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TWO FUROSTANOL SAPONINS FROM *TRIGONELLA FOENUM-GRAECUM**

RAJESH K. GUPTA, DHARAM C. JAIN and RAGHUNATH S. THAKUR

Central Institute of Medicinal and Aromatic Plants, Lucknow 226016, India

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Key Word Index—*Trigonella foenum-graecum*; Leguminosae; fenugreek; furostanol saponins; trigofoenosides B and C.

Abstract—Two new furostanol glycosides, trigofoenosides B and C, have been isolated from the *Trigonella foenum-graecum* seeds as their methyl ethers, B-1 and C-1 respectively. Their structures have been determined as (25*S*)-22-*O*-methyl-5α-furostane-2α,3β,26-triol-3-*O*-α-L-rhamnopyranosyl(1 → 4)-β-D-glucopyranoside 26-*O*-β-D-glucopyranoside (B-1) and (25*R*)-22-*O*-methyl-5α-furostane 2α,3β,26-triol-3-*O*-α-L-rhamnopyranosyl(1 → 4)-[α-L-rhamnopyranosyl (1 → 2)]-β-D-glucopyranoside 26-*O*-β-D-glucopyranoside (C-1).

INTRODUCTION

In continuation of our studies [1, 2] on the saponins of *Trigonella foenum-graecum* seeds, we have isolated two more new furostanol saponins and their structures have been elucidated.

RESULTS AND DISCUSSION

A methanolic extract of seeds was fractionated with *n*-butanol, which yielded a crude mixture of saponins. Further separation was effected using droplet counter current and column chromatography leading to the isolation of pure methyl ethers of the two saponins, designated as B-1 and C-1. In general, furostanol saponins have been reported to yield methyl ethers [1] and these methyl ethers have been employed in our studies to elucidate their structures.

The furostanol nature of these saponins was established through characteristic colour reactions [3], enzymatic hydrolysis and spectral data [4].

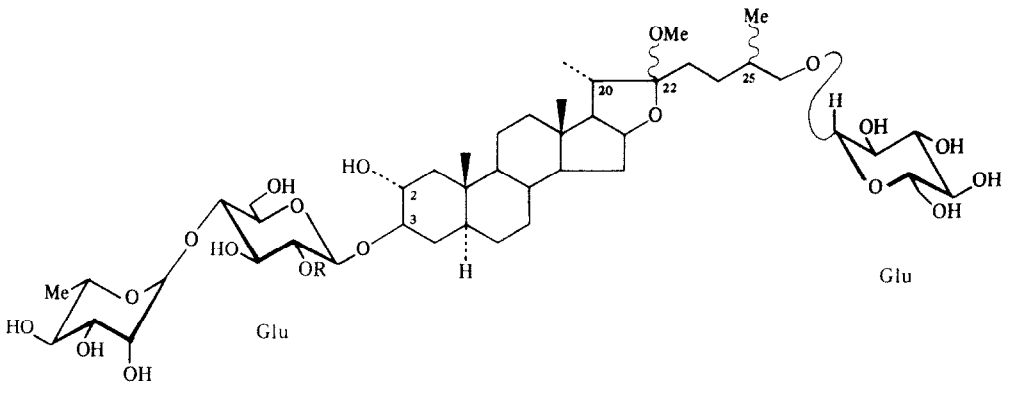
Analysis of trigofoenoside B-1 by fast atom bombardment (FAB)-MS revealed that the molecular weight of B-1 was 934, which was clear from the peaks at *m/z* 1067 $[M+Cs]^+$ and 957 $[M+Na]^+$. A prominent peak at *m/z* 903 $[M+H-MeOH]^+$ was observed due to the loss of methanol involving a methoxy function at C-22, which in turn supported the furostanol skeleton of the compound [5]. The peaks at *m/z* 725 and 741 were due to the loss of one glucose moiety with and without the glycosidic oxygen atom, depicting the terminal position of one glucose, whereas the peak at *m/z* 757 also showed the terminal position of a rhamnose moiety. Two peaks obtained at *m/z* 595 and 579 represented the cleavage at C-3 of the aglycone with and without the adjacent glycosidic oxygen atom, which exhibited the presence of a disaccharide (glucorhamnosyl) moiety at the C-3 position. The peak at *m/z* 563 due to the loss of one rhamnose (C-3) and one glucose (C-26) along with their adjacent oxygens, suggested the presence of one glucose unit in the molecule other than at the C-3 position. The furostanol nature suggested the glycosidation in the neogitogenin molecule at C-26. The peaks at *m/z* 433 and 415 were ascribable to $[aglycone+H]^+$ and $[aglycone+H-H_2O]^+$, respectively. On the basis of these data the probable sequence of sugars in B-1 may be proposed as rhamnose–glucose–aglycone 26-*O*-glucose.

