PERGAMON

Phytochemistry 54 (2000) 853–859

PHYTOCHEMISTRY

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Triterpenoid saponins from *Fagonia cretica*

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Received 23 June 1999; received in revised form 5 May 2000

Abstract

Four new triterpenoid saponins were isolated and identified from the aerial parts of *Fagonia cretica*. They were characterized as 3-*O*-[β-D-glucopyranosyl (1 → 2)-α-L-arabinopyranosyl] hederagenin 28-*O*-β-D-glucopyranosyl ester, 3-*O*-[β-D-glucopyranosyl (1 → 2)-α-L-arabinopyranosyl] oleanolic acid 28-*O*-[β-D-glucopyranosyl (1 → 6)-β-D-glucopyranosyl] ester, 3-*O*-[β-D-glucopyranosyl (1 → 2)-α-L-arabinopyranosyl] 27-hydroxy oleanolic acid 28-*O*-[β-D-glucopyranosyl (1 → 6)-β-D-glucopyranosyl] ester and 3β-*O*-[β-D-glucopyranosyl (1 → 2)-α-L-arabinopyranosyl] olean-12-en-27-al-28-oic acid 28-*O*-[β-D-glucopyranosyl (1 → 6)-β-D-glucopyranosyl] ester. The structures of the saponins were assigned by spectral analyses (FABMS, ^{1}H, ^{13}C NMR, ^{1}H–^{1}H COSY, TOCSY, HMQC and HMBC spectra) and NOE experiments. To the best of our knowledge the genin 3β hydroxy olean-12-en-27-al-28-oic acid is new.

Keywords: *Fagonia cretica*; Zygophyllaceae; Triterpenoid saponins

1. Introduction

The genus *Fagonia* is represented by 18 species in the flora of Egypt (Tackholm, 1974). In an earlier study, flavonoids were identified from some *Fagonia* species (Saleh et al., 1990). The saponin-containing fraction from *Fagonia cretica* showed significant anti-inflammatory activity and considerable analgesic and antipyretic effects (El-Shabrawy et al., 1997).

2. Results and discussion

Fagonia cretica was previously studied by us for its saponin content and three bisdesmosides of hederagenin and oleanolic acid were isolated and identified (Melek et al., 1994). Further examination of this plant led to the isolation of four additional new saponins **1**–**4**. Their structures were determined using mass spectrometry, 1D and 2D NMR spectroscopy as well as NOE experiments. The NMR data are presented in Tables 1 and 2.

On acid hydrolysis, saponin **1** afforded an aglycone that was identified as hederagenin by comparison with an authentic sample, along with sugar components identified as L-arabinose and D-glucose. In the FAB mass spectrum, a quasimolecular ion peak was observed at *m*/*z* 951 corresponding to $[C_{47}\ H_{76}\ O_{18} + Na]^+$. The spectrum also showed an intense ion peak at *m*/*z* 789 corresponding to loss of one hexose unit. In the ^{1}H NMR spectrum, the presence of three sugar residues was deduced from the observation of three anomeric proton signals at δ 5.15 (*d*, $J = 6.0$ Hz), 5.17 (*d*, $J = 8.0$ Hz) and 6.31 (*d*, $J = 8.0$ Hz). The proton system of each sugar unit was analyzed from the TOCSY spectrum and the sequence of protons in each residue was then deduced from ^{1}H–^{1}H COSY experiment. A ^{1}H–^{13}C one bond chemical shift correlation experiment (HMQC) correlated all proton resonances with those of the corresponding carbons.

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0031-9422/00/$ - see front matter
PII: S0031-9422(00)00168-0

