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ISOLATION AND CHARACTERIZATION OF TWO SAPONINS FROM *FAGONIA INDICA*

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Abstract—Two new bisglycosidic triterpenoid saponins were isolated from the ethanolic extract of the aerial parts of *Fagonia indica*. They were characterized as 23,28-di-*O*-β-D-glucopyranosyltaraxer-20-en-28-oic acid and 3β,28-di-*O*-β-D-glucopyranosyl-23-hydroxytaraxer-20-en-28-oic acid. Furthermore, the conversion of the aglycone to 3β,23-dihydroxy-28,20β-taraxastonolide, nahagenin, during the acidic hydrolysis of the new saponins was studied.

INTRODUCTION

Fagonia indica L. [1, 2] is a small spiny undershrub which is widely distributed in Pakistan. It is reputed to be a medicinal plant in scientific and folkloric literature [1, 2]. Extracts of the aerial parts have been used in the indigenous system of medicine for the treatment of various diseases. In previous papers the isolation and identification of different types of compounds from *F. indica* were reported [3, 4]. Our continuing interest in the constituents of *F. indica* has led to the isolation of two new saponins. This paper describes the isolation and structure elucidation of the two saponins and the rearrangement of their aglycone to the earlier isolated nahagenin [3] during acidic hydrolysis.

RESULTS AND DISCUSSION

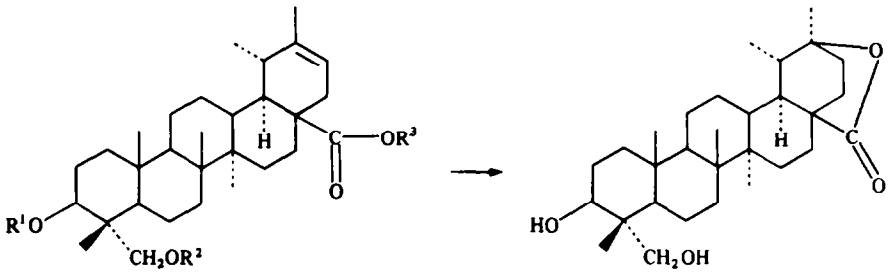
The ethanol extract of the aerial parts of *F. indica* afforded a fraction containing saponins upon precipitation with acetone. One of the saponins was isolated by repeated chromatography on silica gel. It could also be isolated in a better yield as the peracetate after acetylation of the crude product followed by repeated chromatography on silica gel. This saponin was designated as saponin A (**1**).

Another saponin could be isolated after repeated chromatography on Sephadex LH-20 and Biogel P-2. This saponin was designated as saponin B (**2**).

Both saponins were analysed by ^{1}H NMR and ^{13}C NMR spectroscopy, which showed that they each contained a triterpenoic acid and two sugars. On acid hydrolysis of the two saponins, D-glucose and the sapogenin nahagenin (**4**), found earlier after acidic hydrolysis of the ethanol extract [3], were identified. The sugar was analysed as the alditol acetate by GC/MS and nahagenin by comparison with an authentic sample. Analysis of the NMR spectra (Table 1), however, showed that the triterpenoic acid contained a double bond and only two hydroxylated carbons, unlike nahagenin which does not possess any double bond and has three carbons substituted with oxygen. The NMR spectra also showed that one of the sugars is linked to a carboxyl group. This was evident from the signals for the anomeric proton and carbon which had chemical shifts $\delta_H \sim 6.16$ and δ_C 94.9 in saponin A and saponin B. On the other hand, in nahagenin (**4**) the carboxyl group is in the lactone form with the oxygen at C-20. These data indicate that nahagenin is not the sapogenin of saponin A and saponin B but is formed during the acidic hydrolysis by rearrangement of the double bond and formation of the lactone.

