New Steroidal Glycosides from *Balanites aegyptiaca*

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Five new steroidal glycosides were isolated from the roots of *Balanites aegyptiaca*, a widely used African medicinal plant. On the basis of spectroscopic and chemical evidence, their structures were determined as (3*β*,12*α*,14*β*,16*β*)-12-hydroxycholest-5-ene-3,16-diyl bis(*β*-D-glucopyranoside) (**1**), (3*β*,20*S*,22*R*,25*R*)- and (3*β*,20*S*,22*R*,25*S*)-26-(*β*-D-glucopyranosyloxy)-22-methoxyfurost-5-en-3-yl *β*-D-xylopyranosyl-(1 → 3)-*β*-D-glucopyranosyl-(1 → 4)[*α*-L-rhamnopyranosyl-(1 → 2)]-*β*-D-glucopyranoside (**2** and **3**, resp.), and (3*β*,20*S*,22*R*,25*R*)- and (3*β*,20*S*,22*R*,25*S*)-spirost-5-en-3-yl *β*-D-xylopyranosyl-(1 → 3)-*β*-D-glucopyranosyl-(1 → 4)[*α*-L-rhamnopyranosyl-(1 → 2)]-*β*-D-glucopyranoside (**4** and **5**, resp.)

Introduction. – *Balanites aegyptiaca* (L.) DEL. is an economical and medicinal plant commonly used in many regions of Africa [1]. It grows in most arid to sub-humid areas North of Zimbabwe and throughout the Sahel [2][3]. *B. aegyptiaca* is widespread in Northern Kordofan and throughout Northern and Central Sudan. This small to medium-sized tree grows up to 10 m in height. Because of its egg-shaped fruits resembling ripe yellow dates, it is called desert date. In Arabic, the tree is known as Heglig and the fruits as Lalob [4][5]. It is now generally accepted that the genus *Balanites* belongs to the family *Zygophyllaceae* [6] although it has been earlier classified by some taxonomists as a member of the families *Balanitaceae* or *Simaroubaceae* [7]. All parts of the tree have medicinal properties. The fruit is used against stomach pain, as an anthelmintic, and as an oral antidiabetic, while the seed extract is used against bilharzia [1][8][9]. The root is used in the treatment of abdominal pains and asthma, as a purgative, and as an anthelmintic [10]. The bark is a fish poison and is employed as a detergent, as a remedy for malaria and syphilis, and also as an antijaundice agent. The leaf, which has once been regarded as an effective medicine against sleeping sickness, is also employed for wound healing. More recently, the molluscicidal properties of the fruit and bark have been recognized and subsequently recommended as a means for the control of the freshwater snails that act as intermediary hosts of Bilharzia [11]. The molluscicidal properties of *B. aegyptiaca* are attributable to steroidal glycosides [12][13]. Later studies confirmed the presence of steroidal saponins, which mostly derive from diosgenin and yamogenin, in all plant parts [10]. In addition, fatty acids, coumarins, and flavonol glycosides were reported.

These earlier findings prompted us to carry out a phytochemical investigation of the constituents of the root of *Balanites aegyptiaca*, and we here report on the isolation and structure elucidation of five new steroidal glycosides.

Results and Discussion. – The MeOH extract of the roots of *Balanites aegyptiaca* was defatted with petroleum ether and fractionated by a combination of silica gel chromatography, gel filtration on *Sephadex LH-20*, and reversed-phase HPLC to yield compounds **1**–**5**.

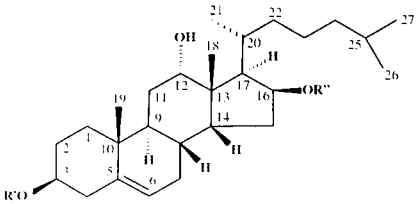
Compound **1** was obtained as a pale-green amorphous powder. Its molecular formula was determined as $C_{39}H_{66}O_{13}$ by MALDI-MS showing an ion at *m*/*z* 747 ($[M - H_2O + Na]^+$). On the basis of spectral evidence, compound **1** was identified as (3*β*,12*α*,14*β*,16*β*)-12-hydroxycholest-5-ene-3,16-diyl bis(*β*-D-glucopyranoside).

