Acylated Triterpenoid Saponins from the Stem Bark of *Foetidia africana*

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Nine new acylated triterpenoid saponins (**4**−**12**) were isolated from the stem bark of *Foetidia africana*. They all possess barringtogenol C as the aglycone, esterified by acetic and/or isovaleric acids. The sugar chain consists of up to three units: D-glucuronic acid (GlcUA) linked to C-3 of the aglycone and substituted by D-galactose (Gal) (at GlcUA C-2) and/or L-rhamnose (Rha) (at GlcUA C-4). The structures were established by acid and alkaline hydrolysis, by NMR experiments including ^{1}H−^{1}H (COSY, HOHAHA, ROESY) and ^{1}H−^{13}C (HSQC, HMBC) spectroscopy, and by mass spectrometry (ESIMS, ESIMS^n).

The genus *Foetidia* belongs to the subfamily of Foetidioideae (Lecythidaceae) and is distributed in Madagascar, Mauritius, and East Africa.[1] *F. africana* Verdc. is the first described species from the african mainland, namely, Tanzania.[2] The aqueous ethanolic extract from the stem bark exhibits a significant antitrypanosomal activity with an IC_{50} value of 1.3 μg/mL on the growth of the parasites.[3] The crude saponin mixture has been shown to significantly stimulate the glycosaminoglycans production from human fibroblastes in the skin (140% of activity at 10 μg/mL).[4] Following this work, we herein report the isolation and structural elucidation of nine new saponins (**4**−**12**) and of three prosapogenins (**1**−**3**), which have not been previously described, from *Foetidia africana* stem bark collected in Tanzania.

Results and Discussion

Dried and powdered stem bark of *F. africana* was extracted with boiling methanol, and after filtration, the solution was evaporated. The solid residue was dissolved in pure methanol and precipitated in acetone. The precipitate was dialyzed against pure water, and then the solution was freeze-dried. A combination of repeated silica gel column chromatography and preparative TLC allowed the purification of nine saponins (**4**−**12**). Preliminary investigation of the NMR spectra showed these compounds to possess acetyl and/or isovaleroyl ester groups and the typical set of methyls of the oleanene skeleton and, consequently, that they consisted of a set of regioisomeric acylated polyhydroxyoleanene triterpene oligoglycosides. To identify the triterpene oligoglycosidic part and to simplify the subsequent analysis of the NMR spectra, an aliquot of the crude saponin fraction was hydrolyzed in basic medium to yield three prosapogenins (**1**−**3**). Similarly, an acid hydrolysis was performed, followed by the isolation of the sugars and their characterization as L-arabinose (Ara), L-rhamnose (Rha), D-galactose (Gal), and D-glucuronic acid (GlcUA).

The triterpene part of the saponins and consequently of the prosapogenins (**1**−**3**) was recognized to be barringtogenol C by ^{1}H and ^{13}C NMR analysis using the set of connectivities observed in H−H COSY, HSQC, and HMBC experiments (Tables 1 and 2). The pertinent signals for seven angular methyl groups, for five deshielded oxygen-bearing positions (H-3, H-16, H-21, H-22, and CH_2-28), and for one trisubstituted ethylenic bond (H-12) were straightforwardly detected. The stereochemistry was ascertained by observation of ROESY interactions between H-3/CH_3-23, H-3/H-5, H-12/H-18, H-18/CH_3-30, H-18/H-28, H-18/H-22, and H-21/CH_3-29. The ^{1}H and ^{13}C assignments were in full agreement with the values published for barringtogenol C.[5−9]

The positive ESIMS of prosapogenin **1** displayed a pair of molecular ion peaks at *m*/*z* 835 $[M+Na]^+$ and 851 $[M+K]^+$, which, in conjunction with the analysis of ^{13}C NMR spectrum, suggested that the molecule had a $C_{42}H_{68}O_{15}$ composition. The MS/MS fragmentation of the $[M+Na]^+$ peak yielded intense product ions at *m*/*z* 689 $[M+Na-146]^+$ and 513 $[M+Na-146-176]^+$, indicating the presence of a diglycoside chain composed of a uronic acid and a terminal desoxyhexose. The identification of an α-L-rhamnopyranosyl unit was readily supported by the characteristic methyl doublet at δ 1.24 (J = 6.2 Hz), the typical singlet of an anomeric proton at δ 4.77, and the triplet for Rha H-4 at δ 3.36 (J = 9.2 Hz). The presence of a β-D-glucuronic acid was deduced from the analysis of COSY and HOHAHA experiments starting from the second anomeric proton at δ 4.38 (d, J = 8.0 Hz) and was supported by the observation of a carbonyl signal at δ 173.6 in the *J*-modulated ^{13}C NMR spectrum. The deshielding of C-3 of barringtogenol C and of C-4 of glucuronic acid allowed to locate the rhamnosyl-(1→4)-glucuronic acid chain at position 3 of barringtogenol C. This sequence was confirmed by the observation of ROE connectivities between H-3 of the aglycone and H-1 of the glucuronic acid and between H-4 of glucuronic acid and H-1 of rhamnose. The corresponding $^3J_{H-C}$ correlations through the glycosidic linkages (H−C−O−C) were also detected in the HMBC spectrum. Thus, compound **1** was 3-*O*-α-L-rhamnopyranosyl(1→4)-β-D-glucuronopyranosylbarringtogenol C.

Comparison of the NMR spectra of prosapogenin **2** and of compound **1** indicated that **2** possessed one supplementary sugar unit. The molecular ion peak observed at *m*/*z* 997 $[M+Na]^+$ in the positive ESIMS and the detection of five CHOH and one CH_2OH in the *J*-modulated ^{13}C NMR spectrum suggested that this sugar was a hexose (Table 3). The proton coupling constants of this hexose were larger than 6.5 Hz, except the coupling between H-3 and H-4 (J = 3.0 Hz), which is characteristic for a β-D-galactose. As in compound **1**, the two other osidic units of **2** were α-L-

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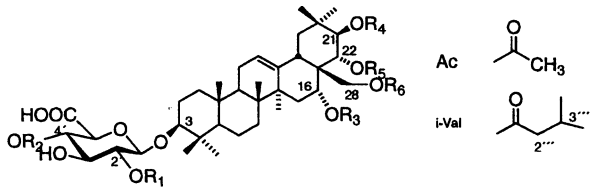

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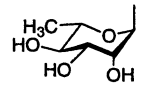
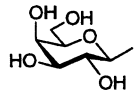
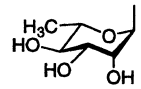
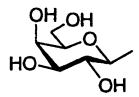
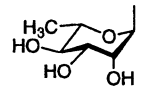
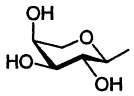
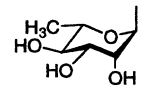
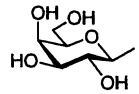
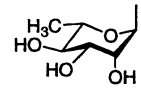
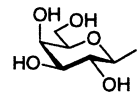
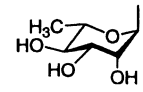
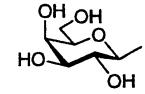
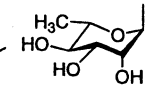
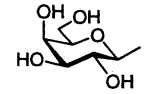
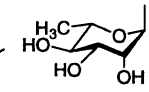
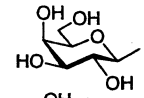
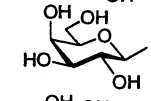
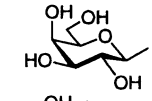
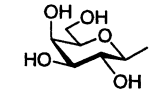
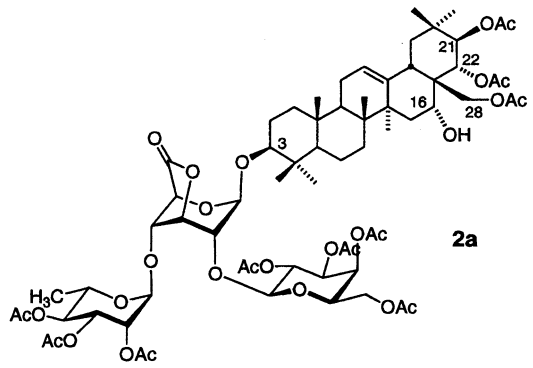
1	H		H	H	H	H
2			H	H	H	H
3			H		H	H
4	H		Ac	Ac	i-Val	H
5			Ac	H	H	H
6			H	H	H	i-Val
7			H	Ac	H	i-Val
8			Ac	Ac	H	i-Val
9		H	H	Ac	i-Val	H
10		H	H	Ac	H	i-Val
11		H	Ac	Ac	i-Val	H
12		H	Ac	Ac	H	i-Val

