ion $[M+Na]^+$ at *m*/*z* 1609 in the FAB mass spectrum and from ^{13}C NMR data and differed from the formula of saponin **1** by 42 mass units. Its spectral features suggested **2** to be another oleanolic acid heptasaccharide. Of the 73 carbons, 30 were assigned to the aglycone part, 41 to the oligosaccharide moiety and the remaining two to an acetyl group. The ^{13}C NMR spectrum of **2** indicated that the sugars were present in two saccharide units, one attached to C-3 and the other at C-28. NMR analysis (HOHAHA, ^{1}H–^{1}H COSY, NOE difference, HMQC and HMBC) of the sugar portion of **1** and **2** revealed the same monosaccharide units and linkages, but the only structural difference between the sugar chains of **1** and **2** was confined to the presence of an acetyl group attached at Glc V C-6 in **2**. This was evident since the resonances due to H_2-6 in **2** (δ 4.53, 4.63) were shifted downfield with respect to the corresponding in **1** (δ 4.07, 4.21). The downfield ^{13}C chemical shift of C-6 at δ 63.7 and the upfield shift of C-5 at δ 73.8 of Glc V in **2** with respect to the corresponding values in **1**, provided additional evidence to the acetylation shift for C-6 position. Further supporting information came from the long-range HMBC coupling between H-6 (δ 4.53) and the acetyl carbonyl carbon (δ 170.6). Acid hydrolysis of **2** afforded oleanolic acid in addition to the sugar components D-glucose, L-arabinose and L-rhamnose. Thus, **2** was assigned the structure of 3-*O*-{β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl-(1→3)-[β-D-glucopyranosyl-(1→2)]-α-L-arabinopyranosyl} oleanolic acid 28-*O*-[α-L-rhamnopyranosyl-(1→4)-β-D-6-*O*-acetyl glucopyranosyl-(1→6)-β-D-glucopyranosyl] ester.

Saponin **3** had a molecular formula $C_{71}H_{116}O_{36}$, identical to that of **1**, based on FAB mass spectrum and ^{13}C NMR data. Its ^{1}H and ^{13}C NMR data indicated that **3** possessed the same aglycone as that of **1** and **2** but differ in the sugar part. The presence of seven sugar units in **3** was indicated from the seven anomeric protons at δ 4.68 (*d*, *J* = 7.4 Hz), 5.36 (*d*, *J* = 8.0 Hz), 5.08 (*d*, *J* = 8.0 Hz), 5.18 (*d*, *J* = 8.0 Hz), 6.21 (*d*, *J* = 8.0 Hz), 4.97 (*d*, *J* = 8.0 Hz) and 5.81 (*br s*). From the assigned aglycone, it was apparent that the seven sugars were present in two saccharide units, one attached to C-3 and the other at C-28. The unambiguous identification of the individual spin systems associated with the seven monosaccharides and the assignments of their NMR resonances were accomplished by NMR analysis. (^{1}H–^{1}H COSY, HOHAHA, NOE difference, HMQC and HMBC). The results revealed that the sugar portion of **3** and **1** had the same monosaccharide units. These observations led us to hypothesize that the structural difference between the sugar chains of **3** and **1** was due to the sugar arrangement. In contrast to the sugar branched connection described above for **1**, a linear sequence of a tetrasaccharide unit linked at C-3 of the aglycone was implied by a set of HMBC correlations. These correlations were between signals due to C-3 (δ 88.8) of the aglycone and Ara H-1 (δ 4.68), Ara C-3

(δ 83.5) and Glc I H-1 (δ 5.36), Glc I C-3 (δ 90.8) and Glc II H-1 (δ 5.08), Glc II C-2 (δ 85.6) and Glc III H-1 (δ 5.18). The connectivities of the sugar moieties were further confirmed from the observed NOEs interactions. The remaining long-range correlations and the observed NOEs across the glycosidic bonds were similar to those observed for **1**, indicating the same trisaccharide chain linked to COOH group at C-28. Acid hydrolysis of **3** afforded oleanolic acid in addition to the sugar components D-glucose, L-rhamnose and L-arabinose. Therefore **3** was characterized as 3-*O*-[β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl-(1→3)-α-L-arabinopyranosyl] oleanolic acid 28-*O*-[α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl] ester.