The ^{13}C-NMR and HSQC spectra of **1** showed the presence of 39 C-atoms *i.e.*, of 5 Me, 12 CH_2, 18 CH, and 4 quartenary C-atoms. The peaks at δ 141.5 and 122.0 were consistent with the presence of 2 olefinic C-atoms. In the ^{1}H-NMR spectrum, two anomeric proton signals are present at δ 4.94 and 4.70, with $J = 8.0$ and 7.7 Hz, respectively, indicating axial orientation of H–C(1) and H–C(2) in the two sugar units, which were identified by their NMR spectra and capillary electrophoreses (CE) experiments as D-glucopyranoses.

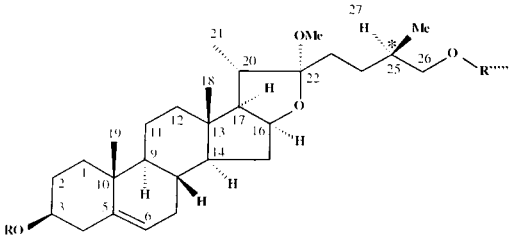
The ^{13}C-NMR chemical shifts of the aglycone of **1** corresponded to the C-resonances of cholesterol (=(3*β*)-cholest-5-en-3-ol), with the exception of downfield shifts for C(12) (δ 80.5), C(16) (δ 82.3), C(13) (δ 46.4), and C(15) (δ 36.9) and an upfield shift for C(14) (δ 47.1) (*Table 1*). The differences in chemical shifts of C(12) and C(16) could be attributed to the presence of the OH group and sugar units at these positions. The ^{13}C-NMR shifts of C(13), C(14), and C(15) indicated a *cis*-fusion of rings C and D. The *β*-configuration of Me(18) and H–C(14) was confirmed by an ROE experiment, where a cross-peak between these two signals was observed. This would not be expected for a *trans*-fusion of rings C and D. The ROE experiment also supported the equatorial orientation of H–C(12), while H–C(3) and H–C(16) were found to be in axial positions. Fragment ions at *m*/*z* 656 ($[M - C_6H_{13}]^-$) and 520 ($[M - Glc - 43]^-$) were consistent with the partial loss of the side chain and of a hexosyl moiety. The fragment at *m*/*z* 384 was attributable to the mass of the aglycone [aglycone + H]$^+$. The attachment of the sugar units to the aglycone was determined with an HMBC correlation experiment. Long-range correlations were observed between anomeric proton H–C(1′) (δ 4.94) and C(3) (δ 78.7) and between the anomeric proton H–C(1″) (δ 4.70) and C(16) (δ 82.3) (*Tables 1* and *2*).

Compounds **2** and **3** were obtained as a 1:1 mixture. A positive assay with *Ehrlich* reagent suggested that they were furostanol saponins [11]. Acidic hydrolysis of these compounds led to the formation of the two expected spirostenes **6**/**7**, which were separated by HPLC and identified on the basis of their NMR data (*Table 3*) as diosgenin (=(3*β*,20*S*,22*R*,25*R*)-spirost-5-en-3-ol) and yamogenin (=(3*β*,20*S*,22*R*,25*S*)-spirost-5-en-3-ol). The ^{1}H- and ^{13}C-NMR chemical shifts of the side chain (C(22) to C(26)) of **2** were in excellent agreement with the data previously obtained by *Debella et al.* [14] for a 26-(*β*-D-glucopyranosyloxy)-22-methoxyfurostan-3-ol saponin with (*R*)-configuration at position C(25). Therefore, **2** was assigned the structure of (3*β*,20*S*,22*R*,25*R*)-26-*β*-D-glucopyranosyloxy)-22-methoxyfurost-5-en-3-yl *β*-D-xylopyranosyl-(1 → 3)-*β*-D-glucopyranosyl-(1 → 4)[*α*-L-rhamnopyranosyl-(1 → 2)]-*β*-D-glucopyranoside. Consequently, the structure of **3** was that of the (3*β*,20*S*,22*R*,25*S*)-isomer. The MeO group at C(22) in compounds **2** and **3** is very likely due to the extraction of the plant material with MeOH according to *Debella et al.* [14].

