Saponins from the Leaves of *Mimusops laurifolia*

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Fifteen new bidesmosidic triterpenoid saponins (**1**−**15**) were isolated from a methanol extract of the leaves of *Mimusops laurifolia*. Their structures were established using one- and two-dimensional NMR spectroscopy and mass spectrometry and determined to be bidesmosides of protobassic acid (**2**−**4**, **11**, **12**, and **15**) and of 16α-hydroxyprotobassic acid (**1**, **5**−**10**, **13**, and **14**).

Mimusops laurifolia (Forssk.) Friis (Sapotaceae), also known as *Binectaria laurifolia* Forssk. and *M. schimperi* A. Rich., is a native species restricted to the mountains surrounding the Red Sea and the Gulf of Aden. The plant is cultivated in Egypt, the Sudan, and Uganda. The seeds have been found in pharaonic tombs as offerings with funeral bouquets of leafy twigs.[1] *M. laurifolia* is an evergreen plant that usually grows up to 15−20 m in height, and the fruit is small and each contains one to three shiny hard seeds. The fruits were considered at one time to be edible but today they are no longer consumed.

Mimusops species also enjoy a considerable reputation in Indian traditional medicine due to their anthelmintic, tonic, and astringent activities.[2] Previously, triterpenoid saponins[2−6] and steroidal glycosides[7] have been found as constituents of this genus. The saponins of fruits were reported to possess anti-inflammatory activity,[8] and those from stem bark of *M. elengi* exhibited antiulcer activity.[9] Since the chemical constituents of *M. laurifolia* have not been previously investigated, the species was selected as part of a research project on saponins from plants growing in Egypt and used in folk medicine. The leaves were collected from the garden of the Egyptian Museum in Cairo. The present paper reports the isolation and the structural elucidation of 15 new triterpenoidal saponins.

Results and Discussion

The dried and powdered leaves of *M. laurifolia* were extracted with MeOH, and the MeOH extract was precipitated by acetone. After dialysis against water, the crude saponin mixture was chromatographed by silica gel vacuum layer chromatography, and further purification was obtained by a combination of reversed-phase C_{18} column and silica gel column chromatography. A last stage of purification by preparative TLC and semipreparative HPLC over C_{18} silica gel afforded saponins **1**−**15**. The sugar composition was determined by TLC after acid hydrolysis of the crude saponin mixture as D-glucose, D-xylose, L-rhamnose, L-arabinose, and D-apiose, after verification of their optical rotation. The structures of the aglycons and of the sugar moieties in the intact saponins were determined from analysis of ^{1}H, ^{13}C, COSY, TOCSY, ROESY, HSQC, and HMBC NMR experiments and confirmed by ESI mass spectrometry.

The aglycon was identified as protobassic acid [δ_{2H-16} 1.66, 2.06 ± 0.03, δ_{C-16} 23.8 ± 0.1] in compounds **2**−**4**, **11**, **12**, and **15** and as 16α-OH protobassic acid [δ_{H-16} 4.50 ± 0.02, δ_{C-16} 74.7 ± 0.1] in compounds **1**, **5**−**10**, **13**, and **14**. These data and other spectral data were found to be in agreement with those reported in the literature.[2,4,11] In all these compounds, the ^{13}C NMR chemical shifts of C-3 and C-28 averaged 83.7 ± 0.1 and 177.5 ± 0.4, respectively, suggesting the presence of two oligosaccharide chains, one attached to C-3 and the other esterifying the acid at position C-28. It is worth noting here that acid-catalyzed hydrolysis techniques yielded bassic acid and 16α-hydroxybassic acid as the sole isolated aglycons, obviously the result of dehydration. Complete assignment of the glycosidic protons in the entire saponins was achieved by analysis of COSY and TOCSY experiments, while those of the corresponding glycosidic carbons were determined through the observation of the direct H−C correlations in the HSQC spectra. Sequencing and points of attachment in the glycosidic chains were established after the correlations observed in HMBC and ROESY NMR experiments.

In saponins **1**−**4** and **15**, the length of the glycosidic ether-linked chain at position 3 of the aglycon was reduced to a single unit, identified as a β-D-glucose with its anomeric proton doublet at δ 4.46 ± 0.01 (J = 7.8 ± 0.1 Hz) and its anomeric carbon at δ 105.3 ± 0.1 (Table 1). Proposition of the usual β-anomeric configuration for this sugar was based on the observation of large $^3J_{H-1,H-2}$ coupling constants (7−8 Hz). The anomeric protons showed HMBC cross-peak correlations with C-3 of the aglycon (δ 83.8). Saponins **5**−**7** were assigned to the same series but contained a two-sugar unit located on C-3 of the aglycon and were characterized by two anomeric carbon signals at δ 105.2 and 111.4, with corresponding protons as doublets at δ 4.50 ± 0.01 (J = 7.5 ± 0.1 Hz) and 5.31 (J = 2.8 Hz) (Table 1). Analysis of 2D-NMR experiments (COSY, TOCSY, and HSQC) showed that these compounds contained a β-D-glucose unit, as in compounds **1**−**4**, whose deshielded C-3 resonance (δ 85.8) indicated a substitution by the second sugar unit of the disaccharide chain. In the COSY experiment, the anomeric proton at δ 5.31 (J = 2.8 Hz) was observed to couple with a vicinal proton at δ 4.02, also a doublet (J = 2.8 Hz) and assigned to H-2. This sugar was further characterized in the ^{1}H NMR spectrum by two other pairs of doublets corresponding to hydroxymethylene

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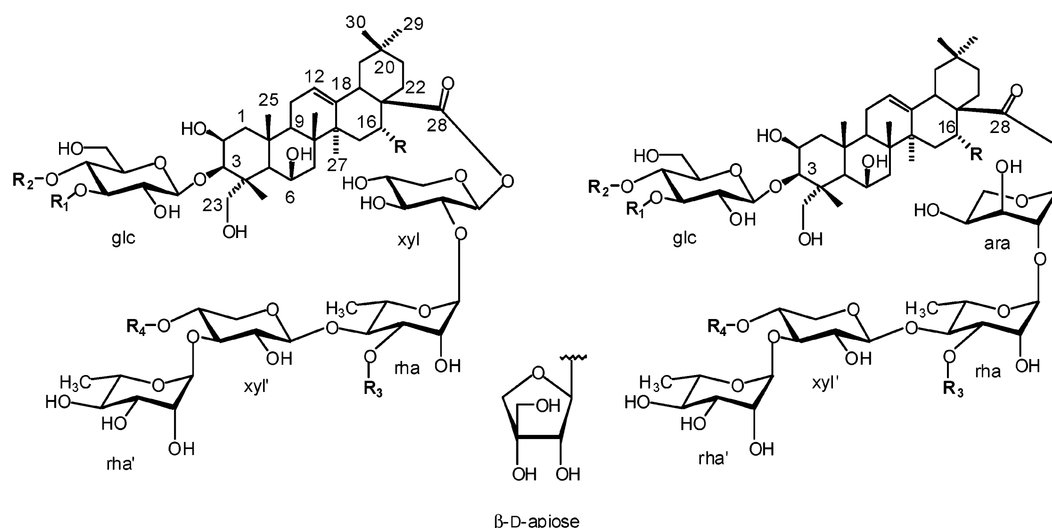
Chart 1



	R	R_1	R_2	R_3	R_4
1	OH	H	H	α-L-rha''	H
3	H	H	H	H	β-D-xyl''
4	H	H	H	α-L-rha''	H
6	OH	β-D-api	H	α-L-rha''	H
7	OH	β-D-api	H	H	β-D-xyl''
10	OH	H	β-D-api	α-L-rha''	H
14	OH	α-L-rha'''	β-D-api	α-L-rha''	H
15	H	H	H	H	H

	R	R_1	R_2	R3	R_4
2	H	H	H	H	β-D-xyl''
5	OH	β-D-api	H	α-L-rha''	H
8	OH	H	β-D-api	α-L-rha''	H
9	OH	H	β-D-api	H	H
11	H	H	β-D-api	α-L-rha''	H
12	H	H	β-D-api	H	H
13	OH	α-L-rha'''	β-D-api	α-L-rha''	H

groups with attached carbons at δ 75 and 63.6 (Table 1). Analysis of HMBC correlations and ROE measurements showed that these elements were best arranged as a β-D-erythroapiofuranose with a quaternary carbon at δ 80.5.[12] Interglycosidic ROEs were further observed between H-1 of glucose (δ 4.50 $\pm$ 0.01) and H-3 of the aglycon (δ 3.59) and between H-1 of apiose (δ 5.31) and H-3 of glucose (δ 3.47 $\pm$ 0.01). The HMBC spectrum confirmed the sequencing deduced from ROEs with observation of cross-peaks between H-1 of apiose and C-3 of glucose (δ 85.8) and between H-1 of glucose and C-3 of the aglycone (δ 83.8). Accordingly, the ether-linked glycosidic chain was identified as the β-D-apiofuranosyl-(1→3)-β-D-glucopyranoside in saponins **5**−**7**.

Saponins **8**−**12** had in common two anomeric carbons at δ 105.2 and 111.1 $\pm$ 0.1 and two anomeric protons at δ 4.48 $\pm$ 0.02 (J = 7.8 Hz) and 5.11 $\pm$ 0.01 (J = 3.6 Hz), which were identified as in the previous manner with a β-D-glucopyranose and a β-D-erythroapiofuranose (Table 2). The only detected difference with the preceding group of compounds was that the apiose was attached to C-4 of the glucose instead of C-3, and that was confirmed by the deshielding of C-4 at δ 79.4 $\pm$ 0.1. The HMBC experiment showed interglycosidic correlations between H-1 of apiose (δ 5.11) and C-4 of glucose (δ 79.4) and between H-1 of glucose (δ 4.49) and C-3 of the aglycon (δ 83.8). ROEs observed between H-1 of apiose (δ 5.11) and H-4 of glucose (δ 3.55) confirmed the nature of the disaccharide chain at position 3 of the aglycon as being β-D-apiofuranosyl-(1→4)-β-D-glucopyranoside for these saponins. Saponins **13** and **14** were shown to contain three glycosidic residues linked to C-3 of the aglycon through an ether bridge. The resonances of the anomeric carbons were observed at δ 105.3, 110.2, and 101.6 $\pm$ 0.1 in the ^{13}C NMR spectrum and were found to correspond to doublets at δ 4.48 (J = 7.8 $\pm$ 0.1 Hz), 5.20 (J = 5 Hz), and 5.48 (J = 1.5 $\pm$ 0.1 Hz), respectively, in the HSQC experiment (Table 2). Analysis of 2D-NMR experiments allowed the sugars to be identified as a β-D-glucose and a β-D-erythroapiofuranose, as in other saponins, and an α-L-rhamnose characterized by a methyl doublet at δ 1.26 correlated with H-5 as a doublet quartet at δ 4.26, and the characteristic H-1, H-2, and H-3 protons linked by small J vicinal coupling constants and the large triplet for H-4 (J = 9.5 $\pm$ 0.1 Hz). The HMBC spectrum showed cross-peaks between H-1 of apiose (δ 5.20) and C-4 of glucose (δ 76), between H-1 of rhamnose (δ 5.48) and C-3 of glucose (δ 78.2), and between H-1 of glucose (δ 4.48) and C-3 of the aglycon (δ 83.8). For saponins **13** and **14**, the triglycosidic ether-linked chain was thus defined as β-D-apiofuranosyl-(1→4)-[α-L-rhamnopyranosyl-(1→3)]-β-D-glucopyranoside.

