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SAPONINS FROM THE STEM BARK OF *FILICIUM DECIPIENS*

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Key Word Index—*Filicium decipiens*; Sapindaceae; stem bark; triterpene saponins; nilic acid; angelic acid.

Abstract—Four new saponins have been isolated from the stem bark of *Filicium decipiens* and identified as 3-*O*-{β-D-glucopyranosyl(1 → 2)-β-D-glucopyranosyl}-28-*O*-{[α-L-arabinopyranosyl(1 → 2)-β-D-xylopyranosyl (1 → 6)] [4-*O*-angeloyloxy-α-L-arabinopyranosyl(1 → 2)-α-L-rhamnopyranosyl(1 → 2)]}-β-D-glucopyranosyl gypsogenic acid, 3-*O*-{β-D-glucopyranosyl(1 → 2)-β-D-glucopyranosyl}-28-*O*-{[α-L-arabinopyranosyl(1 → 2)-β-D-xylopyranosyl(1 → 6)] [4-*O*-angeloyloxy-α-L-arabinopyranosyl(1 → 2)-α-L-rhamnopyranosyl(1 → 2)]}-β-D-glucopyranosyl medicagenic acid, 3-*O*-{β-D-glucopyranosyl(1 → 2)-β-D-glucopyranosyl}-28-*O*-{[α-L-arabinopyranosyl(1 → 2)] [β-D-xylopyranosyl(1 → 4)]α-L-rhamnopyranosyl(1 → 2)-4-*O*-[(3′-hydroxy-2′-methyl-butyroyloxy)-3-hydroxy-2-methyl-butyroyloxy]-β-D-fucopyranosyl} medicagenic acid and 3-*O*-β-D-glucopyranosyl-28-*O*-{[α-L-arabinopyranosyl(1 → 2)] [β-D-xylopyranosyl(1 → 4)] α-L-rhamnopyranosyl(1 → 2)-4-*O*-[(3′-hydroxy-2′-methyl-butyroyloxy)-3-hydroxy-2-methyl-butyroyloxy]-β-D-fucopyranosyl} zanhic acid. These structures were elucidated by analysis of 2D-NMR spectra and of electrospray ionization mass spectra.

INTRODUCTION

Filicium decipiens (Wright and Arn.) Thwaites is a large tree usually found in southern Africa. This species possesses an attractive, glossy, fern-like foliage and a free translation of its botanical name is 'deceptively fern-like' [1]. Previous phytochemical investigations led to the identification of kaempferol, quercetin, 3′,4′-di-*O*-methylquercetin, procyanidin, *P*-hydroxybenzoic acid, vanillic acid and melilotic acid, of tannin and of toxic saponins [2, 3]. We report here on the isolation and structural elucidation of four new saponins from the stem bark of the plant collected in Tanzania.

RESULTS AND DISCUSSION

Primary extraction of dried and powdered stem bark was performed with boiling methanol. The methanolic extract was concentrated and poured into ether to form a precipitate. After dialysis against pure water, the saponin extract was purified by repeated silica gel column chromatography, preparative TLC and/or reversed-phase C-18 column chromatography to give the new saponins **1**, **2**, **3** and **4**.

TLC after acid hydrolysis of the crude saponin extract showed three aglycones, two of which were purified and identified as medicagenic acid [4] and gypsogenic acid [5, 6] according to NMR and mass spectral data. The sugar mixture was analysed by TLC and the identity of its components was established by comparison with authentic samples; D-glucose, D-xylose, L-arabinose, L-rhamnose and D-fucose were detected and their absolute configurations were determined by the measurement of optical rotation after separation by prep. TLC.

The liquid secondary ion mass spectrum (LSIMS) of saponin **1** displayed two ion peaks at *m/z* 1619.6 $[M+Na]^+$ and *m/z* 1635.6 $[M+K]^+$ suggesting an M_r of 1596 amu ($C_{74}H_{116}O_{37}$). These two ions were also observed in the electrospray ionization (ESI) mass spectrum together with a number of diagnostic fragment ions. Losses of terminal pentose and hexose units from $[M+Na]^+$ led to fragment ions at *m/z* 1489.7 and 1457.6, respectively; the loss of a disaccharide unit hexose–hexose in terminal position yielded an ion at *m/z* 1295.6.