rhamnose and β-D-glucuronic acid (Table 3). In this compound, the nature of the uronic acid could not be directly determined by the analysis of the proton NMR spectra because of serious overlap between signals for H-3, H-4, and H-5. In the HMBC experiment, the anomeric proton of galactose showed a correlation with the C-2 of the uronic acid, and the anomeric proton of the uronic acid with C-3 of the aglycone. ROE correlations were observed between the set of uronic protons and the anomeric proton of rhamnose. The ^{13}C chemical shifts of the triosidic chain of **2** were in agreement with those reported in the literature for a β-glucuronic acid disubstituted at positions 2 and 4.[8,10] To confirm the presence of β-glucuronic acid with a rhamnosyl residue located at position 4, compound **2** was acetylated into the derivative **2a**. Its mass spectrum showed a molecular peak $[M+H+Na]^+$ at *m/z* 1400, in agreement with a molecular formula $C_{68}H_{96}O_{29}$ for a decaacetate lactone derivative. The resonance of H-16 at δ_H 4.19 showed that this hindered position of barringtogenol C was not acetylated. The multiplicities and coupling constants of the protons of the terminal β-D-galactose and α-L-rhamnose were distinctly recognized. The COSY and HOHAHA spectra confirmed the presence of the β-D-glucuronic acid moiety of **2**; however, the coupling constants were in agreement with a (3,6)-glucuronolactone unit in which the carboxylic acid has reacted with the hydroxy group at GlcUA C-3, with the subsequent inversion of the pyranoid ring conformation.[11] The chemical shifts of the ^{13}C spectrum of **2a** were obtained from the HSQC and HMBC correlations. The presence of a glucuronolactone was confirmed by the $^3J_{H-C}$ correlation observed between H-3 of this unit and the lactone carbonyl at δ_C 171.0. In the HMBC spectrum, the anomeric proton of galactose showed a correlation with C-2 of glucuronolactone, and H-1 of rhamnose showed correlation with C-4, which corroborated the supposed osidic sequence. Thus, prosapogenin **2** was assigned to be 3-*O*-{[β-D-galactopyranosyl-(1→2)]-α-L-rhamnopyranosyl(1→4)-β-D-glucuronopyranosyl}-barringtogenol C.



Comparison of the NMR spectra of compound **3** and of prosapogenins **1** and **2** indicated that **3** exhibited anomeric signals for a fourth sugar at δ_H 4.22 (d, $J = 7.6$ Hz) and δ_C 106.8 (Table 3). In the ESIMS a negative molecular ion at *m/z* 1105 $[M-H]^-$ and a positive molecular ion at *m/z* 1151 $[M+2Na-H]^+$ indicated a molecular weight of 1106, compatible with a molecular formula of $C_{53}H_{86}O_{24}$. The MS^2 of ion 1105 showed fragment ions at *m/z* 941 $[M-H-\text{rhamnose}-H_2O]^-$, 897 $[M-H-\text{rhamnose}-H_2O-CO_2]^-$, 761 $[M-H-\text{rhamnose}-\text{galactose}-2H_2O]^-$, and 747 $[M-H-\text{rhamnose}-\text{pentose}-2H_2O-CO_2]^-$. These results agreed well with the attachment of a pentose to the aglycone of substructure **2**; it was identified as an α-L-arabinopyranosyl after its proton multiplicities (Table 3). This arabinose was linked to C-21 of the aglycone owing to the observation of a ROESY correlation between the anomeric proton and H-21, itself identified through Overhauser interactions with the α-equatorial CH_3-29 and α-axial H-19. The deshielded value for C-21 at δ 91.4 confirmed that the arabinose unit was linked to O-21 of barringtogenol C. Compound **3** is thus 3-*O*-{[β-D-galactopyranosyl(1→2)]-α-L-rhamnopyranosyl(1→4)-β-D-glucuronopyranosyl}-21-*O*-α-L-arabinopyranosylbarringtogenol C.

Examination of the NMR spectra of saponins **4**−**12** showed that **4** was a triesterified derivative of prosapogenin **1**, while saponins **5**−**8** corresponded to different acylated derivatives of prosapogenin **2**. Saponins **9**−**12** possessed a degradated osidic chain lacking the rhamnose unit; no saponin was isolated containing the elements of prosapogenin **3** (Tables 1−3).

Comparison of the NMR data for **4**−**8** with those of prosapogenins **1** and **2** and the analysis of COSY, HOHAHA, and ROESY experiments allowed the full identification of the α-rhamnose-(1→4)-β-glucuronic acid chain in **4** and of

Table 1. ^{13}C NMR Data (δ) of Triterpene Part for Compounds **1**−**12** in CD_3OD

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
barringtogenol C												
1	39.9	39.9	39.9	40.0	40.0	40.1	40.0	40.3	40.0	40.0	39.9	40.1
2	27.0	27.1	nd	26.5	27.0	27.5	27.1	27.0	27.0	27.0	nd	27.0
3	91.1	91.4	90.9	90.5	90.8	90.9	91.0	90.8	90.9	90.8	90.8	90.8
4	40.2	40.3	40.3	40.2	40.3	40.4	40.4	40.3	40.4	40.4	40.4	40.5
5	57.0	57.0	57.0	56.9	57.0	57.1	57.1	57.0	57.0	57.0	57.0	57.1
6	19.3	19.3	nd	19.2	19.1	19.2	19.3	19.2	19.3	19.3	nd	19.3
7	34.0	34.0	34.0	33.8	34.0	34.1	34.0	34.0	34.0	34.0	33.9	33.9
8	40.9	40.9	40.9	41.1	41.0	41.0	40.9	41.0	41.0	40.9	41.0	41.1
9	48.1	48.0	48.2	48.1	47.9	48.8	48.0	48.0	48.0	48.0	48.0	48.0
10	37.8	37.7	37.7	37.9	37.7	38.8	37.7	37.7	37.7	37.7	37.7	37.9
11	24.6	24.6	24.5	24.7	24.5	24.5	24.6	24.7	24.6	24.6	nd	24.6
12	124.3	124.3	124.3	126.6	125.4	124.3	125.3	126.5	125.3	125.3	126.6	126.4
13	143.8	143.8	143.9	141.0[a]	141.1	143.5	143.0	141.4	143.0	143.0	141.2	142.0
14	42.5	42.5	42.5	41.8	42.3	42.5	42.4	42.1	42.3	42.3	42.0	40.4
15	34.5	34.5	34.5	31.5	31.2	34.6	34.9	31.7	34.7	34.9	31.6	32.2
16	68.6	68.6	68.6	72.5	72.3	69.3	68.8	71.9	69.4	68.8	72.5	72.1[a]
17	47.8	47.8	47.9	47.6	46.7[a]	nd	47.4	46.8	48.4[a]	47.4	47.5	46.0
18	41.6	41.6	41.2	40.6	41.4	41.5	41.0	40.8	40.8	41.0	40.6	40.8
19	48.7	48.7	48.5	48.1	48.2	47.9	47.9	47.9	47.7	47.8	47.6	47.9
20	36.7	36.7	37.6	36.6	36.7	36.7	36.5	36.5	36.8	36.5	36.7	36.6
21	79.7	79.6	91.4	79.7	79.2	79.8	82.5	81.3	80.5	82.5	79.7	81.2
22	77.2	77.1	74.7	73.9	76.3	77.4	72.0	70.4	74.6	72.0	73.9	70.4
23	28.5	28.4	28.4	28.4	28.4	28.5	28.5	28.5	28.5	28.5	28.5	28.5
24	17.0	16.9	16.9	17.0	16.9	17.3	17.0	17.0	17.0	17.0	17.0	17.1
25	16.2	16.1	16.2	16.2	16.2	16.2	16.2	16.2	16.2	16.2	16.2	16.3
26	17.4	17.3	17.3	17.3	17.3	17.4	17.4	17.4	17.3	17.4	17.3	17.6
27	27.5	27.5	27.6	27.3	27.3	28.1	27.6	27.4	27.6	27.6	27.3	27.2
28	68.3	68.2	67.9	64.9	68.2	67.3	66.5	67.0	64.5	66.5	65.0	66.1
29	30.2	30.2	30.0	29.5	30.4	30.2	29.8	29.9	29.6	29.7	29.5	29.8
30	19.1	19.1	19.2	19.4	19.1	19.1	19.3	19.6	20.0	19.9	19.4	19.7
OAc-16												
1′				171.7	171.8			171.3			171.6	171.4
2′				22.0	22.2			22.1			22.0	22.1
OAc-21												
1″				172.7			173.8	173.5	173	173.7	172.6	173.6
2″				21.0			21.3	21.1	21.2	21.2	21.0	21.2
isovaleroyl												
1‴				174.3		175.0	174.5	174.2	175.5	174.5	174.3	174.4
2‴				44.5		44.7	44.5	44.4	44.5	44.5	44.5	44.4
3‴				26.5		27.2	27.0	27.0	26.6	27.1	26.5	27.1
4‴				22.8		22.8	22.7	22.7	22.9	22.7	22.8	22.8
5‴				22.8		22.8	22.8	22.8	22.9	22.8	22.8	22.8

