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New Phytosterols from the Seeds of *Trigonella foenum-graceum* L. of Sudanese Origin

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ABSTRACT : *Phytochemical investigation of Trigonella foenum-graceum L.(Fabaceae) seeds of Sudanese origin furnished with the isolation of four new phytoconstituents characterized as n-dotriacont-10-ene (**4**); p-menthane-2β,3α-diol-7-oic acid (**5**); (24S)-24α-ethyl-cholest-9(11)-en-3α-ol (**6**); (24S)-24α-ethyl cholest-9(11)-en-3α-ol-3β-D-ribopyranosido-(2'→1'')-D-ribopyranosido-(2''→1''')-D-ribopyranoside (**7**) along with three known compound β-sitosterol (**1**), its glucoside (**2**) and D-ribopyranosyl-(2→1')-D-ribopyranoside (**3**).*

KEYWORDS *: Fenugreek, graecumsterol, phytosterols, Trigonella foenum-graecum, trigonellasteryl tririboside.*

I. INTRODUCTION

Fenugreek, *Trigonella foenum-graecum* L. (Family-Fabaceae) is an annual plant, extensively cultivated as a food crop in India, the Mediterranean region, north Africa and Yemen. The plant seeds grown in southern Asia, have health potential with the ability to lower blood glucose and cholesterol levels, and hence in the prevention and treatment of diabetes and coronary heart diseases [1]. N,N'-Dicarbazyl, glycerol monopalmitate, stearic acid, *β*-sitosteryl glucopyranoside, ethyl-*α*-D-glucopyranoside, D-3-O-methyl-chiroinsitol and sucrose [2], *γ*-schizandrin and scopoletin [3], (2*S*, 3*R*, 4*R*)-4-hydroxyisoleucine [4], trioxazonane [5], trigofoenosides A-G [6-8], diosgenin and yamogenin [9] have been reported from the plant. This manuscript reports the isolation and structure elucidation of four new phytoconstituents along with *β*-sitosterol, its glucoside and D-ribopyranosyl-(2→1′)-D-ribopyranoside from *T. foenum-graecum* seeds of Sudanese origin.

II. EXPERIMENTAL SECTION

2.1. General experimental procedures

The melting points were determined on a Perfit apparatus and are uncorrected. The IR spectra were recorded in KBr pellet on Win IR FTS 135 instrument (Biorad, USA). ^{1}H (300 MHz) and ^{13}C (75 MHz) spectra were recorded by Bruker spectrospin NMR instrument in $CDCl_3$ using TMS as internal standard. FAB mass spectra were scanned at 70 eV on a Jeol D-300 instrument (Jeol, USA). Column chromatography was performed on silica gel (Merck, 60–120 mesh) and thin layer chromatography on silica gel G-coated TLC plates (Merck).

2.2. Plant material

The seeds *T. foenum-graecum* L. were purchased from Al-Oshara market, Khartoum city, Sudan, and authenticated by Dr. Wali E. Abdalla, plant taxonomist, Medicinal and Aromatic Plant Research Institute (MAPRI), Khartoum, Sudan. A voucher specimen No: Y91/07 was deposited at the (MAPRI) Herbarium, Khartoum, Sudan.

2.3. Extraction and isolation

T. foenum-graecum L. seeds were dried in an oven at 40° temperature and the material was coarsely powdered. Exhaustive extraction of the seed powder (2 kg) was carried out using 95% methanol in a Soxhlet apparatus for 24 hour. The methanol extract was dried under reduced pressure to yield dark brown viscous mass (275 g, 13.7 %). The viscous mass was dissolved in minimum amount of methanol and adsorbed on silica gel (60-120 mesh) for preparation of slurry. It was dried in air, loaded in silica gel column prepared in petroleum ether and then the column eluted successively in order of increasing polarity with petroleum ether, petroleum ether-chloroform (3:1, 1;1, 1:3 v/v), chloroform, chloroform-methanol (99:1, 49:1, 97:3, 19:1, 9:1, 17:3, 3:1 v/v) and methanol. The fractions collected were subjected to thin layer chromatography to check homogeneity of the fractions. The fractions having the same R_f values were combined together. Purification of the isolated compounds was achieved by crystallization. The following phytoconstituents were isolated:

2.4. □-sitosterol (1)

Elution of the column with chloroform furnished colourless crystalline powder of **1**, recrystallized from acetone, 150 mg (0.0075% yield); R_f: 0.26 (chloroform); m.p.: 136-138°; Co-TLC comparable; +ve ion FAB-MS *m/z* (rel. int.): 414 $[M]^+$ ($C_{29}H_{59}O$), (3.9).