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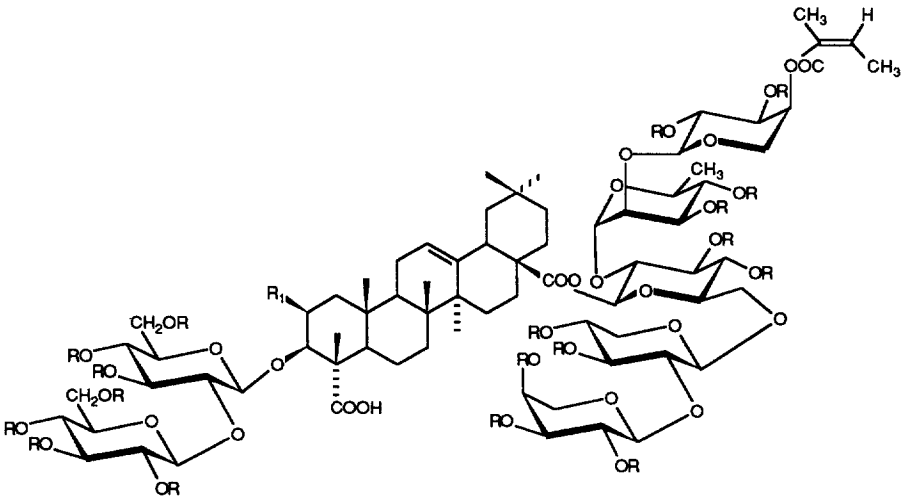
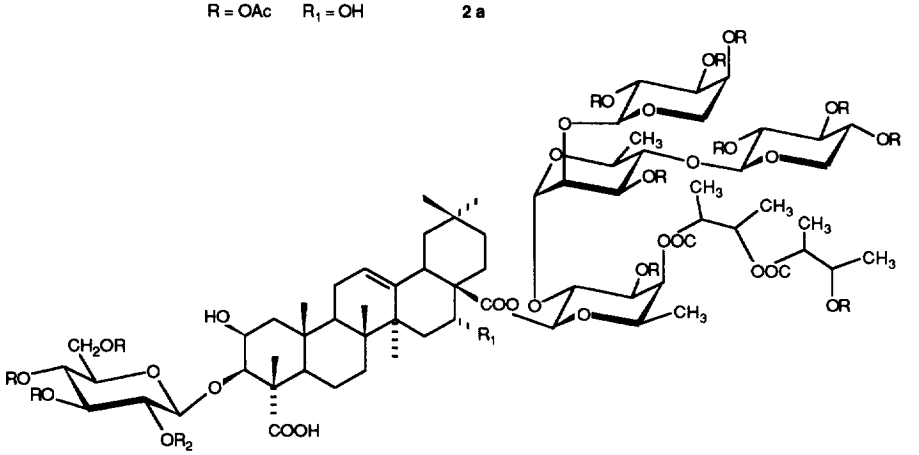


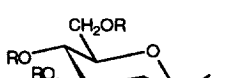
$R = R_1 = H$ **1**

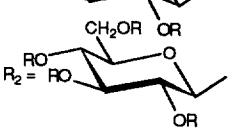
$R = OAc$ $R_1 = H$ **1a**

$R = H$ $R_1 = OH$ **2**

$R = OAc$ $R_1 = OH$ **2a**



$R = R_1 = H$ $R_2 =$  **3**

$R = OAc$ $R_1 = H$ $R_2 =$  **3a**

$R = R_2 = H$ $R_1 = OH$ **4**

$R = R_1 = R_2 = OAc$ **4a**

The structure of the triterpene, gypsogenic acid, was determined by ^1H and ^{13}C NMR analysis (Table 1) using connectivities observed in COSY H–H, HMQC and HMBC spectra. The ^1H NMR spectrum showed five singlets for six tertiary methyl groups, one trisubstituted olefinic proton at δ 5.29, and one hydroxy-bearing methine proton at δ 4.14 (multiplet). This deshielded proton was coupled with high field protons at δ 1.4 in the COSY spectrum. The ^{13}C NMR spectrum revealed the presence of six methyl carbons correlated in the HMQC spectrum with five above mentioned singlets, of a pair of olefinic carbons at δ 129.2 (CH) and 144.8 (C), and of two carboxylic groups at δ 178 and 182.5. This latter carbon and the methyl at

δ 12.8 were correlated in the HMBC spectrum with the deshielded α-hydroxy proton. The ^{13}C chemical shifts for C-28 at δ 178 (esterified state) and for C-3 at δ 86.2 (glycosidation shift) suggested that sugars were connected to these points. Thus, the ^{13}C NMR shifts of the signals of saponin **1** due to the aglycone part were in close concordance with those reported for bidesmosidic saponins of gypsogenic acid [5].

