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ISOLATION AND CHARACTERIZATION OF A SAPONIN FROM *FAGONIA INDICA*

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Key Word Index—*Fagonia indica*; Zygophyllaceae; structural elucidation, 21,22α-epoxy-23-*O*-β-D-glucopyranosyl-nahagenin; saponin; glycoside.

Abstract—A triterpenoid saponin mixture, obtained from the ethanolic extract of the aerial parts of *Fagonia indica*, was acetylated and a saponin isolated, which was characterized as 21,22α-epoxy-23-*O*-β-D-glucopyranosyl-nahagenin after de-*O*-acetylation. The aglycone was found to be transformed to 21α,22β-dihydroxynahagenin during acidic hydrolysis.

INTRODUCTION

Extracts from the aerial parts of *Fagonia indica* L. are used in Pakistani traditional medicine [1, 2]. In previous papers the isolation and identification of different types of compounds from *F. indica* were reported [3–5].

Our continuing interest in the saponins of *F. indica*, due to their novel structures, has led to the isolation of a new saponin. This paper describes the isolation and structure elucidation of this saponin and the rearrangement of its aglycone 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. After acetylation of the crude product, followed by repeated chromatography on silica gel, an acetylated saponin was isolated. After de-*O*-acetylation and purification by chromatography on silica gel a saponin was obtained, which was designated saponin C (**1**).

Analysis of the ^{1}H and ^{13}C NMR spectra showed that saponin C contained a pentacyclic triterpenoid and one sugar residue. On acid hydrolysis of saponin C, D-glucose and a sapogenin (**2**) were obtained. The sugar was analysed as the alditol acetate by GC/MS and the absolute configuration determined by GC of the glycosides obtained by reaction with (+)-2-butanol and trimethylsilylation [6].

The sapogenin (**2**) could be isolated from saponin C (**1**) after hydrolysis with acid and purification by silica gel chromatography. The IR spectrum of the sapogenin showed an absorption at 1740 cm^{-1} which indicated the presence of a six-membered ring lactone and a prominent absorption at 3460 cm^{-1} showed the presence of hydroxy groups.

The sapogenin was further analysed by ^{1}H and ^{13}C NMR spectroscopy. Some ^{13}C-DEPT experiments [7] showed that the sapogenin contained six Me, nine CH_2, eight CH and seven quaternary carbons. The ^{1}H NMR spectrum showed signals for five methyl groups attached to quaternary carbons (singlets) and one methyl group to a methine carbon (doublet). The spectra also

indicated the presence of five carbons carrying oxygen. As evident from the ^{13}C NMR chemical shifts (Table 1) and DEPT and ^{1}H NMR spectra (Table 2) one carbon was involved in a methoxy group and one was a quaternary carbon bound to a lactone oxygen. This was found earlier in sapogenins isolated from *F. indica* [3, 5], whereas the other three methine carbons were hydroxylated. By comparison of the NMR spectra with those from nahagenin, a sapogenin earlier isolated from the crude extract [3, 5], it could be concluded that the new sapogenin was a hydroxylated form of nahagenin. This was supported by the molecular ion *m/z* 504 $[M]^+$ in the mass spectrum which corresponds to nahagenin with two additional hydroxyl groups.

To assign the different spin systems and to find the position and configuration of the two hydroxyl groups, H,H-COSY experiments, resolution enhancement of the ^{1}H NMR spectrum to observe the long-range couplings [8], and NOE-difference spectroscopy [9, 10] were performed. The results from these experiments confirmed the presence of the same skeleton as found in nahagenin. Furthermore the hydroxyl group at C-3 is equatorially oriented, which is evident from the coupling constants observed for the H-3 signal (Table 2). The additional hydroxyl groups are substituted at C-21 and C-22. This was determined from the observation of a long-range coupling between H-19 and H-21 (Table 2) and the results of the NOE-difference experiments (Table 3). In these experiments signals from protons close in space to the irradiated proton, will be enhanced. By irradiation of H-22, enhancement was observed for the signals from H-21 and H-18α demonstrating the α-configuration of H-22 and consequently the β-configuration of the hydroxyl group. The α-configuration of the hydroxyl group at C-21 was confirmed by the ^{4}J-*W* coupling path from H-21 to H-19.

In the ^{13}C NMR spectrum of saponin C (**1**), however, only signals from one methine carbon substituted with a hydroxyl group was obtained (Table 1). Instead two signals at δ59.70 ppm and 57.90 ppm were observed. Those chemical shifts indicated an epoxide ring [11] which was transformed to two hydroxyl groups during the acidic hydrolysis. This was confirmed by the ^{13}C NMR spectrum of the saponin as the two C-21 and C-22 signals were shifted to δ83.81 and 71.24 ppm, respectively. The assignment of these signals was performed by selective heteronuclear decoupling experiments. The 21,22α-configuration of the epoxide in saponin C was confirmed by a long-range coupling of 2.0 Hz between H-21 and H-19, which is derived from a *W*-arrangement of these protons.