[a] Values obtained from HMBC correlations. nd: Not determined.

the [β-galactose-(1→2)][α-rhamnose-(1→4)]-β-glucuronic acid chain in **5**−**8** (Table 3). At variance with compounds **5**−**8**, the NMR spectra of saponins **9**−**12** did not show signals for a 6-desoxyhexose, and the detection of two anomeric osidic carbons at δ 105.3 and 106.3 in the *J*-modulated ^{13}C NMR spectrum suggested that these compounds contained a single disaccharide chain (Table 3). A single osidic carbon resonated above 80 ppm, assigned to C-2 of the glucuronic acid according to the HSQC experiment and displaying an HMBC correlation with the anomeric proton of the galactose. This indicated a β-galactose(1→2)-β-glucuronic acid sequence which was linked to position 3 of aglycone by the observation of a ROE interaction between H-3 of barringtogenol C and the anomeric proton of glucuronic acid. In the *J*-modulated ^{13}C NMR spectra and HMBC spectra of saponins **5** and **6**, another carbonyl signal was detected above 171 ppm, showing that these compounds were monoesters. In a similar fashion, it was shown that saponins **7**, **9**, and **10** were diacylated and that saponins **4**, **8**, **11**, and **12** had three acid chains (Table 1).

The positive and negative ESI-mass spectra of saponin **5** gave molecular ions at *m*/*z* 1039 $[M+Na]^+$ and 1015 $[M-H]^-$, respectively, which differed with those of prosapogenin **2** by 42 mass units. The ^1H and ^{13}C NMR spectra of **5** showed signals for a single acetyl ester at δ_H 2.06 and δ_C 22.2 (CH_3) and 171.8 (CO). In comparison with **2**, the chemical shift of position 16 in **5** was deshielded at δ_H 5.35 and δ_C 72.3 ($\Delta\ \delta_C = +3.7$), while C-15 was shielded at δ_C 31.2 ($\Delta\ \delta_C = -3.3$) (Tables 1 and 2). The deshielding of H-16 was obviously linked to the acetylation, and it sufficed to locate the locus of the esterification. However, in the ROESY spectrum, in compound **5** and in the series, an interaction was detected between the methyl protons of the acetate and the α-axial H-21 of aglycone (Figure 1). Thus, the monoacylated saponin **5** was 3-*O*-{[β-D-galactopyranosyl(1→2)]α-L-rhamnopyranosyl(1→4)-β-D-glucuronopyranosyl}-16-*O*-acetylbarringtogenol C. The same NMR signals and ROESY interaction in the spectra of saponins **4**, **8**, **11**, and **12** indicated an acetate at position 16 in their structures [δ_H 2.14 ± 0.08 and δ_C 22.1 ± 0.2 (CH_3) and 171.5 ± 0.3 (CO)] (Tables 1 and 2).

Saponins **4** and **6**−**12** possessed an isovaleroyl (or 3-methylbutyroyl) unit. The ^1H NMR signals for this appendage consisted of two nonequivalent methyl doublets near δ 0.96, which correlated in the COSY spectrum with a single proton at δ 2.08, the X part of an ABX system with two doublets of doublets near δ 2.20 and 2.25 (Table 2). In the HMBC experiment, the three protons of the ABX system exhibited correlations with a carbonyl near δ_C 174.5. In saponin **6**, the isovalerate was the only ester group attached to the substructure of prosapogenin **2**, as shown by the molecular ions observed at *m*/*z* 1081 $[M+Na]^+$

