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Yamogenin 3–O–*b*–D-glucopyranosyl (1 → 4)–O–*a*–D-xylopyranoside from the seeds of *Trigonella foenum-graecum*

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Abstract. *Trigonella foenum-graecum* (NO – Leguminosae) is cultivated in many parts of India. It has been found to possess significant medicinal value. Its leaves are used both internally and externally to reduce swelling, prevent falling of hair and in treating burns. Its seeds are carminative, antipyretic, anthelmintic and diuretic, and are also useful in treatment of dropsy, chronic cough, heart diseases, bronchitis, piles and small pox. This plant also possesses antidiabetic property.

The present communication deals with the isolation and identification of steroidal saponin SA-III, characterised as yamogenin 3–O–*b*–D-glucopyranosyl (1 → 4)–O–*a*–D-xylopyranoside, which was isolated from the seeds of *Trigonella foenum-graecum*.

Keywords. *Trigonella foenum-graecum*; Leguminosae; steroidal saponin; yamogenin 3–O–*b*–D-glucopyranosyl (1 → 4)–O–*a*–D-xylopyranoside.

1. Introduction

Trigonella foenum–graecum[1–3] (NO – Leguminosae) and is cultivated in many parts of India. It has been found to possess significant medicinal value. Its leaves are used both internally and externally to reduce swelling, prevent falling of hair and in the treatment of burns. Its seeds are carminative, antipyretic, anthelmintic and diuretic and are also useful in dropsy, chronic cough, heart diseases, bronchitis, piles and small pox. This plant is also found to possess antidiabetic property.

Earlier workers[4–8] have already reported the presence of diosgenin, gitogenin, neogitogenin and yamogenin in the seeds of *Trigonella foenum-graecum*.

2. Results and discussion

Defatted seeds of *Trigonella foenum-graecum* were extracted with MeOH and a concentrated extract was subjected to column chromatography over alumina. On elution with benzene, it gave a compound SA-III analysed as $C_{38}H_{60}O_{12}$, M^+ – 708, m.pt.– 242–244°C. Characteristic bands at n_{max}^{KBr} 982, 914, 892, 850 cm^{-1} (intensity 915 > 896) in the IR spectrum of SA-III shows the spiroketal nature of SA-III. It shows all characteristic colour reactions of steroidal saponin[9,10] and also gives positive Molisch test for glycoside.

Compound SA-III on acid hydrolysis yielded SA-III (A), $C_{27}H_{42}O_3$, m.pt. – 198–200°C, and sugar moieties as D-glucose and D-xylose (*Rf* – 0·20, 0·29).

Permethylation[11] by Kuhn procedure followed by acid hydrolysis of SA-III yielded aglycone SA-III (A) and methylated sugars, identified as 2,3,4,6-tetra-O-methyl glucose and 2,3-di-O-methyl xylose, showing the presence of D-glucose in pyranose form and also that the C-4 OH group of D-glucose is linked to the C-1 OH group of D-glucose.

Enzymatic hydrolysis of the glycoside with almond emulsion gave proaglycone SA-III (B) and D-glucose (by Co-PC) indicating *b*-linkage between SA-III (B) and D-glucose. The SA-III (B) on hydrolysis with takadiastase yields the aglycone SA-III (A) and D-xylose confirming the *a*-linkage between SA-III (A) and D-xylose.

Sodium metaperiodate oxidation of the glycoside consumed 2·82 moles of periodate and liberated 1·05 moles of formic acid, indicating the presence of both the sugars and aglycone in equimolar ratio and also confirming that the both sugars are present in the pyranose form. Hydrolysis of SA-III with Kil-

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liani mixture liberated D-glucose first followed by D-xylose, which suggests that D-glucose was the terminal sugar and the D-xylose was linked to the aglycone SA-III (A).

The IR spectrum of SA-III (A) shows a peak at 3402·5 cm^{-1} which indicates the presence of –OH group (*s*) in it. SA-III (A) was found to form mono acetyl derivative, $C_{29}H_{44}O_4$, [M^+] 256, m.pt. 180–182°C. Estimation of acetyl group (10·32%) by Wiesenberger method[12] as described by Belcher and Godbert[13] indicates the presence of one –OH group in the SA-III (A).

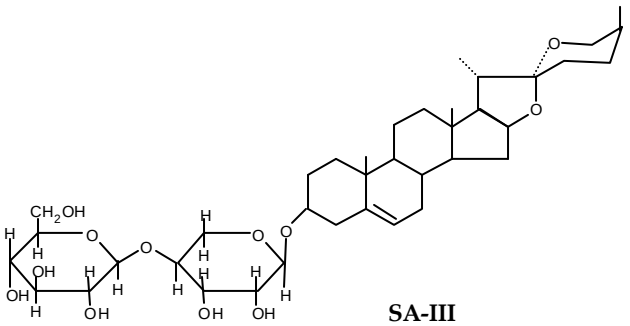
The Cr_2O_3/pyridine oxidation of SA-III (A) yielded a ketone, $C_{27}H_{40}O_3$, [M^+] 412, m.pt. 191–193°C, giving a positive Zimmerman test[14] for the C-3 keto group, the confirming the presence of one OH at C-3 and further indicating its secondary nature in SA-III (A).

