Oleanane Saponins from *Stylosanthes erecta*

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Received February 13, 2007

Five new oleanane saponins (**1**−**5**) together with four know flavonol glycosides were isolated from the aerial parts of *Stylosanthes erecta*. Their structures were elucidated by 1D and 2D NMR experiments including 1D-TOCSY, DQF-COSY, ROESY, HSQC, and HMBC spectroscopy, as well as ESIMS analysis. The aglycone moieties of **1**−**4** were assigned as new oleanane derivatives.

Stylosanthes erecta P. Beauv. (synonym *Stylosanthes guineensis* Schum. et Thonn.) (Fabaceae) is an herbal plant, 20−40 cm in height, occurring mainly in the West African countries Mali, Senegal, Nigeria, and Congo.[1] The decoction of the aerial parts is used in Malian folk medicine, where it is known as "segoufali", for the treatment of urinary and intestinal diseases and particularly against schistosomiasis.[2,3] A study on the characterization of cysteine proteases in Malian medicinal plants revealed that *S. erecta* whole plant acetone-defatted crude extract showed a high papain-like activity,[4] but, to date, no comprehensive phytochemical study on this plant has been done. As part of an ongoing investigation on Malian medicinal plants aimed at Traditional Improved Drugs,[5,6] we have carried out a chemical study on the aerial parts of *S. erecta*, resulting in the isolation and structural characterization of five new oleanane saponins on the basis of extensive spectroscopic and spectrometric analysis, including 2D NMR and ESIMS spectra. Four known flavonol glycosides were also isolated and characterized.

Results and Discussion

The methanol extract of the aerial parts of *S. erecta* was subjected to Sephadex LH-20 column chromatography, followed by RP-HPLC, to afford five new oleanane saponins (**1**−**5**) and four known flavonol glycosides. Compound **1** was assigned a molecular formula of $C_{48}H_{76}O_{18}$, as determined by positive HRESIMS (*m*/*z* 963.5023, $[M + Na]^+$). The ESIMS of **1** showed an $[M - H]^-$ ion at *m*/*z* 939 and prominent fragments at *m*/*z* 921 $[M - H - 18]^-$, 877 $[M - H - 18 - 44]^-$, 731 $[M - H - 18 - 44 - 146]^-$, and 569 $[M - H - 18 - 44 - 146 - 162]^-$, due to the loss of H_2O and CO_2 fragments and one deoxyhexose and one hexose unit, respectively. Compound **1** displayed 48 carbon resonances in its ^{13}C NMR spectrum (Tables 1 and 2), of which 30 could be assigned to the resonances of the aglycone and 18 to the sugar moiety, including three anomeric carbons (δ 102.0, 102.1, and 105.5) and one carboxylic function at δ 177.0. The ^{13}C NMR spectrum of the aglycone portion exhibited eight tertiary methyl resonances at δ 16.3, 17.0, 17.7, 20.3, 26.5, 28.5, 28.7, and 31.9, two sp^2-hybridized carbons at δ 125.7 and 141.8, resonances for two hydroxymethine groups (δ 68.0 and 91.0), and one carbonyl function (δ 219.8). These data, when coupled with information from the ^1H NMR (Table 1) [eight methyl singlets at δ 0.90, 0.94, 1.02, 1.04, 1.07, 1.12, 1.17, and 1.18, one olefinic proton at δ 5.55, and a pair of oxymethyne protons at δ 3.20 and 4.18], indicated that the aglycone of **1** is based on a 3,22-dihydroxyolean-12-ene skeleton.