Table 2. ^{1}H NMR Data of Triterpene Part for Compounds **1**−**12** in CD_3OD

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
barringtogenol C												
1	0.97/1.62 m	0.99/1.63 m	0.95/1.60 m	0.98/1.61 m	0.97/1.61 m	0.97/1.61 m	0.95/1.59 m	0.96/1.60 m	1.02/1.62 m	1.03/1.61 m	1.02/1.63 m	1.04/1.61 m
2	1.68/1.85 m	1.70/1.86 m	1.67/1.92 m	1.68/1.92 m	1.68/1.92 m	1.63/1.91 m	1.67/1.90 m	1.67/1.89 m	1.70/2.02 m	1.69/2.02 m	1.70/2.02 m	1.69/2.03 m
3	3.17 dd (*11.6, 4.3*)	3.17 dd (*11.0, 3.2*)	3.13 dd (*11.0, 3.6*)	3.14 dd (*10.9, 4.1*)	3.14 dd (*12.0, 4.5*)	3.14 dd (*12.1, 4.2*)	3.15 dd (*11.7, 3.8*)	3.14 dd (*11.7, 3.8*)	3.21 brd (*12.0*)	3.21 dd (*11.7, 4.3*)	3.20 dd (*11.7, 3.9*)	3.20 dd (*12.1, 4.7*)
5	0.79 d (*11.0*)	0.78 d (*11.5*)	0.76 d (*11.9*)	0.78 d (*11.5*)	0.76 d (*11.3*)	0.76 d (*11.5*)	0.76 d (*11.4*)	0.79 d (*10.9*)	0.78 d (*11.4*)	0.78 d (*11.4*)	0.78 d (*10.8*)	0.78 d (*11.4*)
6	1.42/1.56 m	1.42/1.58 m	1.42/1.58 m	1.41/1.56 m	1.42/1.54 m	1.41/1.57 m	1.40/1.57 m	1.39/1.59 m	1.40/1.57 m	1.41/1.58 m	1.41/1.58 m	1.42/1.57 m
7	1.36/1.62 m	1.35/1.61 m	1.33/1.62 m	1.34/1.67 m	1.32/1.57 m	1.34/1.63 m	1.33/1.61 m	1.32/1.52 m	1.33/1.67 m	1.35/1.61 m	1.30/1.53 m	1.32/1.59 m
9	1.66 m	1.65 m	1.65 m	1.64 m	1.64 m	1.62 m	1.62 m	1.62 m	1.65 m	1.67 m	1.66 m	1.64 m
11	1.90 m	1.90 m	1.88 m	1.90 m	1.90 m	1.86 m	1.87 m	1.90 m	1.91 m	1.90 m	1.94 m	1.90 m
12	5.28 brt (*3.0*)	5.28 brt (*3.5*)	5.30 brt (*2.7*)	5.44 brt (*4.4*)	5.36 m	5.26 brt (*3.3*)	5.28 brt (*3.3*)	5.36 brt (*3.2*)	5.35 m	5.28 brt (*3.3*)	5.44 brt (*3.6*)	5.37 brt (*3.1*)
15	1.36 m	1.37 dm (*15.0*)	1.34 m	1.42 m	1.46 dd (*16.6, 2.1*)	1.40 m	1.41 m	1.51 dm (*15.8*)	1.34 m	1.42 m	1.41 m	1.51 m
15	1.82 m	1.84 dd (*15.0, 4.0*)	1.78 dd (*12.5*)	1.81 m	1.86 dd (*16.6, 4.1*)	1.77 m	1.77 dd (*15.5, 4.3*)	1.81 dd (*15.8, 4.6*)	1.68 m	1.78 dd (*14.4, 5.1*)	1.81 dd (*12.7, 4.1*)	1.81 dd (*16.1,5.3*)
16	4.22 brs ($W_{1/2} = 8.0$)	4.22 brs ($W_{1/2} = 9.7$)	4.30 brs ($W_{1/2} = 9.0$)	5.14 brs ($W_{1/2} = 8.3$)	5.35 m	4.08 brs ($W_{1/2} = 8.9$)	4.08 brs ($W_{1/2} = 9.0$)	5.20 brs ($W_{1/2} = 9.6$)	3.95 brs ($W_{1/2} = 8.4$)	4.08 brs ($W_{1/2} = 8.3$)	5.14 brs ($W_{1/2} = 8.0$)	5.20 brs ($W_{1/2} = 8.5$)
18	2.28 dd (*13.9, 3.6*)	2.28 dd (*13.4, 3.5*)	2.32 dd (*13.9, 4.5*)	2.61 dd (*14.3, 4.3*)	2.32 m	2.47 m	2.50 dd (*13.5, 3.7*)	2.57 dd (*14.0, 4.1*)	2.56 dd (*13.5, 3.8*)	2.50 dd (*14.0, 3.2*)	2.62 dd (*13.8, 3.2*)	2.57 dd (*12.6, 5.7*)
19	2.45 t (*13.8*)	2.46 t (*13.4*)	2.53 t (*13.9*)	2.40 t (*14.3*)	2.32 m	2.49 m	2.60 t (*13.2*)	2.43 t (*14.0*)	2.56 t (*13.7*)	2.60 t (*13.5*)	2.40 t (*13.9*)	2.43 t (*13.0*)
19	1.04 dd (*13.8, 4.0*)	1.04 dd (*13.3, 3.7*)	1.04 m	1.32 m	1.17 d (*7.6*)	1.09 m	1.12 dd (*13.0, 3.8*)	1.24 m	1.15 dd (*13.7, 3.9*)	1.13 dd (*13.0, 3.6*)	1.31 m	1.24 m
21	3.93 d (*9.7*)	3.93 d (*9.7*)	4.08 d (*9.9*)	5.37 d (*10.3*)	3.57 d (*10.0*)	3.94 d (*9.7*)	5.51 d (*10.3*)	5.30 d (*10.1*)	5.77 d (*10.1*)	5.52 d (*9.9*)	5.57 d (*10.3*)	5.30 d (*10.1*)
22	3.75 d (*9.7*)	3.75 d (*9.7*)	3.83 d (*9.9*)	5.46 d (*10.3*)	3.76 d (*10.0*)	3.72 d (*9.7*)	3.87 d (*10.3*)	3.87 d (*10.1*)	5.44 d (*10.1*)	3.88 d (*9.9*)	5.46 d (*10.3*)	3.90 d (*10.1*)
23	1.05 s	1.07 s	1.06 s	1.04 s	1.05 s	1.06 s	1.06 s	1.06 s	1.07 s	1.06 s	1.06 s	1.06 s
24	0.85 s	0.86 s	0.84 s	0.83 s	0.84 s	0.85 s	0.85 s	0.84 s	0.86 s	0.86 s	0.86 s	0.86 s
25	0.97 s	0.97 s	0.96 s	0.96 s	0.96 s	0.96 s	0.95 s	0.96 s	0.97 s	0.95 s	0.98 s	0.97 s
26	0.93 s	0.93 s	0.93 s	0.94 s	0.94 s	0.93 s	0.93 s	0.95 s	0.93 s	0.93 s	0.94 s	0.95 s
27	1.41 s	1.41 s	1.42 s	1.33 s	1.28 s	1.41 s	1.43 s	1.31 s	1.46 s	1.43 s	1.33 s	1.31 s
28	3.21 d (*10.6*)	3.20 d (*10.7*)	3.23 d (*10.5*)	3.08 d (*11.0*)	3.28 d (*10.5*)	3.72 d (*10.6*)	3.70 d (*11.1*)	3.79 d (*10.6*)	2.93 d (*11.1*)	3.72 d (*10.0*)	3.08 d (*11.1*)	3.79 d (*10.6*)
28	3.39 d (*10.6*)	3.39 d (*10.7*)	3.36 d (*10.5*)	3.27 d (*11.0*)	3.47 d (*10.5*)	3.86 d (*10.6*)	3.87 d (*11.1*)	3.96 d (*10.6*)	3.23 d (*11.1*)	3.88 d (*10.0*)	3.27 d (*11.1*)	3.96 d (*10.6*)
29	0.93 s	0.93 s	1.03 s	0.88 s	0.98 s	0.95 s	0.84 s	0.87 s	0.84 s	0.84 s	0.89 s	0.88 s
30	0.90 s	0.90 s	0.99 s	1.05 s	0.92 s	0.93 s	1.00 s	1.00 s	1.03 s	1.00 s	1.05 s	1.01 s
OAc-16												
2′				2.20 s	2.06 s			2.18 s			2.21 s	2.18 s
OAc-21												
2″				1.97 s			2.08 s	2.08 s	1.98 s	2.09 s	1.97 s	2.07 s
isovaleroyl												
2‴				2.04 dd (*14.0, 7.0*)		2.18 dd (*14.4, 7.1*)	2.19 dd (*14.5, 7.2*)	2.21 dd (*14.8, 7.4*)	2.13 dd (*14.7, 7.1*)	2.19 dd (*14.5, 7.1*)	2.09 dd (*14.8, 7.3*)	2.21 dd (*14.9, 7.5*)
2‴				2.09 dd (*14.0, 7.0*)		2.23 dd (*14.4, 7.2*)	2.24 dd (*14.5, 7.3*)	2.26 dd (*14.6, 7.4*)	2.20 dd (*14.6, 7.4*)	2.25 dd (*14.5, 7.2*)	2.04 dd (*14.9, 7.5*)	2.26 dd (*15.1, 7.6*)
3‴				2.00 m		2.08 sep (*6.6*)	2.08 m	2.10 m	2.05 m	2.10 m	2.00 m	2.10 m
4‴				0.91 d (*6.4*)		0.95 d (*6.1*)	0.96 d (*6.6*)	0.96 d (*6.7*)	0.94 d (*6.6*)	0.97 d (*5.9*)	0.91 d (*6.4*)	0.97 d (*6.0*)
5‴				0.90 d (*6.5*)		0.95 d (*6.1*)	0.96 d (*6.6*)	0.96 d (*6.7*)	0.94 d (*6.6*)	0.97 d (*5.9*)	0.90 d (*6.5*)	0.97 d (*6.0*)

