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Saponins from *Fagonia glutinosa*

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Twelve triterpenoid saponins, including six new, were isolated and identified from the aerial parts of *Fagonia glutinosa*. The new saponins were characterised as 3-*O*-[β-D-glucopyranosyl(1→2)][β-D-glucopyranosyl(1→3)]-α-L-arabinopyranosyl-27-hydroxy oleanolic acid 28-*O*-β-D-glucopyranosyl ester, 3-*O*-[β-D-glucopyranosyl(1→3)]-α-L-arabinopyranosyl ursolic acid, 3-*O*-α-L-arabinopyranosyl ursolic acid 28-*O*-β-D-glucopyranosyl ester, 3-*O*-[β-D-xylopyranosyl(1→2)][β-D-glucopyranosyl(1→3)]-α-L-arabinopyranosyl ursolic acid, 3-*O*-[β-D-glucopyranosyl(1→2)][β-D-glucopyranosyl(1→3)]-α-L-arabinopyranosyl ursolic acid 28-*O*-β-D-glucopyranosyl ester and 3-*O*-[β-D-glucopyranosyl(1→2)][β-D-glucopyranosyl(1→3)]-α-L-arabinopyranosyl-27-hydroxy ursolic acid 28-*O*-β-D-glucopyranosyl ester. The structures of the saponins were established by spectral and chemical evidences. The assignments of the NMR signals were performed by means of HOHAHA, ^{1}H-^{1}H COSY, ROE, HMQC and HMBC experiments.

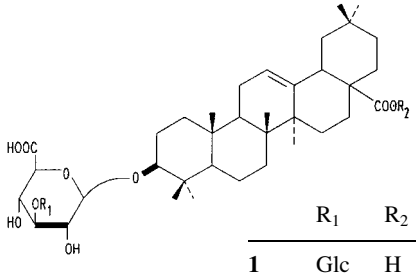
1. Introduction

Fagonia glutinosa (Zygophyllaceae) is widely distributed in Egypt and used in folk medicine for treatment of skin lesions. The chemistry of flavonoids and diterpenes of this plant was previously reported [1–3]. In continuation with our investigations on saponins from the genus *Fagonia* [4–6], we report the isolation of 12 saponins from the aerial parts of *F. glutinosa.* Six of these saponins are new compounds.

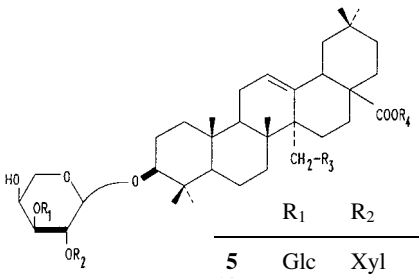
2. Investigations, results and discussion

The saponin containing fraction eluted with dichloromethane-methanol mixture gave after VLC, two fractions. The less polar fraction was chromatographed on a polymer gel column (Mitsubishi Diaion HP20) and the absorbed material was eluted with methanol to afford, after HPLC, the saponins **5–7, 9–12.** The other fraction was passed through an amberlite IR-120 column followed by methylation with diazomethane. The methylated derivatives of saponins **1–4** and **8** were obtained after CC and HPLC. The NMR data of the new saponins **6, 9–12** and **8** methyl ester are shown in Tables 1 and 2.

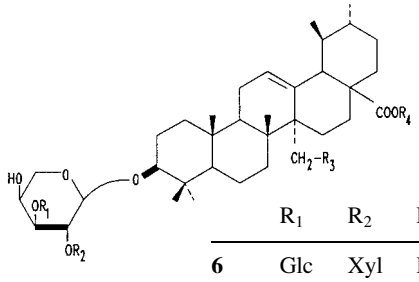
On acid hydrolysis, Saponin **10** yielded ursolic acid and the sugar components L-arabinose and D-glucose. The ^{1}H NMR spectrum showed the presence of four anomeric proton signals at δ 4.79 (d, J = 6.6 Hz), 5.27 (d, J = 8.0 Hz), 5.49 (d, J = 8.0 Hz) and 6.25 (d, J = 8.0 Hz) for L-arabinose and three D-glucose units respectively. Their attached carbons were located in the HMQC spectrum at δ 105.5, 105.0, 104.4 and 95.8, respectively. The proton system of each sugar unit was analysed from the HOHAHA spectrum and the sequence of protons in each residue was then deduced from an ^{1}H-^{1}H COSY experiment. A HMQC experiment correlated all proton resonances with the corresponding carbons. Comparison of the ^{13}C assignments with those of reference methyl glycosides revealed the presence of three terminal β-D-glucopyranosyl units and one 2,3-disubstituted α-L-arabinopyranosyl moiety. Although the glucose units need not to be determined for their sequencing, the site of attachment of each unit was determined from the HMBC spectrum. The observed correlations were between signals at δ 176.2 (C-28 of Ag)/6.25 (H-1 of Glc III); 89.1 (C-3 of Ag)/4.79 (H-1 of Ara); 105.0 (C-1 of Glc I)/4.31 (H-3 of Ara) and 104.4 (C-1 of Glc II)/4.72 (H-2 of Ara). These observations in-