[7] The orientation of the hydroxy groups was assigned as 3β and 22α mainly on the basis of ^1H NMR coupling constants and by comparison with those reported for related compounds.[8] The 22α-OH orientation was also confirmed by a ROESY experiment showing a cross-peak between H-22ax (δ 4.18) and axial Me-30ax (δ 1.04). Assignments of all chemical shifts of protons and carbons of the aglycone portion were ascertained from a combination of 1D-TOCSY, DQF-COSY, and HSQC analysis. The substitution sites on the triterpene skeleton were confirmed by an HMBC experiment showing correlation peaks between δ 1.94 (H-15a) and 2.88 (H-15b) and 52.5 (C-17), 141.8 (C-13), and 219.8 (C-16), δ 1.18 (Me-28) and 42.4 (C-21), 47.8 (C-19), and 219.8 (C-16), and δ 4.18 (H-22) and 219.8 (C-16), demonstrating the 16 position of the keto group, and between δ 1.18 (Me-28) and 68.0 (C-22), δ 1.50 (H-21b) and 47.8 (C-19), 52.5 (C-17), and 68.0 (C-22), and δ 2.55 (H-18) and 68.0 (C-22), 141.8 (C-13), and 219.8 (C-16), consistent with the presence of a hydroxy group at C-22. On the basis of this evidence, the aglycone of **1** was defined as the new 3β,22α-dihydroxyolean-12-en-16-one. The sugar portion of **1** exhibited, in the ^1H NMR spectrum (Table 2), three anomeric proton resonances (δ 4.46, d, J = 7.8 Hz; 4.92, d, J = 7.8 Hz; 5.22, d, J = 1.8 Hz) and one methyl doublet (δ 1.28, d, J = 6.5 Hz), suggesting the occurrence of one deoxyhexose unit. The structures of the oligosaccharide moieties were deduced using 1D-TOCSY and 2D NMR experiments, which indicated that one β-glucuronopyranose, one β-glucopyranose, and one α-rhamnopyranose were present (Table 2). The configurations of the sugar units were assigned after hydrolysis of **1** with 1 N HCl. The hydrolysate was trimethylsilated, and GC retention times of each sugar were compared with those of authentic sugar samples prepared in the same manner. The absence of any ^{13}C NMR glycosidation shift for the α-L-rhamnopyranosyl moiety suggested that this sugar was the terminal unit. Glycosidation shifts were observed for C-2_{glu} (δ 78.6) and C-2_{glc} (δ 79.5) (Table 2). Direct evidence for the sugar sequence and their linkage sites to the aglycone was derived from the results of the HMBC experiment that showed unequivocal correlations between resonances at δ 4.46 and 91.0 ppm (H-1_{glu}−C-3), δ 4.92 and 78.6 ppm (H-1_{glc}−C-2_{glu}), and δ 5.22 and 79.5 ppm (H-1_{rha}−C-2_{glc}). Thus, compound **1** was identified as 3-*O*-[α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl-(1→2)-β-D-glucuronopyranosyl]-3β,22α-dihydroxyolean-12-en-16-one.

