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Triterpenoid saponins from *Oreopanax guatemalensis*

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Abstract

Seven oleanane-type saponins were isolated from the leaves and stems of *Oreopanax guatemalensis,* together with ten known saponins of lupane and oleanane types. The new saponins were respectively characterized as 3-*O*-α-L-arabinopyranosyl echinocystic acid 28-*O*-[α- L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl] ester, 3-*O*-β-D-glucopyranosyl 3β-hydroxy olean-11,13(18)-dien-28-oic acid 28-*O*-[α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β- D-glucopyranosyl]ester, 3-*O*-[α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl]3β-hydroxy olean-11,13(18)-dien-28-oic acid 28-*O*-[α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl] ester, 3-*O*-[α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl]3β, 23 dihydroxy olean-18-en-28-oic acid 28-*O*-[α-L-rhamnopyranosyl-(1→4)-β-D-6-*O*-acetyl glucopyranosyl-(1→6)-β-D-glucopyranosyl]ester, 3-*O*-[α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl] hederagenin 28-*O*-{α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-[β-D-xylopyranosyl-(1→2)-]β-D-glucopyranosyl}ester, 3-*O*-[α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl]hederagenin 28-*O*-{α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-[β-D-glucopyranosyl-(1→2)-]β-D-glucopyranosyl} ester and 3-*O*-[α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl] hederagenin 28-*O*-{α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-[α-L-arabinofuranosyl-(1→2)]-β-D-glucopyranosyl} ester. The structures were determined by spectral analyses. The NMR assignments were made by means of HOHAHA, ^{1}H–^{1}H COSY, HMQC, HMBC spectra and NOE difference studies.

Keywords: *Oreopanax guatemalensis*; Araliaceae; Triterpenoid saponin

1. Introduction

Oreopanax species (Araliaceae) are ornamental trees cultivated in public gardens in Egypt and known to be rich in saponins with biological and molluscicidal activities. There have been no detailed phytochemical reports on these species. As a part of our continuing search for bioactive saponins from natural sources (Miyase et al., 1996; Abdel-Khalik et al., 2000; Melek et al., 2000) we have isolated seven new compounds and ten known oleanane and lupane-type triterpene saponins. Their structures were established on the basis of spectral data.

2. Results and discussion

The crude saponin mixture, obtained from the methanolic extract of the leaves and stems of *O. guatemalensis* Decne. & Planch, was chromatographed on silica gel. The eluate was dissolved in water, passed through a porous polymer gel Mitsubishi HP-20 column and the adsorbed materials were eluted with methanol. The methanolic eluate was separated using HPLC technique to afford 17 saponins, including seven new **1–7** and 10 known ones **8–17**. The NMR data of the new saponins are presented in Tables 1–3.

The FAB mass spectrum of **1** exhibited a quasi-molecular ion peak $(M+Na)^+$ at m/z 1097, corresponding to a molecular formula $C_{53}H_{86}O_{22}$, and a prominent fragment ion at m/z 951 due to loss of a deoxyhexose unit. Its spectral features suggested **1** to be a triterpenoid saponin. Of the 53 carbons, 30 were assigned to the aglycone part and 23 to the sugar units. The ^{1}H and ^{13}C NMR spectra displayed signals due to the aglycone moiety, characteristic for echinocystic acid. The chemical shift of C-3 (δ 88.8) and C-28 (δ 176.0) suggested that **1** was a bisdesmosidic glycoside. Acid hydrolysis of **1** afforded sugar components identified by GC analysis as L-arabinose, D-glucose and L-rhamnose. The ^{1}H NMR spectrum of **1**

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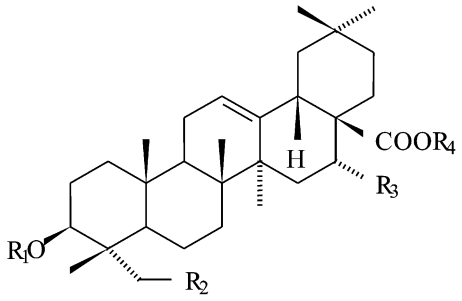


	R_1	R_2	R_3	R_4
1	Ara	H	OH	Glc^{6}---^{1}Glc^{4}---^{1}Rha
5	Rha^{1}---^{2}Ara	OH	H	Glc^{6}---^{1}Glc^{4}---^{1}Rha $\vert^{2}_{1}$ Xyl
6	Rha^{1}---^{2}Ara	OH	H	Glc^{6}---^{1}Glc^{4}---^{1}Rha $\vert^{2}_{1}$ Glc
7	Rha^{1}---^{2}Ara	OH	H	Glc^{6}---^{1}Glc^{4}---^{1}Rha $\vert^{2}_{1}$ Ara (f)
8	Rha^{1}---^{2}Ara	H	OH	Glc^{6}---^{1}Glc^{4}---^{1}Rha $\vert^{6}$ Ac
12	Ara	H	H	Glc^{6}---^{1}Glc^{4}---^{1}Rha
13	Rha^{1}---^{2}Ara	H	H	Glc^{6}---^{1}Glc^{4}---^{1}Rha
14	Rha^{1}---^{2}Ara	H	H	Glc^{6}---^{1}Glc^{4}---^{1}Rha $\vert^{6}$ Ac
15	Rha^{1}---^{2}Ara	H	OH	Glc^{6}---^{1}Glc^{4}---^{1}Rha
16	Rha^{1}---^{2}Ara	OH	H	Glc^{6}---^{1}Glc^{4}---^{1}Rha
17	Rha^{1}---^{2}Ara	OH	H	Glc^{6}---^{1}Glc^{4}---^{1}Rha $\vert^{6}$ Ac

