



## Triterpenoid saponins from *Fagonia indica*

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### Abstract

Two new triterpenoid saponins, 3-*O*-{[ $\beta$ -D-glucopyranosyl-(1  $\rightarrow$  2)]-[ $\alpha$ -L-arabinopyranosyl-(1  $\rightarrow$  3)]- $\alpha$ -L-arabinopyranosyl}-ursolic acid-28-*O*-[ $\beta$ -D-glucopyranosyl] ester (indicasaponin A), 3-*O*-{[ $\beta$ -D-glucopyranosyl-(1  $\rightarrow$  2)]-[ $\alpha$ -L-arabinopyranosyl-(1  $\rightarrow$  3)]- $\alpha$ -L-arabinopyranosyl}-oleanolic acid-28-*O*-[ $\beta$ -D-glucopyranosyl] ester (indicasaponin B) and two known triterpenoid saponins, 3-*O*-[ $\beta$ -D-glucopyranosyl-(1  $\rightarrow$  3)]- $\alpha$ -L-arabinopyranosyl]-ursolic acid-28-*O*-[ $\beta$ -D-glucopyranosyl] ester, 3-*O*-[ $\beta$ -D-glucopyranosyl-(1  $\rightarrow$  3)]- $\alpha$ -L-arabinopyranosyl]-oleanolic acid-28-*O*-[ $\beta$ -D-glucopyranosyl] ester have been isolated from *Fagonia indica*. The structures were determined primarily by NMR spectroscopy. The assignment of NMR signals was performed by means of <sup>1</sup>H–<sup>1</sup>H COSY, NOESY, ROESY, TOCSY, HMQC and HMBC experiments. © 1999 Elsevier Science Ltd. All rights reserved.

**Keywords:** *Fagonia indica*; Zygophyllaceae; Triterpenoid saponins; Indicasaponin A and B

### 1. Introduction

*Fagonia indica* Burm. f. (Zygophyllaceae) is a small spiny shrub widely distributed in Egypt and Pakistan. Plants belonging to the genus *Fagonia* are often used in folk medicine, mainly as a popular remedy for the treatment of various skin lesions. Additionally, the plant is claimed to be a remedy for cancer in its early stages (Chapra, Handa, & Kapur, 1956; Chapra, Nayar, & Chopra, 1956; Rahman, 1983). This prompted us in the frame of our continuing search for triterpenoid saponins (Pöllmann, Elgamal, Shaker, & Seifert, 1997; Pöllmann et al., 1998) to investigate the plant constituents of *F. indica*.

Two taraxast-20-en-28-oic acid saponins have been isolated from *F. indica* (Ansari, Kenne, & Rahman, 1987). The sapogenins hederagenin and ursolic acid could be detected after hydrolysis of the EtOH extract of the aerial parts of *F. indica* (Rahman, & Ansari, 1984). In this report we describe the isolation and structure determination of two new and two known