	R_1	R_2
1	Glc	H
2	Xyl	H
3	H	Glc
4	Glc	Glc



	R_1	R_2	R_3	R_4
5	Glc	Xyl	H	Glc
12	Glc	Glc	OH	Glc



	R_1	R_2	R_3	R_4
6	Glc	Xyl	H	H
7	Glc	H	H	Glc
8	Glc	H	H	H
8a	Glc	H	H	Me
9	H	H	H	Glc
10	Glc	Glc	H	Glc
11	Glc	Glc	OH	Glc

Xyl = β–D–Xylopyranosyl
Glc = β–D–Glucopyranosyl

Table 1: ^{1}H NMR data of compounds 6, 8a, 9–12 in pyridine-d_5

H	6	8a	9	10	11	12
1α	0.86	0.90		0.87	0.96 (dt, 13.7, 3.3)	0.94 (dt, 12.0, 2.4)
1β	1.50	1.53	1.52 (d, 12.0)	1.52 (d, 12.0)	1.53 (d, 12.0)	1.45 (brd, 12.0)
2α	2.10	2.12		2.04	2.01	1.97
2β	1.87	1.82		1.82	1.83	1.78
3	3.27 (dd, 12.0, 6.0)	3.38 (dd, 13.3, 4.0)	3.36 (dd, 13.3, 4.6)	3.26 (dd, 12.0, 4.0)	3.15 (dd, 12.0, 4.0)	3.11 (dd, 12.7, 4.7)
5	0.79 (brd, 12.0)	0.83	0.82	0.77 (d, 12.0)	0.90	0.85
6α		1.53		1.49	1.53 (brd, 12.0)	1.50 (brd, 12.5)
6β	1.33	1.34		1.30	1.32	1.32
7α			1.51 (t, 12.0)	1.53 (t, 12.0)	2.06	1.84
7β				1.34	1.53 (brd, 12.0)	1.41 (brd, 12.0)
9	1.66 (dd, 12.0, 8.0)	1.60 (dd, 12.0; 8.0)	1.59 (dd, 12.0, 8.0)	1.56 (dd, 12.0, 8.0)	2.11 (brt, 10.0)	2.17 (dd, 12.0, 8.6)
11	1.90	1.90		1.93	2.0	1.93
12	5.45 (t, 3.0)	5.38	5.46 (t, 3.0)	5.45 (t, 3.0)	5.74 (t, 3.0)	5.79 (t, 3.0)
15α		1.12 (brd, 13.3)		1.17	1.82	1.45 (brd, 12.0)
15β			2.45 (dt, 13.3, 4.0)	2.43 (dt, 14.6, 4.0)	2.39 (dt, 13.3, 4.0)	2.33 (dt, 13.3, 4.0)
16α			2.11 (dt, 13.3, 4.0)	2.09 (dt, 13.6, 4.0)	2.17 (dt, 13.5, 4.0)	2.11 (dt, 13.3, 2.7)
16β				1.98 (brd, 12.0)	2.04 (brd, 12.0)	1.97 (brd, 12.0)
18	2.61 (d, 11.4)	2.43 (d, 12.7)	2.54 (d, 12.0)	2.53 (d, 12.0)	2.64 (d, 12.7)	3.28 (dd, 14.7, 4.0)
19α	1.47	1.42	1.44	1.43	1.47	1.73 (brt, 13.3)
19β						1.32 (brd, 13.3)
21α				1.50	1.25 (dt, 12.0, 2.7)	1.29 (dt, 12.0, 4.0)
21β				1.38	1.33 (brd, 11.0)	1.07
22α				1.95 (brd, 12.0)	1.91 (brd, 13.3)	1.73 (brd, 13.3)
22β			1.75 (dt, 13.3, 4.0)	1.75 (dt, 14.7, 4.0)	1.75 (dt, 14.6, 4.7)	1.86
23	1.28 (s)	1.33 (s)	1.30 (s)	1.27 (s)	1.19 (s)	1.20 (s)
24	1.07 (s)	1.01 (s)	0.98 (s)	1.09 (s)	1.08 (s)	1.07 (s)
25	1.00 (s)	0.90 (s)	0.91 (s)	0.87 (s)	0.90 (s)	0.85 (s)
26	0.84 (s)	0.84 (s)	1.15 (s)	1.13 (s)	1.17 (s)	1.10 (s)
27	1.23 (s)	1.20 (s)	1.23 (s)	1.20 (s)	4.02	3.76 (d, 10.5), 4.03
28 OMe		3.70 (s)	–	–	–	–