Compound **2** was obtained as an amorphous powder, with the molecular formula $C_{47}H_{74}O_{17}$, as deduced from the $[M + Na]^+$ peak at *m*/*z* 933.4794 by HRESIMS and confirmed by ^{13}C NMR and ^{13}C DEPT data. The ESIMS of compound **2** showed a prominent fragment at *m*/*z* 933 $[M + Na]^+$ and a fragmentation pattern similar to that of **1**. The spectroscopic data of the aglycone moiety of **2** were identical to those of **1**. The proton coupling network within each sugar residue was established, using a combination of 1D-

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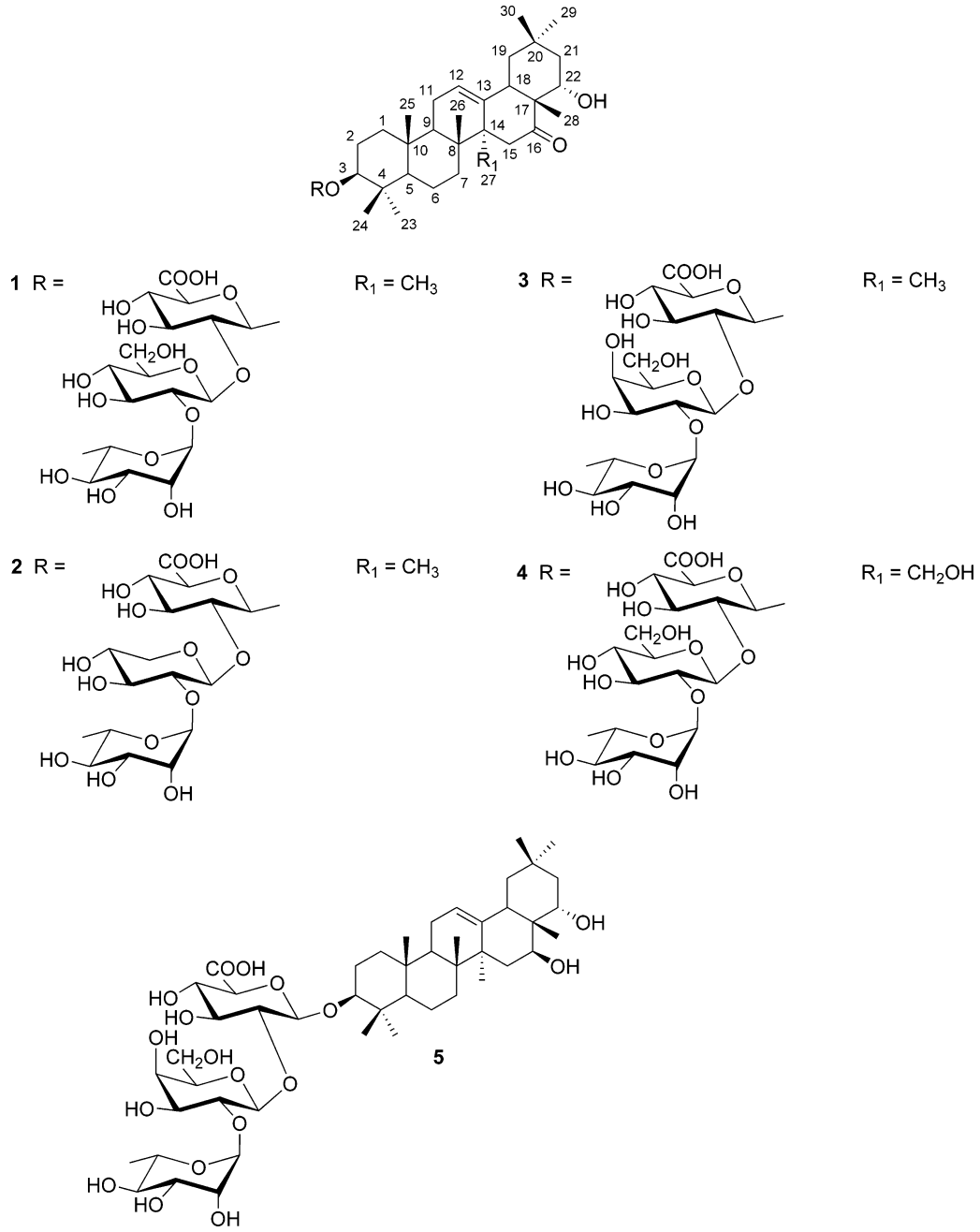
10.1021/np0700671 CCC: $37.00
Published on Web 05/11/2007

Chart 1



TOCSY, DQF-COSY, and HSQC experiments. Once again, direct evidence for the sugar sequence and the linkage sites was derived from the HSQC and HMBC data. Comparison of NMR data of the sugar moiety (Table 2) of **2** with that of **1** indicated that **2** differed from **1** only by the presence of one xylopyranosyl unit instead of one glucopyranosyl moiety. The configuration of the sugar units was determined as reported for compound **1**. Thus, compound **2** was defined as 3-*O*-[α-L-rhamnopyranosyl-(1→2)-*β*-D-xylopyranosyl-(1→2)-*β*-D-glucuronopyranosyl]-3*β*,22α-dihydroxyolean-12-en-16-one.

Compound **3** (molecular formula $C_{48}H_{76}O_{18}$) showed a quasi-molecular ion peak at *m*/*z* 939 $[M - H]^-$ in the negative ESIMS. Furthermore, fragment ions were superimposable with those of compound **1**, suggesting that **1** and **3** were two isomers. Analysis of NMR data of compound **3** and comparison with those of **1** showed that the glucopyranosyl moiety of **1** was replaced by one galactopyranosyl unit in **3** (Table 2). The configuration of the sugar units was determined as reported for compound **1**. Therefore, the structure 3-*O*-[α-L-rhamnopyranosyl-(1→2)-*β*-D-galactopyranosyl-(1→2)-*β*-D-glucuronopyranosyl]-3*β*,22α-dihydroxyolean-12-en-16-one was assigned to compound **3**.