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

	1	**2**	**3**	**4**
Aglycone				
1	1.03	0.92	0.89	0.85
	1.56	1.47	1.52	1.52
2	1.97	1.80	1.83	1.78
	2.16	2.01	2.08	2.06
3	4.14	3.07 (*dd*, 12.0, 4.0)	3.20 (*dd*, 12.7, 4.0)	3.11 (*dd*, 11.7, 3.9)
5	1.58	0.82	0.73 (*brd*, 12.0)	0.56 (*brd*, 12.0)
6	1.36	1.33	1.32	1.22
	1.70	1.44	1.45	1.36
7	1.27	1.44	1.31	1.21
		1.83		1.56
9	1.75	2.15	1.60 (*dd*, 12.0,7.5)	1.72
11	1.93	1.95	1.92	2.08
12	5.43 (*t*,3.3)	5.79 (*t*,3.3)	5.42 (*t*,3.3)	5.94 (*t*, 3.5)
15	1.10 (*brd*,13.3)	1.44	1.16 (*brd*, 14.0)	2.17
	2.31 (*dt*, 13.3, 3.5)	2.30 (*dt*, 14.0, 3.3)	2.31 (*dt*, 14.0, 3.3)	2.27 (*brd*, 14.7)
16	1.96	1.96	1.97	1.98
	2.04 (*dt*,13.3, 3.0)	2.10 (*dt*,14.0, 3.3)	2.10	2.15
18	3.19 (*dd*,14.7,4.0)	3.28 (*dd*, 14.7, 3.3)	3.20 (*dd*, 12.7, 4.0)	3.22 (*dd*, 14.3, 3.9)
19	1.23	1.32	1.26	1.06
	1.75	1.72	1.78	1.20
21	1.08	1.10		1.02
	1.33	1.28		1.18
22	1.73	1.73	1.75	1.70
	1.84	1.94	1.94	1.85 (*dt*, 15.0, 4.0)
23	3.73 (*d*, 10.0)	1.16 (*s*)	1.22 (*s*)	1.09 (*s*)
	4.20			
24	1.02 (*s*)	1.02 (*s*)	1.04 (*s*)	0.98 (*s*)
25	0.97 (*s*)	0.90 (*s*)	0.90 (*s*)	0.85 (*s*)
26	1.12 (*s*)	1.11 (*s*)	1.10 (*s*)	1.10 (*s*)
27	1.18 (*s*)	3.75 (*d*,10.4)	1.24 (*s*)	9.90 (*s*)
		4.01		
29	0.90 (*s*)	0.83 (*s*)	0.90 (*s*)	0.76 (*s*)
30	0.89 (*s*)	0.91 (*s*)	0.90 (*s*)	0.85 (*s*)
C-3 sugar				
Ara				
1	5.15 (*d*, 6.0)	4.92 (*d*, 6.0)	4.96 (*d*, 6.0)	4.90 (*d*, 5.9)
2	4.55 (*t*, 6.3)	4.55 (*t*, 6.0)	4.57 (*t*, 6.0)	4.55 (*t*, 6.5)
3	4.24	4.35	4.35	4.35
4	4.29	4.37	4.37	4.37
5	3.70 (*d*, 11.3)	3.8 (*d*, 10.0)	3.78 (*d*,10.0)	3.78 (*d*, 10.0)
	4.27	4.28	4.28	4.28
GlcI				
1	5.17 (*d*, 8.0)	5.16 (*d*, 8.0)	5.17 (*d*, 8.0)	5.16 (*d*, 7.8)
2	4.05 (*t*, 8.8)	4.07 (*t*, 8.7)	4.07 (*t*, 8.0)	4.07 (*t*, 9.0)
3	4.15 (*t*, 9.0)	4.17 (*t*, 9.0)	4.17 (*t*, 9.0)	4.17 (*t*,9.5)
4	4.20 (*t*, 8.0)	4.31 (*t*, 9.5)	4.30 (*t*, 8.5)	4.29 (*t*, 9.0)
5	3.8 *m*	3.80 *m*	3.8 *m*	3.8 *m*
6	4.34 (*dd*, 10.7, 3.0)	4.41 (*d*, 3.3)	4.41 (*d*, 3.3)	4.39 (*d*, 3.3)
	4.44(*brd*, 10.7)			
C-28 sugar				
GlcII				
1	6.31 (*d*, 8.0)	6.26 (*d*, 8.0)	6.24 (*d*, 8.5)	6.27 (*d*, 7.8)
2	4.17 (*t*, 8.0)	4.14 (*t*, 8.0)	4.13 (*t*, 8.0)	4.13 (*t*, 8.3)
3	4.25 (*t*, 9.0)	4.21 (*t*, 8.5)	4.20 (*t*, 8.5)	4.21 (*t*, 9.0)
4	4.33 (*t*, 8.0)	4.31 (*t*, 8.5)	4.31 (*t*, 8.5)	4.32 (*t*, 9.0)
5	4.01 *m*	4.13 *m*	4.11 *m*	4.13 *m*