Similarities of proton and carbon resonances in saponins **2**, **5**, **8**, **9**, and **11**−**13** suggested that the second oligosaccharide chain, connected to the C-28 position of the aglycon

Table 1. NMR Data for C-3 Saccharide Portions of Saponins **1**−**7** and **15** (CD_3OD)

position	**1**		**2**		**3**		**4**	
	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C
3-*O*-*β*-D-glucose								
1	4.46 (d, 7.8)	105.4	4.47 (d, 7.8)	105.3	4.47 (d, 7.7)	105.4	4.46 (d, 7.9)	105.2
2	3.31 (brt, 8)	75.5	3.30 (t, 9)	75.5	3.31 (t, 8)	75.4	3.33 (t, 8)	75.5
3	3.39 (m)	78.2	3.38 (m)	78.2	3.39 (t, 7)	78.2	3.39 (t, 7)	77.2
4	3.40 (m)	71.1	3.38 (m)	71.1	3.39 (m)	71.1	3.39 (m)	71.1
5	3.31 (m)	77.8	3.31 (m)	77.8	3.32 (m)	77.7	3.33 (m)	78
6	3.72 (dd, 11.7, 4.7)	62.3	3.72 (dd, 11.7, 4.9)	62.3	3.72 (dd, 11.7, 4.6)	62.2	3.72 (dd, 11.8, 4.6)	62.3
6	3.82 (dd, 12, 2.4)		3.83 (dd, 12, 2)		3.83 (brd, 11.5)		3.83 (brd, 11.5)	

position	**5**		**6**		**7**		**15**	
	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C
3-*O*-*β*-D-glucose								
1	4.49 (d, 7.5)	105.2	4.50 (d, 7.5)	105.2	4.51 (d, 7.4)	105.2	4.46 (d, 7.7)	105.3
2	3.44 (t, 9)	75	3.44 (t, 9)	75	3.44 (t, 8)	75	3.31 (t, 9)	75.5
3	3.47 (m)	85.8	3.46 (m)	85.8	3.48 (m)	85.8	3.40 (m)	78.2
4	3.44 (t 9)	69.7	3.44 (m)	69.7	3.45 (t, 8.5)	69.7	3.39 (m)	71.1
5	3.32 (m)	77.4	3.32 (m)	77.4	3.33 (m)	77.5	3.30 (m)	77.8
6	3.73 (dd, 12, 3.4)	62.2	3.72 (dd, 11.6, 5.5)	62.4	3.73 (dd, 11.8, 4.5)	62.2	3.72 (dd, 11.9, 4.7)	62.3
6	3.83 (dd, 12, 3)		3.83 (dd, 11.6, 5.8)		3.83 (dd, 11.9, 2.2)		3.83 (dd,12, 2)	
β-D-apiose (at C-3 Glc)								
1	5.31 (d, 2.8)	111.4	5.31 (d, 2.8)	111.4	5.31 (d, 2.8)	111.4		
2	4.02 (d, 2.8)	77.9	4.02 (d, 2.8)	77.9	4.02 (d, 2.8)	77.9		
3		80.5		80.5		80.5		
4	3.81 (d, 9.4)	75	3.81 (d, 9.4)	74.8	3.83 (d, 9.5)	75		
4	4.15 (d, 9.6)		4.15 (d, 9.6)		4.15 (d, 9.6)			
5	3.61 (d, 11.7)	65.1	3.62 (d, 11.5)	65.1	3.62 (d, 11.7)	65.1		
5	3.62 (d, 10.6)		3.64 (d, 11.0)		3.64 (d, 11.5)			

through an ester bond, contained as a substructure a common tetraglycosidic chain. The ^{13}C NMR spectrum of compounds **9** and **12** showed four signals for anomeric carbons at δ 94.1, 101.4 (or 101.5), 106.4 (or 106.7), and 102.6, respectively, linked to anomeric protons in the HSQC spectrum at δ 5.63 or 5.67 (d, J = 4 Hz), 5.10 or 5.13 (br s), 4.57 or 4.55 (d, J = 7.8 Hz), and 5.17 or 5.16 (d, J = 1.4 or 1.5 Hz) (Table 3). The analysis of the COSY and TOCSY spectra revealed the presence of an α-L-arabinose in a predominant $^{1}C_4$ conformation ($J_{H-1,H-2,H-3}$ = 3−5 Hz),[13] as well as two α-L-rhamnoses detected by the observation of $^{3}J_{H-H}$ COSY correlations between two methyl doublets at δ 1.32 and 1.27 and protons at δ 3.75 and 4.03, and a fourth sugar unit assigned to a *β*-D-xylose, characterized by large *trans*-diaxial proton coupling constants and by 1,3 and 1,5 diaxial relationships in the ROESY spectrum. The linkage of the sugar units and their sequencing were established using the following HMBC correlations: H-1 (δ 5.17) of the terminal rhamnose with C-3 (δ 84) of xylose, H-1 of xylose (δ 4.57) with C-4 (δ 83.1) of the inner rhamnose, and H-1 (δ 5.10) of the inner rhamnose with C-2 (δ 75.6) of arabinose. The attachment of the tetrasaccharide chain to C-28 of the aglycon was based on the observation of a correlation between H-1 (δ 5.63) of arabinose and C-28 (δ 177.1). ROEs observed across the glycosidic bonds confirmed the previous assignments of the HMBC spectrum. These data agreed with the presence of the α-L-rhamnopyranosyl-(1→3)-*β*-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside as the tetraglycosidic ester chain of saponins **9** and **12**. Saponins **5**, **8**, **11**, and **13** displayed extra signals for a fifth glycosidic unit with an anomeric carbon at δ 104.4 (or δ 104.5) linked to an anomeric proton at δ 5.01 (or δ 5.04) in the HSQC spectrum and assigned as a terminal α-L-rhamnose (Table 3). The analysis of HMBC correlations showed that H-1 (δ 5.01) of this second outer rhamnose was attached to C-3 (δ 81.4) of the inner rhamnose of the tetrasaccharide chain, and consequently the ester chain of saponins **5**, **8**, **11**, and **13** was α-L-rhamnopyranosyl-(1→3)-*β*-D-xylopyranosyl-(1→4)-[α-L-rhamnopyranosyl-(1→3)]-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside. In addition to the basic tetrasaccharidic chain, saponin **2** possessed a supplementary sugar residue that the 2D-NMR experiments (COSY, TOCSY, and HSQC) helped to determine as a terminal *β*-D-xylose, with anomeric carbon at δ 103.3 and anomeric proton at δ 4.27 (d, J = 7.6 Hz). The HMBC spectrum showed a cross-peak between H-1 (δ 4.27) of this fifth sugar (terminal xylose) and the deshielded C-4 (δ 74.9) of the inner xylose of the tetrasaccharide chain. ROEs were also observed between H-1 (δ 4.27) of this terminal xylose and H-4 (δ 3.75) of the inner xylose. Accordingly, saponin **2** included the α-L-rhamnopyranosyl-(1→3)-[*β*-D-xylopyranosyl-(1→4)]-*β*-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl chain at position C-28 of the aglycon.

Saponins **1**, **3**, **4**, **6**, **7**, **10**, and **14** contained a slightly different but isomeric ester-linked glycosidic chain attached to C-28 of the aglycon (Table 4). The 2D-NMR experiments allowed the detection of the proton and carbon resonances of the tetrasaccharide chain of the previous group of saponins except for the first sugar, detected as a *β*-D-xylose [δ_{H-1} 5.48 ± 0.04 (d, J = 6.2 ± 0.5 Hz), δ_{C-1} 95.4] instead of an α-L-arabinose. The values of the proton coupling constants and the ROEs observed between axial H-1, H-3, and H-5 indicated that this xylose predominantly adopted a $^{4}C_1$ conformation. Saponins **1**, **4**, **6**, **10**, and **14** displayed five anomeric proton signals for this chain near δ 5.49, 5.29, 4.54, 5.18, and 5.04, bound to carbons at δ 95.5, 101.4, 105.2, 102.6, and 104.2 ± 0.3 in the HSQC spectrum. Analysis of 2D-NMR experiments revealed the presence of two *β*-D-xyloses (xylose and xylose′) and three α-L- rhamnoses (named in Table 4 rhamnose, rhamnose′, and rhamnose″). The attachment of the glycosidic chain to C-28 of the aglycon was based on a correlation between H-1 (δ 5.49) of xylose and C-28 (δ 177.1) of the aglycon. The HMBC spectrum showed interglycosidic correlations for the tet-

Table 2. NMR Data for C-3 Saccharide Portions of Saponins **8**–**14** (CD_3OD)

position	**8**		**9**		**10**		**11**	
	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C
3-*O*-β-D-glucose								
1	4.49 (d, 7.8)	105.2	4.49 (d, 7.8)	105.2	4.50 (d, 7.8)	105.2	4.48 (d, 7.8)	105.2
2	3.36 (t, 8.5)	75.2	3.37 (dd, 9, 8)	75.2	3.37 (t, 7.9)	75.2	3.35 (t, 8.2)	75.2
3	3.50 (t, 8.8)	76.6	3.50 (t, 8.8)	76.6	3.50 (t, 8.9)	76.6	3.50 (t, 8.8)	76.6
4	3.55 (t, 8.8)	79.4	3.55 (t, 9.4)	79.4	3.55 (t, 9)	79.4	3.55 (t, 9.5)	79.5
5	3.41(dd, 7, 2.5)	76.4	3.41 (dd, 9.3, 3.2)	76.4	3.41 (m)	76.4	3.40 (dd, 9.5, 3)	76.4
6	3.78 (dd, 11, 3)	61.4	3.78(dd, 11.5, 3.5)	61.4	3.80 (m)	61.4	3.79 (dd, 11.5, 3.5)	61.4
6	3.82 (d, 11)		3.82 (d, 11.5)		3.80 (m)		3.82 (d, 11.5)	
β-D-apiose (at C-4 Glc)								
1	5.11 (d, 3.6)	111.1	5.12 (d, 3.6)	111.1	5.12 (d, 3.6)	111.1	5.11 (d, 3.6)	111.2
2	3.95 (d, 3.6)	77.7	3.95 (d, 3.6)	77.7	3.95 (d, 3.5)	77.7	3.94 (d, 3.3)	77.8
3		80.2		80.2		80.4		80.2
4	3.83 (d, 9.6)	75	3.83 (d, 9.5)	75	3.83 (d, 9.7)	74.9	3.83 (d, 9.4)	75
4	4.18 (d, 9.7)		4.18 (d, 9.6)		4.18 (d, 9.6)		4.18 (d, 9.6)	
5	3.56 (d, 11.4)	64.6	3.56 (d, 11.5)	64.6	3.56 (d, 11)	64.7	3.56 (d, 11)	64.7
5	3.59 (d, 11.4)		3.59 (d, 11.5)		3.59 (d, 11)		3.59 (d, 11.4)	