2.5. □–Sitosterol glucoside (2)

Elution of the column with chloroform-methanol (19:1) gave colourless amorphous powder of **2**, recrystallized from methanol, 181 mg (0.0090 % yielded); R_f: 0.35 (chloroform -methanol, 9:1); m.p: 256-257°; +ve ion FAB-MS *m/z* (rel. int.): 577 $[M+H]^+$ ($C_{35}H_{60}O_6$) (11.3), Co-TLC comparable with an authentic sample.

2.6. Diriboside (3)

Elution of the column with chloroform-methanol (17:3) mixture furnished colourless, crystalline mass of **3**, recrystallized from methanol, 397 mg (0.019 % yield); R_f: 0.28 (chloroform-methanol, 3:1); m.p: 159-160°; IR ν_{max} (KBr): 3510, 3450, 3320, 2925, 2850, 1465, 1210, 1012 cm^{-1}; ^1H NMR (DMSO-d_6): δ 5.18 (1H, d, *J*=7.2 Hz, H-1′), 4.90 (1H, d, *J*=7.0 Hz, H-1), 4.38 (1H, m, H-2), 4.26 (1H, m, H-2□), 3.87 (1H, m, H-3), 3.68 (1H, m, H-3′), 3.56 (1H, m, H-4), 3.41 (1H, m, H-4′), 3.21 (1H, d, *J*=12.9 Hz, H_2-5a), 3.16 (1H, d, *J*=12.9 Hz, H_2-5b), 3.09(1H, d, *J*=13.2 Hz, H_2-5′a), 3.05 (1H, d, *J*=13.2 Hz, H_2-5′b); ^{13}C NMR (DMSO-d_6): δ 104.04(C-1′), 91.75(C-1), 82.56 (C-2), 77.03(C-4′), 74.29 (C-4), 72.84 (C-2′), 71.64 (C-3), 69.85 (C-3′), 62.10 (C-5), 60.50 (C-5′); +ve ion FAB-MS *m/z* (rel. int.): 282$[M]^+$ (C_{10} H_{18} O_9) (11.6), 228(12.2), 192 (41.7), 176 (19.8), 104 (100).

2.7. *n*-dotriacont-10-ene (4)

Elution of the column with petroleum ether-chloroform (3:1) gave colourless crystals of **4**, recrystallized from petroleum ether-chloroform (1:1), 355 mg (0.017 % yield); R_f: 0.50 (chloroform -methanol, 1:1); m.p: 75-76 °C; IR ν_{max} (KBr): 2920, 2850, 1640, 1470, 1310, 1205, 1120,911, 892, 718 cm^{-1}; ^1H NMR ($CDCl_3$): δ 5.26 (1H, m, H-10), 5.23 (1H, m, H-11), 2.21(2H, m, H_2-9), 1.96 (2H, m, H_2-12), 1.50 (2H, m, H_2-8), 1.22 (20 H, brs,10 x CH_2), 1.18 (30 H, brs,15 x CH_2), 0.80 (3H, t, *J*=6.1 Hz, Me-1), 0.77 (3H, t, *J*=6.1 Hz, Me-32); ^{13}C NMR ($CDCl_3$): δ 129.93 (C-10), 128.04 (C-11), 34.16 (C-9), 31.94 (C-11), 31.51 (C-8), 29.94 (9 x CH_2), 29.38 (12 x CH_2), 27.26 (CH_2), 25.61 (CH_2), 24.92 (CH_2), 22.68 (CH_2), 14.09 (Me-1, Me-32); +ve ion FAB-MS *m/z* (rel.int): 448$[M^+]$ (C_{32} H_{64}) (21.7), 433 (11.6), 321 (26.1), 153 (73.8), 127 (42.5).