Saponin **4** exhibited a quasi-molecular ion $[M+Na]^+$ at *m*/*z* 1583 ($C_{71}H_{116}O_{37}$) which is consistent with a triterpene glycoside containing one pentose, one deoxyhexose and five hexoses and an aglycone with molecular mass 472. Comparison of the NMR data of **4** (1D and 2D NMR spectra) and those of **3**, indicated identical saccharide chains at C-3 and C-28 and structural similarity to those of oleanolic acid except C-16 and low frequency shift of the axial methyl group at C-14 (H_3-27) implying that there is an additional axial hydroxyl group at C-16. Comparison of the ^{13}C NMR spectral data of **4** with those reported for various echinocystic acid glycosides (Nagao et al., 1993) confirmed the identity of the aglycone part as echinocystic acid. Acid hydrolysis of saponin **4** afforded echinocystic acid in addition to the sugar components L-arabinose, D-glucose and L-rhamnose. Therefore the structure of saponin **4** was assigned as 3-*O*-[β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl-(1→3)-α-L-arabinopyranosyl]echinocystic acid 28-*O*-[α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl] ester.

3. Experimental

3.1. General

Optical rotations were measured with a JASCODIP-1000 digital polarimeter. MS were measured on JEOL JMS-SX 102 mass spectrometer. NMR spectra were recorded on JEOL GSX-500 FT NMR spectrometer. Chemical shifts are given on the δ scale with TMS as internal standard. HPLC was performed on a JASCO system 800 instrument. GC analysis was carried out on a HITACHI G-3000 gas chromatograph.

3.2. Plant material

Leaves and stems of *M. lanceolata* hort was collected from a public garden 60 km west of Alexandria, Egypt, in 1998. Plant identification was confirmed by Mrs T. labib, head specialist for plant identification in El-Orman public garden, Cairo, Egypt.

3.3. Extraction and isolation

Dried leaves and stems of *M. lanceolata* (690 g) were extracted with chloroform twice then extracted with methanol at room temperature twice. The combined methanolic extract was concentrated and diluted with acetone to precipitate the crude saponin mixture (60 g). The mixture was dissolved in water and the solution was passed through a porous polymer gel column (Mitsubishi Diaion HP-20). The column was washed with water and the adsorbed materials were eluted with methanol. The methanol eluate (20 g) was subjected to CC on silica gel (900 g) with $CHCl_3$–MeOH–H_2O (65:33:2) with increasing the proportion of methanol to give 13 fractions. The fractions were combined into two groups. A part (1.0 g) of the material from the less polar group was chromatographed on HPLC Develosil PhA column [2×25 cm, acetonitrile–water + 0.05 TFA, 6.5 ml/min monitored at 205 nm] to give **1** (37 mg), **2** (8 mg), **3** (13 mg) **4** (19 mg) and **5** (20 mg).

3.4. Saponin (*1*)

Amorphous powder; $[\alpha]_D^{23} = -4.3^\circ$ ($c = 1.43$, MeOH); FAB-MS (*m*/*z*): 1567 [$C_{71}H_{116}O_{36}$ + Na], 1421. ^1H and ^{13}C NMR: see Tables 1 and 2.

3.5. Saponin (*2*)

Amorphous powder; $[\alpha]_D^{23} = -0.9^\circ$ ($c = 0.75$, MeOH); FAB-MS (*m*/*z*): 1609 [$C_{73}H_{118}O_{37}$ + Na], 1463. ^1H and ^{13}C NMR: see Tables 1 and 2.

3.6. Saponin (*3*)

Amorphous powder; $[\alpha]_D^{23} = +3.3^\circ$ ($c = 1.32$, MeOH); FAB-MS (*m*/*z*): 1567 [$C_{71}H_{116}O_{36}$ + Na], 1421. ^1H and ^{13}C NMR: see Tables 1 and 2.

3.7. Saponin (*4*)

Amorphous powder; $[\alpha]_D^{23} = -12.8^\circ$ ($c = 1.90$, MeOH); FAB-MS (*m*/*z*): 1583 [$C_{71}H_{116}O_{37}$ + Na], 1437. ^1H and ^{13}C NMR: see Tables 1 and 2.

3.8. General method for acid hydrolysis (Hara et al., 1986)

Each saponin (2 mg) dissolved in dioxane (100 μl) and 2M HCl (100 μl) was heated at 100 °C for 1 h. The reaction mixture was diluted with H_2O and extracted twice with EtOAc. From the EtOAc layer, the aglycone