Table 3. NMR Data of Osidic Part for Compounds **1**−**12** in CD_3OD

glucuronic acid	**1**		**2**		**3**[a]		**4**		**5**		**6**		**7**		**8**		**9**		**10**		**11**		**12**	
	H	C	H	C	H	C	H	C	H	C	H	C	H	C	H	C	H	C	H	C	H	C	H	C
1	4.38 d (*8.0*)	106.9	4.52 d (*7.4*)	105.4	4.42 d (*7.2*)	105.4	4.31 d (*7.8*)	107.5	4.42 d (*7.0*)	105.2	4.42 d (*7.1*)	105.4	4.43 d (*6.6*)	105.5	4.42 d (*6.7*)	105.5	4.45 d (*6.8*)	105.3	4.44 d (*7.6*)	105.3	4.44 d (*7.4*)	105.3	4.44 d (*7.5*)	105.4
2	3.27 t (*8.5*)	75.6	3.61 m	82.4	3.59 m	83.2	3.28 m	75.8	3.59 m	83.1	3.58 m	83.1	3.59 m	83.1	3.59 m	83.1	3.53 m	83.2	3.53 m	83.2	3.53 m	83.2	3.53 t (*7.4*)	83.2
3	3.43 t (*9.0*)	76.5	3.68 m	76.5	3.58 m	78.1	3.38 t (*9.1*)	76.9	3.57 m	76.7	3.58 m	76.8	3.60 m	76.8	3.61 m	76.8	3.59 t (*8.4*)	78.1	3.58 t (*8.9*)	78.1	3.58	78.1	3.58 t (*8.1*)	78.1
4	3.63 t (*8.6*)	80.9	3.68 m	80.2	3.65 m	80.8	3.62 t (*9.0*)	81.5	3.67 m	80.7	3.67 m	80.8	3.67 m	80.7	3.66 m	80.7	3.47 m	73.5	3.46 t (*9.0*)	73.5	3.45	73.5	3.46 t (*8.2*)	73.6
5	3.81 d (*8.5*)	nd	3.68 m	76.5	3.62 m	76.8	3.59 d (*9.5*)	78.6	3.62 m	76.8	3.63 m	74.4	3.64 m	76.8	3.61 m	76.8	3.55 m	76.6	3.53 m	76.5	3.54 m	76.5	3.52 t (*7.8*)	76.4
6		173.6		nd		174.3		176.7		175.3		176.7		nd		nd		nd		176.7		nd		176.8
rhamnose																								
1	4.77 brs ($W_{1/2}$ = *5.0*)	102.6	4.78 brs	102.5	4.81 d (*1.5*)	102.3	4.80 brs	102.5	4.81 brs ($W_{1/2}$ = *3.5*)	102.1	4.82 d (*1.2*)	102.4	4.81 brs	102.3	4.81 brs	102.4								
2	3.79 m	72.3	3.78 m	72.3	3.85 m	72.2	3.85 dd (*3.5, 1.6*)	72.3	3.85 m	72.2	3.85 m	72.2	3.85 m	72.2	3.85 brd (*3.1*)	72.2								
3	3.62 dd (*9.2, 2.7*)	72.1	3.61 dd (*9.0, 3.0*)	72.2	3.63 dd (*9.7, 3.6*)	72.0	3.64 dd (*9.5, 3.6*)	72.2	3.63 dd (*9.3, 3.5*)	72.0	3.63 dd (*9.5, 3.4*)	72.0	3.63 brd (*9.7*)	72	3.63 dd (*9.5, 3.4*)	72.1								
4	3.36 t (*9.2*)	73.8	3.37 t (*9.2*)	73.7	3.34 t (*9.7*)	73.9	3.34 t (*9.6*)	73.7	3.34 t (*9.3*)	73.9	3.34 t (*9.5*)	74.1	3.34 t (*9.5*)	73.9	3.34 t (*9.5*)	73.9								
5	3.98 dq (*9.5, 6.2*)	70.4	3.98 dq (*9.5, 6.2*)	70.4	3.97 dq (*9.7, 6.5*)	70.1	3.98 dq (*9.5, 6.3*)	70.4	3.97 dq (*9.3, 6.0*)	70.1	3.97 dq (*9.5, 6.2*)	70.1	3.97 dq (*9.5, 6.0*)	70.1	3.98 dq (*9.5, 6.0*)	70.1								
6	1.24 d (*6.2*)	17.8	1.22 d (*6.1*)	17.8	1.21 d (*6.2*)	17.8	1.22 d (*6.2*)	17.8	1.21 d (*6.2*)	17.8	1.21 d (*6.2*)	17.8	1.21 d (*6.2*)	17.9	1.21 d (*6.2*)	17.8								
galactose																								
1			4.58 d (*7.6*)	105.9	4.55 d (*7.6*)	106.2			4.55 d (*7.6*)	106.2	4.55 d (*7.6*)	106.2	4.56 d (*7.6*)	106.2	4.55 d (*7*)	106.2	4.53 d (*7.5*)	106.3	4.52 d (*7.6*)	106.3	4.52 d (*7.7*)	106.3	4.52 d (*7.6*)	106.3
2			3.57 dd (*9.8, 7.6*)	73.9	3.58 t (*7.5*)	74.1			3.58 dd (*10.0, 7.9*)	74.1	3.58 dd (*9.7, 7.6*)	78.4	3.58 dd (*9.5, 7.7*)	74.1	3.58 dd (*9.7, 7.4*)	74.1	3.59 t (*8.4*)	74.1	3.59 dd (*9.7, 7.6*)	74.1	3.59 t (*8.5*)	74.1	3.59 dd (*9.7, 7.8*)	74.1
3			3.49 dd (*9.8, 3.3*)	74.8	3.49 dd (*7.0, 3.6*)	74.5			3.49 dd (*10.0, 3.0*)	74.7	3.49 dd (*9.7, 3.3*)	74.7	3.49 dd (*9.5, 3.3*)	74.7	3.48 dd (*9.7, 3.2*)	74.7	3.48 dd (*9.2, 2.5*)	74.7	3.48 dd (*9.7, 3.4*)	74.7	3.47 dd (*9.4, 3.1*)	74.7	3.47 dd (*9.7, 3.3*)	74.7
4			3.85 d (*3.0*)	69.8	3.85 m	69.8			3.86 m	69.8	3.86 m	69.8	3.85 m	69.8	3.85 m	69.8	3.85 d (*2.9*)	69.8	3.86 d (*2.8*)	69.8	3.85 d (*2.7*)	69.8	3.85 d (*2.7*)	69.8
5			3.47 t (*6.8*)	76.9	3.45 m	76.7			3.47 dd (*5.5, 3.0*)	76.7	3.46 t (*7.0*)	76.7	3.47 t (*6.2*)	76.8	3.46 t (*6.3*)	76.7	3.46 t (*6.0*)	76.8	3.47 m	76.8	3.46 t (*5.7*)	76.8	3.46 m	76.8
6			3.67 m 3.72 m	61.9	3.70 d (*6.8*)	61.7			3.70 d (*6.0*)	61.7	3.70 d (*6.6*)	61.8	3.70 d (*6.5*)	61.9	3.70 d (*6.3*)	61.7	3.70 d (*6.2*)	61.7	3.71 m	61.7	3.70 d (*6.3*)	61.7	3.70 d (*6.3*)	61.8

[a] Values of arabinose for **3**: H-1: 4.22 (d, *7.6*), H-2: 3.59 (m), H-3: 3.50 (dd, *5.4, 3.4*), H-4: 3.80 (m, $W_{1/2}$ = *7.5*), H-5: 3.88 (dd, *13.0, 2.4*) and 3.61 (dd, *13.0, 3.6*); C-1: 106.8, C-2: 73.1, C-3: 74.5, C-4: 70.1, C-5: 67. nd: Not determined.

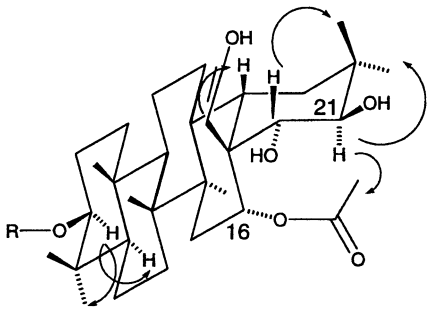


Figure 1. Important ROESY NMR correlations observed for **5**.

and 1057 $[M-H]^-$ and the fragments at 997 (positive) and 973 (negative) corresponding to the loss of 84 mass units of the isovalerate unit. The deshielding of the chemical shifts of H-28 at δ_H 3.72 and 3.86 allowed linking of this ester to C-28. Thus, saponin **6** was 3-*O*-{[*β*-D-galactopyranosyl(1→2)]α-L-rhamnopyranosyl(1→4)-*β*-D-glucuronopyranosyl}-28-*O*-isovaleroylbarringtogenol C. As it had been established for saponin **6**, the isovaleric acid esterified the hydroxymethylene C-28 in saponins **7**, **8**, **10**, and **12**. This result was deduced from the observation of a HMBC correlation between H-28 (δ 3.75 and 3.91 ± 0.05) and the carbonyl of isovalerate at δ_C 174.3 ± 0.2.