Acid hydrolysis of B-1 and C-1 gave neogitogenin and gitogenin, respectively. The sugar components were D-glucose and L-rhamnose for both compounds in the molar ratio of 2:1 and 1:1, respectively.

Methylation studies, periodate oxidation and partial hydrolysis results suggested the sugar sequence at the C-3 position as L-rhamnopyranosyl (1 → 4)-D-glucopyranose for compound B-1 (also supported by FAB-MS results) and L-rhamnopyranosyl (1 → 4)-[L-rhamnopyranosyl (1 → 2)]-D-glucopyranose for compound C-1. D-Glucose was shown to be located at the C-26 position in both compounds (also supported by enzymic hydrolysis results). The anomeric configurations of D-glucose and L-rhamnose were established as β and α, respectively, which was determined by the application of Klyne's rule [6] and ^1H NMR spectral data. In addition enzymatic hydrolysis with a β-hydrolysing enzyme suggested the β-configuration for the C-26 glucose. The attachment of the sugar chain in both the compounds at the 3β-hydroxycarbon of the aglycone was further confirmed by making their acetylated products. On mild acid hydrolysis these compounds produced a 2α-acetoxy compound rather than a 3β-acetate [7].

Accordingly, the structure of B-1 was elucidated as (25*S*)-22-*O*-methyl-5α-furostane-2α,3β,26-triol-3-*O*-α-L-rhamnopyranosyl (1 → 4)-β-D-glucopyranoside 26-*O*-β-D-glucopyranoside (**1**) and (25*R*)-22-*O*-methyl-5α-furostane-2α,3β,26-triol-3-*O*-α-L-rhamnopyranosyl (1 → 4)-[α-L-rhamnopyranosyl (1 → 2)]-β-D-glucopyranoside 26-*O*-β-D-glucopyranoside for C-1 (**2**).

*Part 10 in the series "Plant Saponins". For Part 9 see ref. [2].



1 R = H (B-1); 25-Me = axial

2 R = α-L-rhamnopyranoside (C-1); 25-Me = equatorial

EXPERIMENTAL

Mps are uncorr. TMS was used as an internal standard in CD_3OD–$CDCl_3$ and DMSO-d_6 for ^1H NMR (400 MHz and FT-80A). Column chromatography was on silica gel (60–120 mesh) (BDH). Whatman No. 1 paper was used for PC. 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*, *n*-BuOH–pyridine–H_2O (6:4:3); solvent *d*, C_6H_6–Me_2CO (3:1); solvent *e*, C_6H_6–Me_2CO (17:3); solvent *f*, *n*-BuOH–EtOH–H_2O (5:1:4); solvent *g*, hexane–EtOAc (2:3); solvent *h*, EtOAc–C_6H_6 (3:17). Spraying reagents, 10% H_2SO_4, Ehrlich's reagent and Liebermann–Burchard reagent. Sugars and their methylated derivatives were located on PC (descending) by aniline hydrogen phthalate and ammoniacal $AgNO_3$ soln: GLC of sugars, dual FID, column 6′, 3% OV-17 chromosorb-W, N_2 as a carrier gas, condition (a), temp. programming—initial hold at an initial temp. 125° for 4 min and then at the rate of 10°/min to a final temp. of 265°. Condition (b) same column—initial hold at an initial temp. 150° for 2 min and then at the rate of 10°/min to a final temp. of 275°. DCCC was performed using the DCC-A apparatus of Tokyo Rikakikai, Tokyo (Japan). 300 tubes were used. The solvent system used was $CHCl_3$–MeOH–H_2O (7:13:8), upper layer (water layer) as stationary phase, in descending mode. FAB-MS, JMS DX300 Mass spectrometer.

