Journal of Natural Products
Vol. 47, No. 1, pp. 44-46, Jan-Feb 1984

SAPONINS FROM *TRIGONELLA FOENUM-GRAECUM* LEAVES

I.P. VARSHNEY,[1] D.C. JAIN,[2]

Department of Chemistry, University of Garhwal, Srinagar-Garhwal 246174, India

and H.C. SRIVASTAVA

Chemistry Division, Ahmedabad Textile Industry's Research Association, Ahmedabad 380 015, India

ABSTRACT.—From the leaves of *Trigonella foenum-graecum* five new spirostanol saponins have been isolated and named as graecunin-B, -C, -D, -E, and -G. The structures of graecunin-G and graecunin-E have been shown to be (diosgenin) 3-O-α-*D*-glucopyranosyl (1↦2)α-*L*- rhamnopyranosyl (1↦-6)α-*D*-glucopyranoside and (diosgenin) 3-O-α-*D*-glucopyranosyl (1↦4)α-*D*-glucopyranosyl (1↦2)α-*L*-rhamnopyranosyl (1↦6)α-D-glucopyranoside, respectively.

Trigonella foenum-graecum L. (Leguminosae, sub-family Papillionaceae) is commonly known as methi in Hindi. Although various parts of the plant have been studied for steroidal saponins and sapogenins, little work has been done on the structure of the leaf saponins (1, 2) and sapogenins (3); hence, a study has been made of them.

RESULTS AND DISCUSSION

T. foenum-graecum leaves yielded a mixture of steroidal saponins which, on tlc examination, showed seven spots. The crude mixture was purified by column chromatography to give five pure saponins, named graecunin-B, -C, -D, -E, and -G. (Two trace compounds were named graecunin-A and -F but were not isolated in sufficient quantities to characterize them.)

The ir spectra of all the saponins showed the absorption of a spirostanol side-chain (*i.e.*, ir ν max (KBr) 980, 920, 900, 860 cm^{-1}). None of the saponins on tlc examination reacted with Ehrlich's reagent. These results indicate that all the saponins are spirostanol glycosides. Each saponin was separately acid-hydrolyzed to yield diosgenin (mp, mmp, tlc, ir) and sugars, which were identified by pc and glc, and their relative proportions estimated by glc (Table 1).

The sequence and interlinkage of the sugars in each saponin were determined by methylation using Hakomori's method (4) followed by Purdie methylation (5). The fully methylated saponins were subjected to methanolysis (6) with methanolic HCl and the products identified by glc (Table 1).

The saponins were partially hydrolyzed with 0.1 N H_2SO_4, and the sugars, liberated after 0.5 h, 1 h, and 2 h, were characterized. The prosapogenins obtained after 2 h were separated, hydrolyzed with 2 N H_2SO_4, and the sugars obtained were characterized (Table 1).

None of saponins on treatment with β-*D*-glucosidase liberated any sugar, which showed the absence of a β-*D*-configuration in a *D*-glucose molecule. The anomeric configuration of each monosaccharide has been assigned by the application of Klyne's rule (7).

Graecunin-G and -E were treated with 0.05M $NaIO_4$ solution, and no sugars were liberated, which indicated the absence of branching in the sugar chain; this is also supported by methanolysis results, as no dimethyl sugar was obtained.

---

[1]Present address: Manager, R&D, Mehta Pharmaceuticals Pvt. Ltd., Chheharta, Amritsar 143105, India.

[2]Present address: Central Institute of Medicinal & Aromatic Plants, Lucknow 226016, India.

TABLE 1. Important Characteristics of Graecunin Saponins.

Graecunin	A	B	C	D	E	F	G
Rf[a] value	0.16	0.20	0.28	0.40	0.56	0.78	0.82
mp	—	154-6°	147-9°	141-2°	136-8°	—	123-5°
$[\alpha]_D$	—	−46.0	+ 9.5	+49.0	+24.6	—	− 3.0
Diosgenin %	—	25.5	32.0	28.0	37.5	—	45.0
Sugars and molar ratio	—	G:X:R[b] 4:1:2	G:R 4:1	G:X:R 4:1:1	G:R 3:1	—	G:R 2:1
Methyl sugars	—	—	—	—	(i)3,4-di-O-Me-*L*-rhamnose (ii)2,3,4,6, tetra-O-Me-*D*-glucose (iii)2,3,6-tri-O-Me-*D*-glucose (iv)2,3,4, tri-O-Me-*D*-glucose	—	(i)3,4-di-O-Me-*L*-rhamnose (ii)2,3,4,6-tetra-O-Me-*D*-glucose (iii)2,3,4-tri-O-Me-*D*-glucose
Hydrolysis products ($0.1N\ H_2SO_4$) after							
0.5 h	—	G	G	G	G	—	G
1 h	—	G	G	G	G	—	G
2 h	—	G,R	G,R	G	G,R	—	G,R
Sugars from prosapogenin	—	G,X,R	G	G,X,R	G	—	G
Calculated molecular rotation	—	—	—	—	+280.38	—	−28.08
Observed molecular rotation	—	—	—	—	+257.32	—	−26.52

[a]Rf values in the solvent system ($CHCl_3$:MeOH:H_2O:60:40:10).

[b]G=glucose; X=xylose, R=rhamnose.

On the basis of the above data, the structure of the new glycoside graecunine-G has been assigned as (diosgenin) 3-O-α-*D*-glucopyranosyl (1↦2) α-*L*-rhamnopyranosyl (1↦6) α-*D*-glucopyranoside.