The ^{1}H NMR chemical shifts and the large coupling constants ($J_{1,2} \sim 7.8$ Hz) for the anomeric protons of the two D-glucose residues proved these to be β-D-glucopyranosyl groups. As discussed above, one of these is substituted to the carboxyl group but the other is substituted to the oxygen at C-3 or C-23 of the aglycone or to the ester-linked β-D-glucopyranosyl group forming a disaccharide at C-28. Analysis of the region δ60–80 of the ^{13}C NMR spectra from saponin A (**1**) and saponin B (**2**) showed that the signals given by the sugars had almost identical chemical shifts. However, the signals obtained from C-3 and C-23 (Table 1) gave different values when compared to those obtained from nahagenin. The chemical shift differences indicate that saponin A and saponin B are substituted in C-23 and C-3, respectively, with the other β-D-glycopyranosyl group. Similar chemical shift differences for C-3 and C-23 were recently observed from saponins in which hederagenin, having the same configuration in ring A, was substituted at C-3 or C-23 by sugar residues [5].

The substitution at C-23 in saponin A was further supported by ^{1}H NMR studies on the acetylated saponin A. Using two-dimensional techniques such as *J*-resolved, COSY and NOESY, all the signals could be assigned (Table 2) and the dipolar interaction between the anomeric proton (δ4.36) and H-23' (δ2.93) observed. Such interactions are observed for protons which are close to each other. That only interaction to one of the two protons on C-23 was observed could be due to the fact that in the optimum conformation of the glycosidic bond, H-23 lies away from the anomeric proton. This observation together with the low chemical shifts of the protons



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	R^1	R^2	R^3
1	H	β-D-Glc*p*	β-D-Glc*p*
2	β-D-Glc*p*	H	β-D-Glc*p*
3	β-D-Glc*p*	H	H

Scheme 1.

Table 1. ^{1}H NMR and ^{13}C NMR spectral data* of some selected signals of saponin A, and saponin B isolated from *Fagonia indica* together with the prosapogenin from saponin B and nahagenin. Coupling constants ($J_{H,H}$) are given in parentheses

Compound	^{1}H NMR (δ and *J*)			^{13}C NMR chemical shifts (δ)						
Saponin A (1)	H-1'†	H-1"†	H-21	C-1'†	C-1"†	C-3	C-23	C-20	C-21	C-28
	6.168	4.800	5.334	94.9	104.7	71.3	74.0	142.7	117.1	173.8
	(7.8)	(7.6)	(6.1; n.r.)‡							
Saponin B (2)	6.151	5.138	5.330	94.9	104.6	81.9	68.7	142.6	117.1	173.9
	(8.1)	(7.8)	(6.6; n.r.)							
Prosapogenin		5.154	5.418							
from saponin B (3)			(6.3; n.r.)							
Nahagenin (4)						73.6	68.2	83.4	~27.2	175.9

*Spectra were obtained in pyridine-d_5 at 80° (^{1}H) and 60° (^{13}C).
†H-1' = H-1 from the 28-*O*-β-D-Glc*p* group; H-1" = H-1 from the 3- or 23-*O*-β-D-Glc*p* group etc.
‡n.r., non-resolved coupling.

Table 2. ^{1}H NMR spectral data of some selected signals of nahagenin and acetylated saponin A (1) isolated from *Fagonia indica* (coupling constants in parentheses)

Residue	H-1	H-2	H-3	H-4	H-5	H-6	H-6'	H-21	H-23	H-23'
23-*O*-β-D-Glc*p*	4.36	5.02	5.22	5.07	3.64	4.12	4.23			
	(7.9)	(9.8)	(9.2)	(10.3)	(2.6; 4.8)	(−12.4)				
28-*O*-β-D-Glc*p*	5.64	5.14	5.26	5.11	3.79	4.05	4.26			
	(8.1)	(9.7)	(9.2)	(10.4)	(2.5; 5.0)	(−12.4)				
Aglycone of saponin A			4.72					5.24	3.56	2.93
			(4.9; 11.1)					(5.0; 9.6)	(−9.9)	
Nahagenin (4)			3.63						3.72	3.42
			(6.5; 9.0)						(−10.3)	

on C-23, showing that these are not acetylated, support the evidence for substitution of the β-D-glucopyranosyl group to C-23 of the sapogenin.

