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STRUCTURAL STUDIES ON A SAPONIN ISOLATED FROM *NIGELLA SATIVA*

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Key Word Index—*Nigella sativa*; Ranunculaceae; saponin; structural elucidation; hederagenin.

Abstract—A saponin, isolated from an ethanolic extract of the seeds of *Nigella sativa*, was characterized as 3-*O*-[β-D-xylopyranosyl-(1-3)-α-L-rhamnopyranosyl-(1-2)-α-L-arabinopyranosyl]-28-*O*-[α-L-rhamnopyranosyl-(1-4)-β-D-glucopyranosyl-(1–6)-β-D-glucopyranosyl]-hederagenin.

INTRODUCTION

Nigella sativa L. (Ranunculaceae) is a herbaceous plant which grows in Mediterranean countries and is cultivated in Pakistan. It is used as a spice and for the treatment of various diseases [1–3]. The isolation and identification of different alkaloids from *N. sativa* have been reported [4–8]. Our interest in the saponins from medicinal plants used in traditional medicine in Pakistan has led to the analysis of various saponin extracts from these plants. This paper describes the isolation and structural elucidation of a triterpenoid saponin from the seeds of *N. sativa*.

RESULTS AND DISCUSSION

The ethanol extract of the seeds of *N. sativa* afforded a fraction containing saponins after partition between hydrochloric acid and *n*-butanol. A saponin was isolated from this fraction by chromatography on Sephadex LH-20 and silica gel. The saponin was analysed by ^1H and ^{13}C NMR spectroscopy, which showed that it contained a triterpenoic acid and six sugars.

Acid hydrolysis of the saponin yielded hederagenin, indistinguishable from an authentic sample (TLC, NMR). The monosaccharides simultaneously released were analysed as their alditol acetates by GC/MS and their absolute configuration by GC after reaction with (+)-2-butanol and trimethylsilation [9]. L-Rhamnose, D-glucose, D-xylose and L-arabinose in the relative portions 2:2:1:1 were the only sugars detected. The linkages by which the sugar residues are connected were determined by methylation analysis [10]. The methylated sugars obtained are listed in Table 1. By using sodium borodeuteride as a reducing agent, 2- and 4-linked pentopyranosyl residues could be distinguished by MS.

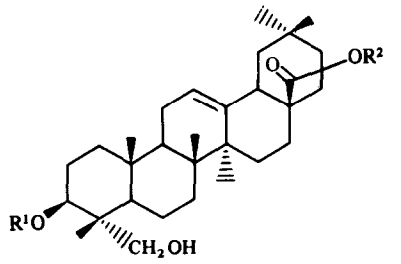
A linked scan FABMS spectrum, observing negative ions, of the saponin (**1**) showed the fragments *m/z* 881, 749, 603 and 471 in addition to the molecular ion at *m/z* 1351 [M−1]. The fragments correspond to the consecutive loss of a trisaccharide, consisting of two hexoses

Table 1. Methylation analysis of the saponin (**1**), the prosapogenin (**2**) and the reduced trisaccharide (**3**) released by alkaline hydrolysis

Methylated sugar	T*	Residue/mol 1	2	3
2,3,4-Rha†	0.70	1		1
2,3,4-Xyl	0.70	1	1	
1,2,3,4,5-Glc	0.75			1
3,4-Ara	0.91	1	1	
2,4-Rha	0.93	1	1	
2,3,6-Glc	1.28	1		1
2,3,4-Glc	1.34	1		

*Retention time of the corresponding alditol acetate relative to 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol.

†2,3,4-Rha = 1,5-di-*O*-acetyl-2,3,4-tri-*O*-methyl-L-rhamnitol, *etc.*



1 R^1 = β-D-Xyl*p*-(1-3)-α-L-Rha*p*-(1-2)-α-L-Ara*p*-(1-
R^2 = α-L-Rha*p*-(1-4)-β-D-Glc*p*-(1-6)-β-D-Glc*p*-(1-

2 R^1 = β-D-Xyl*p*-(1-3)-α-L-Rha*p*-(1-2)-α-L-Ara*p*-(1-
R^2 = H

and one deoxyhexose, a pentose, a deoxyhexose and a pentose.

Alkaline hydrolysis of the saponin followed by reduction yielded a prosapogenin (**2**) and a reduced trisaccharide (**3**) which were analysed by sugar and methylation analysis (Table 1). The latter three fragments in the FABMS spectrum originate from the trisaccharide of the prosapogenin and gave, in conjunction with the methylation analysis, the sequence: D-Xyl*p*-(1–3)-L-Rha*p*-(1–2)-L-Ara*p*-(1-. This sequence was also confirmed by NOE difference spectroscopy. Irradiation of the anomeric signal of the rhamnosyl residue enhances the H-2 signal of the arabinopyranosyl residue and its own H-2 signal, inferring its own α-configuration. The anomeric configurations of the sugar residues in the trisaccharide were determined with ^{1}H and ^{13}C NMR spectroscopy to be β-D-xylopyranose (δ_H 4.49 *J* 7.3 Hz; δ_C 106.8), α-L-rhamnopyranose (δ_H 5.21 *J* 1.8 Hz; δ_C 101.7) and α-L-arabinopyranose (δ_H 4.54 *J* 6.0 Hz; δ_C 104.8). Such a trisaccharide linked to hederagenin in the 3-position has been reported earlier [11] and ^{13}C NMR spectra of these compounds were identical.