29	1.00 (d, 6.5)	0.95 (d, 6.5)	0.96 (d, 6.5)	0.95 (d, 6.5)	1.05 (d, 6.5)	0.85 (s)
30	0.95 (d, 6.5)	0.85 (d, 6.5)	0.92 (d, 6.5)	0.92 (d, 6.5)	0.83 (d, 6.5)	0.91 (s)
3-*O*-sugar						
Ara				Ara		
1	4.77(d, 6.8)	4.75 (d, 6.5)	4.72 (d, 6.6)	4.79 (d, 6.6)	4.73 (d, 6.6)	4.74 (d, 6.8)
2	4.66 (t, 7.0)	4.58 (t, 7.0)	4.52 (t, 7.0)	4.72 (t, 7.0)	4.69 (t, 7.0)	4.70 (t, 7.0)
3	4.28 (dd, 6.8, 3.5)	4.22 (dd, 6.8, 3.5)	4.02 (dd, 6.8, 3.5)	4.31 (dd, 6.8, 3.5)	4.31 (dd, 6.8, 3.5)	4.31 (dd, 6.8, 3.5)
4	4.52 (m)	4.43 (m)	4.49 (m)	4.49 (m)	4.48 (m)	4.48 (m)
5	3.68 (d, 11.4), 4.18 (d, 11.4)	3.74 (d, 11.0), 4.21	3.74 (d, 11.0), 3.78 (d, 11.0)	3.66 (d, 11.0), 4.18 (d, 11.0)	3.66 (d, 11.0), 4.18	3.70 (d, 11.0), 4.18 (d, 11.0)
Glc				Glc I		
1	5.27 (d,7.8)	5.37 (d,8.0)		5.27 (d,8.0)	5.27 (d,8.0)	5.27 (d,8.0)
2	4.0 (t, 8.0)	4.02 (t, 8.0)		3.98 (t, 8.0)	3.98 (t, 8.0)	3.98 (t, 8.0)
3	4.22 (t, 8.0)	4.24 (t, 8.0)		4.21 (t, 8.0)	4.20 (t, 8.0)	4.20 (t, 8.0)
4	4.18 (t, 8.0)	4.21 (t, 8.0)		4.18 (t, 8.0)	4.18 (t, 8.0)	4.19 (t, 8.0)
5	3.93 (m)	3.98 (m)		3.93 (m)	3.92 (m)	3.92 (m)
6	4.31 (dd, 12.0, 4.5), 4.47 (dd, 12.0, 2.0)	4.37 (dd, 12.0, 4.5), 4.53 (dd, 12.0, 2.5)		4.30 (dd, 12.0, 4.5), 4.47 (dd, 12.0, 2.0)	4.30 (dd, 12.0, 4.5), 4.46 (dd, 12.0, 2.0)	4.31 (dd, 12.0, 4.0), 4.47 (dd, 12.0, 2.0)
Xyl				Glc II		
1	5.38 (d, 7.5)			5.49 (d, 8.0)	5.48 (d, 8.0)	5.49 (d, 8.0)
2	4.02 (t, 7.5)			4.03 (t, 8.0)	4.02 (t, 8.0)	4.03 (t, 8.0)
3	4.11 (t, 7.5)			4.18 (t, 8.0)	4.18 (t, 8.0)	4.19 (t, 8.0)
4	4.22 (t, 7.5)			4.15 (t, 8.0)	4.14 (t, 8.0)	4.15 (t, 8.0)
5	3.45 (brt, 9.0),4.22			3.70 (m)	3.70 (m)	3.70 (m)
6				4.25 (dd, 12.0, 4.5), 4.34 (dd, 12.0, 2.0)	4.25 (dd, 12.0, 4.0), 4.34 (d, 12.0)	4.25 (dd, 12.0, 4.0), 4.34 (d, 12.0)
28-*O*-sugar						
Glc				GlcIII		
1			6.26 (d, 8.0)	6.25 (d, 8.0)	6.30 (d, 8.0)	6.34 (d, 8.1)
2			4.19 (t, 8.0)	4.19 (t, 8.0)	4.20 (t, 8.0)	4.21 (t, 8.0)
3			4.27 (t, 8.0)	4.27 (t, 8.0)	4.30 (t, 8.0)	4.28 (t, 8.0)
4			4.31 (t, 8.0)	4.33 (t, 8.0)	4.33 (t, 8.0)	4.35 (8.0)
5			4.02 (m)	4.02 (m)	4.03 (m)	4.03 (m)
6			4.38 (dd, 12.0, 4.5), 4.44 (d, 12.0, 2.0)	4.37 (dd, 12.0, 4.5), 4.44 (dd, 12.0, 2.0)	4.38 (dd, 12.0, 4.5), 4.46 (d, 12.0)	4.38 (dd, 12.0, 4.0), 4.46 (d, 10.0)