The prosapogenin (3) of saponin B could be isolated after treatment with base and purification on a column of Sephadex LH-20. A ^{1}H NMR spectrum (Table 1) of the prosapogenin showed that the double bond was still intact after the alkaline treatment during which the ester-linked β-D-glucopyranosyl group was removed.

Evidence for the position of the double bond at C-20/C-21 in the aglycone is obtained from the ^{1}H NMR and ^{13}C NMR spectra. There is only one proton connected to the olefinic carbons, giving a signal at δ_H 5.33 and δ_C 142.7 and 117.1 supporting the structure CH=C. The proton

chemical shift of one of the methyl groups δ1.67 in saponin A and 1.69 in the prosapogenin of saponin B, indicating a vinylic methyl group, is shifted to 1.32 in nahagenin. Also, the methyl group (C-30) giving a doublet at δ1.05 (J = 6.3 Hz) in saponin A, δ1.04 (J = 6.6 Hz) in saponin B and δ1.09 (J = 6.3 Hz) in the prosapogenin (3) of saponin B is shifted to δ1.00 (J = 7.1 Hz) in nahagenin (4). The increase of the coupling constant could reflect the conformational changes involved in the formation of the lactone and changing the form of ring E. The only position in the aglycone where the double bond can be placed in the light of above-mentioned properties is between C-20 and C-21. The formation of nahagenin can then be explained either by direct attack by the carboxyl group on the double bond or by the initial hydration of the double bond forming a tertiary alcohol followed by lactone formation during acidic hydrolysis.

In order to understand the mechanism of the conversion of the aglycone to nahagenin (4), hydrolysis in different labelled solvents (D_2O and $H_2{}^{18}O$) was performed. Analysis by mass spectrometry of the nahagenin formed showed that one deuterium had been incorporated (m/z 473, 455, 437 and 425) but no ^{18}O (m/z 472, 454, 436 and 424).

Nahagenin (4) was also formed during methanolysis of acetylated saponin A. These experiments show that nahagenin is formed directly by attack of the carboxyl group at the double bond and not through a hydroxylated intermediate (Scheme 1).

EXPERIMENTAL

Concentrations were performed under red. pres. at bath temps. not exceeding 55°. ^{1}H NMR spectra were obtained at 400 MHz and ^{13}C NMR spectra at 100 or 25 MHz on JEOL GX-400 and JEOL FX-100 spectrometers using tetramethylsilane (organic solvents), sodium 3-trimethylsilyl-propanoic acid-d_4 (^{1}H NMR, D_2O) and 1,4-dioxane (^{13}C NMR, D_2O; δ67.4) as internal references. Electron impact mass spectra (EIMS) were recorded on a Varian MAT-311A instrument using the direct inlet for sapogenins and GC for the alditol acetates. Separations were performed on SE-54 fused-silica capillary columns using authentic hexitol hexaacetates as references.

Plant material. Plants were collected in the neighbourhood of Karachi, Pakistan, and voucher specimens have been deposited at the Department of Botany, University of Karachi. The aerial parts of fresh plants (8 kg) were finely chopped and soaked in EtOH (2 × 20 l.) for 15 days. Extracts were concentrated in a cyclone evaporator maintaining the temp. between 50° and 55°. A dark-green semi-solid residue (53 g) was obtained, which was dissolved in MeOH (200 ml). Addition of Me_2CO (400 ml) resulted in precipitation and the ppt. was filtered, washed with petrol (40–60°; 500 ml), $CHCl_3$ (500 ml) and EtOAc (250 ml) and then dried, yielding a powder (18 g).

Isolation of saponin A (1). Crude product (10 g) containing saponins was fractionated by flash chromatography on silica gel (360 g), elution being carried out with $CHCl_3$–MeOH–EtOAc (4:5:7) and $CHCl_3$–MeOH–EtOAc–H_2O (4:5:7:2).

Evaporation of a fraction obtained with the first solvent contained a mixture of saponins (2.15 g) of which one was present in major quantity. Another fraction from the second solvent afforded, after evaporation, a mixture of saponins and pinatol (5.72 g). The product from the first fraction was rechromatographed on a silica gel column (140 g), elution being carried out with $CHCl_3$–MeOH–EtOAc (4:4:7) giving almost pure saponin A (0.5 g), of which a part was further purified by preparative TLC yielding pure saponin A (65 mg), $[\alpha]_{578}^{22}$ +1° (c 0.4; MeOH).