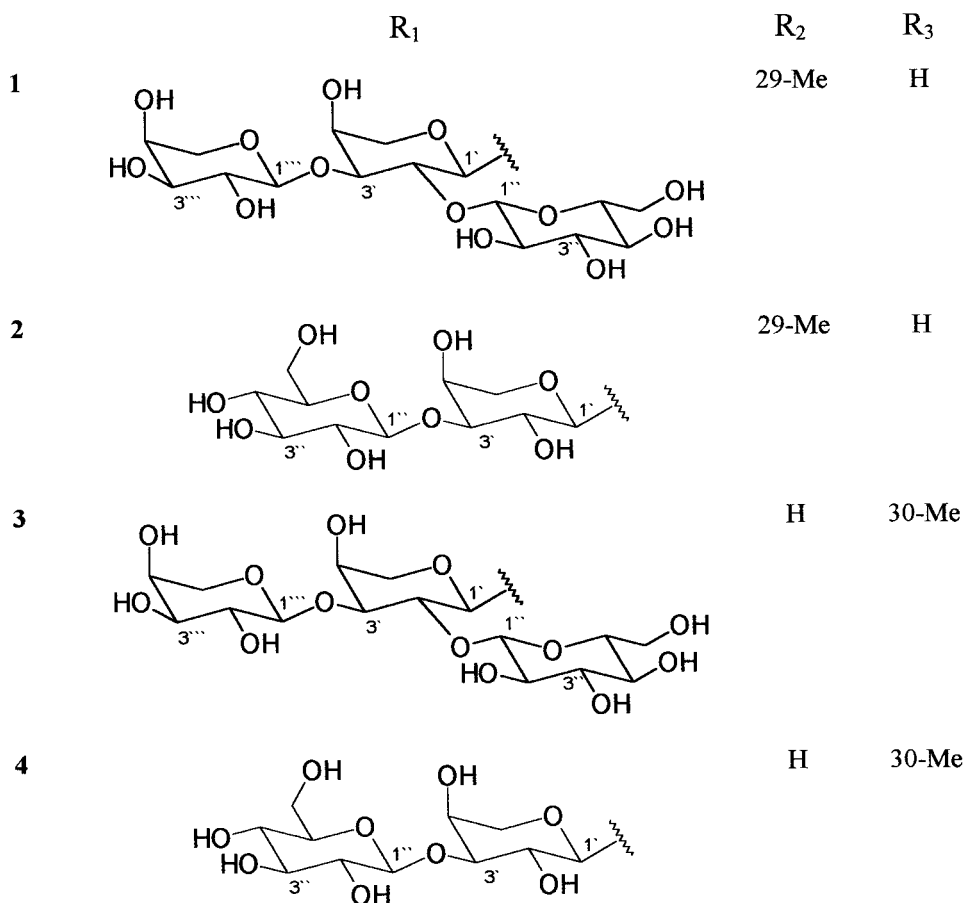
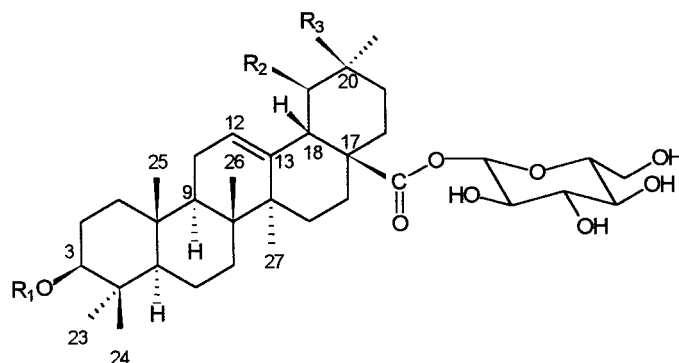
triterpenoid saponins with ursolic and oleanolic acid as aglycones.

### 2. Results and discussion

The butanol extract of the whole plants of *F. indica* were obtained as described in Section 3. The crude saponins were subjected to column chromatography on silica gel to be eluted successively with CHCl<sub>3</sub>, CHCl<sub>3</sub>–MeOH and CHCl<sub>3</sub>–MeOH–H<sub>2</sub>O with increasing amounts of MeOH and H<sub>2</sub>O. Four saponins have been isolated after further purification by column chromatography on Sephadex LH-20 followed by preparative HPLC on RP-18 material.

The LSI mass spectrum of **1** exhibited [M–1]<sup>–</sup> ion at *m/z* 1043. The fragment ions at *m/z* 881 [M–1–162]<sup>–</sup> and *m/z* 719 [M–1–2 × 162]<sup>–</sup> showed the loss of one or two hexose moieties, respectively. The fragment ions at *m/z* 587 [M–1–2 × 162–132]<sup>–</sup> and *m/z* 455 [M–1–2 × 162–2 × 132]<sup>–</sup> were proof for the elimination of one or rather two pentose moieties. The [M–1]<sup>–</sup> ion together with <sup>1</sup>H and <sup>13</sup>C NMR data allowed us to propose the molecular formula C<sub>52</sub>H<sub>84</sub>O<sub>21</sub>.