2.8. Trigonella-*p*-menthanoic acid (5)

Elution of the column with chloroform afforded colourless crystals of **5**, recrystallized from acetone, 197 mg (0.0098% yield); R_f: 0.5 (chloroform-methanol, 19:1), m.p: 153-154°; UV λ_{max} (MeOH): 241nm (log ε 2.5); IR ν_{max} (KBr); 3510, 3320, 2920, 2845, 1701, 1474, 1406, 1345, 903 cm^{-1}; ^1H NMR ($CDCl_3$): δ 3.85 (1H, dd, *J*=11.4, 3.0 Hz, H-2*β*), 3.20 (1H, dd, *J*=11.4, 11.4 Hz, H-3*α*), 1.87 (1H, m, H-1), 1.70 (1H, m, H-4), 1.68 (1H, m, H-8), 1.30 (2H, brs, H_2-6), 1.28 (2H, m, H_2-5), 1.08 (3H, *J*=6.3 Hz, Me-9), 1.05 (3H, d, *J*=6.6 Hz, Me-10); ^{13}C NMR ($CDCl_3$): δ 54.72 (C-1), 67.99 (C-2), 67.12 (C-3), 53.40 (C-4), 20.36 (C-5), 21.86 (C-6), 181.06 (C-7), 37.13 (C-8), 12.85 (C-9), 10.82 (C-10); +ve ion FAB-MS *m/z* (rel.int): 202 $[M^+](C_{10}H_{18}O_4)$ (18.3), 187 (21.6), 184 (11.6), 169 (81.2), 166 (22.5), 154 (14.2), 122 (27.8).

2.9. Graecumsterol (6)

Further elution of the column with chloroform yielded colourless crystals of **6**, recrystallized from acetone, 137 mg (0.0068 % yield); R_f : 0.24 (chloroform); m.p. 117-118°; IR ν_{max} (KBr): 3450, 2915, 2845, 1645, 1470, 1360, 1235, 1120, 1015 cm^{-1}; ^1H NMR ($CDCl_3$): δ 5.26 (1H, m, H-11), 3.32 (1H, w1/2=10.5 Hz, H-3*β*), 0.96 (3H, brs, Me-19), 0.91 (3H, d, *J*=6.1 Hz, Me-21), 0.83 (3H, d, *J*=5.9 Hz, Me-26), 0.81 (3H, d, *J*=6.0 Hz, Me-27), 0.79 (3H, d, *J*=5.8 Hz, Me-29), 0.65 (3H, brs, Me-18); ^{13}C NMR ($CDCl_3$): 37.23 (C-1), 31.56 (C-2), 68.22 (C-3), 40.01 (C-4), 47.82 (C-5), 18.79 (C-6), 30.03 (C-7), 34.17 (C-8), 139.75 (C-9), 35.12 (C-10), 118.52 (C-11), 38.34 (C-12), 43.17 (C-13), 54.41 (C-14), 23.65 (C-15), 26.86 (C-16), 53.61 (C-17), 6.73 (C-18), 20.78 (C-19), 37.51 (C-20), 19.06 (C-21), 33.69 (C-22), 25.96 (C-23), 40.37 (C-24), 29.58 (C-25), 17.29 (C-26), 17.84 (C-27), 22.05 (C-28), 9.81 (C-29); +ve ion FAB-MS *m/z* (rel. int): 414 $[M]^+$ ($C_{29}H_{50}O$) (26.8), 399 (100), 396 (29.1), 384 (22.8), 381 (21.2), 369 (11.6), 302 (4.6), 288 (2.1), 274 (12.5), 273 (9.9), 271 (13.8), 255 (23.7), 253 (12.39), 240 (11.0), 237 (16.2), 223 (8.3), 213 (33.4), 211 (18.6), 198 (26.9), 191 (11.8), 177 (12.3), 140 (39.3), 126 (17.8), 112 (20.6), 96 (99.8).

2.10. Trigonellasteryl tririboside (7)

Elution of the column with chloroform-methanol (9:1) produced colourless, amorphous powder of **7**, recrystallized from methanol, 108 mg (0.0054 % yield); R_f: 0.6 (chloroform-methanol, 9:1); m.p: 281-282°; IR ν_{max} (KBr): 3557, 3516, 3481, 3448, 3329, 3265, 3233, 2921, 2855, 1645, 1458, 1382, 1120, 1024 cm^{-1};^1H NMR

