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FUROSTANOL GLYCOSIDES FROM *TRIGONELLA FOENUM-GRAECUM* SEEDS

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Key Word Index—*Trigonella foenum-graecum*; Leguminosae; fenugreek; furostanol glycosides, trigofoenosides A and D.

Abstract—Two new furostanol glycosides trigofoenosides A and D have been isolated from the *Trigonella foenum-graecum* seeds as their methyl ethers, A-1 and D-1. Their structures have been determined as (25*S*)-22-*O*-methyl-furost-5-ene-3β,26-diol, 3-*O*-α-L-rhamnopyranosyl (1 → 2)-β-D-glucopyranoside; 26-*O*-β-D-glucopyranoside (A-1) and (25*S*)-22-*O*-methyl-furost-5-ene-3β,26-diol, 3-*O*-α-L-rhamnopyranosyl (1 → 2)-[β-D-glucopyranosyl (1 → 3)]-β-D-glucopyranoside; 26-*O*-β-D-glucopyranoside (D-1).

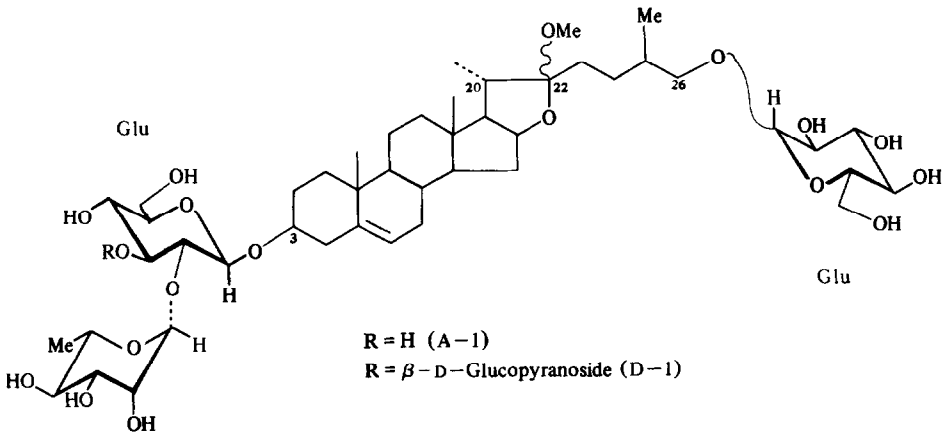
INTRODUCTION

In continuation of our studies [1] on the seeds of *Trigonella foenum-graecum* saponins, we have further 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 two saponins, designated as A-1 and D-1. The formation of methyl ethers in the case of furostanol saponins has been reported earlier [1] and for convenience these have been employed in our studies to elucidate their structures. The furostanol nature of these saponins was established through characteristic colour reactions [2], enzymatic hydrolysis and spectral data [3].

Inspection of the Fast Atom Bombardment (FAB) mass spectrum of trigofoenoside A revealed that the molecular weight of A is 902 which was clear from the peaks at *m/z* 1036 $[M+H+Cs]^+$, 925 $[M+Na]^+$ and 941 $[M+K]^+$. A prominent peak at *m/z* 885 $[M+H-H_2O]^+$ was observed due to loss of water involving the hydroxy function at C-22, which also suggested the furostanol structure of this saponin [4]. The peaks at *m/z* 723 $[(M+H-H_2O)-162]^+$ and 739 $[(M+H-H_2O)-146]^+$ resulted from the loss of glucose and rhamnose, respectively. The peak at 707 $[(M+H-H_2O)-178]^+$ was assigned to the loss of one glucose with an adjacent oxygen atom indicating the outer position for one glucose unit. Two signals corresponding to *m/z* 577 and 561 represented the cleavage of a glucorhamnosyl (308) unit at C-3 with and without an oxygen atom. Similarly the peak at *m/z* 545 $[(M+H-H_2O)-340]^+$ was assigned to the



loss of one rhamnose (146 + 16) and one glucose (162 + 16) units along with their glycosidic oxygen atoms which indicated that a glucose unit was linked to the genin molecule at C-26 which was the only alternative position available for glycosidation. The peaks at m/z 415 and 397 were ascribable to the $[aglycone + H]^+$ and $[(aglycone + H) - H_2O]^+$.