Isolation of saponin A as the peracetate. Crude product (160 g) was acetylated with Ac_2O (75 ml) in pyridine (75 ml) at room temp. for 24 hr, concentrated to dryness and fractionated by flash chromatography on silica gel (300 g). Elution was carried out with EtOAc–petrol, 40–60° (1:4) (6 l.) followed by increasing amounts of EtOAc. A fraction (1.0 g, after evaporation to dryness) obtained with the solvent mixture 3:2 contained acetylated saponin A, which was further purified by chromatography on silica gel (80 g), using EtOAc–n-hexane (1:1) as solvent, yielding pure acetylated saponin A (0.2 g), $[\alpha]_{578}^{22}$ +7° (c 0.8; $CHCl_3$).

Isolation of saponin B (2). Crude product (5 g) was chromatographed on a column of Sephadex LH-20 (2.6 × 80 cm). Elution was performed with EtOH–H_2O (1:1). The first fraction contained a mixture of saponins. The mixture was passed through a silica gel (30 g) column eluted with $CHCl_3$–MeOH–EtOAc (4:5:7) to remove the yellow colour. On evaporation of the solvent, a white saponin mixture (1.0 g) was obtained. By chromatography on first Biogel P-2 and then Sephadex LH-20 eluted with H_2O, a fraction containing pure saponin B (35 mg) was obtained, $[\alpha]_{578}^{22}$ +1° (c 0.3; MeOH).

Acid hydrolysis of saponin A and saponin B. Saponins A and B (20 and 12 mg, respectively) were refluxed with 20% HCl in EtOH (1:1, 2 ml) for 2 hr, diluted with H_2O, concentrated to remove the EtOH and extracted with $CHCl_3$. The obtained sapogenin was purified by silica gel chromatography using $CHCl_3$–MeOH (24:1) as solvent. The sapogenin was identical to nahagenin (4) as shown by co-chromatography, MS and NMR spectroscopy.

The aq. layer was further hydrolysed with 0.5 M HCl, neutralized with Ag_2CO_3 and filtered. $NaBH_4$ (10 mg) was added and the soln made acidic by addition of Dowex 50 (H^+) after 2 hr. The soln was filtered and concentrated to dryness, co-distilled with MeOH (2 × 2 ml) and the alditols were acetylated with Ac_2O in pyridine (1:1, 1 ml) at 100° for 30 min. The alditol acetates were analysed by GC/MS.

Alkaline treatment of saponin B. Saponin B (10 mg) was heated under reflux with 2.5% NaOH in H_2O for 2 hr. The soln was made acidic with 0.5 M HCl and extracted with H_2O-saturated n-BuOH. On evaporation of the organic phase, the prosapogenin (3) was obtained, which was purified on a column of Sephadex LH-20 eluted with H_2O–EtOH (1:1), yielding the pure product (5 mg).

Hydrolysis in D_2O. Acetylated saponin A (15 mg) was refluxed in 20% DCl in D_2O and CD_3OD (1:1, 2 ml) for 3 hr, diluted with H_2O and extracted with $CHCl_3$. The sapogenin was purified by a small column of silica gel using $CHCl_3$–MeOH (24:1) as solvent.

Hydrolysis in $H_2{}^{18}O$. A mixture of saponins A and B (40 mg) in 1 M methanolic HCl (1 ml) and $H_2{}^{18}O$ (0.5 ml; 50% isotopic purity) was heated to 80° for 12 hr. The acid was then removed by repeated evaporation with MeOH. The product was partitioned between $CHCl_3$ and H_2O and the organic layer was evaporated. The nahagenin obtained was purified by chromatography on silica gel using $CHCl_3$–MeOH (24:1) as solvent.

Methanolysis. Acetylated saponin A (12 mg) was treated with 1 M methanolic HCl (2 ml) at 80° for 12 hr. The acid was removed by repeated evaporation with MeOH and the nahagenin was obtained a described above.

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