Compound **4** was obtained as an amorphous powder. The HRESIMS showed an accurate $[M + Na]^+$ ion at *m*/*z* 979.4835, in accordance with an empirical formula $C_{48}H_{76}O_{19}$, which was supported by the ^{13}C NMR and DEPT data. Data from the ^{13}C NMR spectrum (Table 1) suggested a triterpenoid glycoside structure. The ^{13}C NMR spectrum showed 48 resonances, of which 30 were assigned to a triterpenoid moiety and 18 to the glycosyl portion. The following NMR data suggested the structural features of the new aglycone 3*β*,22α,27-trihydroxyolean-12-en-16-one for compound **4**: seven tertiary methyl resonances (*δ* 0.92, 0.93, 1.02, 1.04, 1.14, 1.16, and 1.18), resonances at *δ* 3.29 (dd, *J* = 12.0, 3.5 Hz) and 4.16 (dd, *J* = 11.0, 4.0 Hz), ascribable, respectively, to the 3α- and 22*β*-protons on hydroxymethine carbons, one hydroxymethylene (*δ* 3.25 and 4.15, each a doublet, *J* = 12.0 Hz), and a resonance for an olefinic hydrogen at *δ* 5.57 (t, *J* = 3.5 Hz). The presence of a 14α-CH_2OH moiety accounted for elimination and fragmentation patterns observed in the mass spectrometry.[9] The

Table 1. ^{1}H and ^{13}C NMR Data of the Aglycone Moieties of Compounds **1** and **4** (CD_3OD, 600 MHz)[a]

	1		**4**	
position	δ_H	δ_C	δ_H	δ_C
1a	1.06[b]	39.8	1.08[b]	39.6
1b	1.70[b]		1.68[b]	
2a	1.74[b]	26.5	1.72[b]	26.4
2b	2.05 m		2.07 m	
3	3.20 dd (12.0, 3.5)	91.0	3.29 dd (12.0, 3.5)	92.2
4		40.5		41.0
5	0.83 br d (11.0)	57.0	0.98 br d (11.0)	57.3
6a	1.48[b]	19.5	1.50[b]	19.7
6b	1.68[b]		1.60[b]	
7a	1.36[b]	33.7	1.56[b]	33.4
7b	1.53[b]		1.97[b]	
8		41.7		40.0
9	1.57 br d (12.0)	48.1	1.57 br d (12.0)	48.0
10		37.7		38.1
11	2.04 m	24.6	2.01 m	24.0
12	5.55 t (3.5)	125.7	5.55 t (3.5)	125.5
13		141.8		140.0
14		47.8		49.5
15a	1.94 d (13.0)	43.8	1.93 d (13.0)	44.5
15b	2.88 d (13.0)		2.88 d (13.0)	
16		219.8		218.0
17		52.5		52.3
18	2.55 dd (13.0, 4.5)	50.3	2.58 dd (13.0, 4.0)	51.4
19a	1.18[b]	47.8	1.18[b]	48.0
19b	1.55[b]		1.55[b]	
20		30.5		30.4
21a	1.40[b]	42.4	1.42[b]	41.4
21b	1.50[b]		1.48[b]	
22	4.18 dd (11.0, 4.0)	68.0	4.16 dd (11.0, 4.0)	68.6
23	1.12 s	28.5	1.18 s	28.0
24	0.90 s	16.3	0.92 s	16.4
25	1.02 s	17.0	1.04 s	17.8
26	1.07 s	17.7	1.14 s	18.0
27a	1.17 s	26.5	3.25 d (12.0)	64.0
27b			4.15 d (12.0)	
28	1.18 s	20.3	1.16 s	20.0
29	0.94 s	31.9	0.93 s	32.4
30	1.04 s	28.7	1.02 s	28.6

[a] *J* values are in parentheses and reported in Hz; chemical shifts are given in ppm; assignments were confirmed by DQF-COSY, 1D-TOCSY, HSQC, and HMBC experiments. [b] Overlapped signals.

HSQC spectrum correlated the proton resonances with those of the corresponding one-bond coupled carbons, and the HMBC experiment provided unambiguous correlations between H-15−C-13, H-15−C-16, H-15−C-17; H-27a−C-13, H-27a−C-15; and Me-28−C-16, Me-28−C-18, Me-28−C-22. In addition to the aglycone resonances, the ^{13}C NMR spectrum exhibited 18 resonances attributable to the sugar portion made up of two hexopyranosyl and one deoxyhexopyranosyl unit. Analysis of the glycosidic NMR data of compound **4** demonstrated that they were superimposable with those of **1**. Thus, the structure of **4** was established as 3-*O*-[α-L-rhamnopyranosyl-(1→2)-*β*-D-glucopyranosyl-(1→2)-*β*-D-glucuronopyranosyl]-3*β*,22α,27-trihydroxyolean-12-en-16-one.