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The  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra of **1** showed the presence of ursolic acid as aglycone. The signals of the axial and equatorial oriented protons of the ursolic acid were assigned by ROESY experiments. Four anomeric proton signals at  $\delta$  4.38 ( $J=7.1$  Hz), 4.57 ( $J=7.6$  Hz), 4.67 ( $J=7.8$  Hz) and 5.32 ( $J=8.2$  Hz) indicated the presence of four saccharide units, one bonded as a glycosylester ( $\delta$  5.32) and the others as glycosides ( $\delta$  4.38, 4.57, 4.67). By use of the coupling constants of the anomeric protons ( $J=7.1$ – $8.2$  Hz),

$^1\text{H}$ – $^1\text{H}$  COSY-45 and TOCSY spectra and the determination of the D-form for glucose and the L-form for arabinose (as described in Section 3) the individual saccharides were identified as two  $\beta$ -D-glucopyranoses and two  $\alpha$ -L-arabinopyranoses. The linkage of one arabinose and one glucose moiety to the aglycone was determined by means of HMBC spectra. The cross peaks of the  $^3J$  long range couplings between H-1' (inner arabinose)  $\rightarrow$  C-3 aglycone and H-1'' (glucosyl ester)  $\rightarrow$  C-28 aglycone indicated the points of linkage

Table 1  
 $^1\text{H}$  and  $^{13}\text{C}$  NMR spectral data for the aglyca of saponins **1–4** in  $\text{CD}_3\text{OD}$

	<b>1</b>		<b>2</b>	<b>3</b>		<b>4</b>
	$^1\text{H}$ ax/eq	$^{13}\text{C}$	$^{13}\text{C}$	$^1\text{H}$ ax/eq	$^{13}\text{C}$	$^{13}\text{C}$
1	0.98/1.37	39.9	39.8	0.99/1.62	39.9	40.2
2	1.73/1.82	27.1	26.9	1.72/1.82	27.2	27.3
3	3.10	91.0	90.3	3.10	91.2	90.5
4	—	40.1	40.0	—	40.5	40.8
5	0.73	56.9	56.9	0.76	57.1	56.9
6	1.37/1.50	19.1	19.1	1.40/1.53	19.3	19.5
7	1.50/1.37	34.1	34.1	1.67/1.31	33.5	34.0
8	—	40.8	40.8	—	40.7	40.0
9	1.51	49.0	49.0	1.55	48.9	48.9
10	—	37.7	37.7	—	37.9	37.9
11	1.92/2.07	23.5	23.6	1.91/1.91	24.0	23.9
12	5.23	127.1	127.0	5.25	123.8	122.9
13	—	138.9	138.9	—	144.8	144.8
14	—	43.1	43.1	—	42.9	42.9
15	1.91/1.10	29.1	29.1	1.73/1.06	28.9	28.8
16	2.15/1.78	24.2	24.2	2.09/1.71	24.6	24.9
17	—	49.0	49.0	—	48.0	48.2
18	2.20 d 11.0 Hz	54.0	54.0	2.85 dd 4.2/14.2 Hz	42.6	42.8
19	1.40	40.2	40.1	2.09/1.70	47.2	47.5
20	0.99	40.3	40.2	—	31.5	31.6
21	1.26/1.38	30.7	30.5	1.99/1.21	34.6	34.9
22	1.74/1.65	37.3	37.3	1.62/1.59	33.1	33.5
23	1.04 s	28.1	28.3	1.04	28.3	28.4
24	0.83 s	16.5	16.9	0.83	16.7	17.0
25	0.96 s	16.0	16.0	0.94	16.0	15.9
26	0.82 s	17.5	17.5	0.79	17.7	17.5
27	1.10 s	23.8	23.8	1.14	26.3	26.4
28	—	177.8	177.7	—	178.0	178.0
29	0.89 d 6.6 Hz	17.7	17.7	0.92	33.5	33.9
30	1.32 d 6.1 Hz	21.4	21.4	0.90	24.0	24.0

to the sapogenin. The HMBC cross peaks between H-1' (glucose)  $\rightarrow$  C-2' (inner arabinose) and H-1''' (terminal arabinose)  $\rightarrow$  C-3' (inner arabinose) prove the interglycosidic linkage of glucose at position C-2' and terminal arabinose at position C-3' of the inner arabinose.

The LSI mass spectrum of **3** showed the same fragmentation as **1**. The  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra of **3** exhibited the presence of oleanolic acid as aglycone instead of ursolic acid in the case of indicasaponin A (**1**). The  $^1\text{H}$  NMR,  $^{13}\text{C}$  NMR, HMQC, HMBC, NOESY and ROESY data of the sugar moieties of **1** and **3** are in good agreement indicating the same saccharide part for both saponins.