The presence of seven sugar residues was deduced from the observation of signals for seven anomeric carbons at δ 95.5, 101.1, 103.3, 103.7, 104.4, 105.6 and 107.5 attached to proton doublets at δ 5.41, 5.42, 4.48, 4.51, 4.59, 4.61 and 4.45, respectively, in the HMQC spectrum. A methyl carbon at δ 18.2 was assigned to a 6-desoxyhexose, and six hydroxymethyls between δ 62.9 and 66.7 corresponded to six hexoses and/or pentoses. COSY H–H and HOHAHA experiments allowed the full identification of the spin systems of one β-D-glucose starting from the ester anomeric doublet, of one α-L-rhamnose from the doublet methyl at δ 1.22, and of one α-L-arabinose which possessed a deshielded H-4 at δ 5.08 suggesting that this pentose was esterified (Table 2). Signal superimpositions of some proton resonances at 300 MHz did not allow determination of the nature of the last four sugars with certainty. Analysis of the peracetylated derivative **1a** with the help of 2D correlated spectra allowed identification of a rhamnose substituted in position C-2 (δ_H 4.01, *br d*, J = 3 Hz), of three pentoses and of three β-D-glucoses among which the ester sugar disubstituted in positions C-2 (δ_H 3.9, *t*, J = 8 Hz) and C-6 (δ_H 3.57, *dd*, J = 11, 3 Hz and 3.82, *dd*, J = 11, 2.5 Hz), one peracetylated terminal glucose, and a third glucose substituted in position C-2 (δ_H 3.76, *dd*, J = 9, 8 Hz). The three pentoses were resolved into two terminal α-L-arabinoses and one β-D-xylose substituted in position C-2 (δ_H 3.58, *dd*, J = 6.5, 4.5 Hz). This latter sugar was in a conformation intermediate between $^1C_4$ and $^4C_1$ as attested by its vicinal coupling constants [7].

Sequencing of the two sugar chains in saponin **1** was achieved by analysis of HMBC and ROESY experiments. HMBC spectrum of **1** displayed correlations for the gypsogenic acid part, namely between C-28 (δ_C 178) and H-1 of the glucose ester, and between C-3 (δ_C 86.2) and H-1 of a glucose (δ_H 4.51). A carbon assigned as C-2 of rhamnose by its HMQC correlation (δ_C 81.6) showed a ^3J coupling with H-1 of esterified arabinose (δ_H 4.45); this link was confirmed by the observation of an intense ROE (rotating Overhauser effect) between the corresponding protons. Two other heteronuclear interosidic couplings were detected from the HMBC map between H-1 of terminal arabinose (δ_H 4.61) and C-2 of xylose (δ_C 82.8), and between H-1 of xylose (δ_H 4.48) and C-6 of glucose (δ_C 69.1). These conclusions were secured and sequencing was completed by ROEs observed on derivative **1a**. The six anomeric protons (sugar ester anomeric proton excluded) gave ROE correlations with the appropriately shielded protons which characterized the points of substitution, or with H-3 of gypsogenic acid.

Saponin **1** also contained a C_5 acid which was attributed to an angelic acid and characterized by a carbonyl at δ_C 169, by two vinylic methyl groups at δ_H 1.96 and 2.05 and δ_C 21.1 and 16.6, and by one ethylenic methine at δ_C 139.9 and δ_H 6.2 (*qd*, J = 7, 1 Hz). This ester is placed on O-4 of arabinose substituting the rhamnosyl residue to account for the deshielding of arabinose H-4 on the underivatized saponin **1**. On the basis of the above findings, the structure of the new saponin **1** was concluded to be 3-*O*-{β-D-glucopyranosyl(1 → 2)-β-D-glucopyranosyl}-28-*O*-{[α-L-arabinopyranosyl(1 → 2)-β-D-xylopyranosyl(1 → 6)] [4-*O*-angeloyloxy-α-L-arabinopyranosyl(1 → 2)-α-L-rhamnopyranosyl(1 → 2)]}-β-D-glucopyranosyl gypsogenic acid.

