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Steroidal saponins from *Asparagus africanus*☆

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Abstract

The structures of two new monodesmosidic spirostanosides and a new bisdesmosidic furostanol glycoside isolated from the roots of *Asparagus africanus* Lam. (Liliaceae) have been elucidated as (25*R*)-3β-hydroxy-5β-spirostan-12-one 3-*O*-{β-D-glucopyranosyl-(1 → 2)-[α-L-arabinopyranosyl-(1 → 6)]-β-D-glucopyranoside} (**1**), (25*R*)-5β-spirostan-3β-ol 3-*O*-{β-D-glucopyranosyl-(1 → 2)-[α-L-arabinopyranosyl-(1 → 6)]-β-D-glucopyranoside} (**2**) and 26-*O*-β-D-glucopyranosyl]-22α-methoxy-(25*R*)-furostan-3β,26-diol 3-*O*-{β-D-glucopyranosyl-(1 → 2)-[β-D-glucopyranoside} (**3**), respectively, by the combined use of one and two dimensional NMR experiments. The complete ^{13}C and ^{1}H assignments of the peracetyl spirostanosides and the furostanol oligoside were derived. The interconversions between the methoxyl and hydroxyl group at C-22 of the furostanol glycoside was investigated and the genuine furostanol oligoside of *A. africanus* appears to be the hydroxyl type based on the comparative study of the methanol, pyridine and dioxane extracts.

Keywords: *Asparagus africanus*; Liliaceae; Spirostanol glycoside furostanol glycoside; Gloriogenin; Smilagenin

1. Introduction

Asparagus africanus (Liliacae) (local names '*Seriti*' or '*K'estancha*') is a widely spread thorny shrub about 1 to 3 m long distributed along the great rift valley of Ethiopia. It is used in the indigenous system of medicine for various health ailments (Watt, & Breyer-Brandwijk, 1962; Abate, 1989; Abebe, & Ayehu, 1993). Recent phytochemical investigation of the root of this plant has led to the isolation of antiprotozoal compounds muzanzagenin and (+)-nysol (Oketch-Rabah et al., 1997). This paper describes the isolation, structural elucidation and complete ^{13}C and ^{1}H chemical shifts assignments of two new spirostanosides and a new oligofurostanoside from the methanol extract of the roots of this plant.

2. Results and discussion

Solvent partition and chromatography of the saponin mixture obtained from the methanol extract of the roots of *A. africanus* produced three major saponins **1**, **2** and **3**. Compounds **1** and **2** showed spiroacetal absorption in their IR spectra whereas compound **3** showed no spiroacetal absorption but gave an intense red colour with Ehrlich reagent (Stahl, 1965; Kiyosawa et al., 1968; Tschesche, Tjoa, Wulff, & Noronha, 1968). Structural determinations of the three saponins were mainly based on 1D (^{1}H, ^{13}C, ^{13}C-DEPT, selective ^{1}H-NOE) and 2D NMR experiments (^{1}H, ^{1}H-COSY, ^{1}H, ^{1}H-TOCSY, ^{1}H, ^{13}C-HSQC-TOCSY, ^{1}H, ^{13}C-HSQC, ^{1}H, ^{13}C-HMBC) and on NOE experiments. For structure determination of **1** and **2** we prepared the corresponding peracetyl derivatives. This gave enhanced signal dispersion in the carbohydrate region and allowed unambiguous signal assignment. With a ^{1}H, ^{13}C-HMBC experiment we could determine the monosaccharide sequence. Because of heavy signal overlap it was not possble to assign the proton and

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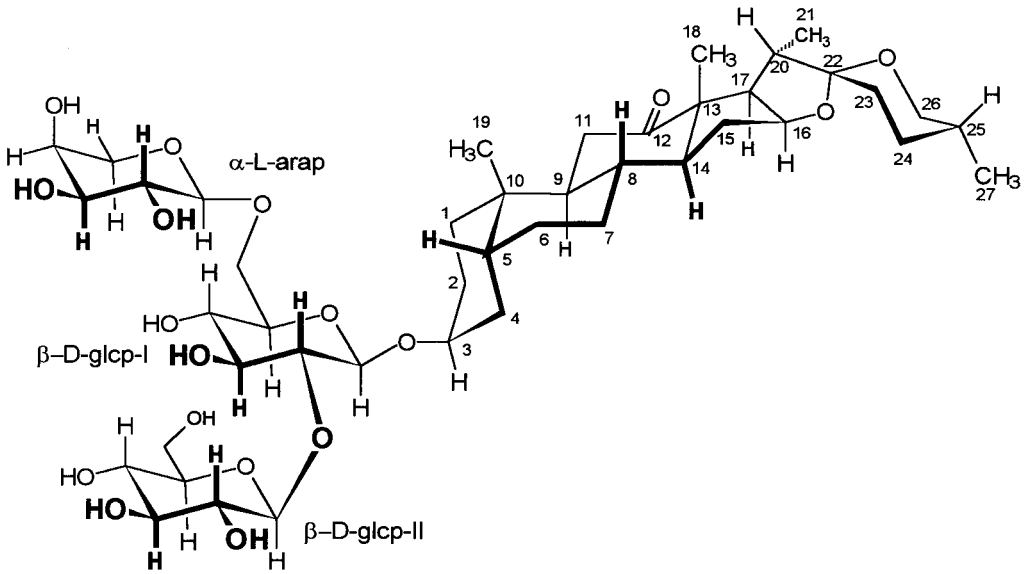

