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FUROSTANOL GLYCOSIDES FROM *TRIGONELLA FOENUM-GRAECUM* SEEDS*

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Key Word Index—*Trigonella foenum-graecum*, Leguminosae (Fabaceae), fenugreek, furostanol glycosides

Abstract—Two new furostanol glycosides, trigofoenosides F and G, have been isolated as their methyl ethers from the methanolic extract of *Trigonella foenum-graecum* seeds (Leguminosae) The structures of the original glycosides have been determined as (25*R*)-furost-5-en-3β,22,26-triol, 3-*O*-α-L-rhamnopyranosyl (1 → 2)β-D-glucopyranosyl (1 → 6)β-D-glucopyranoside, 26-*O*-β-D-glucopyranoside and (25*R*)-furost-5-en-3β,22,26-triol, 3-*O*-α-L-rhamnopyranosyl (1 → 2) [β-D-xylopyranosyl (1 → 4)]β-D-glucopyranosyl (1 → 6)β-D-glucopyranoside, 26-*O*-β-D-glucopyranoside, respectively

INTRODUCTION

Seeds of *Trigonella foenum-graecum* are used in the indigenous system of medicine [1] Due to its diosgenin content this plant has been considered as an alternative source of steroids in place of *Dioscorea* species The present investigation was aimed at the study of the saponins of the seeds

RESULTS AND DISCUSSION

Seven furostanol glycosides which have been isolated from *T foenum-graecum* seeds were designated as Trigofoenosides A, B, C, D, E, F and G However, we found that on TLC analysis these furostanol glycosides appeared as a pair comprising of the hydroxy and methoxy compounds It has been observed previously that the furostanol glycosides when extracted with methanol undergo methylation yielding a mixture of 22-hydroxy and 22-methoxy derivatives [2] In order to confirm that the 22-methoxy derivative is a probable artefact, in a separate extraction with pyridine [3] we found that the 22-methoxy compounds were completely absent In the light of the above observation we have designated the hydroxy furostanol glycosides as trigofoenosides F and G and their methyl ethers as F-1 and G-1, respectively

The two trigofoenosides F-1 and G-1 were isolated by droplet counter current chromatography from fraction (II) of the butanol extract Both compounds F-1 and G-1 showed strong absorption (3600–3200 cm^{-1}) due to hydroxyl groups, but no absorption due to spiroketal functions in their IR spectra [4] and they gave positive tests with Ehrlich's reagent Both compounds on hydrolysis with almond emulsin gave D-glucose [5] and spirostanol glycosides identified by the TLC and IR The ^1H NMR spectra of F-1 and G-1 exhibited a methoxy signal [6] which disappeared on refluxing with acetone and water (7 3) indicating conversion of a 22-methoxy group to a 22-hydroxy group

On hydrolysis with 2 N HCl, both compounds (F-1, G-1) gave diosgenin as a common aglycone, together with the monosaccharides, D-glucose and L-rhamnose (3 1) for F-1 and D-glucose, D-xylose and L-rhamnose (3 1 1) for G-1

Methylation of compound F-1 by Hakomori's method [7] afforded the permethylate, which on methanolysis yielded methyl 2,3,4,6-tetra-*O*-methyl-D-glucopyranoside, methyl 2,3,4-tri-*O*-methyl-D-glucopyranoside, methyl 3,4,6-tri-*O*-methyl-D-glucopyranoside and methyl 2,3,4-tri-*O*-methyl-L-rhamnopyranoside

When compound F-1 was treated with 0 05 M sodium metaperiodate solution no sugar was liberated, which indicated the absence of branching in the sugar chain This is also supported by the methanolysis result, as no dimethyl sugar was obtained

