Sulfonated Triterpenoid Saponins from *Fagonia indica*

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Two new triterpenoid saponins, 3-*O*-{[β-D-4-*O*-sulfonylglucopyranosyl-(1→3)]-α-L-arabinopyranosyl}-ursolic acid-28-*O*-[β-D-glucopyranosyl] ester (indicasaponin C) and 3-*O*-{[β-D-4-*O*-sulfonylglucopyranosyl-(1→3)]-[β-D-xylopyranosyl-(1→2)]-α-L-arabinopyranosyl}-ursolic acid-28-*O*-[β-D-glucopyranosyl] ester (indicasaponin D) have been isolated from *Fagonia indica*. The structures were determined primarily by NMR spectroscopy. The assignment of NMR signals was performed by means of ^{1}H–^{1}H COSY, NOESY, ROESY, TOCSY, HMQC and HMBC experiments.

Introduction

Fagonia indica Brum. fil. belongs to the family Zygophyllaceae and is widely distributed in Egypt and Pakistan. The sapogenins oleanolic acid, betulic acid (Rizk *et al.*, 1972), hederagenin and ursolic acid (Rahman and Ansari, 1984) could be detected after hydrolysis of the EtOH extract of the aerial parts of *F. indica*. Two taraxast-20-en-28-oic acid saponins have been isolated from *F. indica* (Ansari *et al.*, 1987). In the course of our investigations on saponins of this plant we have obtained two ursolic acid and two oleanolic acid saponins (Shaker *et al.*, 1999). In this report, we present the isolation and structure elucidation of two new sulfonated triterpenoid saponins.

Results and Discussion

The butanol extract of the whole plants of *F. indica* were obtained as described in the experimental section. The crude saponins were chromatographed by column chromatography on silica gel to be eluted successively with $CHCl_3$, $CHCl_3$–MeOH and $CHCl_3$–MeOH–H_2O with increasing amounts of MeOH and H_2O. The saponins **1** and **2** 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 the $[M-1]^-$ ion at *m/z* 991. The fragment ions at *m/z* 829 $[M-1\text{-}162]^-$ and *m/z* 587 $[M-1\text{-}162\text{-}242]^-$ showed the loss of a hexose moiety and hexose plus sulfonylhexose moiety. The fragment ion at *m/z* 455 $[M-1\text{-}162\text{-}242\text{-}132]^-$ indicated the elimination of a hexose plus sulfonylhexose plus pentose moiety. The $-OSO_3H$ group was characterized by the fragments *m/z* 97 $[SO_4H]^-$ and *m/z* 80 $[SO_3]^-$. The $[M-1]^-$ ion together with ^{1}H and ^{13}C NMR data allowed us to propose the molecular formula $C_{47}H_{76}O_{20}S$.

The ^{1}H and ^{13}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. Three anomeric proton signals at δ 4.26 (^3J = 7.6 Hz), 4.58 (^3J = 7.8 Hz), and 5.32 (^3J = 8.2 Hz) indicated the presence of three saccharide units, one bonded as a glycosylester (δ 5.32) and the two others as glycosides (δ 4.26, 4.58). By use of the coupling constants of the anomeric protons (^3J = 7.6–8.2 Hz), ^{1}H–^{1}H COSY-45 and TOCSY spectra and the determination of the D-form for glucose and the L-form for arabinose (as described in the experimental section) the individual saccharides were identified as β-D-glucopyranose, β-D-4-*O*-sulfonylglucopyranose and α-L-arabinopyranose. The linkage of the 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′ arabinose → C-3 aglycone and H-1′′′′ glucosyl ester → C-28 aglycone indicated the points of linkage to the sapogenin.