Compound **5** was assigned the molecular formula $C_{48}H_{78}O_{18}$, as shown by its HRESIMS data (*m*/*z* 965.5102 $[M + Na]^+$) in combination with the ^{13}C NMR and ^{13}C DEPT spectra. The ^{13}C and ^{13}C DEPT spectra (Experimental Section) showed 48 resonances, of which 30 were assigned to the aglycone and 18 to the glycosyl portion. It was apparent from the ^{1}H and ^{13}C NMR data of **5** that this compound is based on the same sugar as **3**, while the aglycone moieties differed. Comparing the ^{1}H and ^{13}C NMR spectra of compounds **3** and **5** (see Table 1 and Experimental Section), the presence of a hydroxymethine in **5** instead of a carbonyl carbon in **3** was evident. The aglycone of **5** was thus characterized as 3*β*,16*β*,22α-trihydroxyolean-12-ene and reported before only from the species *Lemaireocereus chichipe* (Cactaceae family).[8] Its full NMR data were not available; therefore we now report it in the Experimental Section. Finally, an unambiguous determination of the sequence and linkage sites of the aglycone and sugar moieties was obtained from HMBC correlations. The configuration of the sugar units was determined as reported for compound **1**. Thus, **5** was identified as the new 3-*O*-[α-L-rhamnopyranosyl-(1→2)-*β*-D-galactopyranosyl-(1→2)-*β*-D-glucuronopyranosyl]-3*β*,16*β*,22α-trihydroxy-olean-12-ene.

The known flavonol glycosides were identified as rutin,[10,11] kaempferol 3-rutinoside,[11] quercetin 3-*O*-*β*-D-glucopyranoside,[10,11] and quercetin 3-*O*-*β*-D-glucuronopyranoside[12] by detailed NMR and MS analyses and comparison with literature data.

The presence of oleanane-type saponins showing the presence of a methyl group at C-17, a hydroxy group on the E-ring, and a glucuronic acid residue in the sugar moiety is peculiar of plants belonging to the family Fabaceae;[13−15] the isolation of oleanane saponins **1**−**5** in *S. erecta* is thus in agreement with previous findings. However, the aglycone moieties of **1**−**3** and **4** represent new oleanane frameworks.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a Perkin-Elmer 241 polarimeter equipped with a sodium lamp (589 nm) and a 1 dm microcell. NMR experiments were performed on a Bruker DRX-600 spectrometer at 300 K. All the 2D NMR spectra were acquired in CD_3OD in the phase-sensitive mode with the transmitter set at the solvent resonance and TPPI (time proportional phase increment) used to achieve frequency discrimination in the ω_1 dimension. The standard pulse sequence and phase cycling were used for DQF-COSY, TOCSY, HSQC, HMBC, and ROESY experiments. The NMR data were processed on a Silicon Graphics Indigo2 workstation using UXNMR software. ESIMS (positive and negative mode) were obtained from an LCQ Advantage ThermoFinnigan spectrometer, equipped with Xcalibur software. HRESIMS were acquired in positive ion mode on a Q-TOF premier spectrometer equipped with a nanospray source. Column chromatography was performed over Sephadex LH-20. HPLC separations were conducted on a Shimadzu LC-8A series pumping system equipped with a Waters R401 refractive index detector and Shimadzu injector on a C_{18} μ-Bondapak column (30 cm × 7.8 mm, flow rate 2.0 mL min^{-1}). GC analyses were performed using a Dani GC 1000 instrument on a L-CP-Chirasil-Val column (0.32 mm × 25 m).

Plant Material. The aerial parts of *S. erecta* were collected in Ouélessebougou, Region de Koulokoro, Mali, in December 2002 and were identified by Prof. N'Golo Diarra of the Department Medicine Traditionelle (DMT), Bamako, Mali, where a voucher specimen (No. 0170) is deposited.