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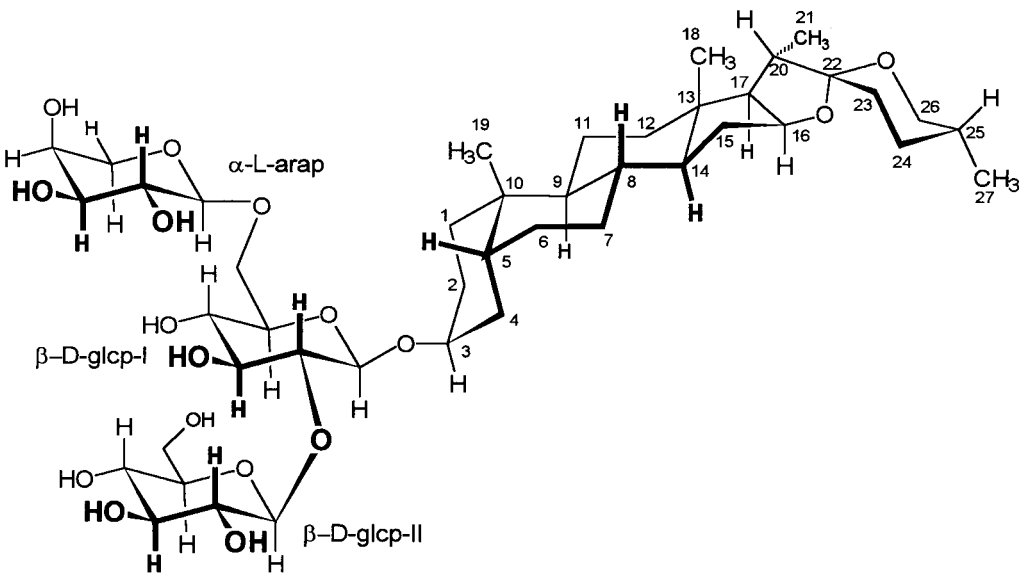
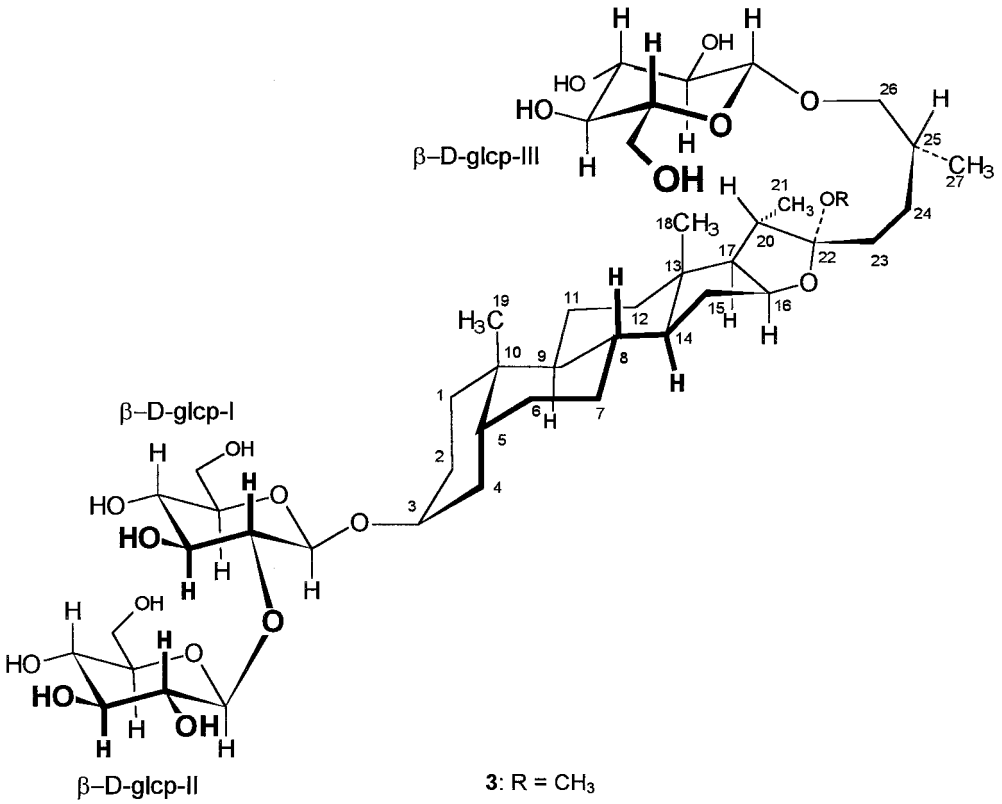