Ara = α-L-arabinopyranosyl
Glc = β-D-glucopyranosyl
Xyl = β-D-xylopyranosyl

Table 2: ^{13}C NMR data of compounds 6, 8a, 9–12 in pyridine-d_5, (ppm)

C	6	8a	9	10	11	12
1	39.1	39.0	39.2	39.1	39.1	38.8
2	26.8	26.8	26.7	26.7	26.7	26.6
3	89.3	88.7	88.9	89.1	88.9	88.9
4	39.8	39.9	39.6	39.7	39.7	39.7
5	56.0	55.9	56.0	56.0	55.9	55.8
6	18.6	18.5	18.6	18.6	18.9	18.6
7	33.6	33.4	33.6	33.6	34.5	33.7
8	40.0	39.9	40.3	40.2	41.1	40.7
9	48.1	48.0	48.1	48.1	48.6	48.7
10	37.1	37.0	37.0	37.0	37.2	37.2
11	23.8	23.6	23.7	23.7	24.1	24.4
12	125.7	126.0	126.2	126.2	130.2	128.0
13	139.3	138.8	138.5	138.5	135.4	139.1
14	42.5	42.3	42.6	42.6	48.4	48.0
15	28.7	28.4	28.7	28.7	23.2	23.9
16	24.9	24.6	24.7	24.8	25.3	23.6
17	48.1	48.3	48.4	48.5	48.2	46.9
18	53.6	53.4	53.4	53.4	53.4	41.6
19	39.4	39.6	39.4	39.4	39.2	45.4
20	39.1	39.2	39.2	39.2	39.2	30.8
21	31.0	30.8	30.8	30.9	30.6	33.9
22	37.0	37.1	37.0	36.8	36.9	32.6
23	27.9	28.2	28.2	28.2	28.0	28.0
24	16.6	17.0	17.0	16.8	16.8	16.7
25	15.7	15.8	15.8	15.8	16.2	16.0
26	17.5	17.2	17.4	17.7	18.9	19.0
27	23.9	23.7	23.8	23.8	64.3	64.5
28	180.0	177.7	176.2	176.2	176.4	176.5
29	17.5	17.4	17.7	17.5	18.0	33.1
30	21.4	21.3	21.3	21.3	21.3	23.8
OMe		51.4				
3-*O*-sugar						
Ara						
1	105.6	107.4	107.4	105.5	105.3	105.4
2	77.5	72.0	72.0	77.5	77.5	77.5
3	83.7	84.2	75.3	83.3	83.3	83.3
4	68.9	69.3	69.3	68.8	68.7	68.7
5	66.1	67.0	67.1	65.9	65.9	65.9
Glc				GlcI		
1	105.1	106.4		105.0	105.0	105.0
2	75.3	75.8		75.3	75.3	75.3
3	79.0	78.7		78.7	78.6	78.7
4	71.4	71.7		71.6	71.6	71.6
5	78.4	78.4		78.3	78.3	78.4
6	62.6	62.8		62.5	62.6	62.6
Xyl				GlcII		
1	105.1			104.4	104.4	104.5
2	76.0			76.2	76.1	76.1
3	78.5			78.5	78.5	78.5
4	71.7			72.5	72.5	72.5
5	67.2			77.5	77.4	77.4
6				63.3	63.3	63.3
28-*O*-sugar				GlcIII		
Glc						
1			95.8	95.8	95.8	95.8
2			74.1	74.1	74.2	74.2
3			78.7	78.9	78.9	79.0
4			71.4	71.4	71.4	71.3
5			79.2	79.2	79.2	79.3
6			62.5	62.6	62.3	62.3

Ara = α-L-arabinopyranosyl
Glc = β-D-glucopyranosyl
Xyl = β-D-xylopyranosyl

dicated that, in the 3-*O*-sugar chain, the β-D-glucopyranosyl unit I is linked to C-3 and the β-D-glucopyranosyl unit II to C-2 of the inner α-L-arabinopyranosyl moiety whereas the β-D-glucopyranosyl unit III is attached to the aglycone C-28. This conclusion was confirmed by ROE experiment. Irradiation of the anomeric proton signal at δ 5.27 (H-1 of Glc I) enhanced the proton signal at δ 4.31 (H-3 of Ara). Irradiation of the anomeric proton signal at δ 5.49 (H-1 of Glc II) enhanced the proton signal at δ 4.72 (H-2 of Ara) and irradiation of the anomeric proton signal at δ 4.79 (H-1 of Ara) enhanced the proton signal at δ 3.26 (H-3 of Ag). Regarding the non glycosidic part the ^{13}C NMR signals were similar to those of ursolic acid except the glycosylation shift observed around C-3 and C-28. The ^{13}C shift assignments were made possible from HMQC and HMBC experiments while proton signals were identified from the combined use of ^{1}H-^{1}H COSY, HMQC and HMBC experiments. The relative orientation of the hydrogen atoms was determined from the values of coupling constant obtained from 1D spectrum or their relative magnitudes from the shape of the cross peaks in the ^{1}H-^{1}H COSY spectrum. The FAB MS showed a molecular ion peak at m/z 1097 $[C_{53}H_{86}O_{22} + Na]^+$, along with a fragment ion at m/z 935 due to loss of glucose unit. From the above studies, saponin **10** was assigned the structure of, 3-*O*-[β-D-glucopyranosyl(1→2)][β-D-glucopyranosyl(1→3)]-α-L-arabinopyranosyl ursolic acid 28-*O*-β-D-glucopyranosyl ester.