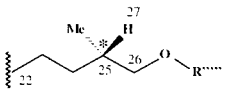
In the ^{13}C-NMR spectrum of **2**/**3** the signal at δ 112.9 ppm was assigned to the acetalic quartenary C-atom which is characteristic of a furostane skeleton possessing an OH or MeO group at C(22) in the 26-*O*-glycosidic form [15]. A molecular formula of $C_{56}H_{92}O_{27}$ determined by MALDI and ESI-MS was not consistent with the NMR data, the latter clearly showing the presence of an additional MeO group bound to C(22). Therefore, the actual molecular formula has to be $C_{57}H_{94}O_{28}$, the observed pseudomolecular ion peak at *m*/*z* 1179 being the result of the loss of the MeO group ($[M - MeOH + H]^+$). Furthermore, NMR data clearly revealed the presence of hexoses (glucose), deoxyhexose (rhamnose), and pentose (xylose) in the ratio of 3:1:1, respectively. The *β*-configuration of the D-glucopyranosyl and D-xylopyranosyl units was established by the



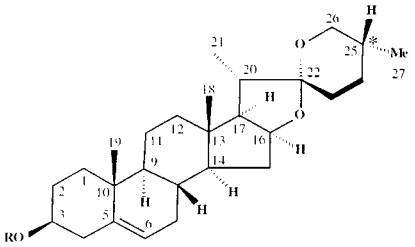
1 R′=R″=β-D-glucopyranosyl (Glcp)



2 R=Xylp-(1→3)-Glcp-(1→4)[Rhap-(1→2)]-Glcp-, R‴″=Glcp

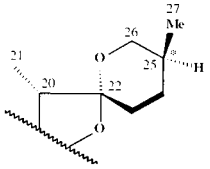


3 R=Xylp-(1→3)-Glcp-(1→4)[Rhap-(1→2)]-Glcp-, R‴″=Glcp



4 R=Xylp-(1→3)-Glcp-(1→4)[Rhap-(1→2)]-Glcp-

6 R=H



5 R=Xylp-(1→3)-Glcp-(1→4)[Rhap-(1→2)]-Glcp-

7 R=H

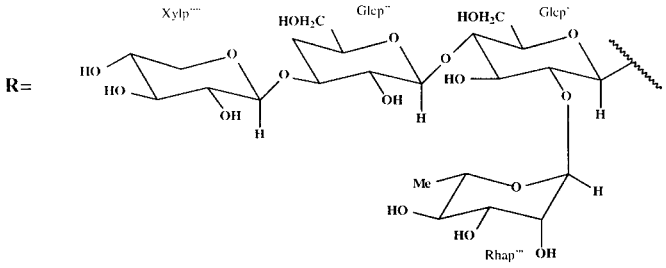


Table 1. *^{1}H- and ^{13}C-NMR Chemical Shifts δ* [ppm] *of the Aglycon Part of Compounds* **1**–**5** *in (D_5) Pyridine at 40°*. At 600 (^{1}H) and 150 or 100 MHz (^{13}C); $SiMe_4$ as internal standard.