Thus the sugar sequence in A could be proposed as rhamnose → glucose → yamogenin; 26-*O*-glucose. Acid hydrolysis of A-1 and D-1 gave the same sapogenin, yamogenin. The sugar components were D-glucose and L-rhamnose for both in the molar ratio of 2:1 for A-1 and 3:1 for D-1.

Methylation studies, periodate oxidation and partial hydrolysis suggested the sugar sequence L-rhamnopyranosyl (1 → 2)-D-glucopyranose for A-1 (also supported by FAB-MS results) and L-rhamnopyranosyl (1 → 2) [D-glucopyranosyl (1 → 3)]-D-glucopyranose for D-1 at the C-3 position of aglycone, whereas the presence of one D-glucose at the C-26 position was confirmed by enzymatic hydrolysis for both compounds.

The anomeric configuration of D-glucose and L-rhamnose was established as β and α respectively which was revealed by the application of Klyne's rule [6] and ^{1}H NMR spectral data. In addition enzymatic hydrolysis with a β-hydrolysing enzyme suggested the β-configuration for the C-26 glucose. Accordingly, the structure of A-1 was elucidated as (25*S*)-22-*O*-methylfurost-5-ene-3β,26-diol, 3-*O*-α-L-rhamnopyranosyl(1 → 2)-β-D-glucopyranoside; 26-*O*-β-D-glucopyranoside and (25*S*)-22-*O*-methylfurost-5-ene-3β,26-diol, 3-*O*-α-L-rhamnopyranosyl (1 → 2)-[β-D-glucopyranosyl (1 → 3)]-β-D-glucopyranoside; 26-*O*-β-D-glucopyranoside for D-1.

EXPERIMENTAL

Mps are uncorr. TMS was used as an internal standard in CD_3OD–$CDCl_3$ and DMSO-d_6 for ^{1}H 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*, BuOH–pyridine–H_2O (6:4:3); solvent *d*, C_6H_6–Me_2CO (3:1); solvent *e*, C_6H_6–Me_2CO (85:15); solvent *f*, BuOH–EtOH–H_2O (5:1:4); solvent *g*, CH_2Cl_2–Me_2CO (49:1); solvent *h*, EtOAc–C_6H_6 (15:85). Spraying reagents, 10% H_2SO_4, Ehrlich's reagent and Liebermann–Burchard reagent. Sugars and 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, conditions for temperature programming: (*a*) initial hold at an initial temp. of 125° for 4 min and then at the rate of 10°/min to a final temp. of 265°; (*b*) same column with initial hold at an initial temp. 150° for 2 min and then at a 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-D × 300 mass spectrometer.

Isolation. Fraction I of the *n*-BuOH 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 the order of increasing polarity. Trigofoenosides A-1 and D-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 A-1. An amorphous solid from MeOH–Me_2CO, R_f 0.84 (solvent system *a*), mp 210–213° (decomp.); IR ν_{max}^{KBr} cm^{-1}; 3600–3200 (OH), 1150–1000 (C–O–C), no spiroketal band; ^{1}H NMR (DMSO-d_6): δ1.76 (*br s*, Me-rha), δ3.24 (3H, *s*, C-22 OMe), 4.25 (*d*, 1H, J = 7.1 Hz), 4.57 (*d*, 1H, J = 7.0 Hz), 5.20 (1H, *br s*); $[\alpha]_D$ −84.18° (pyridine; *c* 1); $[M]_D$ −771.1° (−778°). (Found: C, 59.98 H, 8.39, $C_{46}H_{76}O_{18}$ requires: C, 60.20; H, 8.29%.)

Trigofoenoside A. Amorphous powder, R_f 0.77 (system *a*), mp 219–221° (decomp.), $[\alpha]_D$ −90.1° (pyridine, *c* 1). FAB-MS m/z: 1036 $[M + H + CS]^+$, 925 $[M + Na]^+$, 941 $[M + K]^+$; M_r 902 for $C_{45}H_{74}O_{18}$.