On acid hydrolysis, saponin **11** afforded L-arabinose and D-glucose. The 1D and 2D NMR spectra (HOHAHA, ^{1}H-^{1}H COSY and HMQC) of **11** exhibited signals due to sugar units very similar to those of **10** suggesting that both saponins are identical regarding sugar structures and linkages. Irradiation at each anomeric proton signal in the ROE experiment and the observed cross peaks in the HMBC spectrum supported this conclusion. Comparison between the signals of B and C ring carbons in **11** and **10** revealed that the aglycone moiety of **11** was 27-hydroxy ursolic acid previously found in *F. arabica* [5]. The unambiguous carbon shift assignments were allowed from the HMQC and HMBC spectra. The reversed assignments of C-24 and C-25 became evident on comparison with the assignments initially carried out [5]. The uncertain assignments of C-15, C-16 and C-11, which arose from the close proximity of their signals, could be overcome. In the HMBC spectrum, the carbon signal at δ 23.2 showed correlation with signal at δ 4.02 due to one proton of H_2-27, whereas the carbon signal at δ 25.3 exhibited correlation with one proton signal at δ 2.64 (d, J = 12.7) due to H-18. The former signal was therefore assigned to C-15 and the latter to C-16. The remained signal at δ 24.1 was then assigned to C-11. Its position in the HMQC spectrum was confirmed from the observed correlation with its directly attached protons (coupled to H-12 in the ^{1}H-^{1}H COSY). The differentiation between the signal of the deshielded C-14 (β to the introduced OH) from those of C-9 and C-17, could be achieved. The signals at δ 48.2 and δ 48.6 were assigned to C-17 and C-9 by means of the observed correlations of the former to the signal of H-18 in the HMBC spectrum and the latter to the signal of H-9 (δ 2.11) in the HMQC spectrum. The remained signal at δ 48.4 is therefore attributable to C-14. Although the signal due to C-13 in the ^{13}C NMR spectrum is hidden under solvent signals, its location at δ 135.4 was deduced from the observed correlation with H-18 signal in the HMBC spectrum. Proton signal assignments were made possible from ^{1}H-^{1}H COSY, HMQC and HMBC spectra. In addition to the expected cross peaks corresponding to two and three bond couplings for H-9, H-16α and H-22β protons [7], further correlations were observed in the HMBC spectrum of **11** between the following signals H-7β/C-26, C-5, C-8, C-9; H-15β/C-8, C-27. The downfield position of the

signals due to H-7α (δ 2.06), H-9α (δ 2.11), H-15α (δ 1.82) with reference to the corresponding in ursolic acid saponin **10** (δ 1.53, 1.56, 1.17, respectively) is in full agreement with replacement of α-oriented CH_3 (axial) by CH_2OH at C-14. The FAB MS of **11** displayed $[M + Na]^+$ at m/z 1113 $[C_{53}H_{86}O_{23} + Na]^+$ along with a fragment ion at m/z 951 due to elimination of glucose unit. The presence of a fragment ion, corresponding to loss of CH_3OH, at m/z 1081, was reported from saponins with oleanolic acid skeleton bearing hydroxymethylene function at C-14 [8]. Thus **11** was formulated as 3-*O*-[β-D-glucopyranosyl(1→2)][β-D-glucopyranosyl(1→3)]-α-L-arabinopyranosyl-27-hydroxy ursolic acid 28-*O*-β-D-glucopyranosyl ester. Saponin **11** is the second member with an aglycone possessing 27-hydroxy ursolic acid structure. The first was isolated from *F. arabica* [5].