	1		**2**		**3**		**4**		**5**	
	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)
CH_2(1)	37.4	1.83, 1.24	37.7	1.75, 1.00	37.7	1.75, 1.00	37.4	1.76, 0.99	37.4	1.76, 0.99
CH_2(2)	30.5	1.98, 1.64	30.3	2.10, 1.85	30.3	2.10, 1.85	29.7	2.08, 1.85	29.7	2.08, 1.85
H–C(3)	78.7	3.78	78.5	3.87	78.5	3.87	78.3	3.85	78.3	3.85
CH_2(4)	39.7	2.64, 2.40	39.2	2.75, 2.68	39.2	2.75, 2.68	38.5	2.74, 2.68	38.5	2.74, 2.68
C(5)	141.5		141.1		141.1		141.8		141.8	
H–C(6)	122.0	5.25	122.0	5.32	122.0	5.32	121.8	5.32	121.8	5.32
CH_2(7)	32.3	1.78, 1.49	32.4	1.91, 1.51	32.4	1.91, 1.51	31.8	1.89, 1.49	31.8	1.89, 1.49
H–C(8)	32.1	1.45	31.9	1.56	31.9	1.56	31.3	1.58	31.3	1.58
H–C(9)	44.4	1.78	50.7	0.90	50.7	0.90	50.5	0.91	50.5	0.91
C(10)	37.0		37.4		37.4		37.4		37.4	
CH_2(11)	25.5	2.91, 1.58	21.3	1.42, 1.42	21.3	1.42, 1.42	20.7	1.44, 1.44	20.7	1.44, 1.44
H–C(12) or CH_2(12)	80.5	5.20	40.0	1.70, 1.10	40.0	1.70, 1.10	40.1	1.71, 1.11	40.1	1.71, 1.11
C(13)	46.4		40.2		40.2		40.3		40.3	
H–C(14)	47.1	1.75	56.8	1.04	56.8	1.04	56.2	1.07	56.2	1.07
CH_2(15)	36.9	2.37, 1.74	32.2	1.98, 1.41	32.2	1.98, 1.41	31.6	2.02, 1.44	31.6	2.02, 1.44
H–C(16)	82.3	4.29	81.5	4.45	81.5	4.45	81.3	4.54	81.3	4.51
H–C(17)	54.0	2.28	64.4	1.76	64.4	1.76	62.5	1.80	62.5	1.80
Me(18)	14.6	1.04	16.4	0.83	16.4	0.83	15.9	0.83	15.9	0.83
Me(19)	19.6	0.91	19.5	1.04	19.5	1.04	18.9	1.05	18.9	1.05
H–C(20)	30.8	2.32	40.8	2.23	40.8	2.23	41.5	1.96	42.0	1.91
Me(21)	17.6	1.49	16.3	1.18	16.3	1.18	14.5	1.13	14.5	1.13
CH_2(22) or C(22)	37.3	1.82, 1.26	112.9		112.9		108.9		109.4	
CH_2(23)	25.2	1.58, 1.53	31.1	1.92, 1.84	30.9	1.98, 1.77	31.5	1.70, 1.67	26.0	1.90, 1.45
CH_2(24)	40.3	1.34, 1.23	28.3	1.70, 1.42	28.3	1.78, 1.36	29.0	1.58, 1.58	25.8	2.14, 1.37
H–C(25)	28.4	1.55	34.5	1.88	34.5	1.89	30.2	1.59	27.4	1.60
Me(26) or CH_2(26)	23.2	0.87[a])	75.2	4.06, 3.53	75.4	3.93, 3.60	66.7	3.58, 3.50	65.3	4.06, 3.35
Me(27)	23.1	0.86[a])	17.6	1.04	17.6	1.02	16.8	0.72	16.1	1.09
MeO–C(22)			47.6	3.27	47.6	3.27				

[a]) Assignments are interchangeable.

homonuclear coupling constants (6.1, 7.6, 8.1, and 7.6 Hz) of the anomeric protons [13], whereas the α-configuration of the L-rhamnopyranosyl unit was deduced from the chemical shift of C(5) (δ 69.5) [16] (*Table 2*). The terminal sugar units were identified as one β-D-glucopyranosyl, one α-L-rhamnopyranosyl, and one β-D-xylopyranosyl residues. This was supported by a fragment ion at *m/z* 1033 ($[M - MeOH - 146 + H]^+$) corresponding to the loss of a deoxyhexosyl unit (rhamnose). The peak at *m/z* 577 ($[M - MeOH - 602 + H]^+$) arose from the loss of MeOH and the saccharide moiety bound to C(3). The full sequential assignment of the four sugar units within the carbohydrate moiety and the attachment of the fifth to C(26) was established by HMBC and NOESY experiments.