By analogy with graecunin-G, which has one glucose molecule less than graecunin-E, it is probable that the latter compound is an addition product formed by the combination of graecunin-G and one molecule of glucose. Therefore, graecunin-E has the structure (diosgenin) 3-O-α-*D*-glucopyranosyl (1↦4) α-*D*-glucopyranosyl (1↦2) α-*L*-rhamnopyranosyl (1↦6) α-*D*-glucopyranoside.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points are uncorrected. Chromatography was on Si-gel (BDH), neutral alumina and Whatman No. 1 paper. The following solvents were employed: solvent A, $CHCl_3$-MeOH-H_2O (65:20-40:10); solvent B, C_6H_6-Me_2CO (6:1); solvent C, *n*-butanol-pyridine-H_2O (6:4:3). The spots were revealed by spraying with either 10% aqueous H_2SO_4 or cinnamaldehyde reagent. Gc of the sugars was carried out using a Perkin-Elmer model 3920B with a column of 8% OS-138 on chromosorb-W (NAW), N_2 as carrier gas and a FID (1). Ir spectra were recorded on a Perkin-Elmer isospectrometer.

ISOLATION.—Leaves and stems of *Trigonella foenum-graecum* were separated and air dired. The dried leaves were defatted with light petroleum (40-60°) and then extracted with EtOH. Removal of solvent from the extract gave a dark-colored, syrupy mass, which was successively extracted with different solvents (*i.e.*, light petroleum, Et_2O, CCl_4, $CHCl_3$, EtOAc, and Me_2CO). The dark brown, syrupy residue thus obtained was dissolved in the minimum quantity of MeOH, and the saponins were precipitated in a large volume of Et_2O/Me_2CO a number of times. The crude mixture of saponins was chromatographed on a column of Si-gel (system A). Fractions giving identical spots on tlc were combined and rechromatographed to give five saponins. Each saponin was further purified by column chromatography on neutral alumina using light petroleum, EtOAc, Me_2CO, and MeOH as eluents. This gave five pure saponins. All saponins were recrystallized from MeOH-Me_2CO as amorphous powders.

IDENTIFICATION OF SAPOGENIN AND SUGARS.—The solution of each saponin B, C, and D (100 mg), and E, G (50 mg) in 2 N aqueous H_2SO_4 (25 ml) was refluxed for 4 h. Each reaction mixture was filtered, washed free of acid, and gave dried sapogenin B (25.2 mg), C (32.0 mg), D (28.0 mg), E (18.75 mg), and G (22.5 mg). Each was purified by recrystallization from MeOH, mp 204-5°; ir ν max (KBr) 3200-3400 (OH), 981, 920, 900, 852 cm^{-1}, (intensity 900>920; 25 *R* configuration). This compound was identical to diosgenin (tlc, mmp, ir, and acetate formation). Each of the filtrates was neutralized with $BaCO_3$, deionized with Amberlite IR 120 (H^+) and 400 (OH^-), evaporated to dryness in vacuum, and the residue examined by pc (system C) and, after conversion to the trimethylsilane derivatives, by glc the presence of *D*-glucose, *D*-xylose, and *L*-rhamnose was revealed.

METHYLATION AND IDENTIFICATION OF METHYLATED SUGARS.—Graecunin-G (50 mg) and graecunin-E (50 mg) were separately methylated by the Hakomori method and worked up as usual (4). The partially methylated products were completely methylated using Purdie's procedure (5). Each permethylated saponin (40 mg and 35 mg, respectively) was methanolyzed with dry 3% MeOH-HCl (3 ml) for 3 h and the methylated sugars analyzed by glc as before (Table 1).

ENZYMATIC HYDROLYSIS.—Each saponin (10 mg) was dissolved in (5 ml) sodium acetate buffer solution (pH=5) along with β-*D*-glucosidase (3 mg) and one drop of toluene. The mixture was incubated at 37° for 72 h. A precipitate was collected by filtration, and the filtrate was subjected to pc (system C). No sugar was detected.

PARTIAL HYDROLYSIS.—Each saponin was partially hydrolyzed with 0.1 N H_2SO_4, and the sugars liberated after 0.5 h, 1 h, and 2 h were characterized. The prosapogenin obtained after 2 h, was separated, hydrolyzed with 2 N H_2SO_4, and the sugars obtained characterized by glc, as before.

PERIODATE OXIDATION.—Graecunin-G and -E (10 mg) were treated with 0.05M $NaIO_4$ solution (3 ml). The reaction mixtures were kept in the dark for 48 h at room temperature before the contents were extracted with *n*-butanol. The extracts were evaporated, hydrolyzed with 2 N H_2SO_4 and the hydrolysates subjected to pc (system C).

ACKNOWLEDGMENTS

Thanks are due to the Council of Scientific and Industrial Research for the award of a research fellowship to D.C.J.

LITERATURE CITED

1. I.P. Varshney, D.C. Jain, H.C. Srivastava, and P.P. Singh, *J. Indian Chem. Soc.*, **54**, 1135 (1977).
2. I.P. Varshney and D.C. Jain, *Nat. Acad. Sci. Lett. (India)* **2**, 331 (1979).
3. I.P. Varshney and A.R. Sood, *Indian J. Appl. Chem.*, **34**, 208 (1971).
4. S. Hakomori, *J. Biochem. (Tokyo)*, **55**, 205 (1964).
5. T. Purdie and J.C. Irvine, *J. Chem. Soc.*, 1021 (1903).
6. E. Gordis, *Proc. Soc. Exptl. Biol. Med.* **110**, 659 (1962).
7. W. Klyne, *J. Biol. Chem.*, **47**, 41 (1950).

Received 28 December 1981