displayed sugar anomeric proton signals at δ 4.77 (*d*, $J=6.7$ Hz), 6.22 (*d*, $J=8.0$ Hz) 4.96 (*d*, $J=8.0$ Hz) and 5.82 (*brs*) together with a methyl doublet at δ 1.69 ($J=6.0$ Hz) suggesting the occurrence of four sugar units including one rhamnose unit. The other sugar signals overlapped in the region between δ 3.6 and 4.64. The individual spin systems for the individual monosaccharide units were discerned from the HOHAHA spectrum and the sequence of protons was then deduced from the ^{1}H–^{1}H COSY spectrum. On the basis of the assigned protons, the ^{13}C resonances of each sugar unit were identified by the HMQC spectrum and confirmed by a HMBC experiment. The assignments of the proton and the carbon resonances revealed the presence of a α-rhamnopyranosyl unit, an α-arabinopyranosyl unit and two β-glucopyranosyl units. The anomeric centres of the two glucopyranosyl units were each determined to have a β-configuration based on the large $^{3}J_{H1\text{-}H2}$ values (8.0 Hz). The α-anomeric configuration of the arabinopyranosyl unit was deduced from its $^{3}J_{H1\text{-}H2}$ value (6.7 Hz). The broad singlet of the anomeric proton and the chemical shift values of the signals due to C-3 and C-5 as well as the presence of three-bond strong HMBC correlation between the anomeric proton and both C-3 and C-5 of the rhamnopyranosyl unit, indicated an α-orientation (Jia et al., 1998). The positions of the sugar units were unambiguously defined by the HMBC experiment. A cross peak due to long-range coupling between C-3 (δ 88.8) of the aglycone and H-1 (δ 4.77) of the α-arabinopyranosyl unit, indicated that this pentose unit was linked to C-3 of the aglycone. Similarly, the linkages of the trisaccharide chain at C-28, were established by the cross peaks between H-1 (δ 5.82) of the terminal α-rhamnopyranosyl unit and C-4 (δ 78.4) of the outer β-glucopyranosyl unit, H-1 (δ 4.96) of the outer β-glucopyranosyl unit and C-6 (δ 69.3) of the ester-linked β-glucopyranosyl unit. A cross peak between H-1 (δ 6.22) of the ester-linked β-glucopyranosyl unit and the carbon resonance of the aglycone carbonyl (δ 176.0), provided definitive evidence of an ester linkage between the trisaccharide chain and the aglycone. The same conclusion with regard to the sugar sequence was also drawn from the observed inter-residue NOEs in a NOE difference experiment. Based on the above studies the structure of **1** was concluded to be 3-*O*-α-L-arabinopyranosyl echinocystic acid 28-*O*-[α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl] ester.

Compound **2** exhibited a quasi-molecular ion peak $[M+Na]^{+}$ at *m*/*z* 1109 in its FAB mass spectrum, consistent with a molecular formula $C_{54}H_{86}O_{22}$. Its spectral features suggested **2** to be a triterpenoid saponin. The ^{13}C NMR showed 54 signals, of which 24 were assigned to the saccharide portion and 30 to the triterpene moiety. The ^{1}H NMR spectrum showed, in addition to seven singlets assignable to tertiary methyls at δ 0.85, 0.85, 0.92, 1.01, 1.02, 1.07, 1.33, two lowfield signals at δ 5.71 (*d*, $J=11.0$ Hz) and δ 6.59 (*dd*, $J=11.0$, 3.0 Hz) ascribable to two coupled olefinic protons. Also observed, was a typical signal of axial H-3 at δ 3.42 (*dd*, $J=11.1$, 4.3 Hz). Further features were four signals in the ^{13}C NMR spectrum at δ 125.9 (CH), 127.3 (CH), 132.1 (C) and 137.3 (C) indicative of four sp^2 carbons. These observations suggested that the aglycone moiety possessed a triterpene skeleton of the olean-11,13 (18) type. A signal at δ 175.8 and the carbon resonances of D and E rings indicated the existence of a glycosylated COOH group at C-28. Therefore, the aglycone of **2** was identified as 3β-hydroxy olean-11, 13 (18) dien-28-oic acid. Comparison of the ^{13}C NMR data, due to the aglycone moiety, with the data reported in the literature for a related triterpene, exhibiting the same conjugated diene system (Kojima et al., 1987), showed a good agreement except

Table 1
^{13}C NMR spectral data of compounds **1–7** in pyridine-d_5

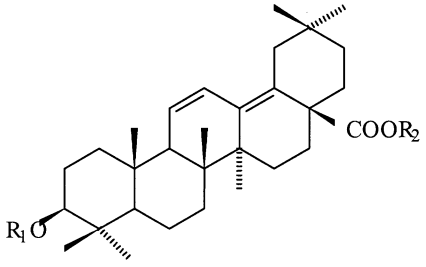
C	1	2	3	4	5	6	7
1	39.0	38.3	38.6	39.4	39.1	39.1	39.1
2	26.7	26.6	26.8	26.4	26.2	26.2	26.2
3	88.8	88.9	88.9	81.2	81.1	81.1	81.2
4	39.6	39.7	39.7	43.7	43.6	43.6	43.6
5	56.1	55.4	55.6	48.2	47.7	47.7	47.9
6	18.5	18.6	18.6	18.1	18.2	18.2	18.3
7	33.5	32.8	32.8	34.6	32.9	33.0	32.9
8	40.2	41.2	41.2	41.1	40.0	40.1	40.1
9	47.3	54.7	54.7	51.6	48.3	48.2	48.3
10	37.2	36.7	36.7	37.1	36.9	36.9	37.0
11	23.9	125.9	125.8	21.3	23.8	23.9	23.9
12	122.8	127.3	127.4	26.4	122.8	122.7	123.0
13	144.5	137.3	137.2	41.3	144.4	144.5	144.2
14	42.1	42.4	42.4	43.0	42.1	42.2	42.3
15	36.2	25.5	25.5	29.9	28.8	29.3	28.8
16	74.4	33.0	33.0	34.0	23.0	23.0	23.3
17	49.3	48.8	48.8	48.8	47.2	47.1	47.1
18	41.3	132.1	132.1	137.9	41.7	41.8	41.8
19	47.3	40.8	40.8	132.9	46.3	46.2	46.2
20	30.8	32.7	32.7	32.3	30.8	30.8	30.7
21	36.0	37.1	37.1	33.6	34.1	34.1	34.1
22	32.3	35.9	35.9	33.8	32.6	32.4	32.3
23	28.3	28.1	27.9	64.1	63.9	63.9	64.0
24	17.0	16.5	16.6	13.7	14.0	14.0	14.0
25	15.8	18.4	18.5	17.5	16.2	16.2	16.3
26	17.7	16.9	16.8	16.4	17.5	17.6	17.5
27	27.3	20.1	20.1	15.3	26.2	26.2	26.0
28	176.0	175.8	175.8	175.4	176.6	176.7	176.5
29	33.2	24.4	24.4	30.7	33.2	33.2	33.2
30	24.7	32.3	32.3	29.2	23.8	23.8	23.8
Sugar moiety at C-3							
	Ara	Glc	Glc	Ara	Ara	Ara	Ara
1	107.4	106.9	105.5	104.2	104.4	104.4	104.3
2	72.9	75.8	80.0	75.9	75.9	75.9	75.9
3	74.6	78.8	77.7	74.5	74.8	74.9	74.6
4	69.5	72.0	72.5	69.2	69.4	69.5	69.3
5	66.6	78.3	78.3	65.4	65.7	65.8	65.6
6		63.2	63.0				
Rha							
1			101.7	101.6	101.6	101.6	101.7
2			72.3	72.4	72.5	72.5	72.4
3			72.5	72.6	72.6	72.6	72.6
4			74.2	74.1	74.2	74.2	74.2
5			69.8	69.7	69.7	69.7	69.7
6			18.7	18.5	18.5	18.5	18.5
At C-28							
Glc							
1	95.8	96.0	96.0	95.9	93.4	93.5	94.4
2	74.0	74.0	74.0	74.2	79.9	78.4	76.3
3	78.7	78.5	78.5	78.9	78.8	78.8	78.6
4	71.0	71.5	71.5	71.0	70.5	70.5	71.0
5	78.1	78.3	78.2	78.1	78.0	78.0	77.9
6	69.3	69.7	69.6	69.7	69.0	69.0	69.0
At C-2 of Glc							
					Xyl	Glc	Ara (f)
1					105.9	104.6	109.1
2					75.7	73.1	82.5
3					78.6	78.3	77.8
4					71.2	70.4	86.0