(DMSO-d_6): δ 5.33 (1H, t, *J*=5.3 Hz, H-6), 3.58 (1H, brm, $w_{1/2}$=18.5 Hz), H-3*α*), 3.24 (2H, brs, H_2-19), 0.96 (3H, d, *J*=6.1 Hz, Me-19), 0.85 (3H, d, *J*=6.0 Hz, Me-26), 0.82 (3H, d, *J*=6.2 Hz, Me-27), 0.79 (3H, d, *J*=5.7 Hz, Me-29), 0.65 (3H, brs, Me-18), 4.88 (1H, d, *J*=7.1 Hz, H-1′), 4.43 (1H, m, H-2′), 3.37 (1H, m, H-3′), 3.77 (1H, m, H-4′), 3.23 (2H, brs, H2-5′), 4.86 (1H, d, *J*=7.3 Hz, H-1″), 4.25 (1H, m, H-2″), 3.35 (1H, m, H-3‴), 3.63 (1H, m, H-4″), 3.19 (2H, brs, H2-5″), 4.83 (1H, d, *J*=7.0 Hz, H-1‴), 3.96 (1H, m, H-2‴), 3.31 (1H, m, H-3‴), 3.61 (1H, m, H-4‴), 3.15 (2H, brs, H2-5‴); ^{13}C NMR (DMSO-d_6): 38.68 (C-1), 31.35 (C-2), 73.71 (C-3), 42.19 (C-4), 144.56 (C-5), 121.63 (C-6), 31.91 (C-7), 31.82 (C-8), 49.58 (C-9), 36.82 (C-10), 20.58 (C-11), 39.77 (C-12), 42.29 (C-13), 56.15 (C-14), 23.84 (C-15), 27.76 (C-16), 55.40 (C-17), 11.74 (C-18), 61.06 (C-19), 35.44 (C-20), 18.91 (C-21), 33.31 (C-22), 25.42 (C-23), 45.11 (C-24), 28.96 (C-25), 19.69 (C-26), 19.76 (C-27), 32.58 (C-28), 11.69 (C-29), 103.94 (C-1′), 80.07 (C-2′), 71.98 (C-3′), 76.59 (C-4′), 66.23 (C-5′), 102.33 (C-1″), 79.86 (C-2″) 71.57 (C-3″), 76.54 (C-4″), 64.93 (C-5″), 100.81 (C-1‴), 78.25 (C-2‴), 70.06 (C-3‴), 73.35 (C-4‴), 62.96 (C-5‴); +ve ion FAB-MS *m/z* (rel.int): 827 $[M+H]^+$ ($C_{44}H_{75}O_{14}$) (1.1), 560 (5.3), 429 (9.6), 414 (20.6), 412 (22.5), 398 (100), 398 (61.3), 383 (23.8), 290 (31.2), 288 (30.9), 271 (13.4), 255 (23.1), 240 (16.2), 213 (11.7), 179 (8.8), 159 (18.9), 153 (37.8), 139 (13.6), 135 (12.5), 133 (15.6).

2.11. Hydrolysis of (7)

Compound **7** (25 mg) was dissolved in 80% aqueous ethanol (10 ml), concentrated HCl (2 ml) added and the solution heated on a steam bath for 1 hour. The reaction mixture was dried under reduced pressure and the residue dissolved in $CHCl_3$ to separate the sterol. The residue was then dissolved in minimum amount of water and chromatographed on silica gel TLC along with standard samples of sugars using n-BuOH-EtOH-H_2O (4:1:2.2) as a solvent system. The sugar was identified as D-ribose, R_f value 0.36.

III. RESULTS AND DISCUSSIONS

The compounds **1**, **2** and **3** are the known compounds identified as *β*-sitosterol (Fig. 1), its glucoside and D-ribopyranosyl-(2→1′)-D-ribopyranoside, respectively.

Compound **4**, was obtained as a colourless crystalline mass from petroleum ether-chloroform (3:1) eluants. It decolorized bromine water indicating unsaturated nature of the molecule. Its IR spectral exhibited a typical absorption band for vinylic linkage (1640 cm^{-1}), and long aliphatic chain (718 cm^{-1}). The mass spectrum of **4** showed a molecular ion peak at *m/z* 448 corresponding to the molecular formula of an alkene, $C_{32}H_{64}$. The generation of the fragment ion peaks due to fission of C_9-C_{10} linkage at *m/z* 127 and due to cleavage of C_{11}-C_{12} bond at *m/z* 153 suggested the location of the vinylic linkage at C_{10}. The ^{1}H NMR spectrum of compound **4** showed two one-proton multiplet at *δ* 5.26 and 5.23 adjusted to vinylic H-10 and H-11 protons, respectively. Two two-proton multiplets at *δ* 2.21 and 1.96 were ascribed to methylene H_2-9 and H_2-12 protons, respectively. Two three-proton triplets at *δ* 0.80 (*J*=6.1 Hz) and 0.77 (*J*=6.0 Hz) were accounted to terminal C-1 and C-32 primary methyl protons, respectively. The remaining methylene protons appeared at *δ* 1.50 (2H), 1.22 (20H) and 1.18 (30H). The ^{13}C NMR of **4** exhibited signals for vinylic carbons at *δ* 129.93 (C-10) and 128.04 (C-11), methyl carbons at *δ* 14.09 and methylene carbons between *δ* 34.16-22.68. The absence of any signal between *δ* 5.23 and 2.21 in the ^{1}H NMR spectrum and between *δ* 128.04-34.16 in the ^{13}C NMR spectrum ruled out the presence of a carbinol group in the molecule. On the basis of foregoing discussion, the structure of **4** has been characterized as *n*-dotriacont-10-ene (Fig. 1).