For the aglycon, the HSQC and HSCQ-TOCSY spectra showed two sets of signals for the resonances of positions C(22) to C(27). The most significant difference was observed for the proton resonances of CH_2(26) suggesting the presence of two epimers resulting from different configurations at C(25).

Compounds **4** and **5**, also obtained as a mixture, showed an absorption in their IR spectrum characteristic for compounds of the spirostanol type. Detailed analysis of 1D- and 2D-NMR spectra suggested a mixture of tetraglycosidic spirostenes with different configurations at C(25). The aglycons were identified as diosgenin (**6**; (25*R*)) and yamogenin (**7**; (25*S*)) on the basis of their ^{13}C-NMR chemical shifts by comparison with reported data [15]. These results were confirmed by acid hydrolysis of **4** and **5** to yield

Table 2. *^{1}H- and ^{13}C-NMR Chemical Shifts* δ [ppm] *of the Carbohydrate Moieties of Compounds* **1**–**5** *in (D_5) Pyridine at 40°*. At 600 (^{1}H) and 150 or 100 MHz (^{13}C); $SiMe_4$ as internal standard; *J* in Hz.

		1		**2/3**		**4/5**	
		δ(C)	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)
Glcp′[a])	H–C(1′)	102.9	4.94 (J = 8.0)	100.3	4.90 (J = 6.1)	100.3	4.90 (J = 8.0)
	H–C(2′)	75.5	3.98	81.8	4.15	81.7	4.16
	H–C(3′)	78.7	4.24	76.4	3.80	76.5	3.80
	H–C(4′)	72.1	4.20	77.6	4.15	77.5	4.16
	H–C(5′)	78.6	3.93	77.6	4.15	77.4	4.16
	CH_2(6′)	63.2	4.49, 4.37	61.9	4.45, 4.40	61.7	4.44, 4.40
Glcp″[a])	H–C(1″)	107.3	4.70 (J = 7.7)	104.9	5.06 (J = 7.6)	104.7	5.06 (J = 7.4)
	H–C(2″)	75.9	3.95	74.1	4.00	74.1	4.02
	H–C(3″)	78.8	4.13	87.5	4.12	87.5	4.12
	H–C(4″)	72.1	4.20	69.2	4.11	69.2	4.12
	H–C(5″)	78.4	3.86	78.1	3.88	78.1	3.89
	CH_2(6″)	63.3	4.47, 4.36	61.9	4.36, 4.22	61.8	4.36, 4.22
Rhap‴[b])	H–C(1‴)			102.0	6.15	101.9	6.16
	H–C(2‴)			72.4	4.67	72.5	4.68
	H–C(3‴)			72.9	4.50	72.8	4.52
	H–C(4‴)			74.2	4.26	74.3	4.27
	H–C(5‴)			69.5	4.87	69.5	4.87
	Me(6‴)			18.8	1.72	18.1	1.73
Xylp⁗[c])	H–C(1⁗)			106.5	5.18 (J = 7.6)	106.3	5.18 (J = 7.3)
	H–C(2⁗)			75.4	3.98	75.2	4.00
	H–C(3⁗)			78.2	4.10	78.2	4.10
	H–C(4⁗)			71.1	4.11	71.0	4.14
	CH_2(5⁗)			67.5	4.28, 3.66	67.5	4.28, 3.68
Glcp⁗′[a])	H–C(1⁗′)			105.5	4.80 (J = 8.1)		
	H–C(2⁗′)			75.4	3.98		
	H–C(3⁗′)			78.8	4.18		
	H–C(4⁗′)			72.1	3.91		
	H–C(5⁗′)			78.6	4.16		
	CH_2(6⁗′)			63.2	4.51, 4.34		