Saponin **2** exhibited an intense $[M + Na]^+$ ion peak at m/z 1635.6 in both the LSI and ESI mass spectra indicating that its M_r was 1612. This result suggested an additional hydroxyl group compared with saponin **1**. The losses of terminal hexose and pentose gave fragment ions at m/z 1473 and 1503, respectively. A fragment ion peak observed at m/z 1421.6 was attributed to the loss of one terminal pentose residue substituted by an angelic or tiglic acid ($[M + Na\text{-pentose-}82]^+$). 1D- and 2D-NMR experiments (^1H, ^{13}C J modulated, COSY, HOHAHA, HMQC) permitted assignments of all ^1H and ^{13}C signals of sugars, and a ROESY experiment provided information about the glycosidic linkages; these data demonstrated that the sugar chains of **1** and **2** were identical (Table 2). By comparison with **1**, the ^1H NMR spectrum of **2** contained one supplementary deshielded signal at δ 4.3 (*m*, $W_{1/2}$ = 10 Hz) correlated in the COSY spectrum with H-3 at δ 4.2. The ^{13}C NMR spectrum of **2** showed a signal for one hydroxymethine at δ 71.6 instead of a methylene at δ 26.8 (Table 1). The genin was thus identified with medicagenic acid; NMR resonances of saponin **2** were in good agreement with those described for saponins containing this aglycone [8–10]. Consequently, the structure of saponin **2** was determined to be 3-*O*-{β-D-glucopyranosyl(1 → 2)-β-D-glucopyranosyl}-28-*O*-{[α-L-arabinopyranosyl(1 → 2)-β-D-xylopyranosyl(1 → 6)] [4-*O*-angeloyloxy-α-L-arabinopyranosyl(1 → 2)-α-L-rhamnopyranosyl(1 → 2)]}-β-D-glucopyranosyl medicagenic acid. The identification was secured by study of peracetylated derivative **2a** (Tables 1 and 2).

The third novel saponin **3** comprised medicagenic acid substituted by two sugar chains at positions C-3 and C-28. Combined analysis of HMQC and HMBC spectra of **3** provided assignments for the ^{13}C NMR resonances which were in accordance with those ascertained for medicagenic acid as in saponin **2** (Table 1). The ^{13}C NMR spectrum showed that **3** contained six sugar residues whose anomeric carbons resonated at δ 95, 100.9, 103.4, 105, 107.1 and 107.2. An HMQC experiment allowed location of anomeric protons at δ 5.38, 5.56, 4.55, 4.6, 4.46 and 4.36, respectively.

Table 1. ^{13}C NMR data of the aglycone moieties of saponins **1–4** in CD_3OD and of derivatives **1a–4a** in $CDCl_3$ (ppm)

C	1	1a	2	2a	3	3a	4	4a
1	40	38.5	45.2	43.1	45	43.1	45	43.2
2	26.8	25.4	71.6	na	71.2	70.3	na	69.8
3	86.2	88.1	85.8	87.3	86	87.2	86.5	86
4	54.5	52.9	na	52.1	53.6	52.1	53.5	52.1
5	54	51.7	53	52	53.5	51.9	53	52.4
6	21.7	20.4	21.5	na	21	na	21.8	na
7	33.6	32.3	33.5	na	33.2	32.2	32	32.5
8	41	39.6	41.1	39.7	41.1	39.7	41.8	40
9	49.3	47.7	49	48	49.8	48.2	na	47.1
10	37.5	36.5	37.3	36.3	37.4	36.3	37.5	36.3
11	24.5	22.7	24.6	na	24.8	22.5	25	23.5
12	129.2	122.3	123.4	122.1	123.6	121.7	123.8	123
13	144.8	143	145	143	144.9	143.8	145	142
14	43	41.9	43.1	42	43.2	42	43.5	41.2
15	29	28.2	29	28.9	29.5	27	30.8	32.1
16	24.5	24.7	24.6	na	24.8	22.4	na	75.9
17	48.7	46.8	47.8	46.8	47.4	46.6	48.2	47.9
18	42.7	41.4	42.6	41.4	42.7	41.9	42.7	41
19	47.5	46	48	na	48	46	na	46.5
20	31.6	30.6	31.6	30.6	31.5	30.6	31.6	30.3
21	35	33.9	34.9	na	34.9	33.8	34	35
22	31	31.8	33.5	na	33.7	31.9	33.2	na
23	182.5	178	178.8	na	183	178.3	na	179.5
24	12.8	11.3	14.4	12.7	14.4	12.8	14.1	12.9
25	16.6	16	17.5	15.8	17.4	16.6	17.4	16.3
26	17.9	16.6	17.9	16.6	17.8	16.8	17.8	16.8
27	26.4	25.6	26.3	26	26.3	25.7	27	26.5
28	178	175.6	178.1	175.4	178.1	175.9	na	174
29	33.6	32.9	33.5	33.2	33.5	33.1	33.4	33.2
30	24.4	23.5	24.4	23.5	24.2	23.8	25	24.4