On acid hydrolysis, saponin **6** yielded ursolic acid and the sugar components L-arabinose, D-glucose and D-xylose. The 1D and 2D spectra allowed identification of α-L-arabinopyranosyl unit, β-D-glucopyranosyl and β-D-xylopyranosyl units. Their anomeric proton signals were located at δ 4.77 (d, J = 6.8 Hz), 5.27 (d, J = 7.8) and 5.38 (d, J = 7.5 Hz) respectively. In the ^{13}C NMR spectrum, the signals due to the aglycone moiety, showed similar shift values to those of ursolic acid [9] except the lower field position of C-3 signal (δ 89.3) due to glycosylation. The shift values due to the sugar carbons, indicated that the β-glucopyranosyl and the β-D-xylopyranosyl units are terminal while the α-L-arabinopyranosyl moiety is inner exhibiting down field shift for C-2 and C-3, (δ 77.5, 83.7), thus suggesting glycosidic linkages at these carbons. The similarity between the sugar carbon signals of **6** and those due to 3-*O*-sugar chain of saponin **5** (NMR data in reference [5]) suggested placement of the β-D-xylopyranosyl and the β-D-glucopyranosyl units at C-2 and C-3 of the inner α-L-arabinopyranosyl moiety respectively. The site of sugar connections was confirmed by ROE experiment. Irradiation of the anomeric proton signal at δ 4.77 (H-1 of Ara) enhanced the proton signal at δ 3.27 (H-3 of Ag). Irradiation of the anomeric proton signal at δ 5.38 (H-1 of xyl) enhanced the proton signal at δ 4.66 (H-2 of Ara) and irradiation of the anomeric proton signal at δ 5.27 (H-1 of Glc) enhanced the proton signal at δ 4.28 (H-3 of Ara). The FAB MS of **6** displayed $[M + Na]^+$ at m/z 905 $[C_{46}H_{74}O_{16} + Na]^+$. Thus **6** was assigned the structure of 3-*O*-[β-D-xylopyranosyl(1→2)][β-D-glucopyranosyl(1→3)]-α-L-arabinopyranosyl ursolic acid.

Saponin **8** methyl ester displayed $[M + Na]^+$ in its FAB MS at m/z 787 $[C_{42}H_{68}O_{12} + Na]^+$. Acid hydrolysis yielded D-glucose and L-arabinose together with ursolic acid. The 1D and 2D NMR spectra indicated the presence of a carbomethoxyl group (δ 51.4), a terminal β-D-glucopyranosyl unit and an inner α-L-arabinopyranosyl moiety [anomeric carbons δ 106.4 and 107.4; anomeric protons δ 5.37 (d, J = 8.0 Hz) and 4.75 (d, J = 6.5 Hz)]. The downfield shift observed on C-3 of the α-L-arabinopyranosyl unit (δ 84.2) suggested the site of attachment to the terminal β-D-glucopyranosyl moiety. The HMBC spectrum confirmed the (1→3) linkage and the observed correlations were between signals at δ 106.4 (C-1 of Glc)/4.22 (H-3 of Ara) and δ 107.4 (C-1 of Ara)/3.38 (H-3 of Ag). ROE experiment added further support and showed that irradiation of the anomeric proton signal at δ 5.37 (H-1 of Glc) enhanced the proton signal at δ 4.22 (H-3 of Ara) and irradiation of the anomeric proton signal at δ 4.75 (H-1 of Ara) enhanced the proton signal at δ 3.38 (H-3 of Ag). The carbon signals of the aglycone moiety were similar to those of ursolic acid methyl ester [10] except the deshielded C-3 signal (δ 88.7) due to glycosylation. Therefore **8** was formulated as 3-*O*-[β-D-glucopyranosyl(1→3)-α-L-arabinopyranosyl] ursolic acid.

On mineral acid hydrolysis, saponin **9** yielded ursolic acid. The sugar units released were identified as L-arabinose and D-glucose. The ^1H NMR spectrum exhibited two anomeric proton signals at δ 4.72 (d, J = 6.6 Hz) and 6.26 (d, J = 8.0 Hz). Their configuration from J values were consistent with α-arabinose and β-glucose. The ^{13}C shift values indicated that the two sugar units exist in the pyranose form. The carbon signals due to the aglycone moiety were in agreement with 28-glycosyl ester of 3-*O*-glycosyl ursolic acid. The signals at δ 95.8, 74.1, 78.7, 71.4, 79.2 and 62.5 were relevant to terminal β-D-glucopyranosyl unit linked to C-28 of the aglycone moiety. Therefore the α-L-arabinopyranosyl moiety is located at C-3. The FAB MS was consistent with the above conclusions and displayed $[M + Na]^+$ at m/z 773 $[C_{41}H_{66}O_{12} + Na]^+$. Therefore **9** was assigned the structure of 3-*O*-α-L-arabinopyranosyl ursolic acid 28-*O*-[β-D-glucopyranosyl] ester.