Isolation. Fraction I of the *n*-butanol extract [1] was chromatographed on a silica gel column with $CHCl_3$–MeOH–H_2O (65:15–35:10). Five major furostanol glycosides, trigofoenosides A-1 to E-1 were isolated in order of their increasing polarity. Trigofoenosides B-1 and C-1 thus obtained were purified by DCCC. Samples were dissolved in 10 ml mixture (1:1) of both upper and lower phases and then chromatographed in a 10 ml sample column. The flow rate was 7–10 ml/hr. The eluents were collected in 5 ml fractions, monitored by TLC, with solvent system *b*.

Trigofoenoside B-1. Crystallized from aq. MeOH as small round crystals, R_f 0.78 (system *a*), mp 198–200° (decomp), $[\alpha]_D$ −62.10° (pyridine; *c* 1), $[M]_D$ −580.01° (−597.24°); IR ν_{max}^{KBr} cm^{-1}: 3500–3200 (OH), 2920, 2860, 1460, 1380, 1200–1000 (C–O–C), no spiroketal bands. ^1H NMR (DMSO-d_6): δ1.67 (3H, *br s*, Me of rhamnose), 3.24 (*s*, 3H, C-22 OMe), 4.28 (*d*, 1H, J = 7.1 Hz), 5.21 1H, *br s*). Found: C, 58.81; H, 8.24, $C_{46}H_{78}O_{19}$ requires C, 59.12; H, 8.35%, FAB-MS *m/z*: 957 $[M+Na]^+$, 1067 $[M+CS]^+$, M_r 934 for $C_{46}H_{78}O_{19}$.

Trigofoenoside C-1. An amorphous powder from MeOH, R_f 0.74 (system *a*), mp 210–212° (decomp), $[\alpha]_D$ −64.1° (pyridine; *c* 1), $[M]_D$ −692.2° (−703°); IR ν_{max}^{KBr} cm^{-1}: 3600–3100 (OH), 2900, 1460, 1380, 1260, 1150–1000 (C–O–C), no spiroketal bands. ^1H NMR (CD_3OD–$CDCl_3$,1:9): δ3.12 (*s*, C-22 OMe), 3.84 (*d*, 1H, J = 7 Hz), 4.02 (*d*, 1H, J = 8 Hz), 4.58 (1H, *br s*), 4.94 (1H, *br s*). Found: C, 57.45; H, 7.98, $C_{52}H_{88}O_{23}$ requires C, 57.77, H, 8.15%.

Enzymatic hydrolysis. Compounds B-1 and C-1 (80 mg and 100 mg, respectively) were dissolved in H_2O (5 ml) and emulsin (almond) containing one drop of toluene was added to each soln. Mixtures were incubated at 37° for 4 and 5 days, respectively. Mixtures were extracted with *n*-BuOH and checked by TLC. The water layers were concd and subjected to PC (system *c*) and TLC (system *b*). The *n*-BuOH concentrate from B-1 gave a single Liebermann–Burchard reagent positive spot, recrystallized from Me_2CO–MeOH as colourless needles (58 mg) named as prosaponin B (PSB) R_f 0.82 (system *a*), mp 231–233° (decomp.), $[\alpha]_D$ −73.7° (pyridine; *c* 1), $[M]_D$ −548.2°; IR ν_{max}^{KBr} cm^{-1}: 3600–3200 (OH), 1460, 1380, 1150–1000 (C–O–C), 980, 922, 900 and 865 (922 > 900, 25*S*, spiroketal). On complete acid hydrolysis it gave neogitogenin and the sugars D-glucose and L-rhamnose (co-PC). The molar ratio of sugars was determined colorimetrically and found to be 1:1. The *n*-BuOH concentrate of C-1 was crystallized from MeOH and designated as PSC. It gave a single Liebermann–Burchard reagent positive spot, R_f 0.76 (system *a*) mp 241–244° (decomp.), $[\alpha]_D^{26}$ −64.97° (Pyridine; *c* 1) $[M]_D$ −569.0°; IR ν_{max}^{KBr} cm^{-1}: 3500–3100 (OH), 981, 920, 902, 863 (902 > 920, 25*R*, spiroketal). On complete acid hydrolysis, it afforded gitogenin and the sugars D-glucose and L-rhamnose (1:2).