Trigofoenoside D-1. An amorphous solid from MeOH–Me_2CO, R_f 0.67 (solvent system *a*), mp 250–253°; IR ν_{max}^{KBr} cm^{-1}: 3600–3250 (OH), no spiroketal; ^{1}H NMR (CD_3OD–$CDCl_3$): δ1.67 (*br s*, Me-rha), 3.12 (*s*, C-22 OMe), 4.21 (*d*, 1H, J = 7.3 Hz), 4.56 (*d*, 1H, J = 7.5 Hz), 4.92 (*d*, 1H, J = 7.8 Hz), 5.10 (1H, *br s*); $[\alpha]_D$ −77.6° (pyridine; *c* 1); $[M]_D$ −836.52° (−844°). (Found: C, 58.0, H; 7.82, $C_{52}H_{86}O_{23}$, requires C: 57.88, H: 7.97.)

Trigofoenoside D. Amorphous powder, R_f 0.55 (system *a*), mp 246–248° (decomp); $[\alpha]_D$ −73.2° (pyridine; *c* 1).

Enzymatic hydrolysis. Compounds A-1 and D-1 (100 mg) were dissolved in H_2O (5 ml) and emulsin (almond, 10 ml) soln and one drop of toluene were added to each soln. Mixtures were incubated at 37° for 96 hr before being extracted with *n*-BuOH and checked by TLC. Water layers were concd and subjected to PC (system *c*) and TLC (system *b*). The *n*-BuOH concentrate from A-1 gave a single Liebermann–Burchard reagent positive spot and recrystallized from MeOH–H_2O as an amorphous powder (PA) mp 198–201° (decomp.); $[\alpha]_D$ −94.7° (pyridine; *c* 1); R_f 0.87 (system *a*); IR ν_{max}^{KBr} cm^{-1}: 3600–3200 (OH), 985, 920, 900, 850 [920 > 900 (25*S*), spiroketal]. PA on silica gel TLC (system *a*) gave a superimposable spot with Pro A of dioscin and on complete hydrolysis furnished D-glucose, L-rhamnose (1:1) and yamogenin.

The *n*-BuOH concentrate of D-1 was crystallized from MeOH. It gave a single Liebermann–Burchard reagent positive spot, R_f 0.70 (system *a*), mp 301–304° (decomp.); $[\alpha]_D$ −86.2° (pyridine; *c* 1); IR ν_{max}^{KBr} cm^{-1}: 3600–3200 (OH), 985, 920, 900, 850 [920 > 900 (25*S*), spiroketal]; PD on TLC exhibited a superimposable spot with gracillin and on complete hydrolysis furnished D-glucose, L-rhamnose (2:1) and yamogenin.

Identification of aglycone and sugars. Compounds A-1 (80 mg) and D-1 (100 mg) in 2 N HCl (H_2O–dioxan, 1:1) were refluxed separately for 4 hr. The ppts were collected and purified by crystallization from Me_2CO–MeOH to afford colourless needles R_f 0.31 (system *h*), mp 201°; MS m/z: 414 $[M]^+$, 139 (base peak) for $C_{27}H_{42}O_3$; $[\alpha]_D$ −129° ($CHCl_3$; *c* 1); IR ν_{max}^{KBr} cm^{-1}: 3600–3200 (OH), 1460, 1380, 980, 921, 898, 861 [921 > 898 (25*S*), spiroketal]. Co-TLC (system *g*) on $AgNO_3$-impregnated plate (2%), three-fold development, gave superimposable spot with yamogenin.

Each filtrate was neutralized with resin (Dowex-3, OH^- form) 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 glucose (R_G 1.00, R_f 0.08) and rhamnose (R_G 2.00, R_f 0.30) in both. GLC of sugar samples as trimethyl silyl derivatives R_t (min): glucose (26.9, 28.9), rhamnose (18.4, 19.7).

The molar ratio of sugars was determined by the help of GLC and calorimetry [7] (phenol–H_2SO_4), which revealed the proportions of glucose and rhamnose to be 2:1 and 3:1 for A-1 and D-1, respectively.