carbon resonances of compound **1** and **2** in pyridine-d-$_5$. The ^{1}H and ^{13}C chemical shifts of the peracetyl derivatives of **1** and **2** and of compound **3** are given in Tables 1 and 2.

The ^{1}H NMR spectra of compounds **1** and **2** displayed three doublets, each belonging to one proton at δ 4.70 (J=7.8 Hz), δ 4.32 (J=6.9 Hz) and δ 4.20 (J=7.8 Hz) for the anomeric sugar protons of compound **1** and at δ 4.74 (J=7.8 Hz), δ 4.37 (J=6.9 Hz) and δ 4.25 (J=7.8 Hz) for the anomeric protons of compound **2**. The coupling constants indicated an axial position of each of the anomeric protons.

Compound **1** ($C_{44}H_{70}O_{18}$) was crystallised in the form of fine needles from methanol. Its FAB mass spectrum gave $[M+Na]^+$ at m/z 909 and $[M+H]^+$ at m/z 887, indicating that the molecular weight was 886. Peaks at m/z=413 and 395 were suggestive of a saturated monohydroxyl spirostane nucleus. The IR spectrum exhibited strong absorption bands at 981, 918, 898 and 868 cm^{-1} characteristic for the spirostane type steroidal sapogenins. Weaker intensity of the band at 918 cm^{-1} than at 898 cm^{-1} showed that **1** belongs to the 25*R* series of spirostanes (Wall, Eddy, Mcclennen, & Klumpp, 1952).

The equatorial orientation of the C-27 methyl was further verified by the axial–axial coupling of H-26 ax



(δ 3.51) and H-25 ax (δ 1.60): $J_{26ax,25ax} = 10.5$ Hz in the ^{1}H NMR and the lower field resonance of C-27 (δ 17.14) as compared to the ^{13}C NMR chemical shift of (25*S*)-spirostanes (δ16–16.5) (Agrawal, Jain, & Pathak, 1995). The absorption peak at 1696 cm^{-1} in the IR spectrum of **1** and a ^{13}C resonance at δ 211.9 in the ^{13}C NMR confirmed the presence of a carbonyl carbon and its position at C-12 was determined in the HMBC correlation with H-11 (δ 2.20, 2.30) and CH_3-18 (δ 1.09). The ^{13}C NMR spectrum Table 1 in combination with ^{13}C-DEPT and HSQC-DEPT showed signals for 44 carbons, 27 of which arose from the aglycone moiety. The spectrum displayed, among others, a signal corresponding to an acetalic quaternary carbon at δ 109.7 that indicated a spirostane structure. The proton and carbon resonances were assigned by different homonuclear and heteronuclear correlation experiments (DQF-COSY, TOCSY, HSQC, HSQC-TOCSY) and these assignments were confirmed by comparison of the chemical shifts with structurally related compounds reported in the literature (Eggret, & Djerassi, 1975; Yan, Ohtani, Kasaki, & Yamasaki, 1996). Acid hydrolysis of **1** gave the aglycone which was identified as gloriogenin [(25*R*)-3β-hydroxy-5β-spirostan-12-one], whose ^{13}C NMR chemical shifts were in good agreement with those reported in the literature (Nakano et al., 1991; Agrawal et al., 1995). The monosaccharides obtained after acid hydrolysis of **1** were analysed as trimethylsilyl derivatives by GC–MS using authentic samples as references. Glucose and arabinose in the relative proportions of 2:1 were detected. The D-configuration of glucose and the L-configuration of arabinose were determined by capillary electrophoresis as complexes with borate and β-cyclodextrin using authentic samples as references (Stefansson, & Novotny, 1993). Fragment ions at *m/z* = 755 $[(M+H)-132]^+$, 133 (arabinosyl), 613 $[(M+Na)$–arabinose–glucose$]^+$, 431 $[(M+H)$–arabinose–2 glucose$]^+$, and 593 $[(M+H)$–arabinose–glucose$]^+$ confirmed the presence of a branched chain trisaccharide, hexosyl–(pentosyl)–hexosyl (1:1:1) linked with aglycone of MW 430 (gloriogenin).