Methylation and methanolysis of PSB and PSC. PSB and PSC (30 mg, each) were methylated according to Hakomori's method [8] and worked up as usual. Permethylates obtained were purified by PLC (system *g*) to afford dull white powders. Methanolysis of permethylates (PSB and PSC) with 3% MeOH–HCl furnished methylated sugars which were examined by PC (system *f*) and TLC (system *d*). Methylated sugars were identified as methyl 2,3,4,-tri-*O*-methyl-L-rhamnopyranoside and methyl 2,3,6-tri-*O*-methyl-D-glucopyranoside in case of PSB whereas methyl 2,3,4-tri-*O*-methyl-L-rhamnopyranoside and methyl 3,6-di-*O*-methyl-D-glucopyranoside in the case of PSC.

Identification of aglycone and sugars. Compounds B-1 (100 mg) and C-1 (80 mg) in 2 N HCl (H_2O–dioxan, 1:1) were refluxed Separately for 4 hr. After usual work up each afforded a genin and a hydrolysate containing sugars.

The aglycone obtained in case of compound B-1 on crystallization from MeOH afforded a colourless crystalline compound.

R_f 0.20 (system *h*), mp 250°, $[\alpha]_D^{26} - 82°$ ($CHCl_3$; *c* 1); IR ν_{max}^{KBr} cm^{-1}: 3600–3200 (OH), 980, 921, 900, 860 (intensity 921 > 900, 25*S*, spiroketal). MS *m/z*: 432 $[M]^+$ 417, 373, 363, 360, 318, 303, 289, 271, 255, 139 (base peak) and 115. The aglycone was acetylated with Ac_2O–pyridine as usual. On crystallization from Me_2CO the acetate gave colourless needles, R_f 0.56 (system *e*) mp 221°, $[\alpha]_D^{26} - 101°$ ($CHCl_3$; *c* 1): IR ν_{max}^{KBr} cm^{-1}: 1735 and 1250, (AcO) 980, 920, 900 and 865 (intensity 920 > 900, 25*S*, spiroketal). MS *m/z*: 516 $[M]^+$, 139 (base peak). On the basis of the above data the aglycone was identified as neogitogenin. The aglycone obtained in the case of compound C-1 on crystallization afforded a crystalline compound R_f 0.20 (system *h*), mp 262°, $[\alpha]_D - 80.8°$ ($CHCl_3$; *c* 1); IR ν_{max}^{KBr} cm^{-1}: 3600–3200 (OH), 980, 921, 900, 862 (intensity 900 > 921, 25*R* spiroketal). MS *m/z*: 432 $[M]^+$, 417, 373, 363, 360, 318, 289, 271, 255, 139 (base peak). On comparison with an authentic specimen it was identified as gitogenin (mmp and co-TLC). Each filtrate was neutralized with Dowex-3 (OH^-) resin and evaporated to dryness *in vacuo*. Each residue was examined by PC (system *c*), TLC (system *b*) and GLC (condition *a*) sugars were identified as D-glucose (R_t 24.78, 28.35) and L-rhamnose (R_t 17.19, 18.29). The molar ratio of sugars was determined with the help of GLC and colorimetry [9] (phenol–H_2SO_4), which revealed the proportions of D-glucose and L-rhamnose to be 2:1 and 1:1 for B-1 and C-1, respectively.

Methylation and methanolysis of B-1 and C-1. Compounds B-1 (75 mg) and C-1 (150 mg) were methylated by the Hakomori's method and worked up as usual. Permethylates of B-1 and C-1 were methanolysed with 3% MeOH–HCl. After usual work up, it afforded the methylated monosaccharides which were examined by TLC (system *d*), PC (system *f*) and GLC (column condition b). These were identified as methyl 2,3,4,6-tetra-*O*-methyl-D-glucopyranosides, methyl 2,3,6-tri-*O*-methyl-D-glucopyranoside, methyl 2,3,4,-tri-*O*-methyl-L-rhamnopyranoside in case of B-1 whereas methyl 3,6-di-*O*-methyl-D-glucopyranoside, methyl 2,3,4,6-tetra-*O*-methyl-D-glucopyranoside and methyl 2,3,4-tri-*O*-methyl-L-rhamnopyranoside in the case of C-1. Identity of these methylated sugars was also confirmed by authentic specimens.

Periodate treatment. Compounds B-1 and C-1 (30 mg each) were taken in H_2O (5 ml each) treated with 0.05 M sodium metaperiodate soln (4 ml) in aq. MeOH and kept in the dark for 48 hr. After usual work up the products were completely hydrolysed which furnished the corresponding aglycone in the precipitate. The filtrates were concentrated and checked on PC (systems *c*). No sugar was detected in compound B-1 but C-1 showed the presence of D-glucose.