Saponin **12** yielded D-glucose and L-arabinose as the sugar components on acid hydrolysis. The ^1H NMR, HOHAHA, ^1H-^1H COSY and HMQC experiments indicated the presence of three terminal β-D-glucopyranosyl units and a 2,3-disubstituted α-L-arabinopyranosyl moiety. Their linkages were identical to those of **10** and **11** from HMBC and ROE experiments. The aglycone moiety was shown from NMR data to be the rare 27-hydroxy oleanolic acid [5, 8]. The unambiguous shift assignments were allowed from 1D and 2D NMR spectra (^1H-^1H COSY, HMQC and HMBC spectra). The signals of C-15 and C-16 with close shift values were distinguished by means of observing cross peaks corresponding to two and three bond couplings (HMBC). The correlations between signals of C-7/H_3 -26 and between C-21/H_3-29 in the HMBC spectrum differentiated the signals of C-7, C-21 and C-22. The FAB MS of **12** displayed $[M + Na]^+$ at m/z 1113 $[C_{53}H_{86}O_{23} + Na]^+$ along with a fragment ion at m/z 951 due to loss of glucose unit. The fragment ion at m/z 1081 which accounted for loss of CH_3OH, confirmed the presence of a CH_2OH group at C-14 of the aglycone moiety [8]. Therefore saponin **12** must be the oleanolic analogue of **11** and formulated as 3-*O*-[β-D-glucopyranosyl(1→2)][β-D-glucopyranosyl(1→3)]-α-L-arabinopyranosyl ursolic acid 28-*O*-β-D-glucopyranosyl ester. This is the second occurrence of the new 27-hydroxy oleanolic acid saponin from the genus *Fagonia* and the third from nature [5, 8]. Sapogenins oxidised at C-27 are known in the family Zygophyllacea. Quinovic acid possessing carboxyl function at C-14 has been found in *F. cretica* [11] and its glycosides in *Zygophyllum* species [12].

The known saponins **1**–**4** were identified from the spectral data of their methyl esters as spinasaponin A [13], Momordin Ic [14, 15], Chikusetsusaponin IVa [16] and 3-*O*-[β-D-glucopyranosyl(1→3) β-D-glucuronopyranosyl]-oleanolic acid 28-*O*-β-D-glucopyranosyl ester [17], respectively. The known saponin **5** was identified by direct comparison with an authentic sample [NMR data, $[\alpha]_D$] isolated from *F. arabica* [5], while **7** was found to be matesaponin [18].

3. Experimental

3.1. Equipment

Optical rotations were measured with a JASCO DIP-1000 digital polarimeter. MS were measured on a JEOL JMS-SX102 mass spectrometer. NMR

spectra were recorded on JEOL α-400 (399.65 and 100.40 MHz) NMR spectrometers. 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

Fagonia glutinosa was collected from the Sinai peninsula, Egypt, in May 1995 and identified by Dr M. El-Gibaly at the taxonomy department, N.R.C., where a voucher specimen was deposited.

3.3. Extraction and isolation

The aerial parts of *F. glutinosa* (2.5 kg) were extracted with CH_2Cl_2 twice and the residue was then extracted with MeOH at room temperature twice. The combined methanolic extract was concentrated and then diluted with Me_2CO to precipitate the crude saponin fraction (30 g). The latter was chromatographed on silica gel using VLC technique [CH_2Cl_2 : MeOH (9 : 1–7 : 3)] to give two fractions. The residue from fraction 1 was dissolved in water and the solution was passed through a porous polymer gel column (Mitsubishi Diaion HP-20). After washing with water, a methanol eluate was obtained as a pale powder (4 g). A part (1.0 g) of the methanolic eluate was chromatographed on a Develosil Lop-ODS column (2x 50 cm) using MeOH-H_2O and Develosil Ph A (2 × 25 cm) column using MeCN-H_2O system to give saponins **5** (51 mg), **6** (6 mg), **7** (40 mg), **9** (15 mg), **10** (16 mg), **11** (8 mg) and **12** (7 mg). Fraction 2 in methanol, was passed through an Amberlite IR-120 column and treated with CH_2N_2. The methylated fraction was chromatographed on a silica gel column with $CHCl_3$-MeOH (9 : 1–4 : 1) and semiprep. HPLC (ODS and PHA : MeCN-H_2O system] to give the methyl esters of **1** (70 mg), **2** (50 mg), **3** (42 mg), **4** (45 mg) and **8** (10 mg).

Compound **6**. Amorphous powder; $[\alpha]_D^{23}$ +14.1° (c = 0.38, MeOH); FABMS (m/z), 905 $[C_{46}H_{74}O_{16} + Na]^+$, 861, 725, 577, 413, 371, 392; ^1H and ^{13}C NMR: see Tables 1 and 2.

Compound **8a**. Amorphous powder; $[\alpha]_D^{23}$ +34.3° (c = 0.22, MeOH); FABMS (m/z), 787 $[C_{42}H_{68}O_{12} + Na]^+$, 453, 393, 307, 298, 262, 203; ^1H and ^{13}C NMR: see Tables 1 and 2.

Compound **9**. Amorphous powder; $[\alpha]_D^{23}$ +17.9° (c = 2.25, MeOH); FABMS (m/z) 773 $[C_{41}H_{66}O_{12} + Na]^+$, 623, 413, 307, 261; ^1H and ^{13}C NMR: see Tables 1 and 2.