The point of attachment of the saccharide part and interglycosidic linkage was established by an HMBC experiment. Long range correlations were observed between H-1 (δ 4.20) of glucose-I and C-3 (δ 74.95) of the aglycone, H-1 (δ 4.32) of arabinose and C-6 (δ 68.0) of glucose-I and H-1 (δ 4.70) of the terminal glucose-II and C-2 (δ 77.8) of glucose-I. All the above data identified **1** as (25*R*)-3β-hydroxy-5β-spirostan-12-one 3-*O*-{β-D-glucopyranosyl-(1 → 2)-[α-L-arabinopyranosyl-(1 → 6)]-β-D-glucopyranoside}.

2 ($C_{44}H_{72}O_{17}$) was crystallised as colourless flakes from methanol. The FAB-MS of **2** gave a $[M+Na]^+$

Table 1
^1H and ^{13}C chemical shifts of the aglycon part of compound **1**–**3** in pyridine-d_5 at 40°C (TMS as internal standard)

Position	Compound					
	1		**2**		**3**	
	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1	30.6	1.86, 1.35	30.8	1.86, 1.55	30.9	1.84, 1.84
2	26.4	1.89, 1.35	26.5	2.01, 1.53	26.9	1.94, 1.51
3	75.0	4.24	75.3	4.30	75.3	4.29
4	30.6	1.79, 1.70	30.6	1.79, 1.79	31.0	1.84, 1.49
5	36.3	2.21	36.7	2.14	37.0	2.16
6	26.5	1.83, 1.21	26.5	1.82, 1.19	27.1	1.86, 1.22
7	26.1	1.32, 0.95	26.5	1.27, 0.95	26.9	1.28, 0.99
8	34.7	1.84	34.4	1.51	35.6	1.51
9	42.1	1.72	42.1	1.28	40.4	1.31
10	36.7		35.8		35.3	
11	37.6	2.20, 2.37	20.9	1.35, 1.21	21.2	1.34, 1.22
12	211.9		40.2	1.68, 1.08	40.3	1.70, 1.08
13	42.7		41.1		41.3	
14	56.1	1.45	56.3	1.08	56.5	1.06
15	31.4	2.13, 1.59	32.1	2.03, 1.41	32.2	1.98, 1.36
16	80.9	4.56	81.1	4.60	81.5	4.51
17	54.4	2.83	63.3	1.86	64.5	1.79
18	16.0	1.09	16.6	0.82	16.5	0.83
19	23.2	1.03	23.9	1.10	24.0	0.99
20	42.6	1.95	41.8	1.97	40.6	2.24
21	13.5	1.36	14.6	1.15	16.3	1.17
22	109.7		109.5		112.7	
23	31.6	1.72, 1.65	31.7	1.71, 1.66	31.0	1.93, 1.85
24	29.0	1.58, 1.58	29.3	1.57, 1.57	28.3	1.72, 1.46
25	30.4	1.60	30.4	1.60	34.5	1.88
26	67.0	3.59, 3.51	66.9	3.58, 3.53	75.0	4.07, 3.54
27	17.1	0.72	17.1	0.71	17.6	1.05
C_{22}–OCH_3					49.7	3.58

at *m/z* 896, indicating that the molecular weight was 872. Peaks at *m/z* 418 and 399 were suggestive of a saturated monohydroxy spirostane nucleus. It was identified as a (25*R*)-spirostanol saponin by its IR spectra that showed a lower intensity peak at 920 cm^{-1} than at around 900 cm^{-1} (Watt et al., 1952, and by the axial–axial coupling of H-26ax (δ 3.53) and H-25ax (δ 1.60) $J_{26ax,25ax} = 11.2$ Hz in the ^1H NMR. This structure was further supported by the ^{13}C NMR chemical shift at δ 17.1 assignable to C-27, which was characteristic for the (25*R*) spirostane (Agrawal et al., 1995). The peak at 1696 cm^{-1} in the IR and a carbonyl resonance at δ 211.9 in the ^{13}C NMR were missing. Comparison of the ^1H and ^{13}C chemical shift data of the aglycone (with the exception of C-12 carbonyl carbon signal absence in **2**) and sugar moieties of **1** and **2** indicated that they were similar compounds.

