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Steroidal saponins from the rhizomes of *Agapanthus africanus* (Linn)

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Two novel steroidal saponins, (25*R*)-5α-spirost-7-ene-2-α,3β,5α-triol-3-O[-α-L-rhamnopyranosyl(1→2)]-β-D-glucopyranoside **1** and (25*R*)-5α-spirost-7-ene-2α,3β,5α, 9α-tetrol-3-O-β-D-glucopyranoside **2** have been isolated from the rhizomes of *Agapanthus africanus* (Linn) and their structures elucidated on the basis of spectral and chemical analysis.

Keywords: *Agapanthus africanus* (Linn), Liliaceae, steroidal saponins

Agapanthus africanus (Liliaceae) is a plant of South African origin[1-3]. The earlier studies on the air dried rhizomes reported β-sitosterol, yuccagenin and spirostan sapogenins[4,5]. Literature survey revealed no biological work has been carried out on the plant. In this communication, is reported the isolation of two novel steroidal saponins, characterized as (25*R*)-5α-spirost-7-ene-2-α,3β,5α-triol-3-O[-α-L-rhamnopyranosyl(1→2)]-β-D-glucopyranoside **1** and (25*R*)-5α-spirost-7-ene-2α,3β,5α,9α-tetrol-3-O-β-D-glucopyranoside **2** from the *n*-butanol fraction of the rhizomes of the *Agapanthus africanus*.

Results and Discussion

n-Butanol fraction of *A. africanus* on repeated column chromatography gave two compounds **1** and **2** (**Figure 1**). Both gave positive test with Liebermann-Burchard reagent, formed soapy lather when shaken with water and gave Fiegel test indicating that compounds **1** and **2** could be steroidal saponins.

Compound **1** gave a molecular ion peak at *m/z* 777 $[M+Na]^+$ corresponding to the molecular formula $C_{39}H_{62}O_{14}$ based on positive FAB-MS and ^{13}C NMR. Its IR spectrum showed bands at 3340 (polyhydroxy system), 2940 (CH stretching), 1638 and 833 cm^{-1} (C=C stretching). Anomeric signals for two sugar units were observed in the ^1H NMR spectrum at δ 4.94 (1H, d, *J*=7.8 Hz, H-1′), 5.60 (1H, d, *J*=7.2 Hz, H-1″) for α-,β-linkages respectively and ^{13}C NMR spectrum showed two anomeric carbons at δ 103.4 and 102.0 assigned to the above protons using DEPT, HMQC and HMBC (**Figure 2**) experiments. Other fragment ions in positive FAB-MS spectrum at *m/z* 630 $[M+Na\text{-rhamnosyl}]^+$ and 467 $[M+Na\text{-H-rhamnosyl-glucosyl}]^+$ were due to the loss of rhamnosyl and simultaneously loss of rhamnosyl and glucosyl. DEPT spectrum showed the presence of 5 methyl, 10 methylene, 19 methine and 5 quaternary carbon atoms. The hydrolysis experiment showed the presence of D-glucose and L-rhamnose by co-paper chromatography with authentic sugars. In IR spectrum the absorption bands at 915 and 895 cm^{-1} with the absorption 895 cm^{-1} being of greater intensity than that of 915 cm^{-1} showed the existence of a 25*R*-spiroketal skeleton[6,7] which was further evident by the presence of a quaternary carbon (C-22) resonance at δ 109.4 in the ^{13}C NMR spectrum[8]. The ^1H NMR spectrum displayed two tertiary C-methyl groups at δ 1.11, 0.76 (3H, each, s), three secondary methyl groups at δ 1.72 (3H, d, *J*=6.2 Hz), 0.91(3H, d *J*=6.5 Hz) and 0.69 (3H, d, *J*=5.3 Hz). ^{13}C NMR signals at δ 83.1 (CH), 70.5 (CH) and 81.2 (CH) were assigned to the oxygenated C-3, C-2 and C-16 carbons of the steroidal ring. The presence of the trisubstituted double bond at Δ^7 was revealed by the characteristic signal[6] in ^1H NMR at δ 5.24 (1H, brs) and ^{13}C NMR signals at δ 140.0 (C) and 116.4 (CH). ^1H NMR signal at δ 1.72 (3H, d, *J*=6.2 Hz) appeared due to the methyl group of 6-deoxy hexapyranose sugar[9]. The above data were consistent with **1** being a (25*R*) spirostanol disaccharide. The hydrolysis product was fractionated and the chloroform fraction showed the presence of (25*R*)-5α-spirost-7-ene-2α,3β,5α-triol as aglycone by comparison of spectral data as those