Table 1 (*continued*)

	1	2	3	4
6	4.37 (*dd*, 10.7, 3.0) 4.45(*dd*, 11.0, 2.0)	4.36 (*dd*, 11.0, 3.0) 4.70 (*dd*, 11.0, 1.5)	4.36 (*dd*, 11.0, 3.0) 4.70 (*dd*, 11.0, 1.5)	4.35 (*dd*, 11.7,3.0) 4.72 (*dd*, 11.7, 1.5)
GlcIII				
1		5.03 (*d*, 8.0)	5.02 (*d*, 8.0)	5.03 (*d*, 8.0)
2		3.99 (*t*, 8.0)	3.99 (*t*, 8.7)	3.99 (*t*, 8.0)
3		4.17 (*t*, 8.5)	4.17 (*t*, 8.5)	4.17 (*t*, 8.5)
4		4.19 (*t*, 9.0)	4.19 (*t*, 9.0)	4.19 (*t*, 9.0)
5		3.88 *m*	3.88 *m*	3.88 *m*
6		4.35 (*dd*, 11.0,3.5) 4.47 (*dd*, 11.0,2.0)	4.35 (*dd*, 12.0, 3.5) 4.47 (*dd*, 12.0, 2.0)	4.35 (*dd*, 11.0, 3.5) 4.47 (*dd*, 11.0, 2.0)

[a] Overlapped signals are presented without coupling constant values. Ara = α-L-arabinopyranosyl; Glc = β-D-glucopyranosyl.

Comparison of ^{13}C assignments with those of reference methyl glycosides revealed the presence of two terminal β-D-glucopyranosyl units and one 2-substituted-α-L-arabinopyranosyl moiety. The ^{13}C NMR signal due to C-28 of the aglycone moiety at δ 176.4 together with signals relevant to terminal β-D-glucopyranosyl ester unit (δ 95.8, 74.2, 78.9, 71.3, 79.3 and 62.4), indicated esterification of the aglycone carboxyl group with this unit. The remaining two sugars must be present as a chain attached at C-3 position (δ 82.3). The (1 → 2) linkage between the terminal β-D-glucopyranosyl unit and the inner α-L-arabinopyranosyl residue was confirmed by observing long range C–H connectivities from heteronuclear multiple bond correlation spectrum (HMBC). The cross peaks observed were between the carbon signal of C-28 of the aglycone moiety at δ 176.4 and the anomeric proton signal of β-D-glucopyranosyl ester unit II at δ 6.31, between the carbon signal of C-3 of the aglycone moiety at δ 82.3 and the anomeric proton signal of the α-L-arabinopyranosyl unit at δ 5.15 and between the carbon signal of C-2 of the α-L-arabinopyranosyl unit at δ 81.4 and the anomeric proton signal of the β-D-glucopyranosyl unit I at δ 5.17. Regarding the hederagenin moiety, most of the proton signals in the ^1H NMR spectrum were located in the high field between 1.0 and 2.3 ppm showing highly complex multiplets. Some shift assignments were made possible by observing cross peaks correlations in the ^1H–^1H COSY spectrum. However, it was found more convenient to assign the proton shifts in conjunction with the ^{13}C data by consideration of HMQC and HMBC spectra. The most intense signals in the HMBC spectrum were correlations from the methyl groups hydrogen. From these many checks on the assignments were allowed. Based on the results of the above studies, saponin **1** was assigned the structure of 3-*O*-[β-D-glucopyranosyl (1 → 2)-α-L-arabinopyranosyl] hederagenin 28-*O*-β-D-glucopyranosyl ester.