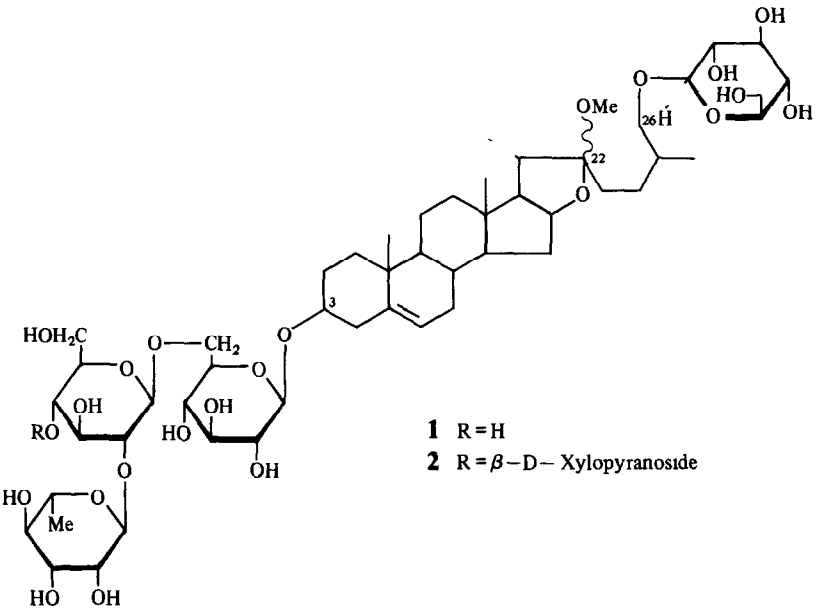
Based upon the above data, the new glycoside F-1 is assigned the structure containing a glucopyranose at C-26 and a trisaccharide [L-rhamnopyranosyl (1 → 2) D-glucopyranosyl (1 → 6) D-glucopyranoside] at the C-3 position of 22-*O*-methyl-furost-5-en-3β,22,26-triol

The configurations at the anomeric centres of glucose and rhamnose were revealed as β and α, respectively, by application of Klyne's rule [8] of molecular rotation The calculated and observed values for $M_{(D)}$ of F-1 (−843 0, −826 8) and G-1 (−952 0, −933 7) were of the same order of magnitude The ^1H NMR spectrum of F-1 displayed signals at δ4 62 (1H, *d*, J = 7 Hz), 4 78 (1H, *d*, J = 7 2 Hz) and 5 95 (1H, *br s*), as ascribable to the anomeric proton of glucose (β-linkage) and the anomeric proton of rhamnose (α-linkage), respectively In addition, the β-configuration of the glucose residue at the C-26 hydroxyl group of F-1 and G-1 was suggested by the results of enzymatic hydrolysis

Accordingly, the structure of F-1 was elucidated as (25*R*)-22-*O*-methyl-furost-5-en-3β,22,26-triol, 3-*O*-α-L-rhamnopyranosyl (1 → 2)β-D-glucopyranosyl (1 → 6)β-D-glucopyranoside, 26-*O*-β-D-glucopyranoside (1) The naturally occurring trigofoenoside F is therefore the corresponding 22-hydroxy compound

On methylation by Hakomori's method G-1 gave a permethylate, which on methanolysis gave diosgenin and methyl 2,3,4,6-tetra-*O*-methyl-D-glucopyranoside, methyl

*Part 6 in the series "Plant Saponins" For Part 5 see Singh, S B, Thakur, R S and Schulten, H R (1982) *Phytochemistry* **21**, 2079



1 R = H
2 R = β-D-Xylopyranoside

2,3,4-tri-*O*-methyl-D-glucopyranoside, methyl 3,6-di-*O*-methyl-D-glucopyranoside, methyl 2,3,4-tri-*O*-methyl-D-xylopyranoside and methyl 2,3,4-tri-*O*-methyl-L-rhamnopyranoside

Compound G-1 was treated with 0 05 M sodium metaperiodate solution and D-glucose was liberated, which showed the presence of branching in the sugar chain at D-glucose, which was also supported by the presence of a dimethyl-D-glucopyranoside