Acid hydrolysis of **2** gave the aglycone smilagenin [(25*R*)-5β-spirostan-3β-ol] identified by co-TLC and comparison of ^{13}C chemical shift with authentic smilagenin and chemical shifts reported in the literature (Eggret, & Djerassi, 1975; Agrawal, Jain, Gupta, & Thakur, 1985). From the aqueous hydrolysates, D-glucose and L-arabinose were obtained in a 2:1 ratio (GC–MS spectrometry). Their configuration was determined by capillary electrophoresis (Stefansson, & Novotny, 1993). Fragment ions at *m/z* 741 $[(M+Na)\text{-}132]^+$, 711 $[(M+Na)\text{-}162]^+$, 579 $[(M+Na)$-glucose–arabinose$]^+$ and 418 $[(M+Na)$-2 glucose–arabinose$]^+$ confirmed the presence of a branched chain trisaccharide, hexosyl–(pentosyl)–hexosyl (1:1:1) linked with an aglycone of MW 416 (smilagenin). The point of attachment of the saccharide part and the interglycosidic linkage was established by HMBC experiments. Long range correlations were observed between H-1 (δ 4.25) of glucose-I and C-3 (δ 75.3) of the aglycone, H-1 (δ 4.37) of arabinose and C-6 (δ 66.8) of glucose-I, and H-1 (δ 4.74) of the terminal glucose-II and C-2 (δ 76.5) of glucose-I. Thus the structure of **2** was assigned as (25*R*)-5β-spirostan-3β-ol 3-*O*-{β-D-glucopyranosyl-

Table 2
^1H and ^{13}C spectral data for carbohydrate moieties in pyridine-d_5 for **3** and in benzene-d_6 for the peracetates of **1** and **2** at 40°C (TMS as internal standard)

	Compound					
	1		**2**		**3**	
	δ_C	δ_H (*J*, Hz)	δ_C	δ_H (*J*, Hz)	δ_C	δ_H (*J*, Hz)
Glucose-I						
1	99.6	4.20, (7.8)	98.7	4.25, (7.8)	101.79	4.93, (7.9)
2	77.8	3.75	76.5	3.78	83.3	4.19
3	76.1	5.33	74.7	5.36	78.4	4.28
4	70.0	5.04	69.0	5.06	71.8	4.13
5	73.6	3.43	72.3	3.45	78.0	3.84
6	68.0	3.90, 3.54	66.8	3.91, 3.56	62.9	4.29, 4.46
Arabinose						
1	101.2	4.32, (6.9)	100.1	4.37, (6.9)		
2	69.6	5.53	68.5	5.54		
3	70.9	5.16	69.5	5.17		
4	68.3	5.24	67.1	5.24		
5	63.3	3.73, 3.02	61.0	3.72, 3.02		
Glucose-II						
1	101.6	4.70, (7.8)	100.4	4.74, (7.8)	106.0	5.35, (7.4)
2	72.1	5.20	71.2	5.25	77.0	4.03
3	73.8	5.37	72.7	5.39	78.0	4.21
4	69.4	5.30	68.1	5.32	71.9	4.26
5	72.7	3.45	71.7	3.46	78.5	3.93
6	62.7	4.48, 4.16	61.6	4.51, 4.18	63.0	4.45, 4.49
Glucose-III						
1					102.0	4.81, (7.4)
2					75.2	3.99
3					78.5	4.17
4					71.9	4.15
5					78.5	3.91
6					63.0	4.51, 4.34

(1 → 2)-[α-L-arabinopyranosyl-(1 → 6)]-β-D-glucopyranoside}.

3 ($C_{46}H_{78}O_{19}$) was proposed to be a furostanol saponin on the basis of the following observations: formation of red colour when the TLC plate was treated with Ehrlich reagent (Stahl, 1965; Kiyosawa et al., 1968), no spiroketal absorption in the IR spectra (Watt et al., 1952; Tschesche et al., 1968) and facile conversion to spirostanol type saponin during acid hydrolysis. The signal at δ 112.7 was assigned to the acetalic quaternary carbon which is characteristic for a furostane skeleton possessing a OH/OMe group at C-22 in the 26-*O*-glycosidic form (Agrawal et al., 1995). The ^{1}H and ^{13}C NMR chemical shifts (Tables 1 and 2) of the furostane skeleton and the sugar moieties of **3** were assigned based on HSQC, HSQC-TOCSY, TOCSY and HMBC experiments. The ^{1}H and ^{13}C shifts of ring A, B, C and D were almost identical to those of **2**. The resonances of the anomeric protons of **3** were all doublets (J = 7.4–7.9 Hz) indicating that all the sugars were pyranoses with axial oriented anomeric protons. Acid hydrolysis of **3** gave the spirostane aglycone smilagenin (Eggret, & Djerassi, 1975; Agrawal et al., 1985).