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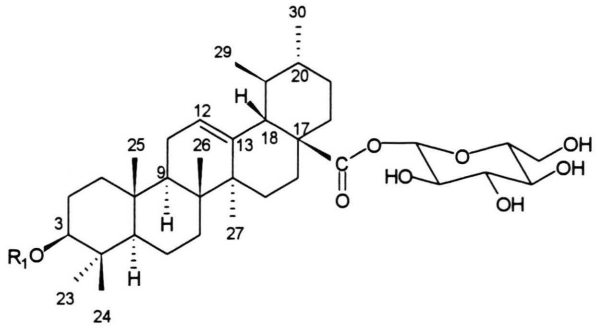
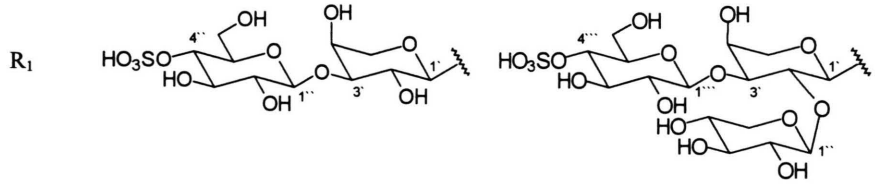


1 **2**



The HMBC cross peaks between H-1″ 4-*O*-sulfonylglucopyranose → C-3′ arabinose proved the interglycosidic linkage of 4-*O*-sulfonylglucopyranose at position C-3′. The downfield shifts of C-4″- (Δδ +6.7) and H-4″-signal (Δδ +0.82) of the sulfonated glucose of **1** in comparison with the glucose of the appropriate non sulfonated saponin were a proof for the sulfate group in position 4″.

The LSI mass spectrum of **2** exhibited the $[M-1]^-$ ion at *m/z* 1123. This together with ^1H and ^{13}C NMR data led to the molecular formula $C_{52}H_{84}O_{24}S$. The $[M-1]^-$ ion and the fragment ions at *m/z* 961 $[M-1\text{-}162]^-$, *m/z* 829 $[M-1\text{-}162\text{-}132]^-$, *m/z* 719 $[M-1\text{-}162\text{-}242]^-$, *m/z* 587 $[M-1\text{-}162\text{-}242\text{-}132]^-$ and *m/z* 455 $[M-1\text{-}162\text{-}242\text{-}2\times132]^-$ of **2** showed an additional pentose moiety compared with **1**.

The ^1H and ^{13}C NMR data of **2** were in agreement with those of **1** referring to the aglycone ursolic acid. One additional anomeric proton signal at δ 4.69 (^3J = 6.5 Hz) showed a further pentose unit. The coupling constants $^3J_{1''',2'''}$ = 6.5 Hz, $^3J_{2''',3'''} = {}^3J_{3''',4'''} = {}^3J_{4''',5ax'''}$ = 9.3 Hz and $^3J_{4''',5eq'''}$ = 4.3 Hz proved the axial position of the protons H-1‴–H-4‴ and thus the presence of a β-xylopyranose. The D-form for xylose was determined as described in the experimental section. The HMBC cross peak H-1‴ xylose/C-2′ arabinose (δ 4.69/δ 77.5) and the downfield shifts of the arabinose signals H-2′ (Δδ +0.17) and C-2′ (Δδ +5.2) in comparison with **1** proved the linkage of xylose in position 2 of arabinose.

Experimental

General

Negative ion MS: MAT 8500 (Finnigan), matrix glycerol. NMR: 500.13 MHz (^1H) and 125.76 MHz (^{13}C), reverse probehead, δ in ppm, solvent CD_3OD, CD_3OD signals were used as int. standard (^1H: 3.30, ^{13}C: 49.0), temp. 290 K, NOESY: phase-sensitive using TPPI, mixing time 300 and 600 msec, TOCSY: phase-sensitive using TPPI, mixing time 134.3 msec (80 MLEV-17 cycles plus 2 trim pulses of 2.5 msec each), HMQC: phase-sensitive using TPPI, BIRD sequence, GARP decoupled, HMBC: using TPPI, delay to achieve long range couplings: 71 msec ($J_{C,H}$ = 14 Hz).