Extraction and Isolation. The dried, powdered aerial parts of *S. erecta* (530 g) were defatted with *n*-hexane and successively extracted for 48 h with $CHCl_3$, $CHCl_3$−MeOH (9:1), and MeOH, by exhaustive maceration (3 × 2 L), to give 12.5, 5.9, and 11.8 g of the respective residues. The MeOH extract was partitioned between *n*-BuOH and H_2O to afford an *n*-BuOH-soluble portion (4.4 g). The *n*-BuOH residue (3.1 g) was submitted to a Sephadex LH-20 column using MeOH as eluent to obtain eight major fractions (1−8) by TLC on silica 60 F_{254} gel-coated glass sheets with *n*-BuOH−HOAc−H_2O (60:15:25) and $CHCl_3$−MeOH−H_2O (40:9:1). Fraction 1 (170 mg) was purified by preliminary SPE followed by RP-HPLC with MeOH−H_2O (5.5:4.5) to afford pure compounds **1** (2.0 mg, t_R = 14 min) and **2** (2.4 mg, t_R = 17 min). Fraction 2 (73 mg) was chromatographed over RP-HPLC with MeOH−H_2O (4.5:5.5) to yield pure compounds **4** (2.5 mg, t_R = 13 min), **3** (1.3 mg, t_R = 17 min), **5** (1.0 mg, t_R = 19 min), **1** (2.5 mg, t_R = 21 min), and **2** (2.5 mg, t_R = 24 min). Fractions 4 (242 mg), 5 (83 mg), and 6 (74 mg) were subjected to RP-HPLC with MeOH−H_2O (2:3) to yield pure rutin (19.7 mg, t_R = 26 min) and kaempferol 3-rutinoside (4.5 mg, t_R = 45 min) from fraction 4, quercetin 3-*O*-*β*-D-glucopyranoside (5.3 mg, t_R = 15 min) from fraction 5, and quercetin 3-*O*-*β*-D-glucuronopyranoside (15.0 mg, t_R = 10 min) from fraction 6, respectively.

Compound 1: amorphous powder; $[\alpha]_D^{25}$ −13 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ϵ) 280 (4.28) nm; ^{1}H and ^{13}C NMR of the aglycone, see Table 1; ^{1}H and ^{13}C NMR of the sugar moieties, see Table 2; ESIMS *m*/*z* 939 $[M - H]^-$, 921 $[M - H - 18]^-$, 877 $[M - H - 18 - 44]^-$,

Table 2. ^{1}H and ^{13}C NMR Data for Glycosyl Moieties of Compounds **1**−**3** (CD_3OD, 600 MHz)[a]

	1		**2**		**3**	
position	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
D-Glc A 1	4.46 d (7.8)	105.5	4.41 d (7.5)	105.5	4.45 d (7.5)	105.4
2	3.74 dd (9.0, 7.8)	78.6	3.61 dd (9.0, 7.5)	78.8	3.71 dd (9.0, 7.5)	78.8
3	3.64 t (9.0)	78.8	3.60 t (9.0)	78.2	3.68 t (9.0)	77.2
4	3.43 t (9.0)	74.3	3.45 t (9.0)	73.6	3.41 t (9.0)	74.1
5	3.57 d (9.0)	76.6	3.55 d (9.0)	76.8	3.44 d (9.0)	76.7
6		177.0		176.8		177.0
D-Glc 1	4.92 d (7.8)	102.1				
2	3.42 dd (9.0, 7.8)	79.5				
3	3.48 t (9.0)	78.3				
4	3.08 t (9.0)	73.1				
5	3.28 m	78.1				
6a	3.85 dd (12.0, 3.0)	64.0				
6b	3.57 dd (12.0, 5.0)					
D-Xyl 1			4.87 d (7.5)	102.7		
2			3.43 dd (9.0, 7.5)	78.9		
3			3.53 t (9.0)	76.6		
4			3.44 m	71.4		
5a			3.87 dd (11.0, 2.5)	66.3		
5b			3.15 dd (11.0, 5.0)			
D-Gal 1					4.83 d (7.0)	102.6
2					3.64 dd (9.0, 7.0)	78.6
3					3.55 dd (9.0, 3.5)	76.5
4					3.66 m	70.9
5					3.60 m	75.4
6a					3.80 dd (11.0, 2.5)	62.9
6b					3.68 dd (11.0, 5.0)	
L-Rha 1	5.22 d (1.8)	102.0	5.21 d (1.8)	101.8	5.20 d (1.5)	101.9
2	3.95 dd (3.0, 1.8)	72.6	3.93 dd (3.0, 1.8)	71.9	3.94 dd (3.0, 1.5)	71.9
3	3.77 dd (9.0, 3.0)	72.2	3.77 dd (9.5, 3.0)	71.8	3.77 dd (9.0, 3.0)	71.9
4	3.47 t (9.0)	74.2	3.42 t (9.5)	73.8	3.44 t (9.0)	73.7
5	4.16 m	69.7	4.13 m	69.4	4.18 m	69.0
6	1.28 d (6.5)	18.5	1.27 d (6.5)	18.0	1.28 d (6.0)	18.2

[a] *J* values are in parentheses and reported in Hz; chemical shifts are given in ppm; assignments were confirmed by DQF-COSY, 1D-TOCSY, HSQC, and HMBC experiments.