position	**12**		**13**		**14**	
	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C
3-*O*-β-D-glucose						
1	4.49 (d, 7.8)	105.2	4.48 (d, 7.8)	105.3	4.48 (d, 7.9)	105.4
2	3.36 (t, 8.5)	75.2	3.49 (dd, 9, 8.1)	76.9	3.49 (dd, 9, 8)	76.9
3	3.50 (t, 8.9)	76.6	3.77 (dd, 9.3, 8.5)	78.2	3.77 (t, 9.3)	78.2
4	3.55 (t, 8.9)	79.4	3.70 (t, 9.4)	76	3.70 (t, 9.4)	76
5	3.41 (dd, 9, 4.5)	76.4	3.38 (dt, 9.5, 2.5)	77.1	3.38 (br dd, 9, 2.5)	77.1
6	3.78 (dd, 11, 4)	61.4	3.83 (dd, 11.5,3)	61.1	3.83 (dd, 11, 3)	61.1
6	3.82 (d, 11)		3.90 (br d, 11.5)		3.90 (br d, 11)	
β-D-apiose (at C-4 Glc)						
1	5.11 (d, 3.6)	111.2	5.20 (d, 5)	110.2	5.20 (d, 5)	110.2
2	3.95 (d, 3.5)	77.8	3.87 (d, 5)	80	3.87 (d, 4.9)	80
3		80.2		79.7		79.7
4	3.83 (d, 9.7)	75	3.74 (d, 10.1)	74.9	3.74 (d, 10.1)	75
4	4.18 (d, 9.6)		4.34 (d, 10)		4.34 (d, 10)	
5	3.56 (d, 10)	64.7	3.54 (s)	64.9	3.53 (s)	64.9
5	3.59 (d, 11.5)		3.54 (s)		3.53 (s)	
α-L-rhamnose‴ (at C-3 Glc)						
1			5.48 (d, 1.5)	101.6	5.48 (d, 1.6)	101.5
2			3.95 (dd, 3.4, 1.7)	72.5	3.95 (dd, 3.3, 1.5)	72.5
3			3.87 (dd, 9.7, 3.2)	71.9	3.87 (dd, 9.4, 3.7)	71.9
4			3.42 (t, 9.4)	74	3.42 (t, 9.6)	74
5			4.26 (dq, 9.5, 6)	69.4	4.26 (dq, 10, 6.1)	69.5
6			1.26 (d, 6)	17.9	1.26 (d, 6.3)	18

rasaccharidic chain between H-1 (δ 5.18) of the terminal rhamnose′ and C-3 (δ 84.4) of the inner xylose′, H-1 (δ 4.54) of the inner xylose′ and C-4 (δ 77.9) of the inner rhamnose, and H-1 (δ 5.29) of the inner rhamnose and C-2 or C-3 (δ 76.6) of xylose (ester), and a supplementary correlation was observed between the fifth H-1 (δ 5.04) of the terminal rhamnose″ and C-3 (δ 81.2) of the inner rhamnose of the tetrasaccharidic chain. Peracetylation of saponin **10** into **10a** and analysis of the COSY spectrum of derivative **10a** showed that the chemical shift of H-2 (δ 3.65) of xylose (ester) was not deshielded since it was not subjected to acetylation. This xylose is in a predominant $^1C_4$ conformation in derivative **10a**. Thus, it was deduced that the inner rhamnose was linked to C-2 of the first xylose (ester) and the ester chain of saponins **1**, **4**, **6**, **10**, and **14** was α-L-rhamnopyranosyl-(1→3)-β-D-xylopyranosyl-(1→4)-[α-L-rhamnopyranosyl-(1→3)]-α-L-rhamnopyranosyl-(1→2)-β-D-xylopyranoside. Saponins **3** and **7** showed five anomeric doublets at δ 5.48 (5.45), 5.40 (5.36), 4.54 (4.58), 5.18, and 4.28, respectively, correlated with carbons in the HSQC spectrum, at δ 95.5 (95.4), 101.3, 107.1 (106.8), 102.3, and 103.4. In comparison with the latter saponins, these two compounds contained a β-D-xylose as the fifth glycosidic unit instead of a rhamnose. The HMBC spectrum showed a cross-peak between H-1 (δ 4.28) of this terminal xylose and C-4 (δ 74.8) of the inner xylose of the tetrasaccharidic chain. ROEs observed across the glycosidic bonds confirmed the sequence of the chain as α-L-rhamnopyranosyl-(1→3)-[β-D-xylopyranosyl-(1→4)]-β-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-β-D-xylopyranoside.

Further support for the sequencing of the sugars was obtained by the MS/MS fragmentation patterns. The compounds may be classified into three series depending on the composition of their glycosidic ester chains. The first group (**1**, **4**, **5**, **6**, **8**, **10**, **11**, **13**, and **14**) possess a pentaglycosidic chain composed of two pentoses and three rhamnoses (chain A; M = 702), the second group (**2**, **3**, and **7**) contains three pentoses and two rhamnoses (chain B; M = 688), and the ester chain of the last group (**9** and **12**) is a tetraglycoside composed of two pentoses and two rhamnoses (chain C; M = 556).

The positive ESIMS of saponins **5**, **6**, **8**, and **10** exhibited an identical quasi-molecular ion peak at *m/z* 1539 $[M + Na]^+$, while, in the negative mode, a quasi-molecular ion was detected at *m/z* 1515 $[M - H]^-$, corresponding to a molecular formula of $C_{69}H_{112}O_{36}$. These four saponins displayed the same MS/MS fragmentation patterns. The MS^2 experiment of the $[M - H]^-$ gave an intense ion peak at *m/z* 1369 $[M - H - 146]^-$ attributed to the loss of the terminal rhamnose, which in the MS^3 yielded a first ion

Table 3. NMR Data for C-28 Saccharide Portions of Saponins **2**, **5**, **8**, **9**, and **11**−**13** (CD_3OD)

	9		12		5		8		11		13		2	
	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C
28-*O*-α-L-arabinose (ester)														
1	5.63 (d, 4)	94.1	5.67 (d, 4)	94.1	5.70 (d, 3.2)	93.8	5.70 (d, 3.1)	93.8	5.74 (d, 3.1)	93.7	5.70 (d, 3.1)	93.8	5.66 (d, 3.9)	94.1
2	3.82 (t, 5)	75.6	3.85 (dd, 5.5, 4)	75.7	3.81 (dd, 5.5, 3)	76	3.81 (dd, 4.5, 3)	76	3.84 (dd, 5, 3)	76.2	3.81 (dd, 5, 3.4)	75.9	3.85 (dd, 5, 3.5)	75.7
3	3.89 (dd, 5, 4)	71.7	3.91 (m)	71.8	3.92 (dd, 5, 3)	70.8	3.92 (dd, 5, 4)	70.8	3.94 (m)	70.9	3.92 (t, 3.5)	70.8	3.91 (m)	71.7
4	3.84 (m)	67.3	3.89 (m)	67.4	3.86 (m)	66.6	3.85 to 3.88 (m)	66.6	3.87 (m)	66.7	3.87 (m)	66.7	3.85 (m)	67.4
5	3.54 (dd, 11.4, 3.9)	64.1	3.54 (d, 11)	64.1	3.52 (dd, 10.9, 4)	63.2	3.52 (d, 10.4)	63.2	3.52 (dd, 11, 4)	63.2	3.52 (dd, 10, 3.5)	63.5	3.54 (dd, 11, 3)	64.1
5	3.94 (dd, 11.5, 6 5)		3.94 (dd, 11, 7)		3.95 (dd, 11, 7.5)		3.95 (dd, 10, 8)		3.94 (dd, 11, 7.5)		3.94 (m)		3.94 (dd, 11, 7)	
α-L-rhamnose (at C-2 Ara)														
1	5.10 (br s)	101.4	5.13 (br s)	101.5	5.00 (d, 1.7)	101.6	5.00 (d, 1.7)	101.5	5.01 (d, 1.5)	101.7	5.00 (d, 1.7)	101.6	5.12 (brs 1)	101.5
2	3.88 (m)	72.3	3.90 (br s)	72.3	3.96 (dd, 3.3, 1.8)	72.2	3.97 (dd, 3.2, 1.8)	72.3	3.98 (dd, 3.5, 1.5)	72.3	3.96(dd, 3.3, 1.8)	72.1	3.89 (brd, 4.5)	72.4
3	3.86 (dd, 9, 3)	72.4	3.88 (dd, 10, 3)	72.5	3.92 (dd, 10, 3)	81.4	3.92 (dd, 10, 3)	81.4	3.94 (dd, 9.5, 3.5)	81.1	3.92 (dd, 9.8, 2.7)	81.4	3.87 (dd, 9, 3)	72.4
4	3.61 (t, 8.7)	83.1	3.58 (t, 9.5)	83.5	3.77 (m)	77.6	3.78 (m)	77.6	3.76 (t, 8.9)	78	3.77 (m)	77.6	3.60 (t, 9)	83.4
5	3.75 (dq, 9, 6)	69	3.77 (dq, 9.5, 6)	69	3.76 (m)	69.4	3.78 (m)	69.4	3.79 (m)	69.4	3.77 (m)	69.4	3.76 (m)	69
6	1.32 (d, 6.2)	18.1	1.31 (d, 6.1)	18.1	1.28 (d, 6)	18.3	1.29 (d, 6.5)	18.3	1.28 (d, 6)	18	1.29 (d, 6)	18.3	1.31 (d, 6)	18.2
β-D-xylose′ (at C-4 Rha)														
1	4.57 (d, 7.8)	106.4	4.55 (d, 7.8)	106.7	4.53 (d, 7.7)	105.1	4.53 (d, 7.7)	105.1	4.53 (d, 7.6)	105.2	4.53 (d, 7.7)	105.1	4.57 (d, 7.6)	106.5
2	3.37 (dd, 9, 8)	76.3	3.35 (t, 8)	76.3	3.33 (t, 8.4)	75.6	3.33 (t, 8.4)	75.6	3.30 (t, 8.5)	75.5	3.33 (t, 8)	75.6	3.39 (t, 8.4)	76.9
3	3.49 (t, 8.8)	84	3.49 (t, 8.8)	84.2	3.45 (t, 8.5)	84	3.45 (t, 8.9)	84	3.44 (t, 8.9)	84.4	3.45 (t, 8.9)	84	3.65 (t, 7.9)	80.3
4	3.55 (m)	69.8	3.55 (m)	69.8	3.57 (td, 9, 5.5)	70.1	3.57 (m)	70.1	3.58 (m)	70.1	3.57 (m)	70.1	3.75 (m)	74.9
5	3.25 (dd, 11.5, 10.3)	67.2	3.24 (t, 11.3)	67.2	3.19 (dd,11.5,10.4)	67	3.19 (dd, 11.5,10.4)	67	3.19 (t, 10.9)	66.9	3.19 (dd, 11.3, 10.6)	67	3.32 (t, 11)	64.5
5	3.90 (dd, 11.3, 5.1)		3.90 (dd, 11.4, 5.2)		3.90 (dd, 11.8, 5.8)		3.90 (dd, 12, 6)		3.90 (dd, 11.8, 5.1)		3.90 (dd, 11, 5.5)		4.07 (dd, 11.8, 5.1)	
α-L-rhamnose′ (terminal at C-3 Xyl′)														
1	5.17 (d, 1.4)	102.6	5.16 (d, 1.5)	102.6	5.19 (d, 1.7)	102.5	5.19 (d, 1.6)	102.5	5.18 (d, 1.3)	102.6	5.19 (d, 1.4)	102.5	5.18 (d, 1.3)	102.2
2	3.98 (dd, 3.3, 1.7)	72.2	3.98 (dd, 3.3, 1.8)	72.3	3.96 (dd, 3.3,1.8)	72.3	3.97 (dd, 3.2, 1.8)	72.2	3.98 (dd, 3.5, 1.5)	72.2	3.96 (dd, 3.3, 1.8)	72.2	3.95 (dd, 3.4, 1.4)	72.3
3	3.73(dd, 9.5, 3.3)	72.3	3.73 (dd, 9.4, 3.4)	72.2	3.73 (dd, 9.5, 3.4)	72.2	3.73 (dd, 9.5, 3.4)	72.2	3.73 (dd, 9.4, 3.4)	72.2	3.73 (dd, 9.5, 3.5)	72.4	3.77 (dd, 9.4, 3.4)	72.2
4	3.41 (t, 9.6)	74	3.42 (t, 9.6)	74	3.41 (t, 9.5)	74	3.42 (t, 9.5)	74	3.42 (t, 9.5)	74	3.42 (t, 9.5)	74	3.39 (t, 9.6)	74.1
5	4.03 (dq, 9.5, 6)	70	4.03 (dq, 10.2, 6)	70	4.02 (dq, 9.5, 6.1)	70.1	4.02 (dq, 10, 6)	70.1	4.01 (dq,10, 6)	70.1	4.02 (dq, 10, 6)	70.1	4.27 (dq, 9.5, 6.5)	69.5
6	1.27 (d, 6.2)	17.9	1.27 (d, 6.2)	17.9	1.27 (d, 6.3)	17.9	1.27 (d, 6.2)	17.9	1.27 (d, 6)	17.9	1.27 (d, 6.4)	18	1.24 (d, 6.2)	18.1
α-L-rhamnose″ (terminal at C-3 Rha)														
1					5.01 (d, 1.5)	104.5	5.01 (d, 1.6)	104.4	5.05 (d, 1)	104.4	5.01 (d, 1.4)	104.4		
2					4.08 (dd, 3.3, 1.7)	72.1	4.08 (dd, 3.3, 1.7)	72.1	4.08 (dd, 3.3, 1.7)	72.1	4.08 (dd, 3.3, 1.7)	72.1		
3					3.77 (dd, 9.7, 3.4)	72.2	3.77 (dd, 9.5, 3.3)	72.3	3.77 (dd, 9.2, 3.8)	72.3	3.77 (dd, 9.4, 3.3)	72.2		
4					3.42 (t, 9.5)	74	3.43 (t, 9.5)	74	3.43 (t, 9.2)	74	3.42 (t, 9.4)	74		
5					3.85 (dq, 9.5, 6.1)	70.3	3.85 (dq, 9.5, 6)	70.3	3.85 (dq, 10, 6)	70.3	3.85 (dq, 10, 6)	70.3		
6					1.27 (d, 6.2)	18	1.27 (d, 6.2)	18	1.27 (d, 6.2)	17.9	1.27 (d, 6.4)	18		
β-D-xylose″ (terminal at C4 Xyl′)														
1													4.27 (d, 7.6)	103.3
2													3.18 (dd, 9.2, 7.8)	75
3													3.30 (t, 9)	78
4													3.53 (dt, 9.5, 6)	71.1
5													3.18 (t, 10.9)	67
5													3.90 (dd, 11.3, 5)	