In comparison with the mass spectrum of **6**, the ESI molecular peaks of saponin **7** showed an extra 42 mass units. This acetyl group was placed at position 21 and was characterized by chemical shifts at δ_H 2.08 and δ_C 21.1 (CH_3) and 173.5 (CO). The HMBC experiment exhibited long-range correlations for the carbonyl carbon of the acetyl group with H-21 of the aglycone. The structure of saponin **7** was established to be 3-*O*-{[*β*-D-galactopyranosyl(1→2)]α-L-rhamnopyranosyl(1→4)-*β*-D-glucuronopyranosyl}-21-*O*-acetyl-28-*O*-isovaleroylbarringtogenol C. The presence of the same acetate attached at C-21 in saponins **4** and **8**−**12** was easily detected from the analysis of their NMR spectra [δ_H 2.03 ± 0.06 and δ_C 21.1 ± 0.1 (CH_3) and 173.2 ± 0.6 (CO) (Tables 1 and 2)] and ESIMS. Thus, saponin **8**, exhibiting molecular ions at *m/z* 1165 $[M+Na]^+$ and 1141 $[M-H]^-$, which is 42 mass units more than in **7**, was composed of [prosapogenin **2** + two acetates + one isovalerate], and its structure was 3-*O*-{[*β*-D-galactopyranosyl-(1→2)]α-L-rhamnopyranosyl(1→4)-*β*-D-glucuronopyranosyl}-16,21-di-*O*-acetyl-28-*O*-isovaleroylbarringtogenol C.

The two pairs of saponins **9**−**10** and **11**−**12** displayed molecular peaks $[M+Na]^+$ (and $[M-H]^-$) at *m/z* 977 (953) and 1019 (995), respectively. These observations implicated that **11** and **12** contained one supplementary acetyl group and that each couple was composed of regioisomers. In the HMBC experiments run on compounds **9** and **11**, the deshielded doublet proton of barringtogenol C at δ 5.45 ± 0.01 (J = 10.2 ± 0.1 Hz) was correlated with the carbonyl of the isovalerate near δ_C 175. In the HSQC experiment this proton was correlated to a methine at δ 73.9 (**11**) or 74.6 (**9**). Observation of a HMBC correlation between this carbon atom and the methylene protons H-28 defined this methine as C-22. Consequently, saponin **9** was 3-*O*-{*β*-D-galactopyranosyl(1→2)-*β*-D-glucuronopyranosyl}-21-acetyl-22-isovaleroylbarringtogenol C, and saponin **11** 3-*O*-{*β*-D-galactopyranosyl(1→2)-*β*-D-glucuronopyranosyl}-16-,21-di-*O*-acetyl-22-*O*-isovaleroylbarringtogenol C. As established above for saponins **7** and **8**, the isovaleroyl group was linked to C-28 of aglycone in saponins **10** and **12**. Thus, saponin **10** was identified as 3-*O*-{*β*-D-galactopyranosyl-(1→2)-*β*-D-glucuronopyranosyl}-21-*O*-acetyl-28-*O*-isovaleroylbarringtogenol C, and saponin **12** as 3-*O*-{*β*-D-galactopyranosyl(1→2)-*β*-D-glucuronopyranosyl}-16,21-di-*O*-acetyl-28-*O*-isovaleroylbarringtogenol C.

The last isolated saponin, compound **4** (positive molecular peak $[M+Na]^+$ detected at *m/z* 1003), which corresponded to a triester of prosapogenin **1**, had acetates attached at C-16 and C-21 and one isovalerate at C-22 and was 3-*O*-{α-L-rhamnopyranosyl(1→4)-*β*-D-glucuronopyranosyl}-16,21-di-*O*-acetyl-22-*O*-isovaleroylbarringtogenol C.

Only a few triterpenes or saponins, acylated with an isovaleroyl group, have been described in the literature and usually as mixtures of different esters.[12,13] The set of saponins from *Foetidia africana* represents the first isolation of pure acylated polyhydroxyoleanene oligoglycosides containing this ester. The isolation of prosapogenin **3** after alkaline hydrolysis and of no corresponding four sugar saponin suggests that the saponin mixture is more complex than shown in this study. Further complexity might be due to acyl migration during the extraction and purification stages as recently shown with the acylated saponins from *Gymnema sylvestre*.[14]

Experimental Section

General Experimental Procedures. ^1H and ^{13}C NMR spectra were recorded on a Bruker Avance DRX 500 NMR spectrometer at 500 and 125 MHz, respectively, using $CDCl_3$ or CD_3OD as solvent. Two-dimensional NMR experiments were performed using standard Bruker microprograms (Xwin-NMR version 2.6 software). The EIMS and $ESIMS^n$ were run with a Bruker Esquire LC-MS instrument. Optical rotations were determined with a Perkin-Elmer 241 polarimeter.

Plant Material. The stem bark of *Foetidia africana* was collected by one of us (C.D.), 70 km west from Dar es Salaam near the main road to Morogoro in Tanzania. A specimen was compared with herbarium H.B. 5566 from the Brussels National Botanical Gardens.

Extraction and Isolation. Dried and powdered stem bark (635 g) was boiled in MeOH (7 L) for 3 h. After cooling and filtration, the solution was evaporated to provide a residue (182 g), which was dissolved in MeOH (800 mL). The solution was poured into 4 L of acetone, and the precipitate was collected on a fritted glass and dried over P_2O_5 in vacuo. The filtered solution was evaporated and the residue (63 g) was dissolved in MeOH (400 mL). A second precipitation was achieved by addition of Et_2O (2 L) to the methanolic solution. The second precipitate was filtered and dried over P_2O_5. Each precipitate was dissolved in H_2O (350 and 250 mL, respectively) and dialyzed in cellulose tubing against pure water for 4 days. Freeze-drying of the dialyzed materials left a total residue of 28 g of saponin mixture (yield 4.4%). A part of the saponin mixture obtained from precipitation in Me_2CO (4 g) was purified by reversed-phase RP-18 column chromatography (160 g) using a gradient of MeOH−H_2O (from 1:1 to 8:2). Fractions [6−7] eluted with 1:1 MeOH−H_2O contained saponin **5** (4.5 mg), which was purified by a second RP-18 column chromatography eluted with MeOH−H_2O (from 4:6 to 6:4) and finally by preparative TLC with $CHCl_3$−MeOH−H_2O−AcOH (7.5:4:0.8:0.05). Fractions [10−16] and [24−51], eluted with 1:1 MeOH−H_2O and containing saponins **4** and **6**−**12**, were subjected to ion exchange chromatography on an Amberlite IRN 77 (H^+) resin column and were put together before purification by RP-18 column chromatography, eluted with a gradient of MeOH−H_2O (from 4:6 to 8:2). Fractions [64−77] eluted with 4:6 MeOH−H_2O contained saponin **6**, fractions [302−325] eluted with 55:45 MeOH−H_2O contained saponins **7**, **8**, **9**, and **11**, and fractions [348−359] eluted with 6:4 MeOH−H_2O contained saponins **4**, **10**, and **12**. Preparative TLC with $CHCl_3$−MeOH−H_2O−AcOH (7.5:4:0.8:0.05) of the collected fractions gave pure saponins **4** (4 mg), **6** (4.5 mg), **7** (22 mg), **8** (10 mg), **9** (5.5 mg), **10** (8 mg), **11** (5 mg), and **12** (4 mg).

Acid Hydrolysis of Saponin Mixture. An aliquot of the crude saponin mixture (500 mg) was dissolved in 15 mL of a

mixture (1:1) of 0.02 N H_2SO_4 and 6.5% $HClO_4$ and heated at 140 °C in a sealed tube for 2 h. After cooling, the precipitate was filtered and the acid aqueous solution was neutralized with 1 N KOH and freeze-dried. Five sugars were identified with authentic samples by TLC in MeCOEt−Me_2CH_2OH−Me_2CO−H_2O (20:10:7:6) as glucuronic acid, glucose, galactose, arabinose, and rhamnose. After preparative TLC of the sugar mixture, the optical rotation of each purified sugar was measured: L-rhamnose $[\alpha]^{25}_D$ +5.0° (*c* 0.4, H_2O); D-galactose $[\alpha]^{25}_D$ +35.0° (*c* 0.3, H_2O); D-glucuronic acid $[\alpha]^{25}_D$ +6.0° (*c* 0.2, H_2O).