All the above facts help to conclude that the C-3 OH of SA-III (A) is linked to the C-1 OH of D-xylose through an *a*-linkage and the C-4 OH of D-xylose is linked to the C-1 OH of D-glucose via a *b*-linkage.

A peak at n_{max}^{KBr} 1640 cm^{-1} in the IR spectrum of SA-III (A) indicates unsaturation. On catalytic hydrogenation, with Pd/C a dihydro derivative, $C_{27}H_{44}O_3$ of m.pt. 152–153°C is obtained, indicating the presence of one double bond in it. In ^1H the NMR spectrum of SA-III (A) one proton signal at 5·32 (*m*) is seen, which indicates the presence of a vinyl proton at C-6, owing to the double bond between C-5 and C-6.

The IR spectrum of SA-III (A) shows a peak at n_{max}^{KBr} 2924·4 cm^{-1} for angular methyl groups, which when estimated by Zeisels method (8·67%) confirm the presence of four methyl groups in it. The chemical shift in the ^1H NMR spectrum of SA-III (A) gives a singlet at *d* 0·89 and *d* 1·16, and a doublet at *d* 1·18 and *d* 0·72, confirming the presence of angular methyl groups at C-18, C-19, C-21 and C-27 in SA-III (A). ^{13}C NMR spectrum shows a chemical shift at *d* 16·6, *d* 20·0, *d* 15·2 and *d* 15·0 for C-18, C-19, C-21 and C-27 respectively for methyl group.

On the basis of the above deliberations the saponin SA-III was identified as yamogenin 3–O–*b*–D-glucopyranosyl (1 → 4)–O–*a*–D-xylopyranoside.



SA-III

3. Experimental

3.1 *Plant material*

The seeds of *Trigonella foenum-graecum* were collected locally and identified by a taxonomist.

3.2 *Extraction and isolation*

The seeds (2·0 kg) of *Trigonella foenum-graecum* were air-dried and defatted with petroleum ether in a Soxhlet apparatus. The solvent-free seeds were extracted with MeOH until the extract became colourless. The concentrated mass was shaken with $CHCl_3$ and filtered. The residue was taken up in H_2O and extracted with *n*-BuOH. The *n*-BuOH extract was concentrated under reduced pressure when it yielded a crude compound that was chromatographed over alumina with benzene. Crystallisation from methanol/water and from acetone yielded SA-III (Yield 0·06%) which showed a single homogeneous spot on TLC[15] over silica gel, using chloroform : acetone : formic acid (9 : 2 : 1) as solvent and I_2 vapours as visualising agent.

Compound SA-III: Colourless needles from MeOH, m.pt. 242–244°, $[a]_D^{20}$ –96° $CHCl_3$. IR n_{max}^{KBr} cm^{-1} 3400 (OH), 2928 (CH_3 stretch), 1620 (C=C stret.) 1040 (C–O–C), 982, 914, 892, 850 (intensity 915 > 896, 25 S-spiroketal).[16]

3.3 *Acid hydrolysis of SA-III*

Saponin SA-III 60 mg was refluxed with 10ml of 7% H_2SO_4 for 6 h, cooled and filtered to afford aglycone SA-III(A) (yamogenin),[17–19] colourless needles, m.pt. 180–182°C, [M^+] 414, $[a]_D^{20}$ – 125·0° $CHCl_3$, IR n_{max}^{KBr} cm^{-1}: 3402·5 (OH), 2924·4 (CH_3 stretch.), 1640 (C=C stretch), 1448·8 (C–H bending), 1216 (C–O stretch), 1032·8 (C–O–C stretch) 910·5, 850, 757·3 (intensity 915 > 896, 25 S-spiroketal). EIMS *m*/*z* 414 [M^+], 355, 345, 342, 300, 285, 271, 139 (base peak).

^{13}C-NMR: 37·6 (C-1), 31·9 (C-2), 70·4 (C-3), 42·4 (C-4), 140·2 (C-5), 120·8 (C-6), 32·6 (C-7), 31·6 (C-8), 50·4 (C-9), 37·2 (C-10), 21·4 (C-11), 42·2 (C-12), 40·8 (C-13), 55·8 (C-14), 32·4 (C-15), 81·2 (C-16), 60·8 (C-17), 16·6 (C-18), 20·0 (C-19), 42·8 (C-20), 15·2 (C-21), 109·5 (C-22), 26·4 (C-23), 27·8 (C-24), 25·4 (C-25), 64·8 (C-26), 15·0 (C-27).