The above observations and inspections of the anomeric configurations of each monosaccharide established the structure of G-1 as (25*R*)-22-*O*-methyl-furost-5-en-3β,22,26-triol, 3-*O*-α-L-rhamnopyranosyl (1 → 2) [β-D-xylopyranosyl (1 → 4)] β-D-glucopyranosyl (1 → 6) β-D-glucopyranoside, 26-*O*-β-D-glucopyranoside (**2**) The naturally occurring trigofoenoside is therefore the corresponding 22-hydroxy compound

EXPERIMENTAL

Mps are uncorr TMS was used as an internal standard in DMSO-d_6 for ^{1}H NMR (90 MHz) Chromatography was on silica gel 60–120 mesh (BDH), Whatman No 1 paper The following solvents were employed Solvent a, $CHCl_3$–MeOH–H_2O (65 40 12), solvent b, $CHCl_3$–MeOH–H_2O (65 35 10), solvent c, BuOH–pyridine–H_2O (6 4 3), solvent d, *n*-hexane–EtOAc (2 3), solvent e, C_6H_6–Me_2CO (85 15), solvent f, BuOH–EtOH–H_2O (5 1 4) On TLC (silica gel) the glycosides were detected by Ehrlich's reagent, and by 10% H_2SO_4, sugars and methylated derivatives were located on PC (descending) by ammoniacal $AgNO_3$ soln and aniline hydrogen phthalate GC of sugars, dual FID column 6', 3% OV-17 Chromosorb-W, N_2 as a carrier gas Condition (1) temperature programming, initial hold at 125° for 4 min and then programme at 10°/min to a final temp of 265° Condition (2) same column, temperature programming, initial hold 150° for 2 min and then programme at 10°/min to a final temp of 275° Droplet counter current separation was made on DCC-A apparatus by Tokyo Rikakikai, Tokyo (Japan), 300 tubes were used The solvent system was $CHCl_3$–MeOH–H_2O (7 13 8) in which the stationary phase consisted of the upper layer, hence the apparatus was used in the descending mode

A crude mixture of furostanols was partitioned between H_2O and EtOAc–BuOH (1 1) An upper part consisting of five trigofoenosides A, B, C, D and E, designated as fraction I and a lower part consisting of trigofoenosides F and G designated as fraction II, were obtained Fraction II was chromatographed by DCCC Crude fraction II (500 mg) was dissolved in 10 ml of 1 1 mixture of both upper and lower phases and then injected into the sample column The flow rate was 5–8 ml/hr The eluants were collected in 5 ml fractions, monitored by TLC, with solvent system b After elution of compound F-1 (200 mg) and G-1 (150 mg) the stationary phase containing polar substances was recovered from the columns

Trigofoenoside F-1 An amorphous solid from MeOH–Me_2CO, R_f 0 34 (solvent system a), mp 256–258°, $[\alpha]_D$ −78 9° (pyridine), Ehrlich positive [Found C, 57 7, H, 7 55, $C_{52}H_{23}O_{13}$ requires C, 57 8, H, 7 97%]

Trigofoenoside G-1 An amorphous solid from MeOH–Me_2CO, R_f 0 27 (solvent system a), mp 270–274°, $[\alpha]_D$ −79 2° (pyridine), Ehrlich positive [Found C, 58 75, H, 7 5 $C_{57}H_{94}O_{27}$ requires C, 57 6, H, 7 66%]

C-22 *hydroxy and* C-22 *methoxy derivatives* Compounds F-1 and G-1 (20 mg) were boiled with Me_2CO–H_2O (7 3) for 14 hr On evaporation *in vacuo* amorphous solids were obtained Analysis by ^{1}H NMR showed no methoxy signal Trigofoenoside F, R_f 0 23, mp 233–236° and trigofoenoside G, R_f 0 18, mp 275–278° were obtained When compounds F and G (15 mg) were refluxed with dry MeOH for 6 hr, F-1 and G-1 were regenerated