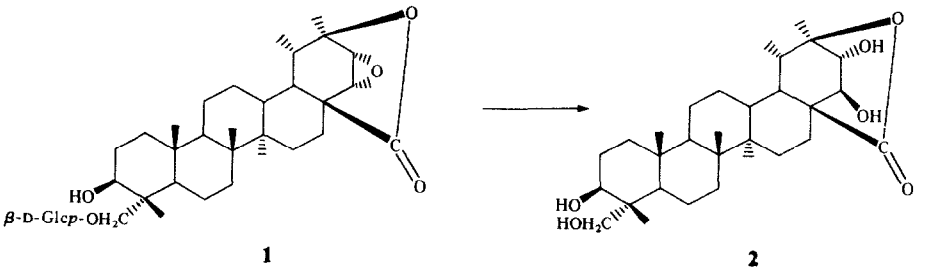
The anomeric configuration and the substitution position of the D-glucose residue could be determined by the ^{1}H and ^{13}C NMR spectra. The signals of the anomeric carbon, δ104.73 ppm, and proton δ4.783 ppm $J_{1,2}$ 7.6 Hz showed that the sugar was linked as a β-D-glucopyranosyl group. From the chemical shifts of the C-3 and C-23 signals in saponin C and the sapogenin, respectively (Table 1), it is evident that the sugar is linked to C-23 as a significant glycosidation shift (5.6 ppm) is observed for the C-23 signal. This is further supported by the induced acetylation shift observed for H-3 (0.6 ppm) when spectra of saponin C and acetylated saponin C are compared. This C-23-position was also substituted by a β-D-glucopyranosyl group in another saponin isolated from *F. indica* [5].

During the removal of the sugar from the saponin by treatment with acid, transformation of the sapogenin

Table 1. ^{13}C NMR chemical shifts of several selected signals of acetylated saponin C, saponin C (**1**) and the sapogenin (**2**)

Compound	^{13}C NMR chemical shifts (δ)						
	C'-1	C-3	C-20	C-21	C-22	C-23	C-28
Acetylated Saponin C*	100.50	74.14	85.65	58.55	56.82	71.37	174.62
Saponin C†	104.73	71.84	87.77	59.70	57.90	72.94	171.19
Sapogenin†	—	73.84	86.66	83.81	71.24	67.31	‡

*Spectrum was obtained in $CDCl_3$ at 22°.
†Spectra were obtained in CD_3OD at 30°.
‡Signal was not obtained.



1 **2**

Table 2. ^{1}H NMR chemical shifts of the methyl groups and selected protons of acetylated saponin C, saponin C (**1**) and the sapogenin (**2**)

Compound	^{1}H NMR chemical shifts (δ)													
	H-3	H-15α	H-15β	H-18	H-19	H-21	H-22	H-23	H-23′	H-24	H-25	H-26	H-27	H-29	H-30	H′-1
Acetylated Saponin C*	4.700 (11.2, 4.6)§					3.312 (4.6, 2.0)	3.174 (4.6)	3.545 (9.9)	2.917 (9.9)	0.707	0.835	0.892	0.986	1.099 (6.8)	1.505	4.343 (7.8)
Saponin C†	4.103 (11.0, 5.4)					3.338 (4.6)	3.203 (4.6)			0.791	0.833	0.878	0.922	1.068 (7.0)	1.421	4.783 (7.6)
Sapogenin‡	3.502 (10.8, 5.7)	2.161	1.846	1.330	1.537	3.724 (4.6, 2.0)	3.895 (4.6)	3.422 (10.8)	3.191 (10.8)	0.586	0.803	0.844	0.881	1.087 (7.0)	1.320	

*Spectrum was obtained in a solution of $CDCl_3$ at 22° with TMS (δ 0.000 ppm) as reference.
†Spectrum was obtained in a solution of pyridine at 85°.
‡Spectrum was obtained in a solution of CD_3OD at 30° with TMS (δ 0.000 ppm) as reference.
§Coupling constants are given in parenthesis.

Table 3. Nuclear Overhauser enhancements (NOE) obtained for the sapogenin as observed by NOE difference spectroscopy*

Irradiated proton (δ)	Enhanced protons (δ)
H-15α	H-13, H-15β, H-16, H-26
H-21	H-22, H-30
H-22	H-18, H-21
H-24	H-23, H-23′, H-25

*Spectra were obtained for solutions in CD_3OD at 30°.

occurred (Scheme 1). This shows that the sapogenin obtained after hydrolysis was not a natural product. In a similar saponin from *F. indica* the observation of the rearrangement of a C-20, C-21 double bond to a lactone was reported [5].