The aqueous hydrolysate contained only D-glucose, the identity of which was confirmed by GC–mass spectrometry and D-configuration was determined by capillary electrophoresis as described above (Stefansson, & Novotny, 1993). The structure was further confirmed by FAB-MS showing peaks at m/z 579 $[(M+H)–MeOH\text{-}325]^+$ and 741 $[(M+H–MeOH\text{-}163]^+$ which arose by the loss of two hexose units at C-3 and one hexose moiety from C-26, respectively.

The interglycosidic linkage was established by long range correlations obtained from the HMBC experiment. Correlations were observed between H-1 (δ 4.93) of glucose-I and C-3 (δ 75.3) of the aglycone, H-1 (δ 5.35) of glucose-II and C-2 (δ 83.3) of glucose-I. Further correlations were observed between H-1 (δ 4.81) of glucose-III and C-26 (δ 75.0) of the aglycone. The ^{1}H signal (δ 3.58, 3H, s, OCH_3) and the ^{13}C peak (δ 49.7, O–CH_3) suggested the presence of a C-22 methoxyl furostanol structure. The configuration of C-22 was confirmed to be α by the observed NOE between the α-oriented methyl protons at C-20 (δ 1.17) and the methoxy protons at C-22 (δ 3.58). On boiling in aqueous acetone, **3** was converted to the more polar **4**. The ^{1}H and ^{13}C NMR of the latter did not show any methoxyl signal. Such interconversion between a hydroxyl and methoxyl groups at C-22 of furostanol oligosides has already been reported in the literature (Kiyosawa et al., 1968; Tschesche et al., 1968). The presence of a methoxyl group in **3** was also supported by FAB-MS, which showed a pseudomolecular ion at m/z 904 $[(M+H)–MeOH]^+$ and the simultaneous loss of MeOH and a hexose that appeared at m/z 741 $[(M+H)–MeOH\text{-}163]^+$. Accordingly, the structure of **3** was determined as 26-*O*-β-D-glucopyranosyl-22α-methoxy-(25*R*)-furostan-3β,26-diol 3-*O*-{β-D-glucopyranosyl-(1 → 2)-β-D-glucopyranoside}. As described above, the structure of 22-methoxy furostanol oligoside from the methanol extract of *Asparagus africanus* has been established. Interconversion between 22-hydroxy and 22-methoxy furostanol oligosides is known to occur, so an attempt was made to obtain the genuine oligoside by extracting the powdered root of *A. africanus* with pyridine and dioxane. TLC of the extract of the plant with pyridine and dioxane showed only the spot corresponding to 22-hydroxyl furostanol oligoside (**4**) with no spot related to 22-methoxyl furostanol oligoside.

3. Experimental

3.1. General

Melting points were determined in open capillaries in an electrothermal melting apparatus. FAB-MS was recorded with a ZAB-E (VG analytical (Micromass)); matrix: thioglycerol and 'magic bullet'.

Proton correlated NMR spectra were recorded with a VARIAN UNITY INOVA 600 NMR spectrometer at 313 K, ^{13}C spectra with a VARIAN UNITY INOVA 400 NMR spectrometer at 313 K. Concentration 60 mmol/l. Tubes: 5 mm diameter (Kontes). Triple resonance probe head with shielded z-gradient (600 MHz); dual probe with shielded z-gradients or broadband probe (400 MHz). Solvents: pyridine-d_5 and benzene-d_6 (99.98% deuterium content) from Uetikon/Switzerland. TMS was used as internal standard. Chemical shifts were given in ppm. Typical parameters are: 1D-^{13}C NMR: (12.5 μs $\pi/2$ pulse), pulse width: 5 μs, WALTZ decoupling, 128 K real data points, 4000 transients. 1D-^{1}H NMR: (7.5 μs $\pi/2$ pulse), pulse width: 4 μs, 32 K real data points, 8 scans, relaxation delay 2 s. 1D-NOESY: mixing time 0.4 s, 32 K real data points, relaxation delay 5 s. In 2D proton detected spectra: 4 K data points in F2, spectral width 7000 Hz, carrier at 2.6 ppm, relaxation delay 1.5 s. 2D-^{1}H, ^{1}H-DQF-COSY: 1400 real data points in F1, 8 transients. 2D-^{1}H, ^{1}H-TOCSY: spinlock 8 kHz, mixing time 60 ms, 800 data points in F1, 4 transients. 2D-^{1}H, ^{13}C-HSQC: 800 data points in F1, decoupler at 76 ppm, spectral width 23000 Hz, 8 transients, GARP decoupling. 2D-^{1}H, ^{13}C-HSQC-TOCSY: spinlock 8 kHz, mixing time 80 ms, 800 data points in F1, decoupler at 76 ppm, spectral width 23000 Hz, 16 transients, GARP decoupling. 2D-^{1}H, ^{13}C-HMBC: 800 data points in F1, decoupler at 100 ppm, spectral width 33000 Hz, 16 transients.