Identification of sapogenin and sugars Compounds F-1 (50 mg) and G-1 (60 mg) in 2 N HCl (10 ml) were refluxed for 3 hr The precipitates were collected by filtration and purified by recrystallization from MeOH to afford colourless needles, mp 206–208°, $[\alpha]_D$ −129° ($CHCl_3$), IR ν_{max}^{KBr} cm^{-1} 3600–3200 (OH), 981, 918, 898, 860 [intensity 900 > 920, (25*R*)-spiroketal] It was identical with diosgenin (mmp, co-TLC and superimposable IR)

Each filtrate was neutralized with $AgCO_3$ and deionized with Amberlite resin IR 120 $(H)^+$ and 400 $(OH)^-$ and evaporated to dryness *in vacuo* Each residue was examined by PC (system c), TLC (system b) and GLC using condition (1) Sugars were identified as glucose, rhamnose and glucose, xylose, rhamnose for trigofoenosides F-1 and G-1, respectively GLC of sugar samples as trimethyl silyl derivatives, R_f (min) glucose (29 0, 30 5), xylose (23 5, 25 6) and rhamnose (20 6, 21 5)

Quantitative determination of sugars was carried out colorimetrically [9] (phenol–H_2SO_4) and by GLC of the silyl derivatives, which showed that the proportions of glucose and rhamnose for F-1 and glucose, xylose and rhamnose for G-1, were 3 1 and 3 1 1, respectively

Enzymatic hydrolysis Compounds F-1 and G-1 (50 mg each) were dissolved in H_2O, emulsin (almond) 10 ml, was added Mixtures were incubated at 37° for 50 hr Solns were extracted with *n*-BuOH Extracts were concd and checked by TLC and IR The aq layer of each was evaporated to dryness *in vacuo* The residues were examined by PC and only D-glucose was detected as the sugar present in each case

Methylation of F-1 Compound F-1 (75 mg) was methylated by the Hakomori method and worked up as usual The methylate was obtained as a brown residue Hydrolysis of the methylate with 3% MeOH–HCl gave diosgenin and methyl 2,3,4,6-tetra-*O*-methyl-D-glucopyranoside, methyl 2,3,4-tri-*O*-methyl-D-glucopyranoside, methyl 3,4,6-tri-*O*-methyl-D-glucopyranoside and methyl 2,3,4-tri-*O*-methyl-L-rhamnopyranoside identified by comparison with authentic samples by PC (solvent system f), TLC (solvent system d) and GLC using condition (2)

Methylation of G-1 Compound G-1 (50 mg) was methylated as in the case of F-1 and the methylate was obtained as brown residue which on methanolysis as described above, furnished methyl 2,3,4,6-tetra-*O*-methyl-D-glucopyranoside, methyl 2,3,4-tri-*O*-methyl-D-glucopyranoside, methyl 3,6-di-*O*-methyl-D-glucopyranoside, methyl 2,3,4-tri-*O*-methyl-L-rhamnopyranoside and methyl 2,3,4-tri-*O*-methyl-D-xylopyranoside (identified by TLC, PC and GLC as in the case of F-1, with authentic samples), some of the methylated sugars were identified by comparison with their R_g and RR_t and periodate oxidation results

Partial hydrolysis Compounds F-1 and G-1 (30 mg each) were partially hydrolysed with 0 1 N HCl and sugars liberated after 0 5 hr, 1 hr and 2 hr were characterized The prosapogenin obtained after 2 hr for both was separated, hydrolysed and only D-glucose was found to be present in each case

Periodate treatment Compounds F-1 and G-1 (20 mg each) were treated with 0 05 N sodium metaperiodate soln (3 ml) Reaction mixtures were kept in the darkfor 48 hr at room temp The mixtures were extracted with *n*-BuOH and the extracts were subjected to PC (solvent system c) No sugar was detected from compound F-1, but compound G-1 showed the presence of D-glucose

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