The compound **5**, designed as trigonella-*p*-menthanolic acid, was obtained as a colourless crystalline mass from chloroform eluants. It gave effervescence with sodium biocarbonate solution indicating the presence of carboxylic group in the molecule. Its IR spectrum showed characteristic absorption bands for hydroxyl (3510 cm^{-1}) and carboxylic (3320, 1701 cm^{-1}) groups. On the basis of its mass and ^{13}C NMR spectra its molecular formula was established at *m/z* 202 corresponding to molecular formula of a monocyclic monoterpenic acid $C_{10}H_{18}O_4$. The important ion fragments arising at *m/z* 187 $[M\text{-}Me]^+$, 158 $[M\text{-}CO_2]^+$, 184 $[M\text{-}H_2O]^+$, 166 $[184\text{-}H_2O]^+$, 122 $[166\text{-}CO_2]^+$, 169 $[184\text{-}Me]^+$ and 154 $[169\text{-}Me]^+$ supported the existence of one carboxylic and hydroxyl functions in the molecule. The ^{1}H NMR spectrum of showed two one-proton double doublets at *δ* 3.85 (*J*=11.4, 3.0 Hz) and 3.20 (*J*=11.4, 11.4 Hz) assigned to *α*-oriented H-2 and *β*-oriented H-3 carbinol protons, respectively. Two three-proton doublets at *δ* 1.08 (*J*=6.3 Hz) and 1.05 (*J*=6.6 Hz) were ascribed correspondingly C-9 and C-10 secondary methyl protons. Three one-proton multiplets at *δ* 1.87, 1.70 and 1.68 were ascribed to H-1, H-4 and H-8 methine protons respectively. The remaining methylene protons appeared as two-proton multiplets at *δ* 1.30 (H_2-6) and 1.28 (H_2-5). The ^{13}C NMR spectrum of compound **5** exhibited 10 carbon signals in molecule and the signals at *δ* 181.06, 67.99 and 67.12 were accounted to C-7 carboxylic and C-2 and C-3 carbinol carbons, respectively, and methyl carbons at δ 12.85 (Me-9) and 10.82 (Me-10). The absence of any signal beyond *δ* 3.85 in the ^{1}H NMR spectrum and between *δ* 181.06-67.59 in the ^{13}C NMR spectrum supported

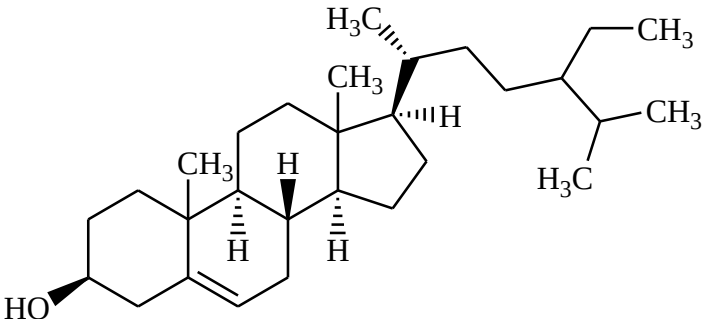
saturated nature of the molecule. On the basis of these evidences the structure of compound **5** has been established as *p*-menthane-2β,3α-diol-7-oic acid (Fig. 1).