Acid hydrolysis of saponin **2** afforded L-arabinose and D-glucose. The ^1H NMR and ^1H–^1H COSY spectra of saponin **2** showed the presence of six methyl singlets at δ 0.83, 0.90, 0.91, 1.02, 1.11, 1.16, two signals for oxymethylene group at δ 3.75 and 4.01 and two deshielded proton signals due to H-18 at δ 3.28 (*dd*, $J = 14.7,3.3$Hz) and H-12 at δ 5.79 (*t*, $J = 3.3$ Hz) characteristic for triterpene acid moiety of olean-12-en-28-oic type. Four anomeric proton signals were also observed. The three doublets at δ 5.03 ($J = 8.0$ Hz), 5.16 ($J = 8.0$ Hz) and 6.26 ($J = 8.0$ Hz) are due to the anomeric protons of D-glucoses. Their coupling constant values determined the β-glycosidic linkage. The doublet remaining at δ 4.92 ($J = 6.0$ Hz) was attributed to the anomeric proton of L-arabinose and confirming the α linkage. In the ^{13}C NMR spectrum, the anomeric carbon signal at δ 95.8 and the signal due to C-28 at δ 176.6 together with the signal at δ 88.8 assignable for C-3 suggested that **2** was a 3, 28 bisdesmoside. The presence of deshielded signals, due to methylene C-6 of glucose and C-2 of arabinose at δ 69.5 and 80.9, respectively, suggested glycosylation at these positions. Alkaline hydrolysis of **2** yielded D-glucose as the sole sugar component. Considering the above observations, saponin **2** possesses a disaccharide chain composed of two glucose units bonded to the C-28 carbonyl group by an ester linkage. The remaining two sugar moieties, linked to C-3 aglycone by a glycosidic bond, are one arabinose and one glucose. Complete assignments of each sugar proton system were achieved by considering TOCSY and ^1H–^1H COSY spectra, while the carbons were assigned from HMQC and HMBC spectra. Comparison of the ^{13}C assignments with those of methyl glycosides disclosed the presence of two terminal β-D-glucopyranosyl units, a 6-substituted β-D-glucopyranosyl and a 2-substituted α-L-arabinopyranosyl moieties. Information about the sequence of the monosaccharide units and the linkage sites was further obtained using NOE experiment. Irradiation of the anomeric proton signal of the β-D-glucopyranosyl unit III at δ 5.03 enhanced the hydroxymethylene proton signals of the β-D-glucopyra-

Table 2
^{13}C NMR spectral data of compounds **1**–**4** in pyridine-d_5[a]

	1	2	3	4
Aglycone				
1	38.9	38.7	38.8	38.5
2	26.1	26.4	26.5	26.4
3	82.3	88.8	88.9	88.4
4	43.5	39.5	39.6	39.4
5	48.0	55.7	55.9	55.8
6	18.3	18.7	18.6	18.3
7	32.9	33.6	33.2	35.7
8	40.0	40.6	40.0	41.9
9	48.2	48.7	48.1	49.7
10	37.0	37.2	37.0	37.4
11	23.9	24.4	23.9	24.1
12	123.0	128.0	123.3	128.4
13	144.2	139.2	144.2	136.9
14	42.2	48.0	42.2	59.1
15	28.3	23.9	28.3	20.9
16	23.5	23.5	23.5	23.4
17	47.0	46.9	47.1	46.7
18	41.8	41.5	41.8	42.2
19	46.3	45.4	46.3	43.6
20	30.8	30.8	30.8	30.6
21	34.1	33.9	34.1	33.6
22	32.6	32.6	32.6	32.0
23	65.0	28.1	28.3	28.0
24	13.4	16.7	16.8	16.7
25	16.2	16.1	15.7	16.2
26	17.6	19.0	17.6	18.3
27	26.0	64.5	26.1	207.3
28	176.4	176.6	176.6	176.3
29	33.1	33.1	33.2	32.9
30	23.7	23.9	23.7	23.9
C-3 sugar				
Ara				
1	103.9	104.7	104.8	104.8
2	81.4	80.9	81.0	81.0
3	73.7	73.4	73.4	73.4
4	68.3	68.2	68.2	68.3
5	65.0	64.8	64.8	65.0
Glc I				
1	106.0	106.0	106.0	106.0
2	76.2	76.3	76.4	76.4
3	78.3	78.1	78.2	78.2
4	71.5	71.7	71.7	71.7
5	78.3	78.2	78.0	78.0
6	62.6	62.6	62.7	62.7
C-28 sugar				
Glc II				
1	95.8	95.8	95.7	95.7
2	74.2	73.9	74.0	74.0
3	78.9	78.8	78.8	78.8
4	71.3	71.1	71.1	71.1
5	79.3	78.0	78.0	78.1
6	62.4	69.5	69.6	69.6
Glc III				
1		105.4	105.3	105.3
2		75.2	75.2	75.2