IR spectra were obtained with a Perkin Elmer 881

IR spectrometer. Optical rotations were measured with a Perkin Elmer 241 MC polarimeter. Gas liquid chromatography was run on a Hewlett-Packard 5890 (series 2 plus) equipped with a FID and Hewlett Packard 5989B Mass spectrometer. UV spectra were obtained from a Shimadizu UV 160A UV-visible recording spectrophotmeter. Capillary electrophoresis was recorded with Hewlett Packard 3D CE using a diode array detector. Paper partition chromatography (PPC) of sugars was conducted on Whatman No. 1 paper using a descending mode and detection was carried out with aniline/hydrogen phthalate as a spraying reagent. Column chromatography was carried out over Silicagel-60 (Merck, mesh 230–240 μm), RP8 (Merck, 40–63 μm) and Sephadex LH-20 (Pharmacia, mesh 25–100 μm).

Homogeneity of fractions was tested on TLC (Silicagel, Merck) and (RP18, Merck). The spots were visualized by spraying with Ehrlich reagent and/or anisaldehyde–sulfuric acid reagent followed by heating at 100°C for 3 min. Unless otherwise noted solvents used for TLC and CC were as the following: A: $CHCl_3$/MeOH (10:1); B: $CHCl_3$/MeOH (5:1); C: $CHCl_3$/MeOH (1:1); D: $CHCl_3$/MeOH/H_2O (65:30:7); E: MeOH/CH_3COCH_3 (9:1); F: MeOH/H_2O (9:1) and G: *n*-BuOH/AcOH/H_2O (5:1:4).

3.2. Plant material

The roots of *Asparagus africanus* were collected near Shashemene, a town 250 km south of Addis Abeba, Ethiopia in August 1997 at an altitude of 1550 m and identified by Dr. Dawit Abebe of EHNRI. Voucher specimen (Herbarium No. 1123) has been deposited at Herbarium of Department of Drug Research (EHNRI, Ethiopia) and at the Institute of Pharmaceutical Chemistry (Karl-Franzens-University, Graz).

3.3. Extraction and isolation of the glycosides

The roots were air dried, coarsely powdered (1.4 kg) and defatted with petroleum ether in a Soxhlet apparatus. The solvent free powder was re-extracted exhaustively with MeOH (4 × 12 h) in a soxhlet to afford a brown gum (68 g), which was taken up in water and extracted with *n*-BuOH (3 × 300 ml). The *n*-BuOH extract after concentration under reduced pressure yielded a saponin mixture (47 g). This mixture was further purified by repeated silicagel column chromatography (solvent A–D) followed by column chromatography on Sephadex LH-20 with MeOH as an eluting solvent and rechromatography on RP-8 silicagel with solvent F to yield colourless compounds: **1** (120 mg), **2** (200 mg) (Ehrlich reagent negative) and **3** (105 mg) (Ehrlich reagent positive).

3.4. Glycosides

(25*R*)-3β-hydroxy-5β-spirostan-12-one 3-*O*-{β-D-glucopyranosyl-(1 → 2)-[α-L-arabinopyranosyl-(1 → 6)]-β-D-glucopyranoside} (**1**): fine needles (methanol); m.p.: uncorr. 206–207.6°C. $[\alpha]_D^{20}$ +52° (CH_2Cl_2, *c* 0.18); UV: λ_{max}^{MeOH} nm 232; IR ν_{max}^{KBr} cm^{-1} 3413, 2927, 1696, 981, 918, 898, 863 (intensity 918 < 898, 25R spiroketal); FAB-MS: *m/z* (rel. int.) 909 $[M+Na]^+$ (12), 887 $[M+H]^+$ (10), 755 $[(M+H)\text{-}132]^+$ (10), 593 $[(M+H)\text{-}132\text{-}162]^+$ (17), 431 $[(M+H)\text{-}132\text{-}324]^+$ (100). ^{13}C and ^1H NMR Tables 1 and 2.