(*continued on next page*)

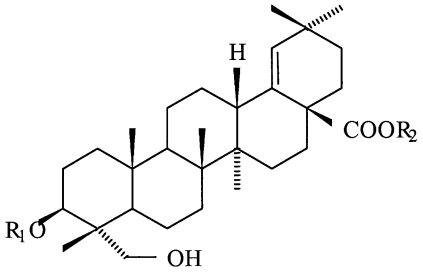
Table 1 (*continued*)

C	1	2	3	4	5	6	7
5					67.3	78.5	62.9
6						64.1	
Outer Glc							
1	105.0	104.9	105.0	105.0	104.9	104.9	104.8
2	75.3	75.3	75.4	75.1	75.4	75.4	75.4
3	76.5	76.6	76.6	76.3	76.5	76.6	76.6
4	78.4	78.8	78.8	79.3	78.6	78.6	78.6
5	77.2	77.2	77.2	73.8	77.2	77.2	77.2
6	61.4	61.5	61.4	63.7	61.4	61.4	61.4
Ac				20.6			
C=O				170.6			
Rha							
1	102.8	102.7	102.7	102.9	102.8	102.8	102.8
2	72.6	72.6	72.6	72.4	72.6	72.6	72.6
3	72.8	72.8	72.8	72.7	72.8	72.8	72.8
4	74.0	74.0	74.2	73.8	74.0	74.1	74.0
5	70.3	70.3	70.3	70.7	70.4	70.5	70.4
6	18.5	18.5	18.5	18.5	18.5	18.6	18.6

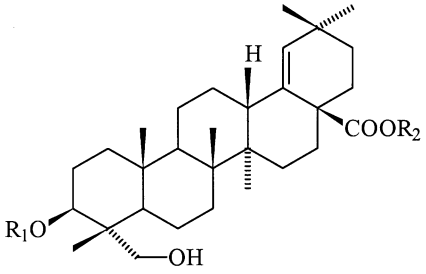
Ara, α-L-arabinopyranosyl; Ara(f), α-L-arabinofuranosyl; Rha, α-L-rhamnopyranosyl; Glc, β-D-glucopyranosyl; Xyl, β-D-xylopyranosyl.



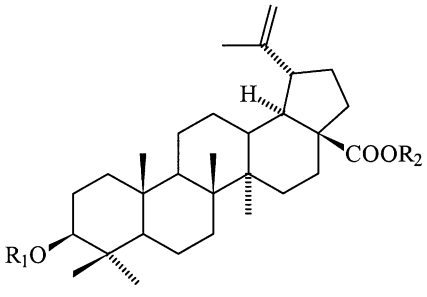
	R_1	R_2
2	Glc	$Glc^{6}{-}{-}{-}Glc^{4}{-}{-}{-}{-}Rha^{1}$
3	$Rha^{1}{-}{-}{-}Glc^{2}$	$Glc^{6}{-}{-}{-}Glc^{4}{-}{-}{-}{-}Rha^{1}$



	R_1	R_2
9	Glc	$Glc^{6}{-}{-}{-}Glc^{4}{-}{-}{-}{-}Rha^{1}$



	R_1	R_2
4	$Rha^{1}{-}{-}{-}Ara^{2}$	$Glc^{6}{-}{-}{-}Glc^{4}{-}{-}{-}{-}Rha^{1}$ $\vert^{6}$ Ac



	R_1	R_2
10	Glc	$Glc^{6}{-}{-}{-}Glc^{1}$
11	Glc	$Glc^{6}{-}{-}{-}Glc^{4}{-}{-}{-}{-}Rha^{1}$