Table 1
^{1}H NMR spectral data for compounds **1**–**4** in pyridine-d_5

	1	**2**	**3**	**4**
Aglycone				
3	3.23 (*dd*, 12.0, 3.0)	2.23 (*dd*, 12.0, 3.0)	3.32 (*dd*, 12.0, 4.0)	3.34 (*dd*, 12.0, 3.0)
12	5.42 (*br s*)	5.39 (*br s*)	5.42 (*t*, 3.0)	5.60 (*t*, 3.0)
16				5.28 (*br s*)
18	3.18 (*dd*, 14.0, 3.0)	3.18 (*dd*, 14.0, 3.0)	3.19 (*dd*, 14.0, 3.0)	3.51 (*dd*, 14.0, 3.0)
23	1.24 (*s*)	1.24 (*s*)	1.29 (*s*)	1.28 (*s*)
24	1.08 (*s*)	1.08 (*s*)	1.00 (*s*)	1.00 (*s*)
25	0.86 (*s*)	0.86 (*s*)	0.91 (*s*)	0.94 (*s*)
26	1.08 (*s*)	1.07 (*s*)	1.09 (*s*)	1.13 (*s*)
27	1.23 (*s*)	1.23 (*s*)	1.26 (*s*)	1.83 (*s*)
29	0.90 (*s*)	0.90 (*s*)	0.91 (*s*)	1.00 (*s*)
30	0.90 (*s*)	0.92 (*s*)	0.91 (*s*)	1.07 (*s*)
C-3-O-sugar				
Ara				
1	4.76 (*d*, 6.2)	4.76 (*d*, 6.2)	4.68 (*d*, 7.4)	4.68 (*d*, 7.4)
2	4.70 (*t*, 8.0)	4.70 (*t*, 8.0)	4.51	4.51 (*t*, 8.0)
3	4.30	4.30	4.19	4.18
4	4.48	4.47	4.37	4.37
5	3.65	3.64 (*brd*, 10.5)	3.68 (*brd*, 10.5)	3.68
5′	4.14	4.14	4.19	4.19
Glc I				
1	5.44 (*d*, 8.0)	5.44 (*d*, 8.0)	5.36 (*d*, 8.0)	5.36 (*d*, 8.0)
2	4.02	4.02	4.15	4.14
3	4.16	4.16	3.96	3.96
4	4.13	4.13	4.04	4.04
5	3.71 (*m*)	3.71 (*m*)	3.86	3.86
6	4.25	4.26	4.20	4.20
6′	4.33	4.35	4.39	4.39
Glc II				
1	5.25 (*d*, 8.0)	5.24 (*d*, 8.0)	5.08 (*d*, 8.0)	5.09 (*d*, 8.0)
2	4.00	4.00	4.06	4.05
3	4.19	4.21	4.27	4.28
4	4.06	4.08	4.10	4.10
5	3.87 (*m*)	3.87	3.96	3.96
6	4.19	4.20	4.19	4.20
6′		4.38	4.51	4.52
Glc III				
1	5.15 (*d*, 8.0)	5.16 (*d*, 8.0)	5.18 (*d*, 8.0)	5.18 (*d*, 8.0)
2	4.02	4.02	4.05	4.05
3	4.16	4.17	4.15 (*t*, 9.0)	4.16
4	4.13	4.13	4.04	4.04
5	3.93	3.93	3.90	3.91
6	4.26	4.24	4.20	4.20
6′	4.50	4.48	4.40	4.42
C-28-O-sugar				
Glc IV				
1	6.22 (*d*, 8.0)	6.21 (*d*, 8.0)	6.21 (*d*, 8.0)	6.22 (*d*, 8.0)
2	4.13	4.12	4.11	4.06
3	4.20	4.19	4.19	4.17
4	4.30	4.25	4.27	4.30
5	4.11	4.11	4.08	4.08
6	4.32	4.33	4.31	4.30
6′	4.65	4.66	4.65	4.64
Glc V				
1	4.97 (*d*, 8.0)	4.98 (*d*, 8.0)	4.97 (*d*, 8.0)	4.96 (*d*, 8.0)
2	3.92 (*t*, 8.5)	3.93	3.92 (*t*, 8.0)	3.91 (*t*, 8.0)
3	4.15	4.06	4.12	4.13
4	4.37	4.09	4.37	4.37
5	3.67	3.83	3.66	3.66
6	4.07	4.53	4.08	4.08
6′	4.21	4.63	4.20	4.20

(*continued on next page*)

Table 1 (*continued*)

	1	2	3	4
Ac		1.93		
Rha				
1	5.81 (*brs*)	5.52 (*brs*)	5.81 (*brs*)	5.81 (*brs*)
2	4.65	4.60	4.64	4.65
3	4.52	4.47	4.52	4.52
4	4.30	4.29	4.29	4.30
5	4.91 (*dq*, 9.0, 6.0)	4.82 (*dq*, 9.0, 6.0)	4.90 (*dq*, 9.0, 6.2)	4.91 (*dq*, 9.0, 6.2)
6	1.69 (*d*, 6.0)	1.69 (*d*, 6.0)	1.68 (*d*, 6.2)	1.69 (*d*, 6.2)

Ara = α-L-arabinopyranosyl; Glc = β-D-glucopyranosyl; Rha = α-L-rhamnopyranosyl.