Table 4. NMR Data for C-28 Saccharide Portions of Saponins **1**, **3**, **4**, **6**, **7**, **10**, **14**, and **15** (CD_3OD)

	1		**4**		**6**		**10**		**14**		**3**		**7**		**15**	
	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C
β-D-xylose (ester)																
1	5.49 (d[a], 6.3)	95.5	5.52 (d[a], 5.7)	95.4	5.49 (d, 6.1)	95.4	5.49 (d[a], 6.1)	95.5	5.49 (d[a], 6.5)	95.4	5.48 (d, 6.5)	95.5	5.45 (d, 6.4)	95.4	5.47 (d[a], 6.7)	95.5
2	3.62 (m)	76.6	3.62 (t, 7.3)	76.8	3.62 (t, 5.9)	76.5	3.59 to	76.6	3.61 (m)	76.6	3.63 (t, 6.6)	76.4	3.60 (t, 7)	76.4	3.63 (t, 7.2)	76.3
3	3.62 (m)	76.6	3.57 to	76.8	3.56 (m)	76.6	3.60 (m)	76.6	3.61 (m)	76.6	3.55 to	77.8	3.57 (t, 7)	77.4	3.59 (t, 8.6)	77.8
4	3.57 (m)	70.1	3.60 (m)	70.2	3.58 (m)	70.1	3.58 (m)	71	3.58 (m)	70.9	3.60 (m)	71.1	3.54 (m)	71[b]	3.56 (m)	71.1
5	3.35 (dd, 11.5, 8.5)	66.6	3.36 (dd, 11, 7)	66.6	3.36 (dd, 11.5, 8)	66.6	3.36 (dd, 12, 8)	66.6	3.36 (dd, 12, 7.5)	66.6	3.32 (dd, 12, 7)	66.9	3.33 (dd, 12, 8.5)	66.8	3.33 (dd, 12.5, 7.5)	66.9
5	3.96 (dd, 11.5, 4.4)		3.96 (dd, 11.6, 4.6)		3.96 (dd, 11.5, 4.5)		3.96 (dd, 12, 5.2)		3.96 (dd, 11.7, 4.6)		3.92 (dd, 11.6, 4.8)		3.93 (dd, 12, 2)		3.92 (dd, 11.7, 4.8)	
α-L-rhamnose (at C-2 Xyl)																
1	5.29 (d, 1.8)	101.4	5.31 (d, 1.5)	101.6	5.29 (d, 1.5)	101.4	5.29 (d, 1.5)	101.1	5.29 (d, 1.7)	101.4	5.40 (d, 1)	101.3	5.36 (d, 1.7)	101.3	5.41 (d, 1.4)	101.3
2	4.04 (dd, 3.2, 1.8)	72.1	4.05 (dd, 3.2, 1.8)	72.2	4.04 (dd, 3, 1.8)	72	4.04 (dd, 3, 1)	72.1	4.04 (dd, 3, 1.9)	72.1	3.96 (dd, 3.1, 1.6)	72.4	3.95 (dd, 3.2, 1.9)	72.2	3.97 (dd, 3.1, 1.7)	72.3
3	3.91 (dd, 9.4, 3.5)	81.2	3.94 (dd, 9.7, 3.5)	80.6	3.91 (dd, 9.3,3.6)	81.2	3.91 (dd, 9.6, 3)	81.3	3.91 (dd, 9.7, 3.5)	81.2	3.88 (dd, 9.3, 3.2)	72.4	3.88 (dd, 9.5, 3.3)	72.3	3.89 (dd, 9.5, 3.2)	72.5
4	3.80 (t, 9)	77.9	3.77 (t, 9.6)	78.5	3.79 (t, 8.7)	77.7	3.80 (m)	77.7	3.80 (m)	77.8	3.58 (t, 9.5)	84.2	3.62 (t, 9.5)	83.6	3.58 (t, 9.7)	84.3
5	3.83 (m)	69.4	3.84 (dq, 9.5, 5.5)	69.3	3.81 (dq, 8.5, 5)	69.4	3.81 (m)	69.4	3.82 (m)	69.4	3.82 (dq, 10, 6)	68.9	3.78 (m)	69	3.83 (dq, 10, 6)	68.9
6	1.31 (d, 5.5)	18.5	1.27 (d, 6.1)	18.5	1.31 (d, 5.5)	18.5	1.31 (d, 5.5)	18.5	1.30 (d, 5.7)	18.5	1.32 (d, 6.5)	18.3	1.35 (d, 6.3)	18.4	1.32 (d, 6.3)	18.3
β-D-xylose′ (at C-4 Rha)																
1	4.54 (d, 7.7)	105.2	4.52 (d, 7.6)	105.4	4.54 (d, 7.6)	105.2	4.54 (d, 7.7)	105.2	4.54 (d, 7.7)	105.2	4.54 (d, 7.6)	107.1	4.58 (d, 7.7)	106.8	4.52 (d, 7.8)	107.1
2	3.37 (dd, 9, 7.5)	75.5	3.32 (t, 8.8)	75.5	3.37 (t, 7.8)	75.6	3.37 (t, 7.9)	75.2	3.37 (t, 7.8)	75.6	3.40 (t, 8)	77.1	3.44 (t, 8.9)	77	3.36 (t, 9)	76.5
3	3.46 (t, 9)	84.4	3.44 (t, 8.5)	85.1	3.46 (t, 9.1)	84.3	3.46 (t, 8.9)	84.3	3.46 (t, 9)	84.4	3.66 (t, 9)	80.4	3.67 (t, 9)	80.2	3.49 (t, 8.9)	84.4
4	3.58 (m)	70.9	3.58 (m)	70.9	3.58 (m)	70.9	3.58 (m)	70.1	3.58 (m)	70.1	3.75 (m)	74.8	3.78 (m)	74.8	3.56 (m)	69.8
5	3.20(dd, 11.6,10.2)	67	3.20 (t, 10.4)	66.9	3.20 (t, 11.3)	67	3.20 (t, 11.3)	70	3.20 (dd, 11.6,10.2)	70	3.32 (t, 10)	64.6	3.34 (t, 11.6)	64.7	3.25 (dd, 11.3,10.5)	67.3
5	3.91 (dd, 11.4, 5.5)		3.90 (dd, 11.4, 5.2)		3.91 (dd, 10, 6)		3.91 (dd, 11.5, 4.5)		3.91 (dd, 11.8, 5.8)		4.08 (dd, 11.7, 5)		4.09 (dd, 11.9, 5.2)		3.90 (dd, 11, 5.4)	
α-L-rhamnose′ (terminal at C-3 Xyl′)																
1	5.18 (d, 1.6)	102.6	5.15 (d, 1)	102.8	5.18 (d, 1.4)	102.6	5.18 (d, 1.3)	102.5	5.17 (d, 1.6)	102.6	5.18 (d, 1.5)	102.3	5.18 (d, 1.5)	102.3	5.17 (d, 1.5)	102.6
2	3.99 (dd, 3.3, 1.8)	72.3	4.01 (dd, 3.3, 1.6)	72.3	3.99 (dd, 3.2, 1.7)	72.2	3.99 (dd, 3.3, 1.8)	72.2	3.99 (dd, 3.3, 1.7)	72.2	3.96 (dd, 3.1, 1.4)	72.2	3.97 (dd, 3.3, 1.6)	72.4	4 (dd, 3.4, 1.8)	72.3
3	3.74 (dd, 9.4, 3.4)	72.2	3.74 (dd, 9.3, 3.4)	72.2	3.73 (dd, 9.7, 3.6)	72.3	3.74 (dd, 9.6, 3.4)	72.3	3.74 (dd, 10, 3.7)	72.3	3.77 (dd, 9.5, 3.3)	72.2	3.77 (dd, 9.4, 3.2)	72.1	3.75 (dd; 9.6, 3.2)	72.3
4	3.42 (t, 9.4)	74	3.43 (t, 9.5)	74	3.42 (t, 9.7)	74	3.42 (t, 9.6)	74	3.42 (t, 9.5)	74	3.39 (t, 9.8)	74.1	3.39 (t, 9.6)	74.1	3.42 (t; 9.5)	74.1
5	4.02 (dq, 9.5, 6)	70.1	3.99 (m)	70.3	4.02 (m)	70.1	4.02 (dq, 9.4, 6.3)	70.1	4.02 (dq, 10, 6)	70.1	4.26 (dq, 9.5, 6.1)	69.6	4.27 (dq, 9.5, 5.8)	69.6	4.03 (dq; 11, 6)	70.1
6	1.28 (d, 6)	17.9	1.28 (d, 6)	17.9	1.27 (d, 6)	17.9	1.28 (d, 6)	17.9	1.28 (d, 6.1)	18	1.25 (d, 6.2)	18.1	1.25 (d, 6.2)	18.1	1.27 (d; 6.2)	17.9
α-L-rhamnose″ (terminal at C-3 Rha)																
1	5.04 (d, 1.5)	104.2	5.11 (d, 1.5)	104.1	5.04 (d, 1.2)	104.2	5.04 (d, 1.5)	104.2	5.04 (d, 1.4)	104.2						
2	4.08 (dd, 3.3, 1.7)	72.3	4.09 (dd, 3, 1.5)	72.1	4.08 (dd, 3, 1.5)	72.1	4.08 (dd, 3, 1.6)	72.2	4.08 (dd, 3.3, 1.7)	72.1						
3	3.78 (dd, 9.5, 3.3)	72.3	3.78 (dd, 9.4, 3.5)	72.2	3.78 (dd, 9.4, 3.1)	72.3	3.77 (dd, 9.6,3.5)	72.3	3.77 (dd, 9.3, 3.3)	72.3						
4	3.43 (t, 9.4)	74	3.43 (t, 9.5)	74	3.44 (t, 9.7)	74	3.43 (t, 9.5)	74	3.43 (t, 9.4)	74						
5	3.86 (dq, 9.5, 6)	70.4	3.83 (dq, 9, 6)	70.4	3.87 (dq, 9.5, 6)	70.4	3.87 (dq, 9, 6)	70.4	3.88 (m)	70.4						
6	1.29 (d, 5.8)	17.9	1.29 (d, 6)	17.9	1.28 (d, 5.7)	17.9	1.29 (d, 5.9)	17.9	1.29 (d, 5.3)	17.9						
β-D-xylose″ (terminal at C-4 Xyl′)																
1											4.28 (d, 7.6)	103.4	4.28 (d, 7.7)	103.4		
2											3.18 (dd, 9.1, 8.8)	75	3.18 (dd, 9, 7.7)	75		
3											3.30 (t, 9.1)	78	3.30 (t, 9.1)	78		
4											3.53 (td, 8.5, 5.3)	71	3.53 (ddd, 11,9,5.2)	71.1[b]		
5											3.18 (dd, 12, 10.9)	67	3.18 (t, 10.9)	67		
5											3.90 (dd, 11, 4.1)		3.91 (dd, 11.3, 5.2)			