731 [M − H − 18 − 44 − 146]$^-$, 569 [M − H − 18 − 44 − 146 − 162]$^-$; HRESIMS *m/z* 963.5023 [M + Na]$^+$ (calcd for $C_{48}H_{76}O_{18}Na$, 963.5032).

Compound 2: amorphous powder; $[\alpha]_D^{25}$ −22 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ϵ) 275 (4.05) nm; ^{1}H and ^{13}C NMR data of the aglycone were superimposable on those of **1**; ^{1}H and ^{13}C NMR of the sugar moieties, see Table 2; ESIMS *m/z* 933 [M + Na]$^+$, 787 [M + Na − 146]$^+$; HRESIMS *m/z* 933.4794 [M + Na]$^+$ (calcd for $C_{47}H_{74}O_{17}Na$, 933.4824).

Compound 3: amorphous powder; $[\alpha]_D^{25}$ +34 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ϵ) 281 (3.95) nm; ^{1}H and ^{13}C NMR data of the aglycone were superimposable on those of **1**; ^{1}H and ^{13}C NMR of the sugar moieties, see Table 2; ESIMS *m/z* 939 [M − H]$^-$, 921 [M − H − 18]$^-$, 877 [M − H − 18 − 44]$^-$, 731 [M − H − 18 − 44 − 146]$^-$; HRESIMS *m/z* 963.5017 [M + Na]$^+$ (calcd for $C_{48}H_{76}O_{18}Na$, 963.5032).

Compound 4: amorphous powder; $[\alpha]_D^{25}$ −16 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ϵ) 278 (3.92) nm; ^{1}H and ^{13}C NMR of the aglycone, see Table 1; ^{1}H and ^{13}C NMR of the sugar moieties were identical to those of compound **1**; ESIMS *m/z* 955 [M − H]$^-$, 937 [M − H − 18]$^-$, 925 [M − H − 30]$^-$, 893 [M − H − 18 − 44]$^-$, 747 [M − H − 18 − 44 − 146]$^-$; HRESIMS *m/z* 979.4835 [M + Na]$^+$ (calcd for $C_{48}H_{76}O_{19}Na$, 979.4878).

Compound 5: amorphous powder; $[\alpha]_D^{25}$ +15 (*c* 0.05, MeOH); UV (MeOH) λ_{max} (log ϵ) 280 (4.21) nm; ^{1}H NMR data of the aglycone (CD_3OD, 600 MHz) δ 0.86 (3H, s, Me-24), 0.92 (3H, s, Me-29), 1.00 (3H, s, Me-25), 1.04 (3H, s, Me-30), 1.08 (3H, s, Me-26), 1.14 (3H, s, Me-23), 1.16 (3H, s, Me-27), 1.24 (3H, s, Me-28), 3.21 (1H, dd, J = 11.5, 4.0 Hz, H-3), 4.18 (1H, dd, J = 10.0, 4.0 Hz, H-22), 4.40 (1H, dd, J = 10.0, 5.0 Hz, H-16), 5.27 (1H, t, J = 3.5 Hz, H-12); ^{13}C NMR data of the aglycon (CD_3OD, 150 MHz) δ 16.6 (C-24), 17.9 (C-25), 18.0 (C-26), 19.5 (C-6), 22.0 (C-28), 24.6 (C-11), 25.5 (C-27), 26.6 (C-2), 28.5 (C-30), 29.0 (C-23), 30.7 (C-20), 32.3 (C-29), 34.0 (C-7), 37.0 (C-15), 37.7 (C-10), 39.9 (C-1), 40.0 (C-4), 40.2 (C-18), 41.6 (C-14), 42.3 (C-8), 43.5 (C-21), 48.1 (C-9), 48.3 (C-19), 53.0 (C-17), 57.1 (C-5), 68.5 (C-22), 74.0 (C-16), 91.0 (C-3), 124.3 (C-12), 144.2 (C-13); ^{1}H and ^{13}C NMR data of the sugar moieties were superimposable on those of **3**; ESIMS *m/z* 941 [M − H]$^-$, 923 [M − H − 18]$^-$, 879 [M − H − 18 − 44]$^-$, 733 [M − H − 18 − 44 − 146]$^-$, 571 [M − H − 18 − 44 − 146 − 162]$^-$; HRESIMS *m/z* 965.5102 [M + Na]$^+$ (calcd for $C_{48}H_{78}O_{18}Na$, 965.5086).