Compound **6**, named graecumsterol, was obtained as a colourless crystalline mass from chloroform eluants. It responded positively to Liebermann-Burchard test for sterols. Its IR spectrum displayed characteristics absorption bands for hydroxyl group (3450 cm^{-1}) and unsaturation (1645 cm^{-1}). On the basis of FAB mass and ^{13}C NMR spectra the molecular ion peak of **6** was determined at *m/z* 414 consistent with molecular formula of sterol, $C_{29}H_{50}O$. The ion fragments arising at *m/z* 399 $[M-Me]^+$, 384 $[399-Me]^+$, 369 $[384-Me]^+$, 396 $[M-H_2O]$ and 381 $[396-Me]^+$ supported the existence of one hydroxyl group in the molecule. The ion peaks generated at *m/z* 273 $[M-C_{10}H_{21}$, side chain$]^+$, 271 $[273-2H]^+$, 255 $[271-Me]^+$, 240 $[255-Me]^+$, 222 $[240-H_2O]^+$, 253 $[271-H_2O]^+$, 211 [253-ring C fission]$^+$, 213 [255-ring C fission]$^+$ and 198 $[213-Me]^+$ indicated the location of saturated side chain and the presence of the vinylic linkage and hydroxyl group in the steroidal nucleus. The ion fragments formed at *m/z* 112, 302 [$C_{5,6}$-$C_{9,10}$ fission]+, 126, 288 [$C_{6,7}$-$C_{9,10}$ fission]$^+$ and 140, 274 [$C_{7,8}$-$C_{9,10}$ fission]$^+$, supported saturated nature of ring A and B and the location of hydroxyl group in ring A which was placed at C-3 on the basis of biogenic consideration. The ion peaks produced at *m/z* 177, 237 [$C_{8,14}$-$C_{12,13}$ fission]$^+$, 191, 223 [$C_{8,14}$-$C_{12,13}$ fission]$^+$ indicated the location of the vinylic linkage C9(11) position. The ^1H NMR spectrum of **6** exhibited a one-proton multiplet at δ 5.26 assigned to vinylic H-11 proton. A one-proton broad multiplet at δ 3.32 with half-width of 10.5 Hz was attributed to β-oriented H-3 carbinol proton. Two three-proton broad singlets at δ 0.65 and 0.96 were ascribed to C-18 and C-19 tertiary methyl protons, respectively. Four three-proton doublets at δ 0.91 (J=6.1 Hz) and 0.83 (J=5.9 Hz), 0.81 (J=6.0 Hz) and 0.79 (J=5.8 Hz) were associated correspondingly with the secondary C-21, C-26, C-27 and primary C-29 methyl protons, respectively. The presence of methyl signals in the range of 0.65-0.94 supported the location of these functionalities on the saturated carbon. The remaining methylene and methine protons resonated between δ 2.73-1.02. The ^{13}C NMR spectrum of **6** displayed 29 carbon signals in the molecule. The signal at δ 139.75, 118.52 and 68.22 were assigned to the unsaturated carbons at C-9, C-11 and to C-3 carbinol carbon, respectively. The ^{13}C and ^1H NMR spectra of **6** were compared with a series of sterols particularly β-sitosterol, stigmasterol and lawsaritol [10-12]. The $\Delta^{9(11)}$-bond substantiated by the chemical shift observed for the H_3-18 at δ 0.65 and H_3-19 at δ 0.94 methyl protons. These values are considerably higher than those for Δ^7 and Δ^5 sterols [10,13]. The sifting of the C-3 carbinol carbon and C-29 methyl carbons in upfield region at δ 68.22 and 9.81, respectively, from the corresponding δc value at δ 71.73 (C-3) and 11.04 (C-29) of **6**. β-Sitosterol indicated α-orientation of the hydroxyl and ethyl groups in the β-configuration of the ethyl group at C-24 had δc at 45.86 and it was observed at 40.37 in **6** supporting α-orientation of the ethyl group. 5α-Stigmast-9(11)-en-3β-ol with 24R-ethyl group (m.p. 132-133º) and 5α-poriferast-9(11)-en-3β-ol (5α-stigmast-9(11)-en-3β-ol) with 24S-ethyl group (m.p. 133-134º) have been isolated from the roots of *Costus speciosus* [14] and the marine red alga *Gracilaria edulis* [15], respectively. On the basis of these evidences the structure of **6** has been elucidated as (24S)-24α-ethyl-cholest-9(11)-en-3α-ol (Fig. 1). This is a new phytosterol isolated from a plant source.