na: not assigned; signal was too weak

Identification of four sugars was directly done with COSY and HOHAHA analysis, and consisted of one β-D-fucose, one α-L-rhamnose, one α-L-arabinose and one β-D-xylose (Table 3). The fucose was engaged in an ester linkage with the aglycone (shielded anomeric carbon at δ 95); its hydroxyl in position C-4 was esterified by an acid residue as the chemical shift of H-4 appeared at δ 5.1. This proton exhibited a $^3J_{H\text{-}C}$ correlation with another carbonyl at δ 175.2 in the HMBC map. Each of the last two sugars possessed a coupled system of seven spins including an AMX pattern with a geminal coupling constant of 13 Hz. The two geminal systems were correlated with two overlapping methylene carbons at δ 62.3. These data proved the presence of two hexoses. Analysis of the COSY and HOHAHA spectra of the acetylated derivative **3a** allowed identification of a β-D-glucose substituted in position C-2 (Table 3). The second hexose had H-1, H-2 and H-3 in axial orientations with vicinal coupling constants larger than 7 Hz, and it was peracetylated and thus terminal. In native saponin **3**, these two hexoses H-4 resonated at δ 3.36 and 3.37 in agreement with what was described in the literature for glucose; a β-D-galactose would have displayed a more deshielded proton chemical shift (δ > 3.8 ppm) [11]. Thus, the two hexose units of saponin **3** were determined as two β-D-glucoses.

Owing to the complexity of the problem, the structural elucidation of compound **3** was pursued on its peracetate **3a**. The CHOR positions of compound **3a** which were not acetylated, were those of H-2 and glucose (δ 3.8), of H-2 of fucose (δ 3.95) and of H-2 and H-4 of rhamnose (δ 3.88 and 3.57). The sequence of the sugars was established through a ROESY experiment. It showed ROEs between H-3 and medicagenic acid and H-1 of substituted glucose (δ 4.48), between H-2 of esterified fucose ester and H-1 of rhamnose (δ 5.14), and between H-2 of rhamnose and H-1 of terminal peracetylated arabinose (δ 4.35). The HMBC spectrum showed interglycosidic correlations between H-1 of fucose and C-28 of the aglycone (δ 175.9), between H-1 of terminal peracetylated arabinose and C-2 of rhamnose (δ 77), and between Hs-1 of terminal peracetylated glucose and xylose (δ 4.57) and C-2 of inner glucose (δ 74) and C-4 of rhamnose (δ 76.6). These three latter sugar carbons were previously assigned by their direct H-C correlation (HMQC experiment). Some interglycosidic links were confirmed in the HMBC spectrum of saponin **3**: between H-1 of arabinose (δ 4.36) and C-2 of rhamnose (δ

Table 2. ^{1}H and ^{13}C NMR data of the osidic moities of saponins **1** and **2** in CD_3OD and of derivatives **1a** and **2a** in $CDCl_3$ (ppm)