[a] A doublet-like proton, X part of an ABX system with H-2 and H-3 overlapping and $J_{H-1/H-3} = 0$. [b] Interchangeable values

at *m/z* 813 [M − H − 2 × 132 − 3 × 146]⁻ assigned to the loss of the ester-linked sugar chain A and a second ion at *m/z* 681 [M − H − 3 × 132 − 3 × 146]⁻ indicating the loss of a supplementary pentose unit. The MS^4 fragmentation of the ion at *m/z* 681 gave an ion at *m/z* 519 [aglycon − H]⁻ due to the loss of a glucose unit and corresponding to the 16α-hydroxyprotobassic acid core. These results confirmed that saponins **5** and **8** contained the same seven sugar units, with the only difference between them being in the position of substitution of the apiose on the glucose as previously discussed. Thus, saponin **5** was deduced to be 3-*O*-(β-D-apiofuranosyl-(1→3)-β-D-glucopyranosyl)-28-*O*-[α-L-rhamnopyranosyl-(1→3)-β-D-xylopyranosyl-(1→4)-[α-L-rhamnopyranosyl-(1→3)-]-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl]-16α-hydroxyprotobassic acid and saponin **8** as 3-*O*-(β-D-apiofuranosyl-(1→4)-β-D-glucopyranosyl)-28-*O*-[α-L-rhamnopyranosyl-(1→3)-β-D-xylopyranosyl-(1→4)-[α-L-rhamnopyranosyl-(1→3)-]-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl]-16α−hydroxyprotobassic acid. As saponins **6** and **10** differed from saponins **5** and **8** in the nature of the most inner pentose in the ester glycosidic chain, saponin **6** was identified as 3-*O*-(β-D-apiofuranosyl-(1→3)-β-D-glucopyranosyl)-28-*O*-[α-L-rhamnopyranosyl-(1→3)-β-D-xylopyranosyl-(1→4)-[α-L-rhamnopyranosyl-(1→3)-]-α-L-rhamnopyranosyl-(1→2)-β-D-xylopyranosyl]-16α-hydroxyprotobassic acid, and saponin **10** as 3-*O*-(β-D-apiofuranosyl-(1→4)-β-D-glucopyranosyl)-28-*O*-[α-L-rhamnopyranosyl-(1→3)-β-D-xylopyranosyl-(1→4)-[α-L-rhamnopyranosyl-(1→3)-]-α-L-rhamnopyranosyl-(1→2)-β-D-xylopyranosyl]-16α-hydroxyprotobassic acid.

The positive ESIMS of saponin **11** showed a quasi-molecular ion peak at *m/z* 1523 [M + Na]⁺, and in the negative-ion mode a quasi-molecular ion peak at *m/z* 1499 [M − H]⁻, 16 mass units less than that of **8**. The MS^n experiments of [M − H]⁻ gave the expected ion peaks at *m/z* 1353 [M − H − 146]⁻, 1207 [M − H − 2 × 146]⁻, and 797 [M − H − 2 × 132 − 3 × 146]⁻, indicating the loss of the ester glycosidic chain A, and at *m/z* 665 [M − H − 3 × 132 − 3 × 146]⁻ assigned to the loss of a terminal apiose unit. The interpretation of both its ESIMS and NMR spectra confirmed that saponin **11** contains the same sugar chain as in saponin **8** but carries protobassic acid as genin, so saponin **11** was deduced to be 3-*O*-(β-D-apiofuranosyl-(1→4)-β-D-glucopyranosyl)-28-*O*-[α-L-rhamnopyranosyl-(1→3)-β-D-xylopyranosyl-(1→4)-[α-L-rhamnopyranosyl-(1→3)-]-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl]protobassic acid.

The positive ESIMS of saponins **13** and **14** both showed a quasi-molecular ion peak at *m/z* 1687 [M + Na]⁺, while, in the negative mode, a quasi-molecular ion was observed at *m/z* 1661 [M − H]⁻, in agreement with a molecular formula of $C_{75}H_{122}O_{40}$. The MS^2 fragmentation of the [M − H]⁻ in these two compounds gave an ion peak at *m/z* 1515 [M − H − 146]⁻ attributed to the loss of terminal rhamnose, confirming that these compounds possess a supplementary rhamnose in comparison with other saponins obtained (**5**, **6**, **8**, and **10**). The MS^2 experiment gave the expected fragments at *m/z* 959 (negative mode) and *m/z* 983 (positive mode) corresponding to the loss of a pentasaccharide unit (chain A) attached to C-28 of the aglycon. The MS^4 experiment gave a positive fragment at *m/z* 793 [M + Na − 2 × 132 − 4 × 146 − CO_2]⁺, confirming the presence of a supplementary terminal rhamnose unit in the chain linked to C-3. The similarity in MS^n fragmentation confirmed that the only difference between saponins **13** and **14** was the nature of the first pentose unit of the ester glycosidic chain. Thus, saponin **13** was assigned as 3-*O*-(β-D-apiofuranosyl-(1→4)-[α-L-rhamnopyranosyl-(1→3)-]-β-D-glucopyranosyl)-28-*O*-[α-L-rhamnopyranosyl-(1→3)-β-D-xylopyranosyl-(1→4)-[α-L-rhamnopyranosyl-(1→3)]-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl]-16α-hydroxyprotobassic acid, and saponin **14**, 3-*O*-(β-D-apiofuranosyl-(1→4)-[α-L-rhamnopyranosyl-(1→3)-]-β-D-glucopyranosyl)-28-*O*-[α-L-rhamnopyranosyl-(1→3)-β-D-xylopyranosyl-(1→4)-[α-L-rhamnopyranosyl-(1→3)]-α-L-rhamnopyranosyl-(1→2)-β-D-xylopyranosyl]-16α-hydroxyprotobassic acid.

Saponins **2** and **3** displayed a quasi-molecular ion peak in the ESIMS positive mode at *m/z* 1377 [M + Na]⁺ and in the negative mode at *m/z* 1353 [M − H]⁻, corresponding to a molecular formula of $C_{63}H_{102}O_{31}$. The MS^2 experiment of [M + Na]⁺ gave a positive fragment at *m/z* 711 assigned to the loss of the glycoside chain consisting of three pentoses and two rhamnoses (ester chain B), while the MS^3 and MS^4 fragmentation gave similar peaks, confirming the isomerism of these two compounds. Saponin **2** was concluded to be 3-*O*-(β-D-glucopyranosyl)-28-*O*-(α-L-rhamnopyranosyl-(1→3)-[β-D-xylopyranosyl-(1→4)]-β-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl)protobassic acid, and saponin **3**, 3-*O*-(β-D-glucopyranosyl)-28-*O*-(α-L-rhamnopyranosyl-(1→3)-[β-D-xylopyranosyl-(1→4)]-β-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-β-D-xylopyranosyl)protobassic acid.

The positive quasi-molecular ion [M + Na]⁺ was detected at *m/z* 1377 for **12** and 1393 for **9**, and their corresponding negative [M − H]⁻ ions were observed at *m/z* 1353 and 1369, respectively. The MS^2 or MS^3 fragmentation of [M − H]⁻ gave an ion analyzed as [M − H − 2 × 132 − 2 × 146]⁻, attributed to the loss of the ester tetraglycosidic chain, and detected at *m/z* 797 (for **12**) and *m/z* 813 (for **9**). The MS fragmentation of these ions produced the loss of the apiose and gave ions at *m/z* 665 and 681, respectively. Finally, the MS^4 fragmentation gave an ion corresponding to the aglycon part at *m/z* 503 for protobassic acid and *m/z* 519 for 16α-hydroxyprotobassic acid, respectively. These results were in agreement with the suggestion that saponin **12** contained the same sugar chain as in saponin **9** but attached to protobassic acid instead of 16α-hydroxyprotobassic acid. Therefore saponin **12** was assigned as 3-*O*-(β-D-apiofuranosyl-(1→4)-β-D-glucopyranosyl)-28-*O*-(α-L-rhamnopyranosyl-(1→3)-β-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl)protobassic acid and saponin **9** as 3-*O*-(β-D-apiofuranosyl-(1→4)-β-D-glucopyranosyl)-28-*O*-(α-L-rhamnopyranosyl-(1→3)-β-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl)-16α-hydroxyprotobassic acid.