(25*R*)-5β-spirostan-3β-ol 3-*O*-{β-D-glucopyranosyl-(1 → 2)-[α-L-arabinopyranosyl-(1 → 6)]-β-D-glucopyranoside} (**2**): colorless flakes (MeOH); m.p. 266–267.3°C; $[\alpha]_D^{20} = +57°$ (CH_2 Cl_2, *c* 0.05); UV: λ_{max}^{MeOH} nm 230; IR ν_{max}^{KBr} cm^{-1} 3434, 2958, 920, 900, 787 (intensity 920 < 900, 25R spiroketal). FAB-MS: *m/z* (rel. int.) 896 $[M+Na]^+$ (20), 741 $[(M+Na)\text{-}132]^+$ (12), 711 $[(M+Na)\text{-}162]^+$ (15), 579, $[(M+Na)\text{-}162\text{-}132]^+$ (10), 418 $[(M+Na)\text{-}324\text{-}132]^+$ (25). ^{13}C and ^1H NMR Tables 1 and 2.

26-*O*-β-D-glucopyranosyl-22α-methoxy-(25*R*)-furostan-3β,26-diol 3-*O*-{β-D-glucopyranosyl-(1 → 2)-[β-D-glucopyranoside]} (**3**) fine needles (acetone); m.p.: uncorr. 160.2–161.4°C; $[\alpha]_D^{20} = -29°$ (MeOH, *c* 0.14); UV λ_{max}^{MeOH} nm 230. IR ν_{max}^{KBr}: cm^{-1} 3434 (no spiroketal absorption). FAB-MS: *m/z* (rel. int.) 936 $[M+H]^+$ (5), 904 $[(M+H)\text{-MeOH}]^+$ (100), 741 $[(M+H)\text{-MeOH-}163]^+$ (20), 579 $[(M+H)\text{-MeOH-}325]^+$ (37), 430 $[(M+H)\text{-MeOH-}488]^+$ (57). ^{13}C and ^1H NMR Tables 1 and 2.

26-*O*-β-D-glucopyranosyl-(25*R*)-furostan-3β,26,22α-triol 3-*O*-{β-D-glucopyranosyl-(1 → 2)-[β-D-glucopyranoside} (**4**): 20 mg **3** were refluxed with 20 ml acetone/H_2O (7:3 v/v) for 10 h, concentrated and cooled to give a TLC homogeneous compound (**4**) as an amorphous solid; $[\alpha]_D^{20} = -24°$(CH_2Cl_2, *c* 0.12); IR ν_{max}^{KBr} cm^{-1} 3400 (OH) (no spiroketal absorption). ^{13}C and ^1H NMR did not show any methoxy signal.

3.5. Acid hydrolysis of compounds *1*, *2* and *3*

The glycosides (30 mg each) were refluxed with 20 ml 7% HCl on a steam bath for 3 h. Extraction with $CHCl_3$ yielded the aglycone. The aglycone of **1** was found to be identical with gloriogenin (comparing the ^{13}C NMR chemical shifts with the published data (Nakano et al., 1991; Agrawal et al., 1995) while the aglycone of **2** and **3** gave smilagenin (co-TLC and comparison of the ^{13}C chemical shifts with authentic sample and published data (Eggret, & Djerassi, 1975; Agrawal et al., 1985). The neutralized ($NaHCO_3$) and concentrated aqueous hydrolysates of compounds **1** and **2** contained glucose and arabinose while compound **3** gave only glucose (PC, solvent G, $R_f = 0.18$

(glucose), 0.21 (arabinose)). GC–MS (column: 5% phenyl and 95% methyl silicone on ultra 2, 0.2×46 m, column temp.: 250°C, carrier gas: He 0.8 ml/min, sample: trimethylsilyl derivatives: RR (min) 15.84, 17.54 (glucose) 10.37, 10.97 (arabinose) for compound **1** and **2** and 15.78, 17.14 (glucose) for **3**. The D-configuration of glucose and the L-configration of arabinose was determined by capillary electrophoresis as complexes with borate and cyclodextrin using authentic samples of D- and L-glucose and D- and L-arabinose.

*3.5.1. Acetylation of compound **1**, **2** and **3***

0.1 ml pyridine and 3 ml of Ac_2O was added to 20 mg of each glycoside and were allowed to stand overnight. Work up as usual gave peracetates of compound **1**, **2** and **3**.

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