Table 2
^{1}H NMR spectral data of compounds **1–4** in pyridine-d_5

H	**1**	**2**	**3**	**4**
1ax	1.00	0.99	0.96	1.00
1eq	1.59	1.76 (*brd*, 12.3)	1.73	1.65
2ax	1.90	1.91	1.94	2.02
2eq	2.15	2.31	2.34	2.22 (*brd*, 12.5)
3	3.37 (*dd*, 12.0, 4.5)	3.42 (*dd*, 11.1, 4.3)	3.38 (*dd*, 11.1, 4.9)	4.24
5	0.86 (*brd*, 12.0)	0.83	0.78 (*brd*, 11.7)	1.62
6ax	1.32	1.37	1.38	
9	1.79	1.99 (*brs*)	1.96 (*brs*)	1.39 (*t*, 12.3)
11	2.00	6.59 (*dd*, 11.0, 3.0)	6.59 (*dd*, 10.5, 2.5)	
12	5.60 (*brt*, 3.5)	5.71 (*d*, 11.0)	5.69 (*d*, 10.5)	ax 1.15 eq 1.59 (*brd*, 12.9)
13				2.62 (*brd*, 11.1)
15ax	2.53 (*dd*, 14.5, 4.0)	2.09 (*dt*, 13.5, 2.5)	2.08 (*dt*, 13.5, 2.5)	2.11 (*dt*, 13.3, 3.0)
15eq	1.77 (*dd*, 14.5, 3.5)	1.11 (*brd*, 13.5)	1.11 (*brd*, 12.3)	1.16
16ax		1.77 (*dt*, 13.5, 3.1)	1.78 (*dt*, 13.5, 3.1)	1.45 (*t*, 12.9)
16eq	5.28 (*brs*)	2.35 (*dt*, 13.5, 3.7, 2.5	2.35	2.49 (*brd*, 12.3)
18	3.51 (*dd*, 14.0, 3.5)			
19ax	2.78 (*t*, 13.7)	2.18 (*d*, 14.1)	2.19 (*d*, 13.5)	5.23 (*brs*)
19eq	1.33 (*dd*, 13.7, 4.0)	2.63 (*d*, 14.1)	2.63 (*d*, 13.5)	
21ax	2.40	1.69	1.69	
21eq	1.27	1.29 (*brd*, 11.7)	1.28	
22ax	2.13 (*dt*, 13.5, 3.5)	1.46 (*dt*, 13.5, 3.7)	1.46 (*dt*, 13.5, 3.7)	
22eq	2.39	2.56 (*brd*, 11.9)	2.57 (*brd*, 11.9)	
23	1.24 (*s*)	1.33 (*s*)	1.28 (*s*)	3.73 (*d*, 11.1), 4.10
24	0.97 (*s*)	1.01 (*s*)	1.20 (*s*)	1.03 (*s*)
25	0.95 (*s*)	0.85 (*s*)	0.86 (*s*)	0.93 (*s*)
26	1.13 (*s*)	1.02 (*s*)	1.02 (*s*)	1.16 (*s*)
27	1.82 (*s*)	1.07 (*s*)	1.06 (*s*)	0.83 (*s*)
29	1.00 (*s*)	0.85 (*s*)	0.84 (*s*)	1.06 (*s*)
30	1.06 (*s*)	0.92 (*s*)	0.92 (*s*)	0.98 (*s*)
3-*O*-sugar	Ara	Glc	Glc	Ara
1	4.77 (*d*, 6.7)	4.95 (*d*, 8.0)	4.96 (*d*, 8.0)	5.11 (*d*, 5.5)
2	4.41 (*t*, 7.0)	4.04 (*t*, 8.0)	4.30	4.54 (*t*, 6.0)
3	4.15	4.25 (*t*, 8.0)	4.30	4.11
4		4.23 (*t*, 8.0)	4.16	4.17
5	3.83 (*brd*, 10.5)	4.02 (*m*)	3.97 (*m*)	3.69 (*brd*, 11.7)
5	4.31			4.25
6		4.41 (*dd*, 12.3, 4.5)	4.36 (*dd*, 12.0, 3.7)	
6′		4.59 (*dd*, 12.3, 2.5)	4.56 (*dd*, 12.0, 2.5)	
Rha				
1			6.57 (*d*, 1.5)	6.19 (*brs*)
2			4.84 (*dd*, 3.0, 1.5)	4.70 (*dd*, 2.5, 1.5)
3			4.69	4.62
4			4.30 (*t*, 9.0)	4.26 (*t*, 9.0)
5			4.80 (*dq*, 9.0, 6.0)	4.65
6			1.71 (*d*, 6.0)	1.63 (*d*, 6.0)
28-*O*-Sugar				
Glc				
1	6.22 (*d*, 8.0)	6.27 (*d*, 8.0)	6.27 (*d*, 8.0)	6.32 (*d*, 8.0)
2	4.06 (*t*, 8.0)	4.06 (*t*, 8.0)	4.06 (*t*, 8.0)	4.07 (*t*, 8.0)
3	4.17 (*t*, 8.0)	4.16	4.15	4.17 (*t*, 8.0)
4		4.13 (*t*, 8.0)	4.10	4.22 (*t*, 8.5)
5	4.08 (*m*)	4.13	4.12 (*m*)	4.08
6	4.30 (*dd*, 12.0, 3.0)	4.22 (*dd*, 12.0, 2.0)	4.23 (*dd*, 12.0, 2.0)	4.30 (*dd*, 12.0, 2.0)
Outer Glc				
1	4.96 (*d*, 8.0)	4.96 (*d*, 8.0)	4.98 (*d*, 8.0)	4.92 (*d*, 8.0)
2	3.91 (*t*, 8.0)	3.91 (*t*, 8.0)	3.92 (*t*, 8.0)	3.93 (*t*, 8.0)
3	4.14 (*t*, 8.9)	4.15	4.16	4.11 (*t*, 8.0)
4	4.37 (*t*, 9.0)	4.37 (*t*, 8.0)	4.39 (*t*, 8.0)	4.08 (*t*, 8.0)
5	3.66 (*m*)	3.68 (*m*)	3.71 (*m*)	3.83 (*m*)

(*continued on next page*)

Table 2 (*continued*)

H	1	2	3	4
6	4.08	4.09 (*dd*, 12.0, 2.0)	4.12 (*dd*, 12.0, 2.0)	4.54 (*dd*, 12.0, 3.0)
6′	4.22	4.22 (*d*, 12.0)	4.23	4.65 (*d*, 12.0)
Ac				1.95 (*s*)
Rha				
1	5.82 (*brs*)	5.81 (*brs*)	5.83 (*d*, 1.5)	5.52 (*brs*)
2	4.66 (*brs*)	4.66 (*dd*, 2.5, 1.5)	4.67 (*dd*, 3.0, 2.0)	4.61
3	4.53 (*dd*, 9.0, 3.0)	4.52 (*dd*, 9.0, 3.0)	4.53 (*dd*, 9.0, 3.0)	4.48 (*dd*, 9.0, 3.0)
4	4.30 (*t*, 9.0)	4.30 (*t*, 9.0)	4.28 (*t*, 9.0)	4.30 (*t*, 9.0)
5	4.92 (*dq*, 9.0, 6.0)	4.91 (*dq*, 9.0, 6.0)	4.92 (*dq*, 9.0, 6.0)	4.82 (*dq*, 9.0, 6.0)
6	1.69 (*d*, 6.0)	1.69 (*d*, 6.0)	1.69 (*d*, 6.0)	1.70 (*d*, 6.0)

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

the reversed assignment of C-15 and C-16. Our assignment was based on long-range C–H correlations in the HMBC spectrum. Glycosylation of the alcoholic function at C-3 and esterification of the 28-COOH group were indicated by their chemical shift values. Acid hydrolysis of **2** yielded the sugar components D-glucose and L-rhamnose. The sugar part contained, in the 1D and 2D spectra, four anomeric signals at δ 4.95 (*d*, $J=8.0$ Hz); 106.9, 6.27 (*d*, $J=8.0$ Hz); 96.0, 4.96 (*d*, $J=8.0$ Hz), 104.9 and 5.81 (*brs*); 102.7. The overall structural assignment was achieved following the same methodology as in **1**. Extensive NMR (HOHAHA, ^{1}H–^{1}H COSY, NOE, HMQC, HMBC) permitted the assignments of the proton and the carbon resonances and indicated the presence of a terminal α-rhamnopyranosyl unit, a terminal β-glucopyranosyl unit, a 6-substituted ester-linked β-glucopyranosyl unit and a 4-substituted β-glucopyranosyl unit. The HMBC correlation between signals due to H-1 (δ 4.95) of the β-glucopyranosyl unit and C-3 (δ 88.9) of the aglycone established the attachment of this hexose unit at C-3 of the aglycone. The other HMBC correlations and the observed inter-residue NOEs were found the same as those previously reported for the 28-*O*-sugar chain of saponin **1**, thus, confirming the existence of identical ester-linked trisaccharide chain in **1** and **2**. Therefore, **2** was assigned the structure of 3-*O*-β-D- glucopyranosyl 3β hydroxy olean-11,13(18)-dien-28 oic acid 28-*O*-[α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl] ester.