Compound **7**, named as trigonellasteryl tririboside, was obtained as a colourless crystalline mass from the chloroform-methanol (9:1) eluants. It responded positively to steroidal glycoside tests. Its IR spectrum showed characteristic absorption bands for hydroxyl groups (3557, 3516, 3481, 3448, 3329, 3265, 3233 cm^{-1}) and unsaturation (1645 cm^{-1}). The mass spectrum of compound **7** exhibited a molecular ion peak at *m/z* 827 $[M+H]^+$ corresponding to the molecular formula of a sterol triglycoside, $C_{44}H_{75}O_{14}$. The important ion peaks generated at *m/z* 133 $[C_5H_9O_4]^+$, 397 $[C_5H_9O_4$-$C_5H_8O_4]^+$, 429 $[M-397]^+$, 414 $[429-Me]^+$, 383 $[414-CH_2OH]^+$, 398 $[429-CH_2OH]^+$, 412 $[M-C_{15}H_{26}O_{13}]^+$, 271 $[412-C_{10}H_{21}$, side chain$]^+$, 255 $[271-Me]^+$, 240 $[255-Me]^+$ and 213 [255-ring C, fission]$^+$ indicated that three C_5-sugar units were attached to the steroidal skeleton which contained saturated side chain and an addition hydroxyl group and vinylic linkage in the steroidal skeleton. The ion fragments arising at *m/z* 139, 290 [$C_{6,7}$-$C_{9,10}$ fission]$^+$, 153 $[C_{7,8}$-$C_{9,10}]^+$, suggested the location of one of the hydroxyl group in the ring A placed at C-3 on the basis of biogenetic consideration and vinylic linkage at C-5. The ion peaks producing at *m/z* 179 [$C_{8,14}$-$C_{9,11}$ fission]$^+$ supported saturated nature of ring C. The ^1H NMR spectrum of **7** showed a one-proton triplets at δ 5.33 (J=5.3 Hz) assigned to vinylic H-6 proton. A one-proton broad multiplet at δ 3.58 with half width of 18.5 Hz was attributed α-oriented oxygenated H-3 proton. Three one-proton doublets at δ 4.88 (J=7.1 Hz), 4.86 (J=7.3 Hz) and 4.83 (J=7.0 Hz) were ascribed to anomeric H-1′, H-1″ and H-1‴, respectively. Two one-proton multiplets at δ 4.43 and 4.25 were accounted to H-2′ and H-2″ carbinol protons and their existence in the deshielded region indicated the location of sugar moieties at C-2′ and C-2″ positions. A two-proton broad singlet at δ 3.24 was accommodated to the H_2-19 hydroxyl methylene protons. The remaining sugar protons appeared in the range of δ 3.69-3.02. A three-proton broad singlet at δ 0.65 was due to tertiary C-18 methyl protons. Four doublets at δ 0.96 (J=6.1 Hz), 0.85 (J=6.0 Hz), 0.82 (J=6.2 Hz) and 0.79 (J=5.7 Hz), integrated for three-protons each, were associated correspondingly to secondary C-21, C-26 and C-27 and primary C-29 methyl protons, all located on the saturated carbons. The ^{13}C NMR spectrum

of compound **7** displayed important signals for vinylic carbons at 144.56 (C-5) and 121.63 (C-6), oxygenated methine carbon at δ 73.71 (C-3), hydroxyl methylene carbon at 61.06 (C-19), methyl carbons at δ 11.74 (C-18), 18.91 (C-21), 19.69 (C-26), 19.76 (C-27), 11.69 (C-29), and anomeric carbons at δ 103.94 (C-1′), 102.33 (C-1″) and 100.81 (C-1‴) and the remaining sugar carbons between δ 80.07-62.96. The presence of C-2′ and C-2″ signals in the deshielded region at δ 80.07 and 79.86, respectively, suggested the attachment of sugar residues on these carbons. The ^{1}H NMR and ^{13}C NMR spectral values of steroidal residue **7** were compared with β-sitosterol others related compounds [6-8,10,11]. Acid hydrolysis of compound **7** yielded a sterol and D-ribose (R_f-comparable). On the basis of spectral data analysis and chemical reactions, the structure of the compound **7** has been elucidated as stigmast-5-en-3β,19-diol-3β-D-ribopyranosido-(2′→1″)-D-ribopyranosido-(2″→1‴)-D-ribopyranoside (Fig. 1). This is a new steroidal glycoside isolated from plant resource for the first time.