Compared with the MS of saponins **9** and **12**, the MS of saponins **1** and **4** were 16 amu different due to the nature of the aglycon: protobassic acid for **4** and 16α-hydroxyprotobassic acid for **1**. Saponin **4** exhibited quasi-molecular ion peaks at *m/z* 1391 [M + Na]⁺ and at *m/z* 1367 [M − H]⁻, corresponding to a molecular formula of $C_{64}H_{104}O_{31}$. The quasi-molecular ion peaks detected at *m/z* 1407 [M + Na]⁺ and *m/z* 1383 [M − H]⁻ for saponin **1** confirmed that this compound possessed an additional hydroxyl in comparison with saponin **4**. The MS^n fragmentation of [M − H]⁻ was similar: the MS^2 experiment gave an intense ion peak at *m/z* 1221 (or 1237) [M − H − 146]⁻, attributed to the loss of terminal rhamnose, and the MS^3 gave a fragment at *m/z* 1075 (or 1091) [M − H − 2 × 146]⁻, indicating a loss of a second terminal rhamnose unit, and at *m/z* 665 (or 681) [M − H − 2 × 132 − 3 × 146]⁻, assigned to the loss of the three rhamnoses and the two pentoses of the ester chain A. Thus, saponin **4** was deduced to be 3-*O*-

(β-D-glucopyranosyl)-28-*O*-(α-L-rhamnopyranosyl-(1→3)-β-D-xylopyranosyl-(→4)[α-L-rhamnopyranosyl-(1→3)]-α-L-rhamnopyranosyl-(1→2)-β-D-xylopyranosyl)protobassic acid, and saponin **1** was assigned to 3-*O*-(β-D-glucopyranosyl)-28-*O*-(α-L-rhamnopyranosyl-(1→3)-β-D-xylopyranosyl-(1→4)[α-L-rhamnopyranosyl-(1→3)]-α-L-rhamnopyranosyl-(1→2)-β-D-xylopyranosyl)-16α-hydroxyprotobassic acid.

Saponin **7** exhibited quasi-molecular ion peaks at *m/z* 1525 $[M + Na]^+$ and *m/z* 1501 $[M - H]^-$, indicating a molecular formula of $C_{68}H_{110}O_{36}$. The MS^2 experiment gave an ion peak at *m/z* 813 $[M - H - 3 \times 132 - 2 \times 146]^-$ attributed to the loss of the ester glycosidic chain composed of three pentose and of two rhamnose units. The MS^3 fragmentation provided the expected ion for the loss of the ester chain B at *m/z* 681 $[M - H - 4 \times 132 - 2 \times 146]^-$ and a second ion at *m/z* 519 $[M - H - 4 \times 132 - 2 \times 146 - 162]^-$ due to the loss of the diglycosidic chain (apiose−glucose) linked at C-3 of 16α-hydroxyprotobassic acid. Saponin **7** was therefore assigned as 3-*O*-(β-D-apiofuranosyl-(1→3)-β-D-glucopyranosyl)-28-*O*-(α-L-rhamnopyranosyl-(1→3)[β-D-xylopyranosyl-(1→4)]-β-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-β-D-xylopyranosyl)-16α-hydroxyprotobassic acid.

The last saponin isolated, compound **15** (ESIMS *m/z* 1245 $[M + Na]^+$), was identified as 3-*O*-(β-D-glucopyranosyl)-28-*O*-[α-L-rhamnopyranosyl-(1→3)-β-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-β-D-xylopyranosyl]protobassic acid, a saponin previously isolated from the roots of *Sideroxylon cubense* and named sideroxyloside A. An examination of the NMR data published for this compound did not produce a perfect match, however, with a major discrepancy being found for the C-2 of the xylose ester: δ 80.5 in ref 10 vs 76.3 for compound **15** (and δ 76.5 ± 0.3 in the series). Given the glycosylation shift (about +6 ppm) due to the rhamnose unit, the 76.3 value was explained by the shielding γ effect arising from the ester carbonyl acting via the oxygen atom of the glycosidic bond (−4 ppm). In chrysantellins A and B[15,16] and in saponins from *Tridesmostemon claessenssi*,[17] the chemical shift of C-2 of the xylose ester substituted at this position was also observed between 76 and 77 ppm. In the absence of a direct comparison between these samples, it was not possible to conclude definitively whether **15** and sideroxyloside A are the same.

Glycosides of protobassic acid seem to have been found exclusively in the family Sapotaceae, while glycosides of 16α-hydroxyprotobassic acid have been isolated from different species of Sapotaceae and Rubiaceae.[18,19] The detection of saponins with the ester chain containing the tetrasaccharide rhamnopyranosyl-(1→3)-xylopyranosyl-(1→4)-rhamnopyranosyl-(1→2)-xylopyranoside is quite rare, and the present elucidation seems to be the third example of this occurrence.[10,17] In contrast the isomeric rhamnopyranosyl-(1→3)-xylopyranosyl-(1→4)-rhamnopyranosyl-(1→2)-arabinopyranoside is a quite common unit in saponins from the fruits of plants in the Sapotaceae and from species of the Asteraceae, Verbenaceae, Lamiaceae, and Cucurbitaceae families.[20]

Experimental Section

General Experimental Procedures. Optical rotations were determined in MeOH or H_2O with a Perkin-Elmer 241 automatic polarimeter. ^1H and ^{13}C NMR spectra were recorded in CD_3OD or $CDCl_3$ on a Bruker Avance DRX-500 spectrometer operating at 500 and 125 MHz, respectively, and 2D-NMR experiments were performed using standard Bruker microprograms. ESIMS and MS-MS experiments were recorded on a Bruker Esquire-LC ion trap mass spectrometer. The samples were introduced by infusion in a solution of MeOH. TLC were carried out on precoated silica gel 60 F 254 (Merck), and spots were visualized by spraying with 50% H_2SO_4. Kieselgel 60 (63−200 μm, Merck) and Lichroprep RP-18 (40−63 μm, Merck) were used for column chromatography. HPLC was performed on a Dionex apparatus equipped with an ASI-100 automated sample injector, a P 580 pump, a STH 585 column oven, a diode array detector UVD 340S, and the Chromeleon software. Prepacked columns (Dionex RP-18, 201 SP, 10 × 250 mm, 5 mm) were used for semipreparative HPLC with a binary gradient or isocratic eluent (solvent A: H_2O−TFA, 0.025%, solvent B: MeCN) and a flow rate of 3 mL min^{-1}; chromatograms were monitored at 205 nm.

Plant Material. The leaves of *M. laurifolia* were collected in the Egyptian Museum gardens, Cairo, Egypt, in July and August 2001. A voucher specimen is kept in the herbarium of Cairo University, CAI. ed. 8, 1990.

Extraction and Isolation. The air-dried powdered leaves of *M. laurifolia* (3 kg) was extracted three times with MeOH to obtain a concentrated extract (450 g). Part of this extract (100 g) was suspended in MeOH (500 mL) and precipitated by addition of a large excess of Me_2CO (3.5 L). The resulting precipitate was filtered and dried over KOH. The dried precipitate (60 g) was dissolved in H_2O and dialyzed against pure distilled H_2O in seamless cellulose tubing with agitation. After 72 h, the contents of the tube were frozen and lyophilized to afford the dialyzed saponin mixture (17 g). The crude saponin was fractionated on silica gel vacuum layer chromatography eluted with $CHCl_3$−MeOH (50:50) and $CHCl_3$−MeOH−H_2O (50:50:5). An aliquot of the saponin mixture (2 g) was chromatographed by RP-18 column chromatography using a gradient of MeOH−H_2O (40:60 to 60:40) to give 80 fractions. Fractions 25 and 26 were purified by preparative TLC with $CHCl_3$−MeOH−H_2O (50:50:7) to give **8** (16 mg) and **13** (10 mg). Fractions 28−30 were chromatographed on a silica gel column eluted with $CHCl_3$−MeOH−H_2O (70:30:4) to yield **5** (42 mg), **9** (8 mg), and **10**. The latter compound was finally purified by semipreparative HPLC with the gradient elution program 25 to 30% B (0 to 30 min) to afford 10 mg of **10**. Fractions 31−35 were purified on silica gel column chromatography using the same solvent as above to yield again **10** (86 mg) and **14** (36 mg). Fractions 36 and 37 were purified by semipreparative HPLC with the isocratic elution program 24% B (30 min) to give **1** (6 mg). Fractions 38−42 were chromatographed by silica gel column chromatography using the same solvent as above to yield **6** (97 mg) and **7** (27 mg). Fractions 52−61 eluted with MeOH−H_2O (55:45) were subjected to preparative TLC with $CHCl_3$−MeOH−H_2O (50:50:7) to give **2** (8 mg). Fractions 62−64 were purified by preparative TLC using the same solvent as above to give **11** (8 mg) and **12** (19 mg). Fractions 67−70 afforded **3** (180 mg). Fractions 75 and 76 were purified by silica gel column chromatography eluted with $CHCl_3$−MeOH−H_2O (70:30:2) to yield **4** (30 mg). Fraction 79 was finally purified on preparative TLC using the same solvent system as above to give **15** (11 mg).

Saponin 1: white powder; $[\alpha]^{21}_D$ − 42.3° (*c* 0.44, MeOH); IR (KBr) ν_{max} 3423, 2927, 1729, 1680, 1454, 1384, 1042 cm^{-1}; ^1H NMR of the aglycon (CD_3OD) δ 0.91 (3H, s, H-29), 0.99 (3H, s, H-30), 1.09 (3H, s, H-26), 1.09 (1H, br d, *J* = 13 Hz, H-19), 1.19 (1H, m, H-21), 1.20 (1H, dd, *J* = 14, 3 Hz, H-1), 1.33 (3H, s, H-24), 1.35 (1H, s, H-5), 1.36 (3H, s, H-27), 1.46 (1H, dd, *J* = 14.7, 3.1 Hz, H-15), 1.56 (1H, dd, *J* = 14, 2 Hz, H-7), 1.65 (3H, s, H-25), 1.67 (1H, dd, *J* = 11.8, 5 Hz, H-9,), 1.83 (1H, m, H-22), 1.84 (1H, dd, *J* = 14, 4 Hz, H-7), 1.85 (1H, dd, *J* = 14, 3 Hz, H-15), 1.91 (1H, m, H-22), 1.92 (1H, m, H-21), 2.00 (1H, ddd, *J* = 18, 5, 4 Hz, H-11), 2.08 (1H, dd, *J* = 14.5, 2.3 Hz, H-1), 2.14 (1H, ddd, *J* = 18, 12, 3 Hz, H-11), 2.31 (1H, dd, *J* = 13, 4 Hz, H-19), 3.03 (1H, dd, *J* = 14.5, 4.2 Hz, H-18), 3.44 (1H, d, *J* = 12 Hz, H-23), 3.6 (1H, d, *J* = 3.1 Hz, H-3), 3.75 (1H, d, *J* = 12 Hz, H-23), 4.37 (1H, br q, *J* = 3.2 Hz, H-2), 4.48 (1H, m, H-6), 4.5 (1H, br t, *J* = 3.5 Hz, H-16), 5.42 (1H, br t, *J* = 3.5 Hz, H-12); ^{13}C NMR of the aglycon (CD_3OD) δ 16.4 (C-24), 19.1 (C-26), 19.3 (C-25), 24.6 (C-11), 25 (C-30), 27.3 (C-27), 31.4 (C-20), 31.7 (C-22), 33.4 (C-29), 36.2 (C-15), 36.5

(C-21), 37.2 (C-10), 39.9 (C-8), 41.5 (C-7), 42.4 (C-18), 43.5 (C-14), 44.1 (C-4), 46.8 (C-1), 47.9 (C-19), 49 (C-9), 49.2 (C-5), 50.3 (C-17), 65.4 (C-23), 68.7 (C-6), 71.4 (C-2), 74.8 (C-16), 83.7 (C-3), 124.1 (C-12), 144 (C-13), 177.1 (C-28); ^{1}H and ^{13}C NMR of the glycosidic part, see Tables 1 and 4; ESIMS (negative-ion mode) m/z 1383.6 $[M - H]^-$; ESIMS-MS MS^2 (1383.6) m/z 1237.6 $[(M - H) - 146]^-$, MS^3 (1237.6) m/z 681.4 $[(M - H) -$ ester chain A $(2 \times 132 - 3 \times 146)]^-$, ESIMS (positive-ion mode) m/z 1407.9 $[M + Na]^+$; ESIMS-MS MS^2 (1407.9) m/z 725.6 [ester chain A $(2 \times 132 + 3 \times 146) + Na]^+$, MS^3 (725.5) m/z 579.4 [ester chain A $- 146 + Na]^+$, 579 [ester chain A $- 132 + Na]^+$, 447 [ester chain A $- 146 - 132 + Na]^+$, 429 $[447 - H_2O]^+$.