	1		**1a**		**2**		**2a**	
	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
β-D-glucose								
1	103.7	4.51 *br d* (7.5)	103.9	4.46 *d* (7.7)	102.9	4.6 *br d* (6)	103.1	4.48 *d* (7.8)
2	82.8	3.43 *m*	74.1	3.76 *dd* (9, 8)	82.7	3.55 *m*		3.8
3	77.3	3.3	75.2	5.16 *t* (9)	77.2	3.3		5.17 *t* (9)
4	71.8	to	70.6	4.91 *t* (9.5)	71.8	to		4.93 *t* (9.5)
5	78.2	3.55	71.6	3.68 *ddd* (9.5, 5, 2)	78.3	3.55		3.65
6	62.7	3.66 *br d* (13)	62.1	4.05 *dd* (12.5, 2)	62.3	3.65 *br d* (11)	61.9	4.05 *br d* (12)
		3.83 *br d* (13)		4.25 *dd* (12.5, 4)		3.82 *dd* (11, 5)		4.25 *dd* (12, 5)
β-D-glucose (terminal)								
1	104.4	4.59 *d* (7)	99.7	4.55 *d* (7)	104.5	4.57 *d* (7)	99.7	4.58 *d* (7.7)
2	71.4	3.29 *m*	71.1	5.08 *dd* (9, 7)	71.4	3.28 *dd* (9, 7)		5.04
3	77.5	3.3	72.3	5.07 *m*	77.3	3.6 *m*		5.1
4	71.4	to	68.5	5.08 *m*	71.5	3.39 *m*		5.1
5	78	3.4	72	3.62 *m*	78.2	3.22 *m*		3.67
6	62.9	3.65 *dd* (12, 4)	61.8	4.13 *dd* (12, 2)	62.8	3.7 *dd* (11, 4)	61.9	4.12 *dd* (12, 2)
		3.89 *br d* (12)		4.28 *dd* (12, 4.5)		3.93 *br d* (11)		4.32 *dd* (12, 5)
β-D-glucose (ester)								
1	95.5	5.41 *d* (7)	93	5.58 *d* (7.5)	95.5	5.39 *d* (7)	92.9	5.59 *d* (7.6)
2	81.6	3.42 *t* (9)	72.6	3.9 *t* (8)	81.6	3.42 *t* (7)		3.9 *t* (8)
3	77	3.5 *m*	75.5	5.27 *t* (9)	77	3.53 *t* (7)		5.27 *t* (9)
4	71.8	3.62 *t* (9)	69	5.06 *t* (9)	71.8	3.55 *m*		5.07
5	79	3.5 *m*	73.1	3.77 *ddd* (9, 4, 2.5)	79	3.48 *m*		3.8
6	69.1	3.74 *dd* (10, 4)	66.5	3.57 *dd* (11, 3)	69	3.74 *dd* (12, 6)		3.59 *dd* (11, 3)
		3.97 *br d* (10)		3.82 *dd* (11, 2.5)		4.02 *dd* (12, 3)		3.8
β-D-xylose								
1	103.0	4.48 *d* (7)	100	4.65 *d* (4.5)	103.3	4.45 *d* (7)	100	4.65 *d* (4.3)
2	82.8	3.42 *t* (8)	76.6	3.58 *dd* (6.5, 4.5)	82.7	3.4 *t* (8)		3.59
3	76.2	3.53 *m*	70.6	5.01 *t* (6.5)	76.2	3.53 *m*		5.02
4	71	3.53 *m*	68.7	4.79 *dt* (6.5, 5)	71.1	3.54 *td* (7, 4)		4.79 *br q* (5)
5	66.6	3.19 *t* (10)	60.4	3.41 *dd* (12, 5.5)	66.6	3.18 *dd* (11, 9)		3.42 *dd* (12.5, 5)
		3.84 *dd* (11, 5)		4.03 *dd* (12, 4)		3.85 *dd* (11, 4)		4.05
α-L-arabinose								
1	105.6	4.61 *d* (6.5)	101.3	4.61 *d* (6)	105.6	4.6 *d* (7)	101	4.61 *d* (6.3)
2	73.1	3.65 *dd* (8, 6)	69.2	5.11 *dd* (9, 6)	73.1	3.66 *dd* (10, 7)		5.11
3	72.8	3.59 *m*	69.8	5.01 *dd* (8.5, 3.5)	73.1	3.59 *t* (9)		5.02
4	69.4	3.83 *br s*	67.2	5.23 *m*	69.5	3.84 *br s*		5.23
5	66.7	3.58 *dd* (12, 4)	62.4	3.6 *dd* (12, 2)	66.9	3.58 *dd* (10, 5)	62.4	3.64
		4.01 *dd* (12, 2)		4.01 *dd* (12, 4)		4.01 *dd* (10, 2)		4.01
α-L-rhamnose								
1	101.1	5.42 *d* (2)	98.3	5.16 *d* (1.5)	101.4	5.4 *br s*	97	5.17 *m* ($W_{1/2}$ 3)
2	81.6	4.03 *br s*	76	4.01 *br d* (3)	81.6	4.02 *dd* (5, 1.5)		4.02
3	70.6	3.82 *m*	68.4	4.9 *dd* (9.5, 3)	70.6	3.82 *m*		4.91 *dd* (10, 4.5)
4	74.1	3.35 *t* (9)	68.5	4.97 *t* (9.5)	74.1	3.35 *m*		4.95
5	70.6	3.7 *m*	66.8	3.92 *dq* (9, 6)	70.9	3.69 *m*		3.91
6	18.2	1.22 *d* (6)	17.3	1.18 *d* (6)	18.4	1.2 *d* (6)	17.3	1.19 *d* (6)
α-L-arabinose (esterified)								
1	107.5	4.45 *d* (7)	102.4	4.35 *d* (7)	107.6	4.42 *d* (7.5)	102.5	4.36 *d* (6.5)
2	73.4	3.65 *dd* (9, 7)	69	5.19 *dd* (9.5, 7)	73.4	3.65 *m*		5.20 *dd* (9, 7)
3	72.6	3.75 *dd* (9, 4)	69.9	5.07 *dd* (9.5, 3)	72.8	3.74 *m*		5.1
4	72.7	5.08 *m* ($W_{1/2}$ 7)	67.4	5.31 *m*	72.8	5.05 *m* ($W_{1/2}$ 8)		5.31
5	66.6	3.58 *dd* (12, 3)	62.9	3.61 *dd* (13, 3)	65	3.67 *dd* (11, 5)	62.8	3.62
		3.99 *br d* (12)		4.04 *d* (13)		3.99 *br d* (11)		4.04
angelic acid								
1	169		167		169			
2	129.2		127.2		129.2		127	
3	139.9	6.2 *qd* (7, 1)	139.3	6.14 *qq* (7, 1.5)	139.9	6.16 *qd* (7, 1.5)	139.3	6.14 *qq* (7, 1.5)
4	16.6	2.05 *br dd* (7, 1)	15.9	2.01 *m*	16.5	2.02 *dd* (7, 1.4)		2.01 *m*
5	21.1	1.96 *m* ($W_{1/2}$ 5)	20.6	1.9 q^t (1.5)	21	1.92 *d* (1.4)		1.01 q^t (1.5)