EXPERIMENTAL

Solns were concd under red. pres. at temps not exceeding 55°. ^{1}H NMR spectra were obtained at 400 or 270 MHz and ^{13}C NMR spectra at 100 or 67.8 MHz TMS (δ_H 0.000) or dioxane (δ_C 67.40) as references. EIMS were recorded for the sapogenin on a Varian MAT-311A instrument using the direct inlet probe. Separation of alditol acetates and 2-butyl glycosides was performed on SE-54 fused-silica capillary columns (25 m × 0.3 mm) at 200° using a Hewlett–Packard 5970, MSD for GC/MS analysis. Authentic samples were used as references.

Plant material. Identification of the plant, and the extraction procedure yielding the crude saponin mixture were previously described [1].

Isolation of saponin C. Crude product (160 g) containing saponins was acetylated with Ac_2O (75 ml) in pyridine (75 ml) at 25° for 24 hr, concd 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 evapn to dryness) obtained with the solvent mixture 3:2 contained acetylated saponin C, which was further purified by chromatography on silica gel (80 g), using EtOAc–*n*-hexane (1:1) as solvent, yielding pure acetylated saponin C (51 mg).

De-O-acetylation of acetylated saponin C. Acetylated saponin C (20 mg) was de-*O*-acetylated with 0.05 M NaOMe in MeOH (10 ml) at room temp. for 14 hr, neutralized with Dowex 50 (H^+), filtered and concd to dryness. The saponin was purified on a column (40 × 1.6 cm) of Sephadex LH-20 eluted with EtOH–H_2O (1:1).

Acid hydrolysis of saponin C. Saponin C (10 mg) was refluxed with 20% HCl in EtOH (1:1, 2 ml) for 2 hr, diluted with H_2O, concd to remove the EtOH and extracted with $CHCl_3$. The sapogenin obtained from the organic phase was purified by chromatography on silica gel using $CHCl_3$–MeOH (24:1) as solvent. The sapogenin was subjected to analysis by MS and NMR spectroscopy.

The aq. layer was further hydrolysed with 2M CF_3COOH and concd to dryness. Part of the material was reduced with $NaBH_4$ in H_2O (10 mg, 1 ml) and the soln made acidic by addition of Dowex 50 (H^+) after 2 hr. The soln was filtered and concd 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.

The other part of the hydrolysate was treated with 1 M HCl in (+)-2-BuOH (0.2 ml) at 100° for 8 hr in a sealed tube. The

mixture was then evapd to dryness, silylated with bis(trimethylsilyl)trifluoroacetamide (BSTFA) in pyridine at 90° for 30 min, concd to dryness, dissolved in $CHCl_3$ and analysed by GC [6].

NMR spectroscopy. 2-D DQF H, H-COSY experiments were performed at 400 MHz. A digital resolution of 4.3 Hz in both dimensions were used and the FID's were multiplied by a non-shifted sine-square function before Fourier transformation. NOE-difference experiments [9, 10] were performed with a JEOL pulse-sequence available in the GSX software.

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LYCOPERSICONOL, A PREGNANE DERIVATIVE FROM TOMATO STOCK ROOTS

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Key Word Index—*Lycopersicon esculentum* × *L. hirsutum*; Solanaceae; tomato stock; lycopersiconol; 3β,16β-dihydroxy-5α-pregnan-20-one.

Abstract—Lycopersiconol was isolated from tomato stock roots and characterized as 3β, 16β-dihydroxy-5α-pregnan-20-one.

INTRODUCTION

In a previous communication [1], a steroid lactone, lycopersiconolide (**3**), was reported as a constituent of roots of a tomato stock (Taibyo shinko No. 1; *Lycopersicon esculentum* × *L. hirsutum*, hybrid, Takii Co. Ltd). A further study of the plant material has now allowed the isolation of a new pregnane derivative, lycopersiconol (**1**).

RESULTS AND DISCUSSION

Lycopersiconol (**1**) was obtained as a crystalline compound; mp 202–204°; IR ν_{max}^{KBr} cm^{-1}: 3380 and 3290 (OH), 1670 (C=O); HRMS: 334.2511 ($C_{21}H_{34}O_3$). The ^{13}C NMR spectrum (Table 1) exhibited 21 signals; three methyls, eight methylenes, seven methines and three quaternary carbons. The chemical shift values of the carbon atoms of rings A, B and C of **1** were found to be very similar to those of **3**, whereas those of ring D showed some differences. The remaining two signals at δ31.7 (Me) and 213.0 (quaternary) arose from the methyl ketone, which was attached to ring D rather than from a γ-lactone ring as found in compound **3**. Compound **1** was acetylated with acetic anhydride–pyridine to yield the diacetate **2**, whose mass spectrum exhibited an ion at *m/z* 419 [M + H]$^+$ and the ^1H NMR spectrum showed two acetyl