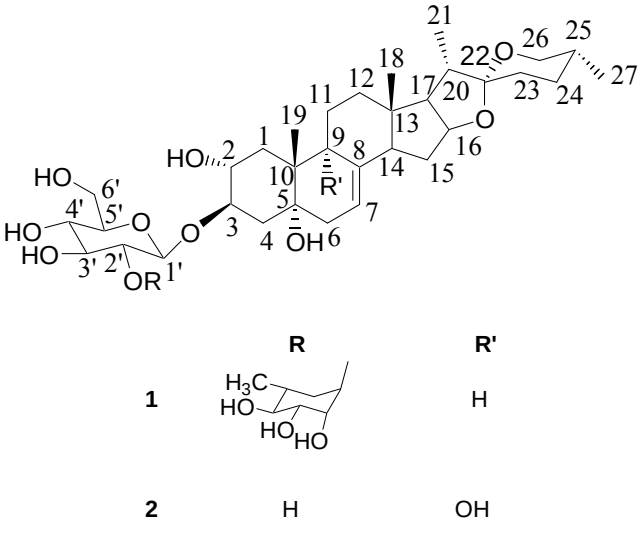

Figure 1 — Compound **1** and **2**

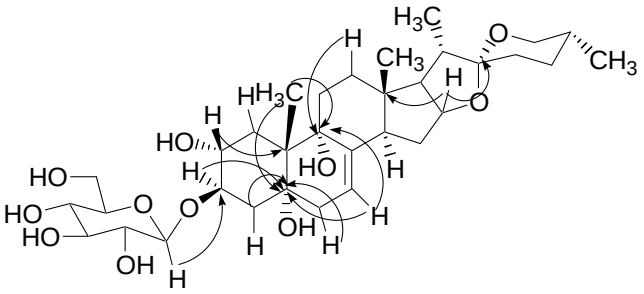

Figure 2 — Selected HMBC ^{3}J and ^{2}J correlations in compound **2**

reported in literature[6]. Assignment of point of linkage of sugar moieties was determined by permethylation[10,11] followed by acid hydrolysis which yielded 3,4,6-tri-O-methyl D-glucose and 2,3,4-tri-O-methyl L-rhamnose by the GC-MS analysis of their alditol acetates[12]. The identified partially methylated sugars correspond to a 1,2 linked glucose and terminal rhamnose. The point of linkage of saccharide grouping at C-3 position of the aglycone was confirmed by the fact in the ^{13}C NMR spectrum of **1** the signal due to C-3 was shifted downfield by 7.6 ppm and signals due to C-2 and C-4 were shifted upfield by 1.9 and 4.67 ppm respectively as compared to the aglycone[6]. From the above spectral analysis **1** was identified as (25*R*)-5α-spirost-7-ene-2α,3β,5α-triol-3-O[-α-L-rhamnopyranosyl(1→2]-β-D-glucopyranoside.