[a]) Glcp = β-D-Glucopyranosyl. [b]) Rhap = α-L-Rhamnopyranosyl. [c]) Xylp = β-D-Xylopyranosyl.

the aforementioned aglycons. In addition, NMR spectroscopy and capillary electrophoresis showed the presence of D-glucose, L-rhamnose, and D-xylose in the ratio of 2:1:1, respectively. The structure and point of attachment of the tetrasaccharide moiety was found to be the same as those of **2** and **3**, based on the HMBC and ROESY spectra. The mass spectra confirmed the structures of **4** and **5** as (3β,20*S*,22*R*,25*R*)- and (3β,20*S*,22*R*,25*S*)-spirost-5-en-3-yl β-D-xylopyranosyl-(1 → 3)-β-D-glucopyranosyl-(1 → 4)[α-L-rhamnopyranosyl-(1 → 2)]-β-D-glucopyranoside, respectively.

The MALDI-MS (positive mode) of **4**/**5** exhibited a peak at *m*/*z* 1017 ($[M+H]^+$) consistent with the molecular formula $C_{50}H_{80}O_{21}$. Other significant peaks (ESI- or MALDI-MS) were at *m*/*z* 997 ($[M+CF_3COOH-132]^-$) corresponding to a loss of a pentosyl unit (xylose), 871 ($[M+H-146]^-$) arising from a

Table 3. *^{1}H- and ^{13}C-NMR Chemical Shifts* δ [ppm] *of Compounds Obtained by Acidic Hydrolysis of* **2** *and* **3** *in Pyridine at 40°*. At 600 (^{1}H) and 150 or 100 MHz (^{13}C); $SiMe_4$ as internal standard.

	6 (Diosgenin)		**7** (Yamogenin)	
	δ(C)	δ(H)	δ(C)	δ(H)
CH_2(1)	37.9	1.82, 1.12	38.0	1.82, 1.12
CH_2(2)	32.9	2.08, 1.78	32.8	2.07, 1.77
H–C(3)	71.6	3.82	71.3	3.82
CH_2(4)	43.7	2.60, 2.60	43.6	2.59, 2.59
C(5)	142.3		142.4	
H–C(6)	121.0	5.39	121.4	5.39
CH_2(7)	32.5	1.94, 1.54	32.5	1.95, 1.53
H–C(8)	31.8	1.63	31.9	1.63
H–C(9)	50.7	0.98	50.7	0.97
C(10)	37.1		37.1	
CH_2(11)	21.3	1.52, 1.48	21.5	1.51, 1.47
CH_2(12)	40.3	1.75, 1.14	40.3	1.74, 1.15
C(13)	40.5		40.5	
H–C(14)	57.0	1.10	56.9	1.09
CH_2(15)	32.4	2.03, 1.44	32.4	2.01, 1.43
H–C(16)	81.7	4.55	81.4	4.51
H–C(17)	63.3	1.83	63.3	1.80
Me(18)	16.6	0.87	16.6	0.85
Me(19)	19.6	1.06	19.7	1.05
H–C(20)	42.7	1.98	42.7	1.91
Me(21)	15.2	1.14	15.0	1.15
C(22)	109.5		109.9	
CH_2(23)	31.8	1.71, 1.67	26.6	1.90, 1.44
CH_2(24)	29.6	1.58, 1.58	26.3	2.14, 1.36
H–C(25)	30.7	1.60	27.7	1.59
CH_2(26)	67.1	3.58, 3.50	65.3	4.05, 3.35
Me(27)	17.4	0.71	16.5	1.08

loss of a deoxyhexosyl unit (rhamnose), 603 ($[Rha - Glc - Glc - Xyl]^-$) for the tetraglycosidic side chain, and 415 ($[aglycone + H]^+$).