Table 2 (*continued*)

	1	2	3	4
3		78.5	78.5	78.5
4		71.7	71.7	71.7
5		78.5	78.5	78.5
6		62.7	62.8	62.7

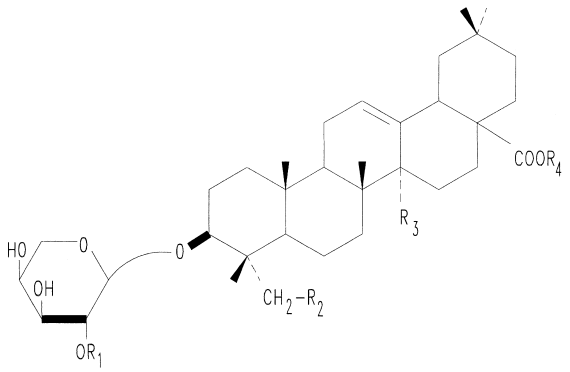
[a] Ara = α-L-arabinopyranosyl; Glc = β-D-glucopyranosyl.

nosyl ester unit II at δ 4.36 and 4.70. Irradiation of the anomeric proton signal of the β-D-glucopyranosyl unit I at δ 5.16 enhanced the proton signal of H-2 of the α-L-arabinopyranosyl unit at δ 4.55. Irradiation of the anomeric proton signal of α-L-arabinopyranosyl unit at δ 4.92 enhanced the proton signal of H-3 of the aglycone moiety at δ 3.67. The above results supported the (1 → 6) connection between the two β-D-glucopyranosyl units of the 28-*O* sugar chain and the (1 → 2) attachment between the terminal β-D-glucopyranosyl and the inner α-L-arabinopyranosyl units in the 3-*O*-sugar chain. This conclusion was further confirmed by HMBC experiment in which the cross peaks observed were between the carbon signal of C-28 of the aglycone moiety at δ 176.6 and the anomeric proton signal of the β-D-glucopyranosyl ester unit II at δ 6.26, between the anomeric carbon signal of β-D-glucopyranosyl unit III at δ 105.4 and the hydroxymethylene proton signals of the β-D-glucopyranosyl ester unit II at δ 4.36 and 4.70, between the carbon signal of C-2 of the α-L-arabinopyranosyl unit at δ 80.9 and the anomeric proton signal of the β-D-glucopyranosyl unit I at δ 5.16 and between the carbon signal of C-3 of the aglycone moiety at δ 88.8 and the anomeric proton signal of the α-L-arabinopyranosyl unit at δ 4.92. The NMR data of the aglycone moiety are almost identical to those of the rare sapogenin 27-hydroxy oleanolic acid previously reported from *Tetrapleura tetraptera* (Maillard et al., 1992) and *Fagonia arabica* (Miyase et al., 1996). Unambiguous assignments of NMR signals were made possible from ^1H–^1H COSY, HMQC and HMBC spectra. The definitive identification of C-11, C-15, C-16 could be achieved. The differentiation between C-7, C-21 was made possible by correlating C-7 with H_3-26 and C-21 by H_3-29 and H_3-30 in the HMBC spectrum. The cross peak correlating the proton signal at δ 3.75 (H-27) with the carbon signal at δ 64.5 (C-27) in the HMQC spectrum and with the carbon signal at δ 23.9 (C-15) in the HMBC spectrum supported the aglycone structure. The FAB mass spectrum exhibited a $[M + Na]^+$ ion at *m/z* 1113 $[C_{53}\ H_{86}\ O_{23} + Na]^+$ along with fragment ion at *m/z* 1081 due to the loss of CH_3OH (Hamburger and Hostettmann, 1986). Therefore, saponin **2** was assigned the structure of 3-*O*-[β-D-glucopyranosyl (1 → 2) α-L-arabinopyranosyl] 27-hydroxy oleanolic acid 28-*O*-[β-D-glucopyranosyl (1 → 6) β-D-glucopyranosyl] ester.