The FAB mass spectrum of compound **3** ($C_{60}H_{96}0_{26}$) showed a quasi-molecular ion-peak $[M+Na]^+$ at m/z 1255; larger than that of **2** by 146 mass units (deoxyhexose unit). On acid hydrolysis, L-rhamnose and D-glucose were released. The 1D and 2D NMR spectra showed signals, due to the aglycone moiety, very close to those of **2** and suggested sugar moieties made up of a trisaccharide chain linked to C-28, identical to that of **1** and **2.** The disaccharide chain at C-3 was composed of a terminal α-rhamnopyranosyl unit (H-1; δ 6.57) attached to C-2 of an inner β-glucopyranosyl unit (H-1; δ 4.96) based on the observed lower field position of the signal due to C-2 (δ 80.0) of the β-glucopyranosyl unit, with reference to the corresponding in **2**. This conclusion was also confirmed from the HMBC correlations and the inter-residue NOEs. Thus, **3** was assigned the structure of 3-*O*-[α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl]3β-hydroxy olean-11,13(18)-dien-28-oic acid 28-*O*-[α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl] ester. Saponins of the family Araliaceae, bearing an aglycone moiety with a conjugated diene group as that of the new saponins **3** and **2**, have only been found in *Tetrapanax papyriferum* (Takai et al., 1977; Asada et al., 1980).

Compound **4** had a molecular formula of $C_{61}H_{98}O_{27}$ as determined from its FAB mass spectrum, which exhibited a quasi-molecular ion peak $[M+Na]^+$ at m/z 1285. The ^{13}C NMR spectrum showed 61 signals of which 30 were assigned to a triterpene moiety, 31 to the saccharide portion, including two due to acetyl group. The ^{1}H NMR spectrum displayed signals characteristic for a triterpene of olean-18-ene type; six singlets for tertiary methyls at δ 1.03, 0.93, 1.16, 0.83, 1.06 and 0.98 assignable to H_3-24, H_3-25, H_3-26, H_3-27, H_3-29, H_3-30, two signals at δ 3.73 (*d*, $J=11.1$ Hz) and 4.10 (overlapped) ascribable to a CH_2OH group and a broad singlet at δ 5.23 ascribable to a H-19 vinyl proton. Further features were two olefinic carbon signals at δ 137.9 and 132.9 in the ^{13}C NMR spectrum assignable to C-18 and C-19, respectively. The rest of the carbon signals, due to the D and E rings, were similar to the corresponding data reported in the literature for 3β- olean-18-en-28-oic acid (morolic acid) (Gonzalez et al., 1981). The equatorial geometry (α) of the CH_2OH at C-4 was established on the basis of the chemical shift value of the signal due to C-24 (δ 13.7) and HMBC correlations. The existence of a glycosylated β-OH group at C-3 was evident from the very close ^{13}C shift values of C-3 in 4 (δ 81.2) and the corresponding one in the spectra of hederagenin saponins occurring in the same plant. Thus, the aglycone of

Table 3
^{1}H NMR spectral data of compounds **5–7** in pyridine-d_5

H	**5**	**6**	**7**
1ax	1.05	1.05	1.05
1eq	1.54	1.54	1.56 (*dt*, 13.5, 3.7)
2ax	1.98	2.18	2.0
2eq	2.19	1.97	2.19
3	4.27	4.26	4.25
5	1.69	1.67	1.71
9	1.77	1.76	1.75
11	1.92	1.92	1.90
12	5.40 (*brt*, 3)	5.40 (*brt*, 3.0)	5.42 (*brt*, 3.5)
18	3.16 (*dd*, 13.5, 3.7)	3.12 (*dd*, 13.5, 3.1)	3.14
19ax	1.73	1.70	1.67
19eq		1.16	
23	3.62, 4.08	3.62, 4.08	3.67 (*d*, 10.0), 4.09
24	1.00 (*s*)	0.99 (*s*)	1.02 (*s*)
25	0.95 (*s*)	0.91 (*s*)	0.98 (*s*)
26	1.06 (*s*)	1.02 (*s*)	1.10 (*s*)
27	1.18 (*s*)	1.15 (*s*)	1.17 (*s*)
29	0.88 (*s*)	0.85 (*s*)	0.85 (*s*)
30	0.91 (*s*)	0.87 (*s*)	0.89 (*s*)
3-*O*-sugar			
Ara			
1	5.10 (*d*, 6.2)	5.10 (*d*, 6.2)	5.12 (*d*, 6.2)
2	4.56 (*t*, 6.5)	4.57 (*t*, 6.5)	4.55 (*t*, 6.5)
3	4.11 (*dd*, 6.5, 3.7)	4.11 (*dd*, 6.5, 3.7)	4.10 (*dd*, 6.5, 3.7)
4	4.17	4.15	4.16
5	3.71 (*d*, 11.0)	3.71 (*d*, 10.0)	3.71 (*dd*, 12.3, 1.3)
5′	4.25 (*dd*, 11.0, 1.5)	4.25 (*dd*, 10.0, 1.8)	4.26
Rha			
1	6.24 (*d*, 1.5)	6.25 (*d*, 1.5)	6.21 (*d*, 1.5)
2	4.69 (*dd*, 2.5, 1.5)	4.70 (*dd*, 2.5, 1.5)	4.71 (*dd*, 2.5, 1.5)
3	4.61 (*dd*, 9.0, 3.0)	4.61 (*dd*, 9.0, 3.0)	4.62 (*dd*, 9.0, 3.0)
4	4.26 (*t*, 9.0)	4.27 (*t*, 9.0)	4.27 (*t*, 9.0)
5	4.65	4.65 (*dq*, 9.0, 6.0)	4.65 (*dq*, 9.0, 6.0)
6	1.64 (*d*, 6.0)	1.64 (*d*, 6.0)	1.64 (*d*, 6.0)
28-*O*-Sugar			
Glc			
1	6.10 (8.0)	6.10 (*d*, 8.0)	6.09 (*d*, 8.0)
2	4.20	4.36 (*t*, 8.0)	4.27 (*t*, 8.0)
3	4.20	4.22	4.22 (*t*, 8.0)
4	4.22	4.20	4.19
5	3.98 (*m*)	3.98 (*m*)	4.04 (*m*)
6	4.28	4.28 (*dd*, 12.0, 2.0)	4.28
6′	4.59 (*dd*, 12.0, 2.5)	4.58 (*d*, 12.0)	4.61
(at C-2 Glc)	Xyl	Glc	Ara (*f*)
1	5.36 (*d*, 7.4)	5.65 (*d*, 8.0)	6.36 (*d*, 2.5)
2	4.03 (*t*, 8.0)	4.04 (*t*, 8.0)	4.97
3	4.14 (*t*, 8.0)	4.24 (*t*, 8.0)	4.90
4	4.23	4.22 (*t*, 8.0)	4.81
5	3.69 (*t*, 12.0)	4.05	4.28 (*dd*, 11.7, 4.3)
5	4.37 (*dd*, 12.0, 4.0)		4.40 (*dd*, 11.7, 3.7)
6		4.34 (*dd*, 12.0, 3.7)	
6′		4.66 (*d*, 12.0)	
Outer Glc			
1	4.95 (*d*, 8.0)	4.95 (*d*, 8.0)	4.96
2	3.93 (*t*, 8.0)	3.94 (*t*, 8.0)	3.94 (*t*, 8.0)
3	4.12 (*t*, 8.0)	4.12 (*t*, 8.0)	4.13 (*t*, 8.0)
4	4.37 (*t*, 8.0)	4.38 (*t*, 8.0)	4.37 (*t*, 8.0)
5	3.67 (*m*)	3.66 (*m*)	3.66 (*m*)