Alkaline Hydrolysis of Saponin Mixture. The crude saponin mixture (600 mg) was boiled in 5% aqueous KOH (60 mL) for 1 day. The cooled solution was neutralized with 2 N HCl and extracted with BuOH. The butanolic layer was evaporated and dissolved in H_2O to achieve dialysis against water for 15 h. After freeze-drying, 310 mg of prosapogenin mixture was obtained, which was purified by reversed-phase RP-18 column chromatography (12.5 g) using a gradient of MeOH−H_2O (from 4:6 to 5:5) as eluent. Fractions [1−3] were submitted to preparative RP-18 TLC in MeOH−H_2O (5:4) to afford prosapogenin **1** (4.5 mg). Fractions [27−35] and [54−65] contained prosapogenins **2** (14 mg) and **3** (7 mg), respectively.

Acetylation of Compound 2. After ion exchange by using an Amberlite IRN 77 (H^+) resin column, 8 mg of **2** was suspended in CH_2Cl_2 (9 mL) and stirred at room temperature for 48 h with Ac_2O (80 μL) and 4-(dimethylamino)pyridine (60 mg). CH_2Cl_2 was added to the reacting mixture, which was rinsed with a $CuSO_4$ aqueous solution and dried with Na_2SO_4 to yield **2a** (8 mg). Final purification was achieved by preparative TLC in $CHCl_3$−Et_2O−MeOH (80:20:2) to afford pure derivative **2a** (1 mg).

Compound 1: $[\alpha]^{25}_D$ −20.6° (*c* 0.15, MeOH); ESIMS (positive) *m/z* 851 $[M+K]^+$, 835 $[M+Na]^+$; MS/MS *m/z* 689 [M+Na−Rha+H]$^+$, 513 [689−glcUA+H]$^+$, 495 [513−H_2O]$^+$; ESIMS (negative) *m/z* 811 $[M-H]^-$; MS/MS *m/z* 647 [M−Rha−H_2O]$^-$, 603 [M−Rha−CO_2−H_2O]$^-$; ^{1}H NMR and ^{13}C NMR, see Tables.

Compound 2: $[\alpha]^{25}_D$ −26.8° (*c* 0.175, MeOH); ESIMS (positive) *m/z* 1013 $[M+K]^+$, 997 $[M+Na]^+$; MS/MS *m/z* 851 [M+Na−Rha+H]$^+$, 513 [851−Gal−glcUA+3H]$^+$; ESIMS (negative) *m/z* 973 $[M-H]^-$; MS/MS *m/z* 809 [M−H−Gal−H]$^-$, 765 [M−H−Gal−CO_2H]$^-$, 629 [809−Rha−2H_2O+3H]$^-$, 603 [765−Rha−H_2O+3H]$^-$, 585 [603−H_2O]$^-$, 557 [629−4H_2O]$^-$, 489 [M−H−Rha−Gal−glcUA+3H]$^-$, 487 [aglycone−H]$^-$; ^{1}H NMR and ^{13}C NMR, see Tables.

Compound 2a: ESIMS (positive) *m/z* 1447 [M+2H+3Na]$^+$, 1415 $[M+K]^+$, 1400 [M+H+Na]$^+$; MS/MS *m/z* 1339 [M+Na−AcOH]$^+$, 1279 [M+Na−2AcOH]$^+$, 1219 [1339−2AcOH]$^+$, 1177 [1219−CH_2CO]$^+$, 1159 [1219−AcOH]$^+$, 1127 [M+H+Na−Rha]$^+$, 1117 [1219−AcOH−CH_2CO]$^+$, 1068 [1279−2AcOH−H_2O−CH_3OCOCH_3]$^+$, 1007 [1339−H−Gal]$^+$; ^{1}H NMR ($CDCl_3$) δ 0.78 (3H, s, H-24), 0.88 (3H, s, H-26), 0.89 (3H, s, H-29), 0.93 (3H, s, H-25), 0.97 (3H, s, H-23), 1.06 (3H, s, H-30), 1.24 (3H, d, J = 6 Hz, Rha-6), 1.40 (1H, brd, J = 15.7 Hz, H-15), 1.47 (3H, s, H-27), 1.76 (1H, dd, J = 15.7, 5.2 Hz, H-15), 1.88 (2H, m, H-11), 3.16 (1H, dd, J = 12, 4.5 Hz, H-3), 3.95 (2H, m, Rha-5 and Gal-5), 4.10 (1H, t, J = 3.6 Hz, glcUA-4), 4.13 (1H, dd, J = 11.5, 5 Hz, Gal-6), 4.15 (1H, d, J = 3.5 Hz, glcUA-2), 4.17 (1H, d, J = 3 Hz, glcUA-5), 4.19 (1H, m, H-16), 4.20 (1H, dd, J = 11.7, 4.5 Hz, Gal-6), 4.66 (1H, t, J = 3.8 Hz, glcUA-3), 4.71 (1H, d, J = 7.9 Hz, Gal-1), 4.95 (1H, d, J = 1 Hz, Rha-1), 5.05 (1H, dd, J = 10.4, 3.5 Hz, Gal-3), 5.09 (1H, t, J = 9.5 Hz, Rha-4), 5.19 (1H, brs, glcUA-1), 5.27 (1H, dd, J = 10.4, 8 Hz, Gal-2), 5.38 (1H, brs, Gal-4), 5.39−5.41 (2H, m, Rha-2 and Rha-3), 5.37 (1H, m, H-12), 5.42 (1H, d, J = 10 Hz, H-22), 5.54 (1H, d, J = 10 Hz, H-21); ^{13}C NMR ($CDCl_3$) δ 15.9 (C-25), 16.9 (C-24), 17.3 (C-26), 17.9 (Rha-6), 19.9 (C-30), 27.3 (C-27), 28.8 (C-23), 29.2 (C-29), 61.5 (Gal-6), 65.2 (Gal-4), 67 (C-28), 67.4 (glcUA-5), 68.1 (Rha-5), 69 (Gal-2), 69.1 (Rha-3), 69.2 (C-16), 70 (Rha-2),70.5 (glcUA-4), 71.3 (Gal-3), 71.5 (Rha-4), 71.7 (Gal-5), 74.2 (C-22), 74.4 (glcUA-3), 77.3 (glcUA-2), 78.8 (C-21), 91.7 (C-3), 96.6 (Rha-1), 102.7 (Gal-1), 103.5 (glcUA-1), 125.3 (C-12), 171 (glcUA-6).

Compound 3: $[\alpha]^{25}_D$ +50.3° (*c* 0.167, MeOH); ESIMS (positive) *m/z* 1151 [M+2Na−H]$^+$; MS/MS *m/z* 987 [M+2Na−Rha+H_2O]$^+$; ESIMS (negative) *m/z* 1105 $[M-H]^-$, 959 [M−Rha]$^-$, 973 [M−Ara]$^-$; MS/MS *m/z* 941 [M−Rha−H_2O]$^-$, 897 [M−Rha−H_2O−CO_2]$^-$, 779 [959−Gal−H_2O−H]$^-$, 761 [941−Gal−H_2O]$^-$, 747 [897−Ara−H_2O+2H]$^-$, 689 [761−4H_2O]$^-$, 557 [689−Ara+H]$^-$; ^{1}H NMR and ^{13}C NMR, see Tables.

Compound 4: $[\alpha]^{25}_D$ −17.0° (*c* 0.1, MeOH); ESIMS (positive) *m/z* 1025 [M+2Na−H]$^+$, 1003 $[M+Na]^+$; MS/MS *m/z* 943 [M+Na−AcOH]$^+$, 857 [M+Na−Rha+H]$^+$, 797 [M+Na−Rha−AcOH+H]$^+$, 681 [M+Na−Rha−glcUA+2H]$^+$, 663 [681−H_2O]$^+$, 621 [681−AcOH]$^+$, 603 [621−H_2O]$^+$, 561 [681−2AcOH]$^+$; ESIMS (negative) *m/z* 979 $[M-H]^-$, 789 [M−H−Rha−Ac]$^-$; MS/MS *m/z* 815 [M−Rha−H_2O]$^-$, 753 [M−Rha−AcOH−H_2O−2H]$^-$, 699 [M−H−Rha−iValOH−CH_3OH+H]$^-$, 693 [753−AcOH]$^+$, 655 [M−H−Rha−glcUA]$^-$, 639 [699−AcOH]$^-$, 595 [655−AcOH]$^+$; ^{1}H NMR and ^{13}C NMR, see Tables.