Compound **10.** Amorphous powder; $[\alpha]_D^{23}$ +16.3° (c = 1.43, MeOH); FABMS (m/z) 1097 $[C_{53}H_{86}O_{22} + Na]^+$, 935, 890, 740, 497, 393, 317; ^1H and ^{13}C NMR: see Tables 1 and 2.

Compound **11.** Amorphous powder; $[\alpha]_D^{23}$ +12.6° (c = 1.49, MeOH); FABMS (m/z) 1113 $[C_{53}H_{86}O_{23} + Na]^+$, 1081, 951, 531, 487, 399; ^1H and ^{13}C NMR: see Tables 1 and 2.

Compound **12**. Amorphous powder; $[\alpha]_D^{23}$ +21.8° (c = 1.50, MeOH); FABMS m/z 1113 $[C_{53}H_{86}O_{23} + Na]^+$, 1081, 951, 531, 487, 399; ^1H and ^{13}C NMR: see Tables 1 and 2.

3.4. General method for acid hydrolysis

Each saponin (2 mg), in dioxane (50 μl) and 2M HCl (100 μl) was heated at 100 °C for 30 min. The reaction mixture was diluted with H_2O and extracted with EtOAc. The produced aglycone was detected by TLC against an authentic sample using various solvent systems and its identity was confirmed by MS. The monosaccharide units in the aqueous layer were analysed according to ref. [19] as follows: the aqueous layer of the reaction mixture 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 then overnight at room temperature. To the reaction mixture, hexamethyldisilazane (20 μl) and trimethylsilyl chloride (20 μl) were added, and the reaction mixture was stirred at 60 °C for 30 min. The supernatant was then analysed by GC, conditions: column, Supelco SPB-TM1 (0.25 mm × 27 m); column temp., 215 °C; carrier gas, N_2; retention times, D-arabinose (11.0 min), L-arabinose (11.9 min), D-xylose (11.7 min), L-xylose (10.8 min), D-glucose (21.6 min) and L-glucose (20.3 min). It was shown for the new saponins that arabinose belongs to the L-form whereas glucose and xylose to the D-series.

References

1 Saleh, N. A. M.; El-Hadidi, M. N.; Al-Wakeel, S. A. M.: Biochem. Syst. ecol. **18**, 49 (1990)
2 Al-Wakeel, S. A. M.: Biochem. Syst. ecol. **20**, 259 (1992)
3 Abdel-Kader, M. S.; Omar, A. A.; Abdel-Salam, N. A.; Stermitz, F. R.: Phytochemistry **36**, 1431 (1994)
4 Melek, F. R.; Abdel-Khalik, S. M.; Miyase, T.; Haggag, M. Y.: Fitoterapia **64**, 963 (1994)
5 Miyase, T.; Melek, F. R.; El-Gindi, O. D.; Abdel-Khalik, S. M.; El-Gindi, M. R.; Haggag, M. Y.; Hilal, S. H.: Phytochemistry **41**, 1175 (1996)
6 Melek, F. R.; Miyase, T.; El-Gindi, O. D.; Abdel-Khalik, S. M.; Haggag, M. Y.: Phytochemistry **42**, 1405 (1996)
7 Schöpke, T.; Wray, V.; Rzazewska, B.; Hiller, K.: Phytochemistry **30**, 627 (1991)
8 Maillard, M.; Adewunmi, C. O.; Hostettmann, K.: Phytochemistry **31**, 1321 (1992)
9 Seo, S.; Tomita, Y.; Tori, K.: Tetrahedron Lett. 1 (1975)
10 Seo, S.; Tomita, Y.; Tori, K.: Tetrahedron Lett. 7 (1975)
11 Miana, G. A.; Al-Hazimi, H. M. G.: Phytochemistry **26**, 225 (1987)
12 Ahmad, V. U.; Ghazala; Uddin, S.: Phytochemistry **31**, 1051 (1992)
13 Shao, C. J.; Kasai, R.; Xu, J. D.; Tanaka, O.: Chem. Pharm. Bull. **37**, 311 (1989)
14 Kawamura, N.; Watanabe, H.; Oshio, H.: Phytochemistry **27**, 3585 (1988)
15 Gafner, F.; Msonthi, J. D.; Hostettmann, K.: Helv. Chim. Acta **68**, 555 (1985)
16 Nie, R. L.; Morita, T.; Kasai, R.; Zhou, J.; Wu, C. Y.; Tanaka, O.: Planta Med. **50**, 322 (1984)
17 Romussi, G.; Bignardi, G.; Falsone, G.; Wendisch, D.: Arch. Pharm. **320**, 153 (1987)
18 Gosmann, G.; Schenkel, E. P.: J. Nat. Prod. **52**, 1367 (1989)
19 Hara, S.; Okabe, H.; Mikashi, K.: Chem. Pharm. Bull. **34**, 1843 (1986)

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