(*continued on next page*)

Table 3 (*continued*)

H	5	6	7
6	4.08 (*dd*, 12.0, 2.0)	4.08	4.09
6′	4.21 (*d*, 12.0)	4.21 (*d*, 12.0)	4.21
Rha			
1	5.81 (brs)	5.82 (*d*, 1.5)	5.82 (*d*, 1.5)
2	4.65 (*dd*, 2.5, 1.5)	4.65 (*dd*, 2.5, 1.5)	4.65 (*dd*, 2.5, 1.5)
3	4.52 (*dd*, 9.0, 3.0)	4.52 (*dd*, 9.0, 3.0)	4.52 (*dd*, 9.0, 3.0)
4	4.30 (*t*, 9.0)	4.30 (*t*, 9.0)	4.30 (*t*, 9.0)
5	4.91 (*dq*, 9.0, 6.0)	4.91 (*dq*, 9.0, 6.0)	4.90 (*dq*, 9.0, 6.0)
6	1.69 (*d*, 6.0)	1.69 (*d*, 6.0)	1.69 (*d*, 6.0)

Ara, α-L-arabinopyranosyl; Ara(f), α-L-arabinofuranosyl; Rha, α-L-rhamnopyranosyl; Glc, β-D-glucopyranosyl; Xyl, β-D-xylopyranosyl.

4 was identified as 3β, 23 dihydroxyolean-18-en-28-oic acid, reported before as ambradiolic acid (Yankov et al., 1980). The bisdesmosidic nature of **4** was revealed from the chemical shift values of signals due to C-3 (δ 81.2) and C-28 (δ 175.4). Acid hydrolysis, yielded the sugar components L-arabinose, D-glucose and L-rhamnose. The 1D and 2D NMR spectra showed the presence of five anomeric signals at δ 5.11 (*d*, $J=5.5$ Hz); 104.2, 6.19 (*brs*); 101.6, 6.32 (*d*, $J=8.0$ Hz) 95.9, 4.92 (*d*, $J=8.0$ Hz); 105.0 and 5.52 (*brs*); 102.9. Extensive NMR analysis (HOHAHA, ^{1}H–^{1}H COSY, NOE, HMQC, HMBC) allowed the assignments of the proton and the carbon resonances and revealed the presence of two terminal α- rhamnopyranosyl units, a 2-substituted α-arabinopyranosyl unit, a 6-substituted ester-linked β-glucopyranosyl unit and a 4-substituted β-glucopyranosyl unit. An acetyl group (δ 1.95) attached to C-6 of the β-glucopyranosyl unit with anomeric proton at δ 4.92, was evident since the resonances due to H_2-6 (δ 4.54, 4.65) were located at low field positions due to acetylation effect. Further supporting information came from the long-range HMBC coupling between H-6 (δ 4.65) and the acetyl carbonyl carbon at δ 170.6. The HMBC correlations between the signals due to H-1 (δ 6.19) of the terminal rhamnopyranosyl unit and C-2 (δ 75.9) of the α-arabinopyranosyl unit and between the signals due to H-1 (δ 5.11) of the α-arabinopyranosyl unit and C-3 (δ 81.2) of the aglycone established the attachment of the disaccharide chain at aglycone C-3. The structure of the trisaccharide unit at C-28 was based on correlations between signals due H-1 (δ 6.32) of the ester-linked β-glucopyranosyl unit and C-28 (δ 175.4) of the aglycone, H-1 (δ 4.92) of the outer β- glucopyranosyl unit and C-6 (δ 69.7) of the ester-linked β- glucopyranosyl unit, H-1 (δ 5.52) of the terminal α-rhamnopyranosyl unit and C-4 (δ 79.3) of the outer β-glucopyranosyl unit. The same conclusion with regard to the sugar sequence was also deduced from NOE studies. Thus, the structure of **4** was elucidated as 3-*O*-[αz-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl] 3β,23-dihydroxy olean-18-en-28-oic acid 28-*O*-[α-L- rhamnopyranosyl-(1→4)-β-D-6-*O*-acetyl glucopyranosyl-(1→6)-β-D-glucopyranosyl] ester. This is the first reported occurrence of an olean-18-en type saponin in family Araliaceae.