Methylation of A-1 and D-1. A-1 (75 mg) and D-1 (100 mg) were methylated by Hakomori's method [5] and worked up as usual. The permethylates were obtained as brown residues. Methanolysis with 3% methanolic HCl gave methyl 2,3,4,6-tetra-*O*-methyl-D-glucopyranoside (R_{TMG} 1.00, R_f 0.61) methyl 2,3,4-tri-*O*-methyl-L-rhamnopyranoside (R_{TMG} 1.01, R_f 0.67) and methyl 3,4,6-tri-*O*-methyl-D-glucopyranoside (R_{TMG} 0.78, R_f 0.55) for A-1 and methyl 2,3,4,6-tetra-*O*-methyl-D-glucopyranoside, methyl 2,3,4-tri-*O*-methyl-L-rhamnopyranoside and methyl 4,6-di-*O*-methyl-D-glucopyranoside (R_{TMG} 0.46, R_f 0.38) for D-1, identified by PC after hydrolysis (system *f*), TLC (system *d*) and GLC (condition *b*) with the help of authentic samples.

Periodate treatment. Compounds A-1 and D-1 (30 mg each) were taken in H_2O (5 ml each) and treated with 0.05 M sodium-*m*-periodate soln (3 ml) in aq. MeOH. The reaction mixtures were kept in the dark for 48 hr at room temp before being extracted with *n*-BuOH. The extracts were concd and completely hydrolysed. On usual work up both saponins furnished yamogenin (co-TLC and mmp) and sugar residues which were subjected to PC (system *c*). No sugar was detected in compound A-1, but D-1 showed the presence of D-glucose.

Partial hydrolytic studies of PA. PA (30 mg) was subjected to partial hydrolysis with 0.1 N HCl in dioxan–H_2O (1:1) for 40 min. It gave a prosapogenin PA_1 (12 mg) and an aglycone after purification by PLC (system *b*). PA_1 (7 mg), R_f 0.89 (system *a*), mp 271–273° (decomp.); IR ν_{max}^{KBr} cm^{-1}: 3600–3250 (OH), 981, 918, 900 and 861 (918 > 900); $[\alpha]_D$ −101.6° (pyridine; *c* 0.5); co-TLC (system *b*) with trillin exhibited a superimposable spot with PA_1 and on complete hydrolysis PA_1 gave D-glucose and yamogenin as an aglycone, identified with the help of authentic samples.

Partial hydrolytic studies of PD. PD (32 mg) on partial hydrolysis with 0.1 N HCl in dioxan–H_2O (1:1) for 40 min yielded three prosapogenins namely PD_1, PD_2 and PD_3 in the order of increasing polarity. PD_1 and PD_2 were found to be identical to PA_1 and PA as on TLC (system *b*) both produced similar spots. These were confirmed by mmp, co-IR and complete hydrolysis of each. PD_3, R_f 0.72 (system *b*), mp 269–272° (decomp.); $[\alpha]_D$ −88.3°. On complete hydrolysis D-glucose was the only sugar detected on PC (system *c*) along with yamogenin in the molar ratio of 2:1.

C-22 *hydroxy and* C-22 *methoxy derivatives.* Compounds A-1 and D-1 (30 mg each) were boiled with Me_2CO–H_2O (7:3) for 21 hr and kept overnight. After evaporation of the solvent (*in vacuo*) an amorphous powder was obtained from each compound. The ^1H NMR spectra of both saponins exhibited no methoxy signal. When these products (A and D) were refluxed with dry MeOH for 11 hr, A-1 and D-1 regenerated.

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REFERENCES

1. Gupta, R. K., Jain, D. C. and Thakur, R. S. (1984) *Phytochemistry* **23**, 2605.
2. Kiyosawa, S. and Hutoh, M. (1968) *Chem. Pharm. Bull.* **16**, 1162.
3. Rothman, E. S., Wall, M. E. and Eddy, C. R. (1952) *J. Am. Chem. Soc.* **74**, 4013.
4. Schulten, H.-R., Singh, S. B. and Thakur, R. S. (1984) *Z. Naturforsch* **39C**, 201.
5. Hakomori, S. (1964) *J. Biochem.* **55**, 205.
6. Klyne, W. (1950) *Biochem. J.* **47**, 12.
7. Dubois, M., Gillis, K., Hamilton, J. K., Rabbers, P. A. and Smith, F. (1956) *Analyt. Chem.* **28**, 350.