Compound **2** had the molecular formula $C_{33}H_{52}O_{11}$ (positive FAB-MS): *m/z* 663 $[M+K]^+$. Its IR spectrum showed broad absorption bands at 3411 (polyhyhdroxy system), 835 and 1641 cm^{-1}(C=C) and characteristic absorption at 897 and 915 cm^{-1} with the absorption of 897 cm^{-1} being of greater intensity than that of 915 cm^{-1}. This indicated the existence of a (25*R*) spiroketal skeleton[6,7] which is further evidenced by the presence of a quaternary carbon (C-22) resonance at δ 110.6 in its ^{13}C NMR spectrum[8]. The ^{1}H NMR spectrum of **2** showed signals for the two tertiary C-methyl groups at δ 1.13, and 0.78 (3H, each, s), two secondary C-methyl groups at δ 0.89 (3H, d, *J*=6.20 Hz), 0.68 (3H, d, *J*=5.1 Hz) and characteristic doublet at δ 5.62 (1H, brs) in ^{1}H NMR and signals at δ 140.0 (C) and 116.0 (CH) in ^{13}C NMR spectrum for trisubstituted double bond[6] at Δ^{7}. The signal at δ 5.96 (1H, d, *J*=6.3 Hz) is assignable to the anomeric proton of the sugar moiety which was also supported by the signal at δ 103 in ^{13}C NMR spectrum. DEPT experiment showed the presence of 4 methyl, 10 methylene, 13 methine and 6 quaternary carbon atoms. Hydrolysis experiment showed the presence of D-glucose by co-paper chromatography with authentic sugar. Positive FAB-MS showed the loss of the glucose unit with *m/z* at 465 $[M+Na\text{-glucosyl-}H_2O\text{-}H]^+$ indicating that glucose is directly attached to aglycone. Sugar attachment at C-3 position was confirmed by HMBC correlation between proton signal at δ 5.96 (H-1′) and carbon signal at δ 83.3 (C-3). From the ^{13}C NMR spectrum and DEPT it is seen that aglycone part (spirostan moiety) of **2** showed close similarity to that aglycone part of **1** except for missing C-H carbon and appearance of a new oxygen bearing quaternary carbon resonance at δ 78.0 indicating the presence of one more tertiary hydroxyl group in addition to the C-5 hydroxyl group of **1**. In the HMBC spectrum of **2**, proton signals at δ 5.62 (H-7), 3.71 (H-3) and 1.13(H-19) showed $^{3}J_{C\text{-}H}$ correlation with carbon signal at δ 76.8 resulting in the assignment of the signal to C-5. Signal for H-2 at δ 3.46 showed correlation with carbon at δ 43.4 resulting in the assignment of the signal to C-10. Signal at δ 5.62 (H-7), 1.48 (H-12, equatorial) and 1.13 (H-19) showed correlation with carbon at δ 78.0 resulting in the assignment of the signal to C-9. The proton signal (H-16) at δ 4.50 showed correlation with carbons at δ 41.3, 46.8 and 110.6 resulting in the assignment of the signals to C-13, C-14 and C-22 respectively. In HMBC spectrum, signals at δ 2.35 (H-4, equatorial) and 1.23 (H-6, equatorial) showed $^{2}J_{C\text{-}H}$ correlation with carbon at δ 76.8 resulting in the assignment of the signal to C-5 (**Figure 2**). Furthermore, in HMQC spectra of **2**

the proton signals at δ 2.04, 1.63 and 1.89 showed correlation with carbons at δ 46.8, 35.4, and 35.1 and were assignable to C-14, C-12 and C-1. These were further confirmed by usual ^{1}H-^{1}H COSY, which were shifted up-field by 8.5, 5.2 and 4.9 ppm relative to those of **1**. Thus, the presence of a 9α hydroxyl group was evident. Glycosylation shift (γ shift) was observed similar to **1** and its position was confirmed by HMBC correlation between anomeric proton of glucose and C-3. From the above spectral analysis the structure of **2** was established as (25*R*)-5α-spirost-7-ene-2α,3β,5α,9α-tetrol-3-O-β-D-glucopyranoside.