CC: silica gel (0.063–0.2 mm); TLC: silica gel (0.25 and 1 mm precoated plates 60 F_{254}, Merck,

0.25 mm precoated plastic sheets SIL G/UV_{254} Macherey-Nagel), the spots were sprayed with 'triterpene reagent' (1% soln. of vanillin in 50% H_3PO_4), 'sugar reagent' (4% ethanolic aniline–4% ethanolic diphenylamine–H_3PO_4, 5:5:1 v/v) and phosphomolybdic acid reagent (Aldrich). For the preparative 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 (H_2 at 50 kPa; 3 min 80°, 80–120° with 3° min^{-1}, 120–170° with 0.5° min^{-1} 170–280° with 5° min^{-1}) was carried out on a Fisons GC 8130 instrument using a fused silica capillary column coated with DB 1 phase (30 m × 0.32 mm, J&W).

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 partitioned between H_2O 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 $CHCl_3$–MeOH–H_2O with increasing amounts of MeOH and H_2O gave three frs.: I (3 g), II (5 g) and III (10 g). I was further chromatographed by means of Sephadex LH-20 eluting with MeOH–H_2O 17:3 v/v and yielded three frs.: IV (1.1 g), V (1.2 g) and VI (100 mg). Prep. HPLC (isocratic, 31% MeCN in H_2O) of 100 mg of fr. V gave pure saponins **1** (5 mg) and **2** (8 mg).

(R)-2-Butylglycosides

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_2 in a sealed vessel. Trimethylsilylation was performed with *N*-methyl-*N*-trimethylsilyltrifluoroacetamide overnight. (*R*)-2-butyl-L-Ara: R_t 39.45, R_i 1777; (*R*)-2-butyl-D-Ara: R_t 38.42, R_i 1765; (*R*)-2-butyl-L-Xyl: R_t 39.21, R_i 1768; (*R*)-2-butyl-D-Xyl: R_t 38.12, R_i 1751; (*R*)-2-butyl-L-Glc: R_t 81.92, R_i 2086; (*R*)-2-butyl-D-Glc: R_t 82.25, R_i 2088. Identification of the sugars were done by comparison of the R_i values and co-injection with the appropriate standard. R_i according to (van den Dool and Kratz, 1963). Consequently it was shown for the two saponins that arabinose belongs to the L-, glucose and xylose to the D-series.

Spectroscopic data

Indicasaponin C (**1**): ($C_{47}H_{76}O_{20}S$, M_r 992); amorphous powder; $[\alpha]_D^{25}$ +18 (MeOH; c 0.15). LSI-MS negative ion mode *m/z* (rel. int.): 991 $[M-1]^-$ (50), 829 $[M-1-162]^-$ (10), 587 $[M-1-162-242]^-$ (12), 455 $[M-1-162-242-132]^-$ (8), 97 $[SO_4H]^-$ (100), 80 $[SO_3]^-$ (79). ^1H NMR and ^{13}C NMR: Tables I and II.

Table I. ^1H and ^{13}C NMR spectral data for the aglyca of saponins **1** and **2** in CD_3OD.

	1		**2**	
	^1H ax/eq	^{13}C	^1H ax/eq	^{13}C
1	0.98/1.39	40.0	0.99/1.38	39.9
2	1.73/1.82	27.3	1.74/1.81	27.3
3	3.10	90.7	3.10	91.3
4	–	40.4	–	40.3
5	0.76	57.0	0.75	57.1
6	1.38/1.52	19.0	1.38/1.51	19.1
7	1.50/1.37	34.2	1.50/1.37	34.2
8	–	41.1	–	41.0
9	1.52	49.0	1.52	49.0
10	–	38.0	–	37.8
11	1.90/2.01	23.8	1.90/1.99	23.7
12	5.23	127.3	5.23	127.2
13	–	138.9	–	138.9
14	–	43.4	–	43.2
15	1.91/1.10	29.2	1.91/1.11	29.2
16	2.14/1.78	24.1	2.14/1.78	24.3
17	–	49.0	–	49.0
18	2.21 *d* 11.5 Hz	54.3	2.21 *d* 11.5 Hz	54.1
19	1.39	40.4	1.40	40.4
20	0.98	40.9	0.98	40.5
21	1.26/1.38	30.9	1.26/1.38	30.8
22	1.75/1.65	37.5	1.75/1.65	37.4
23	1.04 *s*	28.2	1.04 *s*	28.2
24	0.83 *s*	17.2	0.83 *s*	16.7
25	0.96 *s*	16.3	0.95 *s*	16.2
26	0.82 *s*	17.8	0.82 *s*	17.7
27	1.10 *s*	24.1	1.11 *s*	24.0
28	–	177.9	–	177.9
29	0.88 *d* 6.6 Hz	18.0	0.88 *d* 6.6 Hz	17.9
30	1.31 *d* 6.0 Hz	21.7	1.32 *d* 6.0 Hz	21.6