^{1}H-NMR: 1·02 (2H, *m*, H-1), 1·38 (2H, *m*, H-2), 3·81 (1H, *m*, H-3), 2·64 (2H, *m*, H-4), 5·32 (2H, *m*, H-6), 1·94 (2H, *m*, H-7), 1·56 (1H, *m*, H-8), 0·94 (1H, *m*, H-9), 1·45 (2H, *m*, H-11), 1·70 (2H, *m*, H-12), 1·12 (1H, *m*, H-14), 1·52 (2H, *m*, H-15), 4·62 (1H, *m*, H-16), 1·74 (2H, *m*, H-17), 0·89 (3H, *s*, H-18), 1·16 (3H, *s*, H-19), 1·94 (1H, *m*, H-20), 1·18 (3H, *d*, *J* = 6·5 Hz, H-21), 1·68 (2H, *m*, H-23), 1·59 (2H, *m*, H-24), 1·56 (1H, *m*, H-25), 3·54 (2H, *m*, H-26), 0·72 (3H, *d*, *J* = 3·5 Hz, H-27).

The neutralised and concentrated aqueous hydrolysate showed the presence of D-glucose and D-xylose [PC, solvent B:A:W (4:1:5), *Rf* values: 0·20 and 0·29 respectively].

3.4 *Permethylation of SA-III*

The saponin SA-III (10 mg) was treated with methyl iodide (7 ml) and Ag_2O (40 ml) in DMF (5 ml) in a 150 ml conical flask and kept for 4 days at room temperature. The contents were filtered and the residue was washed with DMF. The filtrate was concentrated under reduced pressure to get a viscous mass, which on hydrolysis with HCl gave sapogenin SA-III (A) and methylated sugars. The sapogenin SA-III (A) was separated and the aqueous hydrolysate was neutralised with $BaCO_3$. $BaSO_4$ formed was filtered off and the filtrate was concentrated under reduced pressure. Sugars were examined by paper chromatography,[20] using Whatman filter paper No. 1, BAW (4:1:5) solvent system and aniline hydrogen phthalate as spraying reagent. They were identified as 2,3,4,6-tetra-O-methyl glucose and 2,3-di-O-methyl xylose.

3.5 *Periodate oxidation of SA-III*

Compound SA-III (25 mg) in H_2O (10 ml) was mixed with $NaIO_4$ (250 mg) and the solution was kept in the dark for 48 h. Ethylene glycol was added to decompose excess $NaIO_4$ and the solution was hydrolysed with 10% MeOH-HCl (45 min). It was then filtered and the filtrate upon neutralisation did not show the presence of any monosaccharide.

3.6 *Enzymatic hydrolysis of saponin SA-III*

The saponin (SA-III) (40 mg) was dissolved in MeOH mixed with almond emulsion (30 ml) in a 100 ml conical flask fitted with a stopper. The contents were allowed to stand at room temperature for 48 h and then filtered. The concentrated hydrolysate was examined using paper chromatography for sugar moieties using Whatman filter paper No. 1 and BAW (4:1:5) as the solvent system. The sugars were identified as D-glucose and D-xylose.

A methanolic solution of saponin SA-III (20 mg) was dissolved in MeOH (20 ml) and was mixed with an equal volume of takadiastase solution in a conical flask. The contents were allowed to stand for two days at room temperature and filtered. The hydrolysate on paper chromatographic examination was found to contain D-xylose.

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References

1. Chopra R N, Nayer S L and Chopra I C 1956 *Glossary of Indian medicinal plants* (New Delhi: CSIR) pp 248–249
2. Chaudhary R D 1998 *Herbal drugs industry* (New Delhi: Eastern) p. 80
3. Kirtikar K R and Basu B D 1988 *Indian medicinal plants* 3rd edn (Dehradun: Int. Book Distrib.) vol. 1, p. 700
4. Gupta R K, Jain D C and Thakur R S 1986 *J. Nat. Prod.* **49** 1153
5. Dawidar A M, Saleh A A and Elmotei S L 1973 *Planta Med.* **24** 367
6. Varshney I P and Sharma S C 1966 *J. Indian Chem. Soc.* **43** 564
7. Yoshikawa M, Murakami T, Komatsu H, Murakami N, Yamahara J and Matsuda H 1997 *Chem. Pharm. Bull.* **45** 81
8. Gupta R K, Jain D C and Thakur R S 1984 *Phytochemistry* **23** 2605
9. Salkowski E 1908 *Hoppe Seyl. Z.* **57** 521
10. Libermann C 1885 *Ber. Deustch. Chem. Soc. Ges.* **18** 1804

11. Kuhn R, Trischmann H and Low J 1955 *J. Angew. Chem.* **67** 32
12. Weisenberger E 1947 *Microchem. Microchim. Acta* **33** 51
13. Godbert A L and Belcher R 1954 *Semimicro quantitative organic chemistry* (New York: Longmans Green)
14. Singhal S, Khare M P and Khare A 1980 *Phytochemistry* **19** 2431
15. Egon Stahl 1965 *Thin layer chromatography* (New York, London: Academic Press)
16. Sharma S C and Sharma H C 1984 *Phytochemistry* **23** 645
17. Shashi B M, Niranjan P S and Amar N G 1981 *Phytochemistry* **20** 1943
18. Lin R C, Marie A, Lacaille D, Bernard H, Aria C and Bruno C 1997 *J. Nat. Prod.* **60** 1165
19. Quyen L T, Khoi N H, Suong N N, Schreibber K and Ripperger H 1987 *Planta Med.* **2** 292
20. Lederer E and Lederer M 1957 *Chromatography* (New York: Elsevier)