Experimental Section

The IR spectra were obtained on a Perkin-Elmer 881 spectrometer using KBr pellets. Optical rotations were obtained by Thomas Becker. The FAB mass spectra were recorded on Jeol-SX-120/IDA-6000 mass spectrometer using a beam of argon at 2-8 KeV. EIMS were recorded on Jeol-JMS-D-300 spectrometer at 70 eV with direct inlet system. The ^{1}H and ^{13}C NMR spectra were run on AVANCE DPX 200 and Bruker DRX 300 spectrometers operating at 300 MHz for ^{1}H and 75 MHz for ^{13}C and operating frequency for ^{13}C of aglycone at 50 MHz using TMS as internal standard and chemical shift in δ (ppm). ^{1}H -^{1}H COSY (correlation spectroscopy), ^{1}H-^{13}C HMQC (heteronuclear multiple quantum coherence) and HMBC (heteronuclear multiple bond connectivity) were obtained using Bruker DRX-300 spectrometer. Melting points were determined in open glass capillaries on an electrically heated melting point apparatus and are uncorrected. Silica gel (Qualigens 60-120 mesh) was used for column chromatography. A.S.C. silica gel (250-400 mesh) was used for flash chromatography. Solvents for column chromatography were freshly distilled. TLC was run on precoated silica gel $60F_{254}$ and RP-18 $F_{254}S$ (Merk) plates. The spots were visualized by spraying with 1% $Ce(SO_4)_2$ in 1M H_2SO_4 followed by heating at 110°C. Whatman Paper No.1, standard L-rhamnose, D-glucose (Sigma) and *n*-butanol:acetic acid:water (4:1:5) upper phase were used for ascending paper chromatography. Aniline phthalate was used as spraying agent followed by heating at 110°C.

Plant Material

The rhizomes of the *Agapanthus africanus* were collected from Ootakamund (Tamil Nadu) in March 1995. The collection and authentication were carried out by the Botany Division of CDRI, where the voucher specimen has been preserved.

Extraction and Isolation

The fresh rhizomes (15 kg) were dried in shade, powdered and extracted by percolating it with 95% ethanol (4×15 L) for 24 hr at RT. The whole extract was concentrated under reduced pressure by using rotatory evaporator at 50°C and finally dried *in vacuo* to give crude ethanol extract (800 g). The ethanolic extract 500 g was macerated with *n*-hexane to get *n*-hexane soluble fraction and insoluble residue subsequently macerated with chloroform to get chloroform soluble fraction. The insoluble residue was suspended in water and successively partitioned with *n*-butanol to get *n*-butanol soluble fraction and water soluble fraction. The *n*-butanol fraction (15 g) rich in saponins was subjected to chromatographic separation over silica gel column (60-120 mesh) using gradient elution with chloroform:methanol:water to give fractions F019 (200 mg, 85:14.25:0.75 v/v) and F020 (100 mg, 80:19:1 v/v). The fraction F020 (100 mg) was loaded on a chromatographic column of silica gel (230-400 mesh) and subjected to gradient elution with chloroform:methanol:water to give **1** (35 mg, 85:14.25:0.75 v/v) and F019 (100 mg) was chromatographed over silica gel (230-400 mesh) using gradient elution with chloroform:methanol:water to give **2** (23 mg, 90:9.5:0.5 v/v).

Compound 1. White amorphous powder, $[\alpha]_D^{23}$ -65° (c, 0.10% in MeOH); IR (KBr): 3340, 2940, 1638, 1440, 975, 915, 895, 860, 833, 780 cm^{-1}; ^{1}H NMR (CD_3OD): δ 5.60 (1H, d, *J*=7.2 Hz, H-1″), 5.24 (1H, brs, H-7), 4.94 (1H, d, *J*=7.8 Hz, H-1′), 3.6 (1H, dd, *J*=10.3, 6.7 Hz, H-26a), 3.46 (1H, dd, *J*=10.5, 10.4 Hz, H-26b), 1.72 (3H, d, *J*=6.2 Hz, H-6″), 1.11 (3H, s, H-17), 0.91 (3H, d, *J*=6.5 Hz, H-21), 0.76 (3H, s, H-18), 0.69 (3H, d, *J*=5.3 Hz, H-27); ^{13}C NMR (CD_3OD): δ 40.0 (C-1), 71.0 (C-2), 80.33 (C-3), 35.0 (C-4), 73.1 (C-5), 37.4 (C-6), 116.4 (C-7), 140.0 (C-8), 43.7 (C-9), 39.8 (C-10), 22.0 (C-11), 40.6 (C-12), 41.8 (C-13), 55.3 (C-14), 30.9 (C-15), 81.2 (C-16), 63.0 (C-17), 16.5 (C-18), 19.3 (C-19), 42.6 (C-20), 14.9 (C-21), 109.4 (C-22), 32.4 (C-23), 29.7 (C-24), 30.9 (C-25), 66.9 (C-26), 17.3 (C-27), 103.4 (C-1′), 79.9 (C-2′), 75.2 (C-3′), 69.9 (C-4′), 76.3 (C-5′), 62.8 (C-6′), 102 (C-1″), 72.6 (C-2″), 72.9 (C-3″), 74.2 (C-4″), 69.9 (C-5″), 18.8 (C-6″); FABMS (+ve): *m/z* 793