81.6), between H-1 of xylose (δ 4.46) and C-4 of rhamnose (δ 84.7).

The M_r of saponin **3** was deduced from LSI mass spectrometry showing three ion peaks at m/z 1583.8 $[M+H]^+$, 1605.9 $[M+Na]^+$ and 1621.9 $[M+K]^+$. The sodium adduct was also observed at m/z 1605.7 by electrospray ionization. Thus, the M_r of **3** was 1582 corresponding to a $C_{74}H_{118}O_{36}$ formula. In addition, the ESI spectrum displayed two fragment ion peaks at m/z 849.4 $[M+Na\text{-ester chain}]^+$ and 779.3 $[\text{ester chain}+Na]^+$ corresponding to the fragmentation of the ester function at position C-28. The ion at m/z 849.4 confirmed the presence of a medicagenic acid substituted by two hexose units; the presence of a $C_{10}H_{17}O_4$ organic ester (201 Da) in the second oligosaccharidic moiety was indicated by the second fragment at m/z 779.3. This ester was characterized by two carbonyls at δ 175.2 and 176, and by four methyl groups linked to secondary sp^3 carbon atoms. The protons α to the methyls appeared as quintets at δ 5.16, 3.91, 2.78 and 2.46. The COSY experiment showed that these protons were coupled by pairs. Thus, the C_{10} ester was formed by two symmetrical C_5 acids identified as 3-hydroxy-2-methyl butyric acid, i.e. nilic acid [12, 13]. The mass fragment observed at m/z 1355 $[M+Na\text{-}132\text{-}118]^+$ corresponded to the loss of terminal pentose and of one nilic acid ($C_5H_{10}O_3$) produced by a MacLafferty fragmentation. The chemical shift of the proton α to the oxygen in the first nilate (δ 5.16) indicated that this position was esterified by the second nilic acid. This C_{10} ester is placed on O-4 of the fucose to account for the HMBC correlation observed between H-4 of fucose (δ 5.1) and the ester carbonyl at δ 175.2. The vicinal coupling constants of methine protons were 6.5–7 Hz and corresponded to a *threo*-isomer [14]. Basic hydrolysis of the crude saponin mixture allowed isolation of a small amount of nilic acid with positive optical rotation ($[\alpha]_D = +44°$) suggesting a 2*S*, 3*S*-configuration [12]. Saponin **3** was therefore 3-*O*-{β-D-glucopyranosyl(1 → 2)-β-D-glucopyranosyl}-28-*O*-{[α-L-arabinopyranosyl(1 → 2)] [β-D-xylopyranosyl(1 → 4)] α-L-rhamnopyranosyl(1 → 2)-4-*O*-[(3′-hydroxy-2′-methyl-butyroyloxy)-3-hydroxy-2-methyl-butyroyloxy]-β-D-fucopyranosyl} medicagenic acid.