Compound **5** displayed in its FAB mass spectrum, a quasi-molecular ion peak $[M+Na]^+$ at m/z 1375 suggesting a molecular formula $C_{64}H_{104}O_{30}$. The assigned carbon signals in the ^{13}C NMR spectrum, due to the aglycone moiety, were in good agreement with those of hederagenin. The bisdesmosidic nature of **5** was revealed from the chemical shift values of aglycone C-3 and C-28. On acid hydrolysis, **5** yielded the sugar components L-arabinose, D-glucose, D-xylose and L-rhamnose. The 1D and 2D NMR spectra showed six anomeric signals at δ 5.10 (*d*, $J=6.2$ Hz); 104.4, 6.24 (*d*, $J=1.5$ Hz); 101.6, 6.10 (*d*, $J=8.0$ Hz); 93.4, 4.95 (*d*, $J=8.0$ Hz); 104.9, 5.81 (*brs*); 102.8 and 5.36 (*d*, $J=7.4$ Hz); 105.9. Extensive NMR (HOHAHA, ^{1}H–^{1}H COSY, NOE, HMQC, HMBC) studies enabled the assignments of the proton and the carbon resonances and showed the presence of an α-arabinopyranosyl unit, two β-glucopyranosyl units, two α-rhamnopyranosyl units and a β-xylopyranosyl unit. The β-anomeric configuration of the xylopyranosyl unit was evident from the large $^3J_{H\text{-}1,H\text{-}2}$ value (7.4 Hz). The anomeric configurations for the other sugar units were identical to the corresponding ones in compounds **1**–**4**. The exact positions of the sugar units were defined by the HMBC spectrum. The cross peaks due to long-range coupling between C-3 (δ 81.1) of the aglycone and H-1 (δ 5.10) of the α- arabinopyranosyl unit, C-2 (δ 75.9) of the α- arabinopyranosyl unit and H-1 (δ 6.24) of the terminal rhamnopyranosyl unit established the structure of the 3-*O*-glycosidic chain as α-L-rhamnopyranosyl-(1→2)-α- L-arabinopyranosyl. Similarly, the linkages of the tetrasaccharide chain at C-28, were established from the long-range coupling in the HMBC spectrum between C-28 (δ 176.6) of the aglycone and H-1 (δ 6.10) of the ester-linked β-glucopyranosyl unit, C-2 (δ 79.9) of the ester-linked β-glucopyranosyl unit and H-1 (δ 5.36) of the β-xylopyranosyl unit, C-6 (δ 69.0) of the ester-linked β-glucopyranosyl unit, and H-1 (δ 4.95) of the outer β-glucopyranosyl unit, C-4 (δ 78.6) of the outer

β-glucopyranosyl unit and H-1 (δ 5.81) of the terminal α-rhamnopyranosyl unit. Thus, the terminal β-xylopyranosyl unit and the outer β-glucopyranosyl unit were linked to C-2 and C-6 of the ester-linked β-glucopyranosyl unit respectively, whereas the other terminal α- rhamnopyranosyl unit was attached to C-4 of the outer β-glucopyranosyl unit. The same conclusion, with regard to the sugar sequence was also deduced from the observed inter-residue NOEs. Therefore, **5** was assigned the structure of 3-*O*-[α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl] hederagenin 28-*O*-{α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-[β-D-xylopyranosyl-(1→2)-]β-D-glucopyranosyl}ester.

The FAB mass spectrum of compound **6** showed its $[M+Na]^+$ ion peak at *m/z* 1405, 30 mass units higher than that of **5** and consistent with a molecular formula $C_{65}H_{106}O_{31}$. On acid hydrolysis, the sugar components L-arabinose, D-glucose and L-rhamnose were detected. The spectral features suggested that **6** was also a hederagenin hexasaccharide. Its bisdesmosidic nature was deduced from the chemical shift values of signals due to C-3 (δ 81.1) and C-28 (δ 176.7). The ^1H and ^{13}C NMR spectra coupled with HMQC spectrum showed the presence of six anomeric signals at δ 5.10 (*d*, *J*=6.2 Hz); 104.4, 6.25 (*d*, *J*=1.5 Hz); 101.6, 6.10 (*d*, *J*=8.0 Hz); 93.5, 4.95 (*d*, *J*=8.0 Hz) 104.9, 5.82 (*d*, *J*=1.5 Hz); 102.8 and 5.65 (*d*, *J*=8.0 Hz); 104.6. Signal assignments of the sugar protons and carbons were performed by NMR analysis (HOHAHA, ^1H–^1H COSY, NOE, HMQC, HMBC). Comparison between the sugar signals of **6** and **5** revealed similarity, except that, the signals due to the β-xylopyranosyl unit in **5** were replaced in **6** by a set of signals assigned to a terminal β-glucopyranosyl unit. The HMBC spectrum supported this observation and showed a correlation between H-1 (δ 5.65) of the terminal β-glucopyranosyl unit and C-2 (δ 78.4) of the ester-linked β-glucopyranosyl unit, whereas the other long-range correlations were the same as those previously reported for saponin **5**. Further support was obtained from the observed NOE between H-2 (δ 4.36) of the ester-linked β-glucopyranosyl unit and H-1 (δ 5.65) of the terminal β-glucopyranosyl unit. Likewise, the other inter-residue NOEs reported for saponin **5** were also observed in the NOE experiment of **6**. Based on the previous results the structure of **6** was established as 3-*O*-[α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl]hederagenin-28-*O*-{α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-[β-D-glucopyranosyl-(1→2)-]β-D-glucopyranosyl} ester.

Compound **7** had a molecular formula $C_{64}H_{104}O_{30}$, identical to that of **5**, as determined from the quasi-molecular ion peak $(M+Na)^+$ at *m/z* 1375 in its FAB mass spectrum. NMR analysis indicated that **7** was another hederagenin hexasaccharide partly differed in its sugar part from those of **5** and **6**. On acid hydrolysis, the sugar components L-arabinose, L-rhamnose and D-glucose were detected. The six anomeric signals were located at δ 5.12 (*d*, *J*=6.2 Hz); 104.3, 6.21 (*d*, *J*=1.5 Hz); 101.7, 6.09 (*d*, *J*=8.0 Hz); 94.4, 4.96 (overlapped); 104.8, 5.82 (*d*, *J*=1.5 Hz); 102.8, 6.36 (*d*, *J*=2.5 Hz); 109.1. From the assigned aglycone it was apparent that the six sugar units were present in two saccharide units, one attached to C-3 and the other to C-28. Extensive NMR studies (HOHAHA, ^1H–^1H COSY, NOE, HMQC, HMBC) enabled the assignments of the sugar proton and carbon resonances. Comparison between the assigned signals of **7** and those due to **5** and **6,** indicated that the only difference were the resonances due to the sugar unit attached to C-2 of the ester-linked β-glucopyranosyl unit. These resonances in **7** appeared at δ 6.36 (*d*, *J*=2.5 Hz); 109.1, 4.97; 82.5, 4.90; 77.8, 4.81; 86.0, 4.28 (*dd*, *J*=11.7, 3.7 Hz), 4.40 (*dd*, *J*=11.7, 4.3 Hz); 62.9 and assigned to a terminal α-L-arabinofuranosyl unit. The configuration at the anomeric centre of the arabinofuranosyl unit (*d*, *J*=2.5 Hz) was determined as α-based on the magnitude of the coupling constant of the anomeric proton. The previous information and the HMBC correlations together with the observed NOEs, led us to conclude that the structure of **7** was 3-*O*-[α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl]-hederagenin 28-*O*-{α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-[α-L-arabinofuranosyl-(1→2)-]β-D-glucopyranosyl} ester.