Table I — GC-MS analysis of partially methylated alditol acetates obtained from **1**

Alditol acetate	GC-MS (*m/z*)	R_t (min)
1,5-di-O-acetyl-6-deoxy-2,3,4-tri-O-methyl hexitol	175, 161, 145, 131, 117, 115, 101, 89, 87, 72, 69, 67	32.80
1,2,5-tri-O-acetyl-3,4,6-tri-O-methyl hexitol	223, 189, 161, 159, 157, 143, 129, 117, 101, 99, 87,85,74, 71, 59	37.96

$[M+K]^+$, 777 $[M+Na]^+$, 630 [M+Na-rhamnosyl]$^+$, 467 [M+Na-H-rhamnosyl-glucosyl]$^+$.

Acid hydrolysis of compound 1

Compound **1** (15 mg) was refluxed with 1N HCl in EtOH-H_2O (80:20, 2 mL) for 1.5 hr, diluted with water (0.5 mL) and freed of ethanol by evaporation, it was again refluxed for 1 hr, and then extracted with *n*-butanol. The *n*-butanol layer was washed once with $NaHCO_3$ solution and then with water, evaporated to dryness and the residue chromatographed over silica gel using *n*-hexane:acetone (9:1) to give compound (10 mg). IR(KBr): 3409, 2950, 1633, 1452, 1380, 1245, 1172, 1055, 983, 920, 898, 831, 800 cm^{-1}; ^1H NMR($CDCl_3$): δ 5.07 (1H, brd, H-7), 4.49 (1H, q, *J*=7.7 Hz, H-16), 3.43 (1H, dd, *J*=10.3, 7.2 Hz, H-26a), 3.47 (1H, dd, *J*=10.6, 10.3 Hz, H-26b), 3.8 (1H, m, H-3), 3.5 (1H, m, H-2,), 1.00 (3H, s, Me-19), 0.98 (3H, d, *J*=6.8 Hz, Me-21), 0.78 (3H, d, *J*=6.0 Hz, Me-27), 0.66 (3H, s, Me-18); ^{13}C NMR ($CDCl_3$): δ 39.87 (C-1), 72.9 (C-2), 72.73 (C-3), 39.67 (C-4), 74.3 (C-5), 36.62 (C-6), 115.2 (C-7), 139.7 (C-8), 43.83 (C-9), 40.7 (C-10), 21.8 (C-11), 41.0 (C-12), 42.1 (C-13), 55.05 (C-14), 31.3 (C-15), 80.8 (C-16), 62.40 (C-17), 16.67 (C-18), 19.8 (C-19), 42.47 (C-20), 14.8 (C-21), 109.7 (C-22), 21.73 (C-23), 29.18 (C-24), 30.66 (C-25), 67.28 (C-26), 17.51 (C-27); EIMS: *m/z* 428 [M-H_2O]$^+$, 395, 314, 299, 281, 139, 105.

The aqueous hydrolysate was neutralised with Amberlite IR 410 $(CO_3)^{2-}$ resin, filtered and concentrated and examined by co-paper chromatography BuOH:AcOH:H_2O (4:1:5) and the sugars were identified as D-glucose and L-rhamnose.