Saponin 2: white powder [α]$^{21}_{D}$ − 35.2° (c 0.25, MeOH); IR (KBr) ν_{max} 3423, 2925, 1647, 1384, 1049 cm^{-1}; ^{1}H NMR (CD_3-OD) of the aglycon δ 0.93 (3H, s, H-29), 0.97 (3H, s, H-30), 1.09 (3H, s, H-26), 1.16 (3H, s, H-27), 1.18 (1H, br d, J = 12 Hz, H-19), 1.18 (1H, br d, J = 14 Hz, H-1), 1.23 (1H, m, H-21), 1.34 (3H, s, H-24), 1.34 (1H, s, H-5), 1.42 (1H, td, J = 13, 4 Hz, H-21), 1.54 (1H, br d, J = 14 Hz, H-7), 1.59 (1H, dd, J = 12, 3 Hz, H-22) 1.62 (1H, m, H-9), 1.64 (1H, br d, J = 15.4 Hz, H-16), 1.75 (1H, t, J = 14.7 Hz, H-19), 1.77 (1H, m, H-15), 1.78 (1H, m, H-22), 1.81 (1H, dd, J = 15, 4 Hz, H-7), 2.00 (1H, dt, J = 18, 4.6 Hz, H-11), 2.03 (1H, m, H-16), 2.06 (1H, dd, J = 14, 2 Hz, H-1), 2.15 (1H, ddd, J = 18, 11, 4 Hz, H-11), 2.96 (1H, dd, J = 13.6, 4 Hz, H-18), 3.45 (1H, d, J = 11.5 Hz, H-23), 3.59 (1H, s, H-3), 3.75 (1H, d, J = 11 Hz, H-23), 4.35 (1H, dt, J = 3.5, 3 Hz, H-2), 4.50 (1H, m, $W_{1/2}$ = 7 Hz, H-6), 5.38 (1H, br t, J = 3 Hz, H-12); ^{13}C NMR (CD_3OD) of the aglycon δ 16.4 (C-24), 19 (C-26), 19.3 (C-25), 23.8 (C-16), 24 (C-30), 24.7 (C-11), 26.4 (C-27), 29.1 (C-15), 31.6 (C-20), 33.4 (C-22), 33.5 (C-29), 35 (C-21), 37.2 (C-10), 39.9 (C-8), 41.3 (C-7), 42.8 (C-18), 43.7 (C-14), 44.1 (C-4), 46.7 (C-1), 47.2 (C-19), 48.4 (C-17), 49.2 (C-5), 49.7 (C-9), 65.5 (C-23), 68.6 (C-6), 71.4 (C-2), 83.7 (C-3), 124.3 (C-12), 144.2 (C-13), 177.9 (C-28); ^{1}H and ^{13}C NMR of the glycosidic part, see Tables 1 and 3; ESIMS (negative-ion mode) m/z 1353.7 $[M - H]^-$; ESIMS-MS MS^2 (1353.7) m/z 1221.4 $[(M - H) - 132]^-$, 1207.4 $[(M - H) - 146]^-$, 1075.3 $[(M - H) - 132 - 146]^-$, 665.3 $[(M - H) -$ ester chain B $(3 \times 132 - 2 \times 146)]^-$, MS^3 (665.3) m/z 503.1 $[(M - H) -$ ester chain B $- 162]^-$; ESIMS (positive-ion mode) m/z 1377.8 $[M + Na]^+$; ESIMS-MS MS^2 (1377.8) m/z 711.3 [ester chain B $+ Na]^+$, MS^3 (711.3) m/z 579.2 [ester chain B $- 132 + Na]^+$, MS^4 (579.3) m/z 433.2 [ester chain B $- 132 - 146 + Na]^+$.

Saponin 3: white powder [α]$^{21}_{D}$ − 40.8° (c 0.46, MeOH); IR (KBr) ν_{max} 3416, 2933, 1742, 1647, 1461, 1383, 1046 cm^{-1}; ^{1}H and ^{13}C NMR of the glycosidic part, see Tables 1 and 4;[21] ESIMS (negative-ion mode) m/z 1353.8 $[M - H]^-$; ESIMS (positive-ion mode) m/z 1377.8 $[M + Na]^+$; ESIMS-MS MS^2 (1377.8) m/z 711.5 [ester chain B $+ Na]^+$, MS^3 (711.6) m/z 579.4 [ester chain B $- 132 + Na]^+$, MS^4 (579.4) m/z 433.4 [ester chain B $- 132 - 146 + Na]^+$.

Saponin 4: white powder [α]$^{21}_{D}$ −44.6° (c 0.44, MeOH); IR (KBr) ν_{max} 3406, 2929, 1736, 1655, 1384, 1044 cm^{-1}; ^{1}H and ^{13}C NMR of the glycosidic part, see Tables 1 and 4;[21] ESIMS (negative-ion mode) m/z 1367.4 $[M - H]^-$; ESIMS-MS MS^2 (1367.4) m/z 1221.4 $[(M - H) - 146]^-$, MS^3 (1221.4) m/z 1075.3 $[(M - H) - 2 \times 146]^-$, 665.3 $[(M - H) -$ ester chain A$]^-$; ESIMS (positive-ion mode) m/z 1391.7 $[M + Na]^+$; ESIMS-MS MS^2 (1391.6) m/z 725.3 [ester chain A $+ Na]^+$.

Saponin 5: white powder [α]$^{21}_{D}$ −61.7° (c 0.34, MeOH); IR (KBr) ν_{max} 3414, 2927, 1730, 1647, 1384, 1045 cm^{-1}; ^{1}H and ^{13}C NMR of the glycosidic part, see Tables 1 and 3;[21] ESIMS (negative-ion mode) m/z 1515.4 $[M - H]^-$; ESIMS-MS MS^2 (1515.9) m/z 1369.4 $[(M - H) - 146]^-$, 813.3 $[(M - H) -$ ester chain A$]^-$; ESIMS (positive ion mode) m/z 1539.9 $[M + Na]^+$; ESIMS-MS MS^2 (1539.6) m/z 837.5 $[(M + Na) -$ ester chain A$]^+$, MS^3 (837.7) m/z 793.6 $[(M + Na) -$ ester chain A $- CO_2]^+$.

Saponin 6: white powder [α]$^{21}_{D}$ −58.7° (c 0.52, MeOH); IR (KBr) ν_{max} 3405, 2931, 1729, 1680, 1437, 1384, 1042 cm^{-1}; ^{1}H and ^{13}C NMR of the glycosidic part, see Tables 1 and 4;[21] ESIMS (positive-ion mode) m/z 1539.9 $[M + Na]^+$; ESIMS-MS MS^2 (1539.9) m/z 837.9 $[(M + Na) -$ ester chain A$]^+$, MS^3 (837.8) m/z 793.8 $[(M + Na) -$ ester chain A $- CO_2]^+$.

Saponin 7: white powder [α]$^{21}_{D}$ −50.4° (c 0.5, MeOH); IR (KBr) ν_{max} 3403, 2925, 1736, 1646, 1459, 1381, 1044 cm^{-1}; ^{1}H and ^{13}C NMR of the glycosidic part, see Tables 1 and 4;[21] ESIMS (negative-ion mode) m/z 1501.9 $[M - H]^-$; ESIMS-MS MS^2 (1501.3) m/z 1369.4 $[(M - H) - 132]^-$, 1355.4 $[(M - H) - 146]^-$, 813.3 $[(M - H) -$ ester chain B$]^-$, MS^3 (1355.4) m/z 1223, 813, 681.2 $[(M - H) -$ ester chain B $- 132]^-$, 519.3 [aglycon $- H]^-$; ESIMS (positive-ion mode) m/z 1525.9 $[M + Na]^+$; ESIMS-MS MS^2 (1525.5) m/z 837.6 $[(M + Na) -$ ester chain B$]^+$, MS^3 (837.6) m/z 793.6 $[(M + Na) -$ ester chain B $- CO_2]^+$.

Saponin 8: white powder; [α]$^{21}_{D}$ −67.8° (c 0.39, MeOH); IR (KBr) ν_{max} 3409, 2926, 1733, 1647, 1457, 1383, 1045 cm^{-1}; ^{1}H and ^{13}C NMR of the glycosidic part, see Tables 2 and 3;[21] ESIMS (negative-ion mode) m/z 1515.6 $[M - H]^-$; ESIMS-MS MS^2 (1515.4) m/z 1369.3 $[(M - H) - 146]^-$, 813.3 $[(M - H) -$ ester chain A$]^-$, MS^3 (1369.3) m/z 813.4, 681 $[(M - H) -$ ester chain A $- 132]^-$, MS^4 (681.2) m/z 519.3 [aglycon $- H]^-$; ESIMS (positive-ion mode) m/z 1539.7 $[M + Na]^+$; ESIMS-MS MS^2 (1539.7) m/z 837.5 $[(M + Na) -$ ester chain A$]^+$, 725.3 [ester chain A $+ Na]^+$, MS^3 (837.6) m/z 793.5 $[(M + Na) -$ ester chain A $- CO_2]^+$.

Saponin 9: white powder [α]$^{21}_{D}$ −52.7° (c 0.26, MeOH); IR (KBr) ν_{max} 3404, 2927, 1735, 1655, 1382, 1047 cm^{-1}; ^{1}H and ^{13}C NMR of the glycosidic part, see Tables 2 and 3;[21] ESIMS (negative-ion mode) m/z 1369.6 $[M - H]^-$; ESIMS-MS MS^2 (1369.5) m/z 813.4 $[(M - H) -$ ester chain C$]^-$, MS^3 (813.4) m/z 681.2 $[(M - H) -$ ester chain C $- 132]^-$, 519.2 [aglycon $- H]^-$, MS^4 (681.3) m/z 519.2; ESIMS (positive-ion mode) m/z 1393.8 $[M + Na]^+$; ESIMS-MS MS^2 (1393.9) m/z 837.5 $[(M + Na) -$ ester chain C$]^+$, MS^3 (837.5) m/z 793.6 $[(M + Na) -$ ester chain C $- CO_2]^+$, MS^4 (793.5) m/z 661.4 $[(M + Na) -$ ester chain C $- CO_2 - 132]^+$.