Table II. ^1H and ^{13}C NMR spectral data for the sugar moieties of saponins **1** and **2** in CD_3OD.

	1		**2**	
	^1H	^{13}C	^1H	^{13}C
Ara				
1′	4.26 *d* 7.6 Hz	107.3	4.35 *d* 6.7 Hz	106.2
2′	3.68	72.3	3.85	77.5
3′	3.62	84.1	3.80	83.8
4′	4.02	69.8	4.05	69.8
5′	3.54 ax 3.82 eq	66.9	3.51 ax 3.82 eq	66.4
GlcSO$_3$H				
1″	4.58 *d* 7.8 Hz	105.3	4.63 *d* 7.7 Hz	104.8
2″	3.39	75.4	3.42	76.0
3″	3.66	76.3	3.64	76.5
4″	4.13	77.6	4.12	77.5
5″	3.42	78.4	3.45	78.2
6″	3.84/3.72	62.5	3.85/3.72	62.2
Xyl				
1‴			4.69 *d* 6.5 Hz	104.8
2‴			3.08	76.2
3‴			3.25	78.5
4‴			3.40	70.9
5‴			3.12 ax 3.78 eq	67.1
28-Glc				
1⁗	5.32 *d* 8.2 Hz	95.8	5.32 d 8.2 Hz	95.6
2⁗	3.31	73.6	3.29	73.8
3⁗	3.39	78.6	3.39	78.0
4⁗	3.33	71.6	3.32	71.4
5⁗	3.37	78.3	3.38	78.3
6⁗	3.81/3.68	62.4	3.81/3.68	62.1

Indicasaponin D (**2**): ($C_{52}H_{84}O_{24}S$, M_r 1124); amorphous powder; $[\alpha]_D^{25}$ +27 (MeOH; c 0.18). LSI-MS negative ion mode *m/z* (rel. int.): 1123 $[M-1]^-$ (62), 961 $[M-1\text{-}162]^-$ (10), 829 $[M-1\text{-}162\text{-}132]^-$, 719 $[M-1\text{-}162\text{-}242]^-$ (9), 587 $[M-1\text{-}162\text{-}242\text{-}132]^-$ (8) and 455 $[M-1\text{-}162\text{-}242\text{-}2\times132]^-$ (8), 97 $[SO_4H]^-$ (100), 80 $[SO_3]^-$ (79). ^1H NMR and ^{13}C NMR: Tables I and II.

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Ansari A. A., Kenne L. and Rahman A. U. (1987), Isolation and characterization of two saponins from *Fagonia indica*. Phytochemistry **26**, 1487–1490.

van den Dool H. and Kratz, P. D. (1963), A generalization of the retention index system including linear temperature programmed gas-liquid partition chromatography. J. Chromatog. **11**, 463–471.

Rahman A. U. and Ansari A. A. (1984), Hederagenin, ursolic acid, and pinatol from *Fagonia indica*. J. Nat. Prod. **47**, 186–187.

Rizk A. M., Hammouda F. M. and Abdel-Gawad M. M. (1972), The triterpenoid sapogenins of *Fagonia indica* Brum.. Pharmazie **27**, 534–535.

Shaker K. H., Bernhardt M., Elgamal M. H. A. and Seifert, K. (1999), Triterpenoid saponins from *Fagonia indica*. Phytochemistry **51**, 1049–1053.