Acid Hydrolysis of Compounds 1−5. A solution of each compound [**1**, **2**, and **4** (2.0 mg); **3** and **5** (1.0 mg)] in 1 N HCl (1 mL) was stirred at 80 °C in a stoppered reaction vial for 4 h. After cooling, the solution was evaporated under a stream of N_2. Each residue was dissolved in 1-(trimethylsilyl)imidazole and pyridine (0.2 mL), and the solution was stirred at 60 °C for 5 min. After drying the solution, the residue was partitioned between H_2O and $CHCl_3$. The $CHCl_3$ layer was analyzed by GC using a L-CP-Chirasil-Val column (0.32 mm × 25 m). Temperatures of the injector and detector were 200 °C for both. A temperature gradient system was used for the oven, starting at 100 °C for 1 min and increasing up to 180 °C at a rate of 5 °C/min. Peaks of the hydrolysate were detected by comparison with retention times of authentic samples of D-glucuronic acid, D-glucose, D-xylose, D-galactose, and L-rhamnose (Sigma Aldrich) after treatment with 1-(trimethylsilyl)imidazole in pyridine.

Acknowledgment. The MIUR (Ministero dell'Istruzione, dell'Università e della Ricerca, Roma) is gratefully acknowledged for financial support. The authors are grateful to Dr. Fabrizio Dal Piaz of the Dipartimento di Scienze Farmaceutiche, Università di Salerno, Italy, for registering the HRESIMS spectra.

References and Notes

(1) Kerharo, J.; Adam, J. C. *Pharmacopée Sénégalaise Traditionelle: Plantes Médicinales et Toxicologiques*; Vigot et Frères, Ed.; Paris, 1974; p 1011.
(2) Bah, S.; Diallo, D.; Dembéle, S.; Paulsen, B. S. *J. Ethnopharmacol.* **2006**, *105*, 387−399.
(3) Sanogo, R.; Diallo, D.; Diarra, S.; Ekoumoue, C.; Bougoudogo, C. *Mali Med.* **2006**, *21*, 18−24.
(4) Bah, S.; Paulsen, B. S.; Diallo, D.; Johansen, H. T. *J. Ethnopharmacol.* **2006**, *107*, 189−198.
(5) De Leo, M.; De Tommasi, N.; Sanogo, R.; Autore, G.; Marzocco, S.; Pizza, C.; Morelli, I.; Braca, A. *Steroids* **2005**, *70*, 573−585.

(6) De Leo, M.; De Tommasi, N.; Sanogo, R.; D'Angelo, V.; Germanò, M. P.; Bisignano, G.; Braca, A. *Phytochemistry* **2006**, *67*, 2623−2629.
(7) Doddrell, D. M.; Khong, P. W.; Lewis, K. G. *Tetrahedron Lett.* **1974**, *27*, 2381−2384.
(8) Khong, P. W.; Lewis, K. G. *Aust. J. Chem*. **1975**, *28*, 165−172.
(9) Maillard, M.; Adewunmi, C. O.; Hostettmann, K. *Phytochemistry* **1992**, *31*, 1321−1323.
(10) Agrawal, P. K. *Carbon-13 NMR of Flavonoids*; Elsevier Science Publishers: Amsterdam, 1989.
(11) Kazuma, K.; Noda, N.; Suzuki, M. *Phytochemistry* **2003**, *62*, 229−237.
(12) Moon, J. H.; Tsushida, T.; Nakahara, K.; Terao, J. *Free Radical Biol. Med*. **2001**, *30*, 1274−1285.
(13) Cui, B.; Kinjo, J.; Nohara, T. *Chem. Pharm. Bull*. **1992**, *40*, 2995−2999.
(14) Udayama, M.; Kinjo, J.; Nohara, T. *Phytochemistry* **1998**, *48*, 1233−1235.
(15) Yeşilada, E.; Takaishi, Y. *Phytochemistry* **1999**, *51*, 903−908.

NP0700671