I. FIGURES



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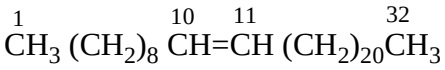

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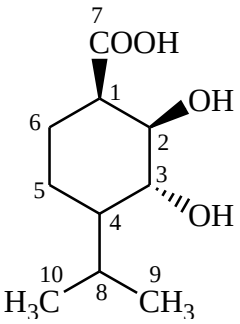

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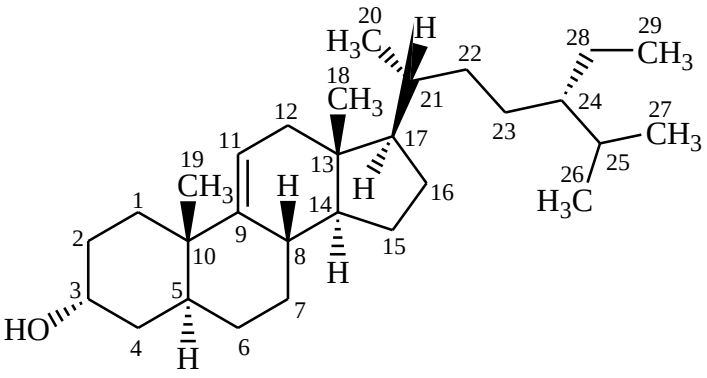

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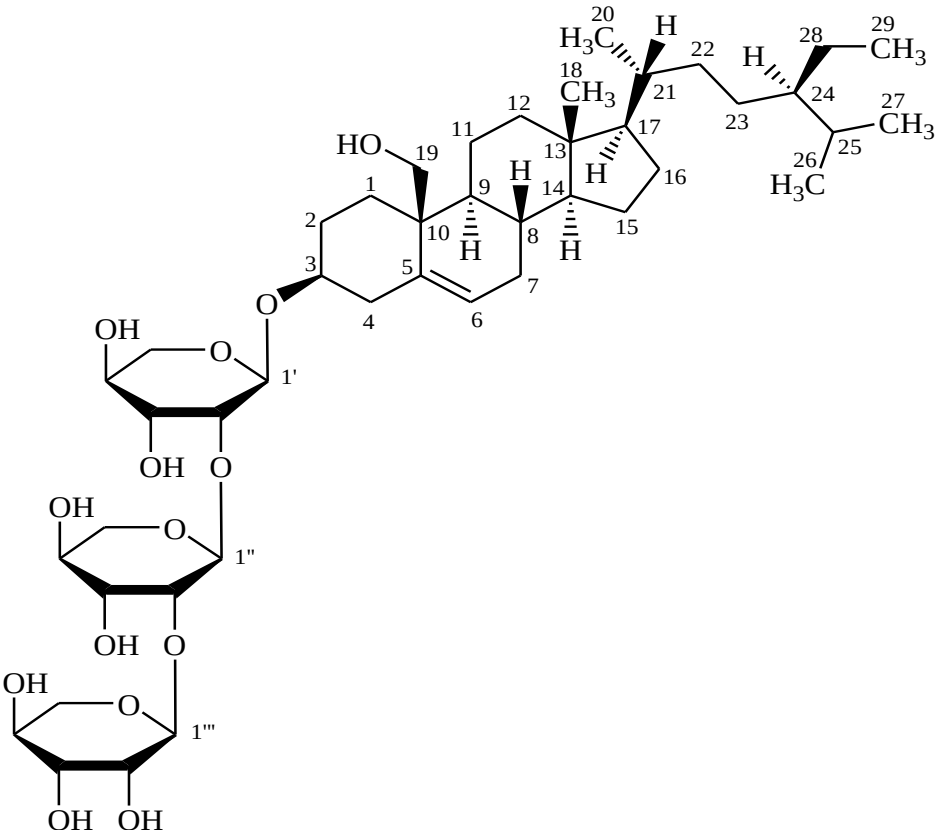

7

Fig. 1. The structures of compounds **1, 4-7.**

IV. CONCLUSION

In present study, the phytochemical investigation of *T. foenum-graceum* seeds of Sudanese origin furnished with the isolation of four new compounds **(4-7)** and compound **(6), (7)** were isolated from a plant source for the first time. These phytoconstituents may have some medicinal importance and will increase the existing knowledge of the titled plant. These phytoconstituents may also be used as chromatographic markers.

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Conflicts of interest

All authors have none to declare.

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