Saponin 10: white powder [α]$^{21}_{D}$ − 62.0° (c 0.51, MeOH); IR (KBr) ν_{max} 3430, 2926, 1735, 1638, 1459, 1387, 1048 cm^{-1}; ^{1}H and ^{13}C NMR of the glycosidic part, see Tables 2 and 4;[21] ESIMS (negative-ion mode) m/z 1515.6 $[M - H]^-$; ESIMS-MS MS^2 (1515.7) m/z 1369.6 $[(M - H) - 146]^-$, 813.6 $[(M - H) -$ ester chain A$]^-$, ESIMS (positive-ion mode) m/z 1539.9 $[M + Na]^+$; ESIMS-MS MS^2 (1539.8) m/z 837.8 $[(M + Na) -$ ester chain A$]^+$, 725.6 [ester chain A $+ Na]^+$, MS^3 (837.8) m/z 793.8 $[(M + Na) -$ ester chain A $- CO_2]^+$.

Saponin 11: white powder [α]$^{21}_{D}$ − 59.8° (c 0.14, MeOH); IR (KBr) ν_{max} 3417, 2929, 1729, 1639, 1595, 1458, 1384, 1047 cm^{-1}; ^{1}H and ^{13}C NMR of the glycosidic part, see Tables 2 and 3;[21] ESIMS (negative-ion mode) m/z 1500.4 $[M - H]^-$; ESIMS-MS MS^2 (1500.1) m/z 1353.7 $[(M - H) - 146]^-$, MS^3 (1353.3) m/z 1207.4 $[(M - H) - 2 \times 146]^-$, 797.4 $[(M - H) -$ ester chain A$]^-$, MS^4 (797.4) m/z 665.3 $[(M - H) -$ ester chain A $- 132]^-$; ESIMS (positive ion mode) m/z 1524.5 $[M + Na]^+$; ESIMS-MS MS^2 (1523.8) m/z 967.5 $[(M + Na) - 2 \times 132 - 2 \times 146]^+$, 821.5 $[(M + Na) -$ ester chain A$]^+$, 725.3 [ester chain A $+ Na]^+$, MS^3 (967.6) m/z 821.5, MS^3 (821.5) m/z 777.5 $[(M + Na) -$ ester chain A $- CO_2]^+$.

Saponin 12: white powder [α]$^{21}_{D}$ −44.8° (c 0.48, MeOH); IR (KBr) ν_{max} 3425, 2930, 1744, 1639, 1459, 1383, 1050 cm^{-1}; ^{1}H and ^{13}C NMR of the glycosidic part, see Tables 2 and 3;[21] ESIMS (negative-ion mode) m/z 1353.7 $[M - H]^-$; ESIMS-MS MS^2 (1353.4) m/z 1207.4 $[(M - H) - 146]^-$, MS^3 (1207.4) m/z 1075.3 $[(M - H) - 146 - 132]^-$, 797.4 $[(M - H) -$ ester chain C$]^-$, MS^4 (797.4) m/z 665.3 $[(M - H) -$ ester chain C $- 132]^-$, 503.2 [aglycon $- H]^-$; ESIMS (positive-ion mode) m/z 1378.1 $[M + Na]^+$; ESIMS-MS MS^2 (1378.5) m/z 821.6 $[(M + Na) -$ ester chain C$]^+$, 579.2 [ester chain C $+ Na]^+$, MS^3 (821.6) m/z 777.5 $[(M + Na) -$ ester chain C $- CO_2]^+$.

Saponin 13: white powder [α]$^{21}_{D}$ −58.9° (c 0.43, MeOH); IR (KBr) ν_{max} 3430, 2924, 1728, 1655, 1457, 1383, 1045 cm^{-1}; ^{1}H and ^{13}C NMR of the glycosidic part, see Tables 2 and 3;[21] ESIMS (negative-ion mode) m/z 1661.5 $[M - H]^-$; ESIMS-MS MS^2 (1661.4) m/z 1515.4 $[(M - H) - 146]^-$, 959.2 $[(M - H) -$ ester chain A$]^-$; ESIMS (positive-ion mode) m/z 1686.6 $[M + Na]^+$; ESIMS-MS MS^2 (1685.8) m/z 983.5 $[(M + Na) -$ ester chain A$]^+$, MS^3 (983.5) m/z 939.5 $[(M + Na) -$ ester chain A $- CO_2]^+$, 837.5 $[(M + Na) -$ ester chain A $- 146]^+$, 793.6 [(M +

Na) − ester chain A − 146 − CO_2]$^+$, MS^4 (793.6) *m/z* 661.5 [(M + Na) − ester chain A − 146−132 − CO_2]$^+$.

Saponin 14: white powder $[\alpha]^{21}_D$ −70.2° (*c* 0.5, MeOH); IR (KBr) ν_{max} 3417, 2929, 1735, 1647, 1458, 1384, 1044 cm^{-1}; ^1H and ^{13}C NMR of the glycosidic part, see Tables 2 and 4;[21] ESIMS (negative-ion mode) *m/z* 1662.4 [M − H]$^-$; ESIMS-MS MS^2 (1662.4) *m/z* 1515.6 [(M − H) −146]$^-$, 1369.4 [M − H) − 2 × 146]$^-$, 959.3 [(M − H) − ester chain A]$^-$, MS^3 (959.2) *m/z* 827.4 [(M − H) − ester chain A − 132]$^-$, 813.4 [(M − H) − ester chain A − 146]$^-$, 681.2 [(M − H) − ester chain A − 132 − 146]$^-$, 519.2 [(M − H) − ester chain A − 132 − 146 − 162]$^-$; ESIMS (positive-ion mode) *m/z* 1686.7 [M + Na]$^+$; ESIMS-MS MS^2 (1686.1) *m/z* 983.6 [(M + Na) − ester chain A]$^+$, MS^3 (983.6) *m/z* 939.5 [(M + Na) − ester chain A − CO_2]$^+$, MS^4 (939.5) *m/z* 793.6 [(M + Na) − ester chain A −146 − CO_2]$^+$, MS^5 (793.6) *m/z* 661.4 [(M + Na) − ester chain A − 132−146 − CO_2]$^+$.

Saponin 15: white powder $[\alpha]^{21}_D$ −28.5 (*c* 0.41, MeOH); IR (KBr) ν_{max} 3406, 2928, 1741, 1639, 1571, 1458, 1383, 1048 cm^{-1}; ^1H and ^{13}C NMR of the glycosidic part, see Tables 1 and 4;[21] ESIMS (positive-ion mode) *m/z* 1245 [M + Na]$^+$.

Acid Hydrolysis of Saponins. The crude saponin mixture (100 mg) was refluxed with 30 mL of 0.02 N H_2SO_4 and 6% $HClO_4$ (1: 1) at 140° for 5 h. After filtration the acid aqueous layer was neutralized with 1 N KOH and freeze-dried. Five sugars were identified and compared with authentic samples by TLC using MeCOEt−isoPrOH−Me_2CO−H_2O (20:10:7:6) as glucose, xylose, arabinose, rhamnose, and apiose. The purification of these sugars was achieved by preparative TLC using Kieselgel 60 plates, which were eluted three times with $CHCl_3$−MeOH:−H_2O (70:30:3) to afford xylose (R_f 0.5, $[\alpha]^{21}_D$ +52 to +12; H_2O; 16 h), arabinose (R_f 0.42, $[\alpha]^{21}_D$ +90; H_2O), and glucose (R_f 0.28, $[\alpha]^{21}_D$ +62; H_2O). Final purification of rhamnose and apiose was performed by preparative TLC eluted four times with $CHCl_3$−MeOH−H_2O (70:30:1) to give the rhamnose (R_f 0.69, $[\alpha]^{21}_D$ +9; H_2O) and apiose (R_f 0.64, $[\alpha]^{21}_D$ +17; H_2O).

Acetylation of Saponin 10. Saponin **10** (10 mg) was suspended in 3 mL of CH_2Cl_2 together with 100 μL of Ac_2O, 50 μL of Et_3N, and 5 mg of DMAP. The suspension was stirred at room temperature, for 48 h, and then was washed with 10% $CuSO_4$ and distilled water until a neutral pH was obtained. This solution was filtered over anhydrous Na_2SO_4 and evaporated to give 8 mg of a wax which was purified by silica gel column chromatography using a gradient of hexane−AcOEt (50:50 to 30:70) to give derivative **10a**: ^1H NMR ($CDCl_3$) δ 1.19 (3H, d, J = 6.3 Hz, rha′-6), 1.21 (3H, d, J = 6.3 Hz, rha″-6), 1.28 (3H, d, J = 5.8 Hz, rha-6), 3.39 (1H, dd, J = 11, 10 Hz, xyl′-5), 3.49 (1H, d, J = 3.8 Hz, H-3), 3.62 (1H, m, glc-5), 3.65 (1H ester, t, J = 3 Hz, xyl-2), 3.7 (1H, t, J = 9.5 Hz, rha-4), 3.71 (1H, dd, J = 9, 7 Hz, glc-4), 3.76 (1H, brd, J = 12 Hz, xyl-5 ester), 3.77 (1H, m, rha-5), 3.79 (1H, d, J = 10 Hz, api-5), 3.83 (1H, dq, J = 9.5, 6 Hz, rha′-5), 3.99 (1H, m, rha″-5), 4.01 (1H, dd, J = 9, 3.5 Hz, rha-3), 4.06 (1H, dd, J = 10, 3 Hz, xyl′-5), 4.09 (1H, d, J = 11 Hz, api-5), 4.1 (1H, d, J = 14 Hz, api-4), 4.12 (1H, m, xyl′-3), 4.13 (1H, dd, J = 11, 3 Hz, xyl-5 ester), 4.22 (1H, d, J = 14 Hz, api-4), 4.24 (1H, dd, J = 12, 8 Hz, glc-6), 4.44 (1H, dd, J = 12, 3 Hz, glc-6), 4.58 (1H, d, J = 8 Hz, glc-1), 4.83 (1H, d, J = 8 Hz, xyl′-1), 4.85 (1H, d, J = 1.5 Hz, xyl-4 ester), 4.91 (2H, d, J = 1.5 Hz, rha-1 and rha″-1), 4.96 (1H, d, J = 3.3 Hz, api-1), 4.98 (1H, t, J = 8 Hz, glc-2), 4.98 (1H, m, xyl′-4), 4.99 (1H, t, J = 8.5 Hz, xyl′-2), 5.0 (1H, s, rha′-1), 5.06 (1H, m, rha-2), 5.06 (1H, t, J = 10 Hz, rha″-4), 5.06 (1H, dd, J = 5, 2 Hz, xyl-3 ester), 5.12 (1H, t, J = 10 Hz, rha′-4), 5.12 (1H, m, rha"-2), 5.12 (1H, m, rha″-3), 5.21 (1H, t, J = 8 Hz, glc-3), 5.21 (1H, dd, J = 9, 3 Hz, rha′-3), 5.22 (1H, d, J = 3 Hz, api-2), 5.52 (1H, dd, J = 3, 1.5 Hz, rha′-2), 5.56 (1H, br t, J = 3 Hz, H-12), 5.63 (1H, m, H-16), 6.12 (1H, d, J = 2 Hz, xyl-1 ester).

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(21) Saponins **3**, **4**, **11**, **12**, and **15**: ^1H and ^{13}C NMR chemical shift values of the aglycon were identical with those described for saponin **2** within ±0.07 and ±0.1 ppm, respectively, and are not described separately. Saponins **5**−**10**, **13**, and **14**: ^1H and ^{13}C NMR chemical shift values of the aglycon are identical with those described for saponin **1** within ±0.08 and ±0.1 ppm, respectively.

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