The triterpenoid glycoside **2** (matesaponin 1) was isolated before from the roots of *Aralia decaisneana* (Araliaceae) (Miyase, Shiokawa, Zhang, & Ueno, 1996). Saponin **4** (guaiacin B) was obtained from the leaves of *Guaiacum officinale* (Zygophyllaceae) (Ahmad, Perveen, & Bano, 1989).

### 3. Experimental

#### 3.1. General

Negative ion MS: MAT 8500 (Finnigan), matrix glycerol. NMR: 500.13 MHz ( $^1\text{H}$ ) and 125.76 MHz ( $^{13}\text{C}$ ), reverse probehead,  $\delta$  in ppm, solvent  $\text{CD}_3\text{OD}$ ,  $\text{CD}_3\text{OD}$  signals were used as int. standard ( $^1\text{H}$ : 3.30,  $^{13}\text{C}$ : 49.0), temp. 290 K, NOESY: phase-sensitive using TPPI, mixing time 300 and 600 ms, TOCSY: phase-sensitive using TPPI, mixing time 134.3 ms (80 MLEV-17 cycles plus 2 trim pulses of 2.5 ms each), HMQC: phase-sensitive using TPPI, BIRD sequence, GARP decoupled, HMBC: using TPPI, delay to achieve long range couplings: 71 ms ( $J_{\text{C,H}} = 14$  Hz).

CC: silica gel (0.063–0.2 mm); TLC: silica gel (0.25 and 1 mm precoated plates 60 F<sub>254</sub>, Merck, 0.25 mm precoated plastic sheets SIL G/UV<sub>254</sub> Macherey-Nagel), the spots were sprayed with 10%  $\text{H}_2\text{SO}_4$  in MeOH, 'triterpene reagent' (1% soln of vanillin in

Table 2

<sup>1</sup>H and <sup>13</sup>C NMR spectral data for the sugar moieties of saponins 1–4 in CD<sub>3</sub>OD

	1		2	3	4	
	<sup>1</sup> H ax/eq	<sup>13</sup> C	<sup>13</sup> C	<sup>1</sup> H ax/eq	<sup>13</sup> C	<sup>13</sup> C
<i>Ara inner</i>						
1'	4.38 d 7.1 Hz	105.9	106.9	4.43 d 7.1 Hz	106.1	106.3
2'	3.86	77.7	70.9	3.91	78.0	71.0
3'	3.76	83.9	83.7	3.77	84.1	84.0
4'	4.02	69.6	69.4	4.10	69.7	69.4
5'	3.85/3.53	66.7	66.5	3.85/3.53	65.4	66.3
<i>Glc</i>						
1''	4.57 d 7.6 Hz	104.9	105.2	4.56 d 7.6 Hz	105.1	104.4
2''	3.30	75.8	75.1	3.30	76.0	75.4
3''	3.30–3.32	78.1	77.5	3.30–3.32	78.3	78.2
4''	3.30–3.32	70.9	70.9	3.30–3.32	71.2	71.2
5''	3.30–3.32	78.0	77.7	3.30–3.32	78.1	77.8
6'	3.82/3.65	62.1	62.1	3.82/3.64	62.1	62.4
<i>Ara terminal</i>						
1'''	4.67 d 7.8 Hz	104.6		4.69 d 7.8 Hz	104.9	
2'''	3.10	70.9		3.12	71.1	
3'''	3.25	75.1		3.26	75.2	
4'''	3.38	70.8		3.39	71.0	
5'''	3.80/3.08	66.4		3.80/3.10	66.5	
<i>28-Glc</i>						
1'''	5.32 d 8.2 Hz	95.5	95.5	5.37 d 8.2 Hz	95.7	95.5
2'''	3.32	73.7	73.7	3.31	73.9	74.0
3'''	3.30–3.32	78.4	78.4	3.30–3.33	78.7	79.0
4'''	3.30–3.32	71.3	71.9	3.30–3.33	71.5	71.4
5'''	3.30–3.32	78.2	78.1	3.30–3.33	78.4	78.8
6'''	3.82/3.65	62.1	62.2	3.82/3.65	62.4	62.3

50% H<sub>3</sub>PO<sub>4</sub>), 'sugar reagent' (4% ethanolic aniline–4% ethanolic diphenylamine–H<sub>3</sub>PO<sub>4</sub>, 5:5:1) and phosphomolybdic acid reagent (Aldrich). For the prep. HPLC a Knauer HPLC system equipped with a variable wavelength monitor together with LiChroprep RP-18 (250 × 8 mm, 5 μm, Knauer) prepacked column was used. GLC (He at 50 kPa; 3 min at 80°C, 80–120°C with 3°C min<sup>-1</sup>, 120–170°C with 0.5°C min<sup>-1</sup>, 170–280°C with 5°C min<sup>-1</sup>) was carried out on a Fisons GC 8000 instrument using a fused silica capillary column coated with DB 1 phase (30 m × 0.32 mm, J&W).