The sequence of the reduced trisaccharide (**3**) is L-Rha*p*-(1–4)-D-Glc*p*-(1–6)-D-Glc-ol. This was also confirmed by NOE difference spectroscopy. Irradiation of the anomeric proton of the rhamnopyranosyl group enhanced the H-4 signal of the glucopyranosyl residue and its own H-2 signal. Irradiation of the H-1 signal of the 4-linked glucopyranosyl residue yielded enhanced signals for H-6 of the glucitol and for its own H-3 and H-5 signals, demonstrating the linkage to the glucitol and the β-configuration, respectively. The anomeric configuration of the sugars were determined from the ^{1}H NMR data, yielding α-configuration for the rhamnopyranosyl group (δ_H 4.89 *J* 1.7 Hz) and β-configuration for the glucopyranosyl residue (δ_H 4.50 *J* 8.1 Hz). The β-configuration for the ester-linked glucopyranosyl residue (δ_H 6.08 *J* 7.5 Hz) was determined from the ^{1}H NMR spectrum of the saponin. The complete trisaccharide structure is thus α-L-Rha*p*-(1–4)-β-D-Glc*p*-(1–6)-β-D-Glc*p*-(1-.

On the basis of the results above, structures **1** and **2** are proposed for the saponin and the prosapogenin, respectively. Several saponins with hederagenin as the aglycone have been found in plants, most of them containing L-arabinose, D-xylose, D-glucose and L-rhamnose, which are glycosically linked to *O*-3 [12]. From *Anemone rivularis* a similar saponin has been reported [13]. The only difference is that the D-xylopyranosyl group is replaced by a D-ribopyranosyl group in the saponin from *A. rivularis*.

EXPERIMENTAL

Solns were concd under red. pres. at temps not exceeding 55°. ^{1}H NMR spectra were obtained at 270 MHz and ^{13}C NMR spectra at 67.8 MHz using TMS, sodium 3-trimethysilyl-tetradeuteriopropionate (TSP) (δ_H 0.00) or dioxane (δ_C 67.40) as ref. NOE difference spectroscopy was performed with a program available in the JEOL GSX-software. Separation of the alditol acetates and partially methylated alditol acetates was done on an SE-54 fused silica capillary column using a temperature program, from 190° (3 min) to 250° at 3°/min. The same system was used for separation of the 2-butyl glycosides but using a constant temp. (200°).

Plant material. Seeds (80 kg) of *N. sativa* were ground and extracted with EtOH in portions under vigorous stirring. The extract was filtered and the EtOH evapd to yield a gum which was then partitioned between 5% HCl and *n*-BuOH. The *n*-BuOH layer was evapd to dryness yielding crude material (600 g) containing saponins.

Isolation of the saponin. Crude material (5 g) was subjected to chromatography on a column of Sephadex LH-20 (80 × 2.6 cm) which was eluted with EtOH–H_2O (1:1). The first fraction contained one major saponin which was further purified by a column of silica gel eluted with $CHCl_3$–MeOH (9:1) yielding pure saponin (90 mg), $[\alpha]_D^{22} -11°$ (H_2O; *c* 0.2).

Acidic hydrolysis of the saponin. Saponin (30 mg) was refluxed with 20% HCl in EtOH (1:1, 3 ml) for 2 hr, diluted with H_2O and concd to remove most of the EtOH and then 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 identical with hederagenin as shown by co-chromatography, MS and NMR spectroscopy.

The aq. layer was further hydrolysed with 2 M CF_3COOH and then concd to dryness. Part of the material was reduced with $NaBH_4$ in H_2O (10 mg, 1 ml) and the soln was made acidic by addition of Dowex 50 (H^+) after 2 hr. The soln was filtered and concd to dryness, co-disd with MeOH (2 × 2 ml) and 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, trimethylsilylated with bis (trimethylsilyl)trifluoroacetamide (BSTFA) in pyridine at 90° for 30 min, concd to dryness, dissolved in $CHCl_3$ and analysed by GC.

Methylation analysis. Samples (1 mg) were methylated according to ref. [14]. The permethylated product was essentially analysed as described in ref. [10] using a Sep-pak C_{18} cartridge for purification [15] and borodeuteride as the reducing agent.

Alkaline treatment of the saponin. Saponin (15 mg) was treated with 0.5 M NaOH in H_2O (2 ml) for 14 hr at 85°. The soln was made acidic with HOAc and extd with H_2O-satd *n*-BuOH. By evapn of the organic solvent and purification on a column of Sephadex LH-20, eluted with H_2O–EtOH (1:1), the prosapogenin (8 mg) was obtained.

The aq. layer was concd to dryness and the product passed through a Bio-Gel P-2 column eluted with H_2O. A fraction containing a trisaccharide (3 mg) was obtained.

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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