Permethylation of compound 1

Compound **1** (5 mg) was dissolved in dry DMSO (0.3 mL). To this was added finally powdered anhydrous NaOH (30 mg) and CH_3I (0.3 mL) followed by stirring for 3 hr at RT. Chloroform (1 mL) was added to the reaction mixture and the mass centrifuged. Chloroform layer was separated and washed free of alkali with water and evaporated to dryness to furnish a viscous mass which gave a positive FAB-MS showing peak at *m/z* 875 $[M+Na]^+$ for permethylated **1** which corresponded to 7 methyl groups.

Preparation of alditol acetates from the per-methylated 1

Permethylated **1** (3 mg) was refluxed with 2 N HCl in EtOH-H_2O (80:20, 10.5 mL) for 4 hr. The hydrolysate was then diluted with water, freed of ethanol by evaporation and further heated at 100°C for 1 hr. It was then neutralized with amberlite IR 410 $(CO_3)^{2-}$ resin, then concentrated to 0.5 mL and stirred with $NaBH_4$ (15 mg) at RT. After 2 hr amberlite IR 120 H^+ resin was added to maintain the *p*H at 3.5 and reaction mixture was filtered, concentrated and co-distilled with 3 portions (5 mL each) of methanol. The resulting mixture was then treated with acetic anhydride and dry pyridine (0.5 mL, each) for 2 hr at 100°C and the reagent was removed by co-distillation with toluene. The residue containing the alditol acetates was subjected to GC-MS using a GLC column containing 3% of OV-1 at 160°C (**Table I**).

Compound **2**. White amorphous power; $[\alpha]_D^{23}$ -56° (c, 0.10% in MeOH); IR (KBr): 3411, 3022, 2929, 1641, 1068, 835, 897, 915, 759, 671 cm^{-1}; ^1H NMR (CD_3OD): δ 5.96 (1H, d, *J*=6.3 Hz, H-1′), 4.74 (2H, brs, H-23), 5.62 (1H, brs, H-7), 4.50 (1H, q, *J*=7.74 Hz, H-16) 4.36-4.37 (2H, m, H-26), 3.71 (1H, m, H-3), 3.46 (1H, m, H-2), 2.35 (1H, dd, *J*=13.9, 5.9 Hz, H-4), 1.48 (1H, brd, *J*=12.8 Hz, H-12), 1.23 (1H, dd, *J*=12.1, 5.3 Hz, H-6), 1.13 (3H, s, Me-19), 0.89 (3H, d, *J*=6.20 Hz, Me-21), 0.78 (3H, s, Me-18), 0.68 (3H, d, *J*=5.1 Hz, Me-27); ^{13}C NMR (CD_3OD): δ 35.1 (C-1), 71.6 (C-2), 83.3 (C-3), 37.2 (C-4), 76.8 (C-5), 37.2 (C-6), 116 (C-7), 140 (C-8), 78.0 (C-9), 43.4 (C-10), 22.5 (C-11), 35.4 (C-12), 41.3 (C-13), 46.8 (C-14), 31.5 (C-15), 79.2 (C-16), 64.5 (C-17), 16.8 (C-18), 19.5 (C-19), 42.6 (C-20), 14.9 (C-21), 110.6 (C-22), 32.4 (C-23), 29.92 (C-24), 32.8 (C-25), 67.9 (C-26), 17.3 (C-27), 103 (C-1′), 83.6 (C-2′), 75.2 (C-3′), 76.3 (C-4′), 72.8 (C-5′), 62.6 (C-6′); FABMS (+ve): *m/z* 663 $[M+K]^+$, 646 $[M+Na-H]^+$, 465 [M+Na-H-glucosyl-H_2O]$^+$.

Hydrolysis of 2

Compound **2** (10 mg) was refluxed with 1N HCl in EtOH-H_2O (80:20, 2 mL) for 1.5 hr, diluted with

water (0.5 mL) and freed of ethanol by evaporation, it was again refluxed for 1 hr, and then extracted with *n*-butanol. After usual work up according to the procedure described for compound **1** there was obtained the sugar solution and a solid mass (6 mg). The sugar was identified as D-glucose by co-paper chromatography with authentic sugar using BuOH:AcOH:H_2O (4:1:5) as mobile phase. The solid mass on TLC revealed it to be a mixture of artifacts that could not be isolated due to the paucity of material.

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