The known saponins **8** (Yakovishin et al., 1999), **9** (Wang et al., 1997), **10**, **11** (Wang et al., 1996), ciwujianoside C_3 **12**, ciwujianoside C_4 **14** (Shao et al., 1988), eleutheroside **13** (Frolova and Ovadov, 1971), **15** (Grishkovets et al., 1996), kalopanaxsaponin B **16** (Shao et al., 1989), **17** (Tomimori et al., 1987), were identified by analysis of their spectral data and by comparison with literature values.

3. Experimental

3.1. General

Optical rotations were measured with JASCO DIP 1000 digital polarimeter. MS were measured on a Jeol-JMX-SX 102 mass spectrometer. NMR spectra were obtained with a Jeol GSX-500 FT NMR spectrometer and chemical shifts are given in ppm with TMS as internal standard. GC was performed on a Hitachi G-3000 gas chromatograph. Preparative and analytical HPLC were performed on a JASCO model 800 instrument.

3.2. Plant material

The leaves and stems of *O. guatemalensis* Decne & Planch were collected from the zoological garden in Giza, Egypt in May 1997. Plant identification was confirmed by Mrs T. Labib, head of specialist for plant identification at El-Orman garden, Giza. The specimen

is deposited in the Chemistry of Natural Products Department, NRC.

3.3. Extraction and isolation

The dried and powdered leaves and stems (1 kg) were extracted twice with MeOH. The concentrated combined extract was diluted by addition of a large excess of Me_2CO to precipitate the crude saponins. The saponin mixture (30 g) was chromatographed on a silica gel column eluting with a stepwise increase of MeOH in $CHCl_3$ to give two fractions. Fraction 1 (10 g) was eluted with $CHCl_3$–MeOH 85:15–80:20. A portion (1.27 g) was subjected to preparative HPLC [column; ODS 5×100 cm, solvent; CH_3CN–H_2O (20:80)–(44:56) linear gradient, flow rate; 45 ml/min, UV; 205 nm and column; ph A-5 2×25 cm, solvent; CH_3CN–H_2O (27.5:72.5) + 0.05% TFA, flow rate; 6.5 ml/min, UV; 205 nm to give **1** (5 mg), **2** (9 mg), **3** (5 mg), **5** (13 mg), **8** (97 mg), **10** (5 mg), **11** (5 mg), **12** (20 mg), **13** (40 mg), **14** (44 mg), **15** (63 mg), **16** (78 mg) and **17** (38 mg). Fraction 2 (15 g) was eluted with $CHCl_3$–MeOH 80:20–73:27. A portion (6.7 g) was dissolved in water and the solution was passed through a porous polymer gel Mitsubishi Diaion HP-20 column (4×16 cm). The column was washed with 2 l H_2O (eluate 1.47 g) and the adsorbed material was eluted with 1.3 l MeOH (eluate 4.77 g). A portion (2.4 g) of the methanol eluate was subjected to preparative HPLC utilizing the same columns and conditions used in separating fraction 1, to give further amounts of **1** (21 mg), **4** (5 mg), **5** (94 mg), **6** (12 mg), **7** (6 mg), **9** (5 mg), **11** (21 mg), **13** (133 mg), **14** (96 mg) together with **16** (418 mg) and **17** (337 mg).

3.4. Saponin (*1*)

Amorphous powder $[\alpha]_D^{23}$ −22.9° (*c* 0.49, MeOH). FAB–MS (*m*/*z*): 1097 $[C_{53}H_{86}O_{22} + Na]^+$. ^1H and ^{13}C NMR: see Tables 1 and 2.

3.5. Saponin (*2*)

Amorphous powder $[\alpha]_D^{23}$ −103.1° (*c* 0.36, MeOH). FAB–MS (*m*/*z*): 1109 $[C_{54}H_{86}O_{22} + Na]^+$. ^1H and ^{13}C NMR: see Tables 1 and 2.

3.6. Saponin (*3*)

Amorphous powder $[\alpha]_D^{23}$ −94.2° (*c* 0.26, MeOH). FAB–MS (*m*/*z*): 1255 $[C_{60}H_{96}O_{26} + Na]^+$. ^1H and ^{13}C NMR: see Tables 1 and 2.

3.7. Saponin (*4*)

Amorphous powder $[\alpha]_D^{23}$ −15.9° (*c* 0.33, MeOH). FAB–MS (*m*/*z*): 1285 $[C_{61}H_{98}O_{27} + Na]^+$. ^1H and ^{13}C NMR: see Tables 1 and 2.

3.8. Saponin (*5*)

Amorphous powder $[\alpha]_D^{23}$ −29.5° (*c* 0.29, MeOH). FAB–MS (*m*/*z*): 1375 $[C_{64}H_{104}O_{30} + Na]^+$. ^1H and ^{13}C NMR: see Tables 1 and 3.

3.9. Saponin (*6*)

Amorphous powder $[\alpha]_D^{23}$ −28.2° (*c* 1.09, MeOH). FAB–MS (*m*/*z*): 1405 $[C_{65}H_{106}O_{31} + Na]^+$. ^1H and ^{13}C NMR: see Tables 1 and 3.

3.10. Saponin (*7*)

Amorphous powder $[\alpha]_D^{23}$ −37.6° (*c* 0.61, MeOH). FAB–MS (*m*/*z*): 1375 $[C_{64}H_{104}O_{30} + Na]^+$. ^1H and ^{13}C NMR: see Tables 1 and 3.

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

Each saponin (2 mg) dissolved in dioxane (50 μl) and 2M HCl (50 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 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, hederagenin from **5–7** (t_R, 7.8 min); echinocystic acid from **1** (t_R, 6.3 min); the aglycone from **2** and **3** (t_R, 14.1 min); the aglycone from **4** (t_R, 7.1 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 (25 μl) and stirred with D-cysteine methyl ester (3.0 mg) for 1.5 h at 60 °C. To the reaction mixture, hexamethyldisilazan (10 μl) and trimethylsilyl chloride (10 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, 0.25×30 m, column temperature; 235 °C, carrier gas; N_2, retention time D-Glc (16.8 min), L-Glc (16.3 min.), D-Xyl (10.1 min), L-Xyl (9.8 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 D-glucose, D-xylose, L-arabinose and L-rhamnose were detected.

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