Experimental Part

1. *General*. TLC: normal silica gel; for visualization, *Camag-UV* monitor. Capillary electrophoresis: *Prince-Technologies* instrument (Emmen, Netherlands) equipped with an on-column UV detector (*Bischoff*, Germany). Anal. HPLC: *LiChrosorb RP-18* column (5 µm, 4.5 × 125 mm; *Merck*, Darmstadt); *Hewlett-Packard 1100* and workstation; UV detection at 205 nm; eluent flow 1 ml/min; MeOH/H_2O 85 :15 for **6** and **7**. Semiprep. HPLC: *LiChrosorb RP-18* column (7 µm, 2.5 × 31.0 cm; *Merck*, Darmstadt), *Series III* pump (*Pharma-Tech. Research Corp.*, Baltimore, USA) and UV detector *K-2500* (*Knauer*, Berlin, Germany); eluent flow 10 ml/min. Melting points: open capillaries; electrothermal melting-point apparatus. UV Spectra: *Shimadzu UV-160A* spectrometer; $\lambda_{max}(\varepsilon)$ in nm. IR Spectra: *Perkin-Elmer 2000FT-IR* spectrometer; in cm^{-1}. NMR: ^{1}H and two-dimensional NMR experiments with *Varian Unity-Inova-600* and *-400* spectrometer according to [14]; δ in ppm, J in Hz. Mass spectra: EI at 70 eV and LSI at 5 kV neg.; ESI platform *LCZ* (*Micromass*), with H_2O/MeCN 1:1 containing 0.1% CF_3COOH as solvent for loop injection (cone voltage 40 eV); for MALDI, *VG* analytical instrument (*Micromass*), with thioglycerol and magic bullet as matrix, in *m/z* (rel. int.).

2. *Plant Material*. The plant material used in this study was collected in November, 1999, in the State of Khartoum, Sudan. This plant was botanically authenticated by taxonomists of the Department of Botany, Faculty of Science, University of Khartoum, and the Medicinal and Aromatic Herb Research Institute, National

Council for Research. Voucher specimens were deposited at the Department of Botany, University of Khartoum and at the Institute of Pharmaceutical Chemistry and Pharmaceutical Technology, Karl-Franzens University, Graz.

3. *Extraction and Isolation.* The coarsely powdered air-dried roots of *Balanites aegyptiaca* were extracted with MeOH and washed with petroleum ether. The defatted extract was then fractionated with BuOH. The extract was subjected to vacuum liquid chromatography (VLC; silica gel, gradient MeOH/$CHCl_3$): subfractions *A*–*E*. The subfraction *B* was further subjected to column chromatography (CC; gradient MeOH/$CHCl_3$), followed by CC (*Sephadex LH-20*, MeOH) and reversed-phase chromatography (*RP-18*): **1**. The subfraction *C* was submitted to CC (silica gel), followed by CC (*Sephadex LH-20*, MeOH) and multiple developments on prep. TLC to give compounds **4** and **5**. The subfraction *D* gave compounds **2** and **3**.

*(3β,12α,14β,16β)-12-Hydroxycholest-5-ene-3,16-diyl Bis(β-*D*-glucopyranoside)* (**1**): Pale-green amorphous powder. M.p. 271–273°. TLC (silica gel, $C_6H_8O_2$/MeOH/H_2O 10:2:1): R_f 0.68; UV fluorescent; anisaldehyde/sulfuric acid (+) yellow. $[\alpha]_D^{21} = +13.3$ ($c = 1.13$, MeOH): UV (MeOH); 213 (1.319). IR (KBr): 3421.6, 1583.1, 1418.4, 957.8. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. MALDI- and ESI-MS: 747 (28, $[M - H_2O + Na]^+$), 656 (9, $[M - C_6H_{13}]^-$), 520 (31, $[M - Glc - 43]^-$).