Compound 5: $[\alpha]^{25}_D$ −10.5° (*c* 0.095, MeOH); ESIMS (positive) *m/z* 1061 [M+2Na−H]$^+$, 1039 $[M+Na]^+$; MS/MS *m/z* 897 [M+2Na−Rha−H_2O]$^+$, 853 [M+2Na−Rha−H_2O−CO_2]$^+$, 837 [M+2Na−Rha−H_2O−AcOH]$^+$, 771 [M+2Na−Gal−AcOH−CH_3OH−2H_2O+H]$^+$, 699 [M+2Na−Rha−Gal−CH_3OH−H_2O−H]$^+$; ESIMS (negative) *m/z* 1015 $[M-H]^-$; MS/MS *m/z* 852 [M−H−Gal]$^-$, 807 [M−H−Gal−CO_2H]$^-$, 747 [807−AcOH]$^-$, 717 [807−AcOH−CH_2O]$^+$, 671 [852−Rha−2H_2O+2H]$^-$, 567 [747−Rha−CH_3OH−H]$^-$, 471 [852−Rha−glcUA−AcOH+3H]$^-$, 439 [471−CH_3OH]$^-$; ^{1}H NMR and ^{13}C NMR, see Tables.

Compound 6: $[\alpha]^{25}_D$ −12.8° (*c* 0.109, MeOH); ESIMS (positive) *m/z* 1104 [M+2Na]$^+$, 1081 $[M+Na]^+$, 997 [M+Na−iVal]$^+$; MS/MS *m/z* 935 [M+Na−Rha+H]$^+$, 597 [M+Na−Rha−Gal−glcUA+3H]$^+$, 507 [M+Na−Rha−Gal−glcUA−iVal−3H]$^+$, 495 [597−iValOH]$^+$, 489 [507−H_2O]$^+$; ESIMS (negative) *m/z* 1057 $[M-H]^-$, 973 [M−H−iVal]$^-$; MS/MS *m/z* 894 [M−H−Gal]$^-$, 849 [M−H−Gal−CO_2H]$^-$, 747 [M−H−Gal−Rha]$^-$, 713 [894−Rha−2H_2O+2H]$^-$, 641 [713−4H_2O]$^-$, 571 [aglycone−H]$^-$, 567 [747−glcUA−3H]$^-$; ^{1}H NMR and ^{13}C NMR, see Tables.

Compound 7: $[\alpha]^{25}_D$ −4.9° (*c* 0.183, MeOH); ESIMS (positive) *m/z* 1145 [M+2Na−H]$^+$, 1123 $[M+Na]^+$; MS/MS *m/z* 977 [M+Na−Rha+H]$^+$, 843 [M+Na−Rha−iValOH−CH_3OH+H]$^+$, 639 [M+Na−Rha−Gal−glcUA+3H]$^+$, 579 [639−AcOH]$^+$, 507 [M+Na−Rha−Gal−glcUA−Ac−iVal−2H]$^+$, 477 [579−iValOH]$^+$, 489 [507−H_2O]$^+$; ESIMS (negative) *m/z* 1099 $[M-H]^-$; MS/MS *m/z* 936 [M−H−Rha−H_2O+2H]$^-$, 831 [M−H−Rha−iValOH−H_2O−H]$^-$, 755 [936−Gal−H_2O]$^-$, 683 [755−4H_2O]$^-$, 651 [831−Gal−H_2O+H]$^-$, 623 [683−AcOH]$^-$, 553 [831−AcOH−Gal−3H_2O−H]$^-$, 521 [623−iValOH]$^-$; ^{1}H NMR and ^{13}C NMR, see Tables.

Compound 8: $[\alpha]^{25}_D$ −13.3° (*c* 0.383, MeOH); ESIMS (positive) *m/z* 1187 [M+2Na−H]$^+$, 1165 $[M+Na]^+$; MS/MS *m/z* 1105 [M+Na−AcOH]$^+$, 1019 [M+Na−Rha+H]$^+$, 959 [1019−AcOH]$^+$, 681 [M+Na−Rha−Gal−glcUA+3H]$^+$, 621 [681−AcOH]$^+$, 561 [621−AcOH]$^+$, 519 [621−iValOH]$^+$, 507 [M+Na−Rha−Gal−glcUA−2Ac−iVal−3H]$^+$; ESIMS (negative) *m/z* 1141 $[M-H]^-$; MS/MS *m/z* 978 [M−H−Rha−H_2O+2H]$^-$, 933 [M−H−Gal−CO_2H]$^-$, 873 [933−AcOH]$^-$, 843 [M−H−Rha−iValOH−CH_3OH−H_2O+H]$^-$, 797 [978−Gal−H_2O]$^-$, 725 [797−4H_2O]$^-$, 665 [725−AcOH]$^-$; ^{1}H NMR and ^{13}C NMR, see Tables.

Compound 9: $[\alpha]^{25}_D$ −9.0° (*c* 0.1, MeOH); ESIMS (positive) *m/z* 1000 [M+2Na]$^+$, 977 $[M+Na]^+$; MS/MS *m/z* 639 [M+Na−Gal−glcUA+2H]$^+$, 579 [639−AcOH]$^+$, 477 [579−iValOH]$^+$; ESIMS (negative) *m/z* 953 $[M-H]^-$; MS/MS *m/z* 773 [M−H−AcOH−iValOH−H_2O]$^-$, 613 [M−H−Gal−glcUA]$^-$; ^{1}H NMR and ^{13}C NMR, see Tables.

Compound 10: $[\alpha]^{25}_D$ +0.9° (*c* 0.109, MeOH); ESIMS (positive) *m/z* 1000 [M+2Na]$^+$, 977 $[M+Na]^+$; MS/MS *m/z* 639 [M+Na−Gal−glcUA+2H]$^+$, 579 [639−AcOH]$^+$, 477 [639−iValOH]$^+$; ESIMS (negative) *m/z* 953 $[M-H]^-$; MS/MS *m/z* 773 [M−H−AcOH−iValOH−H_2O]$^-$, 613 [M−H−Gal−glcUA]$^-$; ^{1}H NMR and ^{13}C NMR, see Tables.

Compound 11: $[\alpha]^{25}_D$ −5.2° (*c* 0.154, MeOH); ESIMS (positive) *m/z* 1041 [M+2Na−H]$^+$, 1019 $[M+Na]^+$, 977 [M+Na−Ac]$^+$, 961 [M+Na−AcOH+2H]$^+$, 796 [M+Na−Gal−AcOH]$^+$; MS/MS *m/z* 959 [M+Na−AcOH]$^+$, 885 [M+Na−iValOH−CH_3-

OH]$^+$, 681 [M+Na−Gal−glcUA+2H]$^+$, 621 [681−AcOH]$^+$, 561 [621−AcOH]$^+$; ESIMS (negative) *m/z* 995 [M−H]$^-$; MS/MS *m/z* 815 [M−H−Gal−H_2O−H]$^-$, 725 [M−H−Gal−CH_3OH−AcOH−H_2O+3H]$^-$, 665 [725−AcOH]$^-$, 655 [725−AcOCH+2H]$^-$; ^{1}H NMR and ^{13}C NMR, see Tables.

Compound 12: $[\alpha]^{25}_D$ −1.6° (*c* 0.064, MeOH); ESIMS (positive) *m/z* 1041 [M+2Na−H]$^+$, 1019 [M+Na]$^+$, 681 [M+Na−Gal−glcUA+2H]$^+$; MS/MS *m/z* 959 [M+Na−AcOH]$^+$, 825 [M+Na−AcOH−iValOH−CH_3OH]$^+$, 681 [M+Na−Gal−glcUA+2H]$^+$, 621 [681−AcOH]$^+$, 561 [621−AcOH]$^+$, 519 [621−iValOH]$^+$; ESIMS (negative) *m/z* 995 [M−H]$^-$; MS/MS *m/z* 953 [M−Ac]$^-$, 911 [M−H−iVal]$^-$, 869 [953−Ac−iVal]$^-$; ^{1}H NMR and ^{13}C NMR, see Tables.

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