Partial hydrolysis of PSB. PSB (20 mg) was subjected to 0.1N HCl in 50% dioxan for 40 min. After usual work up it afforded a prosaponin B_1 (PSB_1) and an aglycone. PSB_1 on crystallization from MeOH gave shining crystals. R_f 0.80 (system *b*), mp 253–255° (decomp.), $[\alpha]_D^{26} - 73.2°$ (pyridine; *c* 0.42), $[M]_D^{26} - 431.1°$; IR ν_{max}^{KBr} cm^{-1}: 3600–3250 (OH), 1150–1000 (C–O–C), 981, 920, 900, 865 (intensity 920 > 900, 25*S*, spiroketal). On complete hydrolysis PSB gave D-glucose and neogitogenin.

Partial hydrolysis of PSC. PSC (25 mg) was subjected to 0.1 N HCl in 50% dioxan for 45 min which afforded three prosaponins PSC_1, PSC_2 and PSC_3 in the order of their increasing polarity. PSC_1 and PSC_2 were found to be identical with prosaponins PSB_1 and PSB of trigofoenoside B-1 confirmed by co-TLC and co-IR, except the difference in the orientation of C-25 methyl group which was equatorial in the case of PSC_1 and PSC_2 whereas axial in the case of PSB_1 and PSB. PSC_3, R_f 0.80 (system *a*), mp 211–213° (decomp.) IR ν_{max}^{KBr} cm^{-1}: 3600–3200 (OH), 981, 920, 898, 861, (898 > 920, 25*R*, spiroketal).

C-22 *Hydroxy and* C-22 *methoxy derivatives.* Compound B-1 (30 mg) and C-1 (50 mg) were boiled with Me_2CO–H_2O (7:3, 12 ml) for 20 hr and kept overnight after evaporation of the solvent *in vacuo* amorphous powders were yielded. The ^1H NMR spectra of both compounds exhibited no methoxy signal, indicating the conversion of the methoxy derivative into the hydroxy form (trigofoenosides B and C, respectively). Trigofoenoside B, R_f 0.69 (system *a*), mp 186–188° (decomp.), $[\alpha]_D^{26} - 54.01°$ (pyridine; *c* 1). Trigofoenoside C, R_f 0.63 (system *a*), mp 234–237° (decomp.), $[\alpha]_D^{26} - 61.5°$ (pyridine; *c* 0.5). When these (B and C) were refluxed with dry MeOH for 14 hr, B-1 and C-1 were regenerated.

Acetylation of B-1 and C-1. B-1 and C-1 (40 mg each) were acetylated with Ac_2O–pyridine (2:1, 10 ml) as usual at room temp. for 24 hr. The acetylated products obtained were purified by preparative TLC and hydrolysed with 1 N HCl for 3 hr. The hydrolysates were neutralized and extracted with $CHCl_3$. Both extracts showed two spots having R_f 0.39 and 0.12 in hexane–EtOAc (3:1) corresponding to aglycones and their 2α-acetoxy compounds, respectively. Acetoxy compounds with R_f 0.12 was separated by preparative TLC and crystallized from hexane.

The acetylated compound obtained from C-1 showed mp 226–227°; IR ν_{max}^{KBr} cm^{-1}; 3560, 1735, 1250 and 1030. ^1H NMR δ: 0.76 (3H, *s*, 18-H_3), 0.79 (3H, *d*, J = 8 Hz, 27-H_3), 0.95 (3H, *s*, 19-H_3), 0.97 (3H, *d*, J = 9 Hz, 21-H_3), 2.02 (3H, *s*, OAc), 3.38 (1H, *m*, H-3αH) and 5.05 (1H, *m*, H-2βH). Identical with gitogenin 2-acetate [9]. Similarly the acetoxy compound obtained from B-1 showed mp 202–204°; IR ν_{max}^{KBr} cm^{-1}: 3540, 1730, 1250, 1025, 981, 920, 900 and 865 (920 > 900; 25*S*, spiroketal). This compound was found to be similar to the 2α-acetoxy getogenin except at the C-25 methyl configuration, which was found to be 25*S* and it was identified as 2α-acetoxy neogitogenin.

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