On acid hydrolysis, saponin **3** yielded oleanolic acid and the sugar components D-glucose and α-arabinose were released. The alkaline hydrolysis afforded D-glucose. Apart from the typical δ values due to 3, 28 disubstituted oleanolic acid moiety, the 1D and 2D NMR spectra (TOCSY, ^1H–^1H COSY, HMQC and HMBC) of **3** showed δ values attributable to sugar units identical to those of **2**. Irradiation at each anomeric proton signal in the NOE experiment and the observed cross peaks in the HMBC spectrum, indicated that saponins **2** and **3** were identical regarding sugar sequencing and connections. The FAB mass spectrum supported these observations and exhibited a $[M + Na]^+$ ion at *m/z* 1097 $[C_{53}\ H_{86}\ O_{22} + Na]^+$. Therefore saponin **3** was formulated as 3-*O*-[β-D-glucopyranosyl (1 → 2)-α-L-arabinopyranosyl] oleanolic acid-28-*O*-[β-D-glucopyranosyl (1 → 6)-β-D-glucopyranosyl] ester.

The ^1H NMR spectrum of saponin **4** showed the existence of six methyl singlets at δ 1.09, 0.98, 0.85, 1.10, 0.76 and 0.85 assigned for H_3-23, H_3-24, H_3-25, H_3-26, H_3-29 and H_3-30 of triterpenoid moiety of oleanane type, respectively. These signals were correlated with their corresponding carbon signals at δ 28.0, 16.7, 16.2, 18.3, 32.9 and 23.9 in the HMQC spectrum. Saponin **4** also exhibited a triplet for olefinic proton H-12 which appeared at low field position (δ 5.94) in its ^1H NMR spectrum, and signals for double bond at δ 128.4 and 136.9 in the ^{13}C NMR spectrum. Two carbonyl signals, resonating at δ 176.3 and 207.3 were also shown in the ^{13}C NMR spectrum and assigned for 28-glycosyl ester carbonyl and aldehydic functions, respectively. These observations led to the conclusion that one of the seven methyl groups generally present in triterpene acid moiety with olean-12-en-28 oate skeleton is in the form of aldehydic function (^1H NMR; δ 9.9). With respect to this type of triterpene, the signals due to C-13 is shifted upfield by 7.3 ppm and the C-12 appeared at 5.1 ppm more deshielded in saponin **4**. Similar shift values were also observed for saponin **2** and other Δ^{12} triterpenes and sapogenins of oleanene type bearing COOH or CH_2OH group at C-14 (Ghulam and Al-Hazimi, 1987). This observation indicated that the aldehyde function is in the close proximity of

the double bond and located at C-14 instead of H_3-27. The glycosyl ester function was proposed to be at C-28 in view of ^{13}C NMR spectrum, which showed δ values for C-16, C-17 and C-22 similar to those of 3, 28 di-*O*-glycoside saponins **1**–**3** present in the same plant. Observation of long range C-H coupling between the carbonyl aldehyde (δ 207.3) and H-15 (δ 2.17) confirmed that H_3-27 was oxidized to CHO. The assignments of NMR signals were allowed following the 2D NMR techniques used in the case of saponin **1**, **2** and **3**. The shift values of C-3 at δ 88.4 and C-28 at δ 176.3 indicated that **4** is 3, 28 bisdesmoside. The stereochemistry at C-3 of the aglycone moiety was determined from the coupling constant values of H-3 at δ 3.11 (*dd*, $J =$ 11.7,3.9 Hz) which indicated axial (α) orientation and therefore equatorial (β) orientation for the substituted OH. The similarity between the shift values of C-3 signal of **4** and the corresponding in **2** and **3**, further supported the deduced stereochemistry. The acid and alkaline hydrolysis of **4** afforded identical sugar components to those obtained from **2** and **3** and the 2D NMR techniques (^1H–^1H COSY, TOCSY, HMQC and HMBC) and NOE experiment suggested identical monosaccharide units, sequencing and linkages. The FAB mass spectrum was consistent with the above conclusions and displayed a $[M + Na]^+$ ion at *m/z* 1111 $[C_{53}\ H_{84}\ O_{23} + Na]^+$; less than that of **2** by 2 mu. The spectrum also showed the presence of fragment ion at *m/z* 1083 accounted for the elimination of CO fragment. Thus the structure of saponin **3** was deduced to be 3 β-*O*-[β-D-glucopyranosyl (1 → 2)-α-L-arabinopyranosyl] olean-12-en-27-al-28-oic-acid 28-*O*-[β-D-glucopyranosyl (1 → 6)-β-D-glucopyranosyl] ester. To the best of our knowledge its sapogenin 3β-hydroxy olean-12-en-27-al-28-oic acid is new.