### 3.2. Isolation

*F. indica* was collected in 1996 near Hurghada Egypt and identified by Dr. M. Elgebaly from the National Research Centre (NRC) Cairo. A voucher specimen of the plant is deposited at the Herbarium of the NRC, Department of Chemotaxonomy. Dried powder of the whole plant of *F. indica* (4 kg) was exhaustively extracted with 80% MeOH. After removal of the solvent by evaporation, the residue was successively parti-

tioned between H<sub>2</sub>O and *n*-BuOH. The butanolic fr. was evaporated under red. pres. at 50°C to obtain a crude saponin mixture (25 g). CC on silica gel eluting with CHCl<sub>3</sub>–MeOH–H<sub>2</sub>O with increasing amounts of MeOH and H<sub>2</sub>O gave three frs.: I (3 g), II (5 g) and III (10 g). I and II were further chromatographed by means of Sephadex LH-20 eluting with MeOH–H<sub>2</sub>O 17:3 followed by prep. HPLC (isocratic, 31% MeCN in H<sub>2</sub>O) to give pure saponins 1 (18 mg), 2 (12 mg), 3 (9 mg) and 4 (7 mg).

### 3.3. (*R*)-2-Butylglycosides

*R*<sub>i</sub> according to (van den Dool, & Kratz, 1963). A sample (ca. 250 μg) of the appropriate saponin was hydrolysed with 0.5 ml 5% HCl for at least 3 h at 80°C. After evaporation of the acid under red. pres., 0.5 ml (*R*)-(-)-2-BuOH was added, dried HCl gas was bubbled through the soln for 30 s and the reaction mixture was heated for 3 h at 80°C under N<sub>2</sub> in a sealed vessel. Trimethylsilylation was performed with *N*-methyl-*N*-trimethylsilyltrifluoroacetamide overnight. (*R*)-2-butyl-L-Ara: *R*<sub>i</sub> 39.41, *R*<sub>f</sub> 1775; (*R*)-2-butyl-D-

Ara:  $R_t$  38.39,  $R_i$  1764; (*R*)-2-butyl-L-Glc:  $R_t$  81.78,  $R_i$  2083; (*R*)-2-butyl-D-Glc:  $R_t$  82.21,  $R_i$  2086. Identification of the sugars were done by comparison of the  $R_i$  values and co-injection with the appropriate standard. Consequently it was shown for the 4 saponins that arabinose belongs to the L- and glucose to the D-series.

### 3.4. Indicasaponin A (1)

( $C_{52}H_{84}O_{21}$ ,  $M_r$  1044); amorphous powder;  $[\alpha]_D^{25} + 23$  (MeOH;  $c$  0.17). LSI-MS negative ion mode  $m/z$  (rel. int.): 1043  $[M-H]^-$  (60), 881  $[M-H-Glc]^-$  (12), 719  $[M-H-2 \times Glc]^-$  (8), 587  $[M-H-2 \times Glc-Ara]^-$  (10) and 455  $[M-H-2 \times Glc-2 \times Ara]^-$  (10).  $^1H$  NMR and  $^{13}C$  NMR: Tables 1 and 2.

### 3.5. Indicasaponin B (3)

( $C_{52}H_{84}O_{21}$ ,  $M_r$  1044); amorphous powder;  $[\alpha]_D^{25} + 24$  (MeOH;  $c$  0.16). LSI-MS negative ion mode  $m/z$  (rel. int.): 1043  $[M-H]^-$  (58), 881  $[M-H-Glc]^-$  (14), 719  $[M-H-2 \times Glc]^-$  (10), 587  $[M-H-2 \times Glc-Ara]^-$  (10) and 455  $[M-H-2 \times Glc-2 \times Ara]^-$  (12).  $^1H$  NMR and  $^{13}C$  NMR: Tables 1 and 2.

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