*(3β,20*S*,22*R*,25*R*)- and (3β,20*S*,22*R*,25*S*)-26-(β-*D*-Glucopyranosyloxy)-22-methoxyfurost-5-en-3-yl β-*D*-Xylopyranosyl-(1→3)-β-*D*-glucopyranosyl-(1→4)-rhamnopyranosyl-(1→2)]-β-*D*-glucopyranoside* (**2** and **3**, resp.): Ratio **2**/**3** 1:1. TLC (silica gel, $C_6H_8O_2$/MeOH/H_2O 10:2:1: R_f 0.44; UV nonfluorescent; anisaldehyde/sulfuric acid (+) yellow; *Ehrlich* reagent (+) purple. $[\alpha]_D^{21} = -1.80$ ($c = 1.67$, MeOH). UV (MeOH): 216 (1.480). IR (KBr): 3386.7, 2932.9, 1069.6, 895.2, 861.0. ^{1}H- and ^{13}C-NMR: *Tables 2* and *3*. MALDI- and ESI-MS: 1179 (53, $[M - MeOH + H]^+$), 1033 (2, $[M - MeOH - 146 + H]^+$), 577 (5, $[M - MeOH - 602 + H]^+$).

*(3β,20*S*,22*R*,25*R*)- and (3β,20*S*,22*R*,25*S*)-Spirost-5-en-3-yl (β-*D*-Xylopyranosyl-(1→3)-β-*D*-glucopyranosyl-(1→4)[α-*L*-rhamnopyranosyl-(1→2)]-β-*D*-glucopyranoside* (**4** and **5**, resp.). Ratio **4**/**5** 2:3. White amorphous powder. M.p. 263–265°. TLC (silica gel, $C_6H_8O_2$/MeOH/H_2O 10:2:1): R_f 0.66; UV fluorescent; anisaldehyde/sulfuric acid (+) yellow; *Ehrlich* reagent (+) purple. $[\alpha]_D^{20} = -1.77$ ($c = 1.70$, MeOH). UV (MeOH): 256 (1.191), 216 (1.429). IR (KBr): 3422.9, 2933.0, 1047.7, 984.2, 919.7, 898.4. ^{1}H- and ^{13}C-NMR: *Tables 2* and *3*. MALDI- and ESI-MS: 1129 (100, $[M + CF_3COOH]^-$), 1017 (42, $[M + H]^+$), 997 (3, $[M + CF_3COOH - 132]^-$), 871 (4.8, $[M + H - 146]^-$), 603 (18, $[Rha - Glc - Glc - Xyl]^-$.

4. *Acid Hydrolysis.* The sample (10 mg) was refluxed in 2N CF_3COOH (10 ml) for 1 h. The mixture was diluted with H_2O (10 ml) and extracted with $CHCl_3$. The upper layer was evaporated. The residue was redissolved in H_2O and then evaporated to remove traces of CF_3COOH. The identification of the aglycones isolated from the $CHCl_3$ layer was based on ^{13}C-NMR analysis and comparison with reported data.

After acid hydrolysis of the mixture **2**/**3**, separation of the aglycons was achieved by semi-prep. HPLC (MeOH/H_2O 85:15) to give diosgenin (**6**) and yamogenin (**7**). ^{1}H- and ^{13}C-NMR: *Table 3*.

5. *Capillary Electrophoresis.* To the residue (*ca.* 5 mg) of the lyophilized aq. phase of hydrolysis, 0.5M 5-aminonaphthalene-2-sulfonic acid (50 µl) was added, and the mixture was heated at 90° for 10 min. Then, aq. $NaBH_4$ soln. (10 µl, 0.3 mg/ml) was added, and the mixture was heated for 60 min at 90°. The samples were then diluted with H_2O to concentrations of 1 mM. For analysis, 1 µl was used. Similar reactions were carried out with authentic sugar samples of D- and L-glucose, L-rhamnose, and D- and L-xylose for comparison. To optimize the resolution, 5 mM cylodextrin and 10 mM borate were used [17].

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