Table 2
^{13}C NMR spectral data for compounds **1**–**4** in pyridine-d_5

	1	2	3	4
Aglycone				
1	38.9	38.9	38.9	39.0
2	26.6	26.5	26.7	26.8
3	89.1	89.1	88.8	88.8
4	39.7	39.7	39.7	39.7
5	56.0	56.0	56.0	56.1
6	18.6	18.5	18.6	18.5
7	33.2	33.2	33.3	33.6
8	40.0	39.9	40.0	40.2
9	48.1	48.1	48.2	47.3
10	37.1	37.1	37.1	37.2
11	23.7	23.8	23.9	23.9
12	123.0	123.3	123.0	122.8
13	144.1	144.1	144.2	144.5
14	42.2	42.2	42.2	42.2
15	28.3	28.3	28.4	36.2
16	23.4	23.4	23.5	74.4
17	47.1	47.1	47.1	49.3
18	41.8	41.7	41.8	42.2
19	46.3	46.3	46.3	47.3
20	30.8	30.6	30.8	30.9
21	34.1	34.1	34.1	36.0
22	32.6	32.7	32.6	32.2
23	28.1	28.1	28.2	28.2
24	16.8	16.7	17.1	17.1
25	15.6	15.6	15.7	15.8
26	17.6	17.5	17.6	17.7
27	26.1	26.1	26.1	27.3
28	176.5	176.4	176.6	176.1
29	33.2	33.2	33.2	33.2
30	23.7	23.8	23.8	24.8
C-3-O-sugar				
Ara				
1	105.5	105.5	107.3	107.3
2	77.3	77.4	71.9	71.9
3	83.3	83.3	83.5	83.5
4	68.7	68.7	69.2	69.2
5	65.9	65.9	66.8	66.8
Glc I				
1	104.3	104.3	104.1	104.1
2	76.2	76.3	73.8	73.8
3	78.6	78.6	90.8	90.9
4	72.5	72.4	69.5	69.5
5	77.4	77.3	77.9	77.9
6	63.3	63.3	62.3	62.3
Glc II				
1	104.6	104.7	103.8	103.8
2	74.0	74.0	85.6	85.6
3	88.0	87.9	77.6	77.6
4	69.8	69.8	71.3	71.4
5	78.1	78.1	78.5	78.5
6	62.5	62.5	62.5	62.5
Glc III				
1	105.4	105.4	107.0	107.0
2	75.4	75.4	76.4	76.5
3	78.1	78.1	77.9	77.9
4	71.6	71.6	71.0	71.1
5	78.6	78.6	79.3	79.4
6	62.3	62.3	62.4	62.4
28-O-sugar				
Glc IV				
1	95.6	95.6	95.7	95.8
2	74.0	74.0	73.9	74.0
3	78.8	78.8	78.8	78.8
4	71.0	71.1	71.0	71.1
5	78.1	78.1	78.1	78.1
6	69.3	69.5	69.3	69.4
Glc V				
1	104.9	104.6	104.9	105.0
2	75.4	75.1	75.4	75.4
3	76.6	76.2	76.6	76.6
4	78.5	79.3	78.5	78.5
5	77.2	73.8	77.2	77.2
6	61.4	63.7	61.4	61.4
Ac		20.6		
C=O		170.6		
Rha				
1	102.8	102.9	102.8	102.8
2	72.6	72.4	72.6	72.6
3	72.8	72.7	72.8	72.8
4	74.0	73.8	74.0	74.0
5	70.3	70.7	70.4	70.4
6	18.5	18.5	18.5	18.5

Ara = α-L-arabinopyranosyl; Glc = β-D-glucopyranosyl; Rha = α-L-rhamnopyranosyl.

was detected by HPLC [column, YMC R & D ODS; 4.6 mm×25 cm, solvent; MeOH–H_2O (9:1) + 0.05% TFA; flow rate; 1 ml/min, detection; UV 205 nm, echinocystic acid (t_R, 6.3 min); oleanolic acid (t_R, 11.0 min)]. The water layer was passed through an Amberlite IRA-60E column (6×60 mm) and the eluate was concentrated. The residue was dissolved in pyridine (50 μl) and stirred with D-cysteine methyl ester (6 mg) for 1.5 h at 60 °C. To the reaction mixture, hexamethyldisilazane (20 μl) and trimethylsilyl chloride (20 μl) were added and the

mixture was stirred for 30 min at 60 °C. The supernatant was then analyzed by GC [column; GL Sciences TC-1, 0.25 mm×30 m, column temperature; 235 °C, carrier gas; N_2, retention time D-Glc (16.8 min), L-Glc (16.3 min), D-Ara (9.7 min), L-Ara (10.2 min), D-Rha (11.4 min), L-Rha (11.6 min). From the new saponins 1–4. D-Glc, L-Ara and L-Rha were detected.

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