	R_1	R_2	R_3	R_4
1-	Glc	OH	CH3	Glc
2-	Glc	H	CH2OH	Glc(1→6)Glc
3-	Glc	H	CH3	Glc(1→6)Glc
4-	Glc	H	CHO	Glc(1→6)Glc

Glc=β–D– glucopyranosyl

3. Experimental

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 JEOL GSX-500 FT NMR spectrometer and chemical shifts were given in ppm with TMS as international standard. HPLC was carried out on a JASCO system 800 instrument.

For plant material extraction, refer to our earlier paper (Melek et al., 1994). The fraction eluted with $CHCl_3$:MeOH:H_2O (67:30:3) yielded saponin mixture. The mixture was chromatographed on HPLC Develosil Ph A-5 column (2 × 25 cm) and Develosil ODS-10 (2 × 25 cm) using MeCN–H_2O system to give saponins **1** (50 mg), **2** (48 mg), **3** (42 mg) and **4** (43 mg).

Saponin **1**. Amorphous powder $[\alpha]_D^{23}$ +30.0° ($c = 2.37$, MeOH). FABMS (*m/z*): 951 $[C_{47}\ H_{76}\ O_{18} + Na]^+$, 789, 743, 437, 248, 203. ^1H and ^{13}C NMR: see Tables 1 and 2.

Saponin **2**. Amorphous powder $[\alpha]_D^{23}$ +4.2° ($c = 1.46$,MeOH). FABMS (*m/z*) 1113 $[C_{53}\ H_{86}\ O_{23} + Na]^+$, 1081, 926, 820, 750, 460. ^1H, ^{13}C NMR: see Tables 1 and 2.

Saponin **3**. Amorphous powder $[\alpha]_D^{23}$ +7.0° ($c = 1.48$ MeOH). FABMS (*m/z*) 1097 $[C_{53}\ H_{86}\ O_{22} + Na]^+$, 964, 774, 439, 307. ^1H, ^{13}C NMR: see Tables 1 and 2.

Saponin **4**. Amorphous powder $[\alpha]_D^{23}$ +42.3° ($c = 0.70$, MeOH). FABMS (*m/z*) 1111 $[C_{53}\ H_{84}\ O_{23} + Na]^+$, 1083, 774, 439, 307. ^1H, ^{13}C NMR: see Tables 1 and 2.

3.1. General method for acid hydrolysis

Each saponin (2 mg), dissolved in dioxane (50 μl) and 2 M HCl (100 μl) was heated at 100°C for 30 min. The reaction mix. was diluted with water and extracted with EtOAc. The produced aglycone was detected by TLC against authentic sample using various solvent systems and its identity was confirmed by MS. The monosaccharide units in the aq. layer were analyzed (Hara et al., 1986) as follows: the aq. layer of the reaction mix. for each saponin was passed through an Amberlite IRA-60E column (6 × 50 mm), and the eluate was concentrated. Each monosaccharide fraction was dissolved in pyridine (50 μl) and stirred with D-

cysteine methyl ester (6 mg) for 1.5 h at 60°C and then overnight at room temperature. To the reaction mix., hexamethyldisilazane (20 μl) and trimethylsilyl chloride (20 μl) were added, and the reaction mix. was stirred at 60°C for 30 min. The supernatant of the mix. was then analyzed by GC, conditions: column, Supelco SPB-TM1 (0.25 mm × 27 m); column temperature, 215°C; carrier gas, N_2; retention times, D-arabinose (11.0 min.), L-arabinose (11.9 min), D-glucose (21.4 min.) and L-glucose (20.5 min). From saponins **1**–**4** L-arabinose and D-glucose were detected.

3.2. General method for alkaline hydrolysis

A solution of saponin (2 mg) in 5% aq. KOH (3 ml) was heated at 100°C for 30 min. The reaction mix. was neutralized with 5% HCL and then extracted with EtOAc. The residual aq. layer containing the sugar components was analyzed as shown earlier.

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