The ^1H NMR spectrum of saponin **4** showed signals for five sugar residues and closely resembled that of saponin **3** (Table 3). Major differences were due to the absence of signals for a second glucose in **4**. Analysis of COSY and HOHAHA experiments gave similar chemical shifts for the four sugar ester chain, for the two nilic acids, and for only one glucose. Acetylated derivative **4a** possessed a terminal peracetylated β-D-glucose with all protons except H-1 (δ 4.61) and H-5 (δ 3.7) deshielded by acetylation. The presence in **4a** of the same sequence of the ester chain of **3a** was confirmed by analysis of COSY, HOHAHA, HMQC, HMBC and ROESY experiments. A ROE was observed between H-1 of glucose and H-3 of aglycone (δ 4.09).

ESI and LSI mass spectra of saponin **4** displayed a major $[M+Na]^+$ ion peak at m/z 1459.7. The presence of an ester chain identical to that of saponin **3** was confirmed by the presence of the fragment ion peak at m/z 779.4. The other part of the molecule was characterized by the m/z 703.4 fragment ion, corresponding to the hydroxylation of medigagenic acid into zanhic acid [15]. The ^1H spectrum of **4** exhibited a supplementary hydroxy methine at δ 4.49 (*m*, $W_{1/2} = 6.5$ Hz) in an equatorial position. This proton was coupled with a high field methylene located at δ 1.42 and 1.69 in the COSY spectrum. Derivative **4a** displayed the corresponding acetoxy methine group at δ_H 5.6 (*m*, $W_{1/2} = 9$ Hz) and δ_C 75.9 in its NMR spectra. The ^{13}C chemical shift values of the genin of saponin **4** and of derivative **4a** were comparable with those described in the literature for zanhic acid [15, 16]. Thus saponin **4** was 3-*O*-{β-D-glucopyranosyl}-28-*O*-{[α-L-arabinopyranosyl(1 → 2)] [β-D-xylopyranosyl(1 → 4)] α-L-rhamnopyranosyl(1 → 2)-4-*O*-[(3′-hydroxy-2′-methyl-butyroyloxy)-3-hydroxy-2-methyl-butyroyloxy]-β-D-fucopyranosyl} zanhic acid.

EXPERIMENTAL

General. ^1H and ^{13}C NMR spectra were recorded at 300 and 75 MHz; 2D experiments were performed using standard Bruker microprograms. ESIMS and LSIMS of native saponins were recorded on ZAB-SPEC-T five-sector tandem spectrometer. The LSIMS experiments were performed using a bombardment ion beam of Cs^+ (30 keV). The matrix was a mixt. of glycerol, *m*-NBA and TFA (50:50:1). ESIMS was achieved by use of methanolic sample solns infused continuously in the ion source at a flow of 10 μl min^{-1} by a syringe pump. FABMS of peracetylated derivatives were recorded on a ZAB2-SEQ mass spectrometer; for acetylated derivatives, $CHCl_3$ soln was added to *m*-NBA or to *m*-NBA with LiCl matrices.

Plant material. The stem bark of *F. decipiens* was collected in the north of Tanzania, in Kimboza Forest. A specimen was compared to herbarium HB 5580 from the Brussels National Botanical Garden.

Extraction and isolation of saponins. Dried and powdered stem bark (730 g) was macerated for 2 hr in 7 l. of MeOH; the mixt. was boiled under reflux for 3 hr, and was filtered after cooling. Evapn of solvent yielded a residue (92.3 g) which was suspended in 380 ml of MeOH. The solubilized fr. was added to 2 l. Me_2CO and the ppt. was filtered and dried over KOH *in vacuo* (19.2 g). After evapn, the filtrate was again pptd by 1300 ml Me_2CO to afford a second ppt. dried over KOH (3.6 g) and then by 800 ml of Et_2O to give a third ppt. which was dried over P_2O_5 *in vacuo* (38.3 g). The ppts were dissolved in pure H_2O and dialysed against H_2O in seamless cellulose tubing. After 4 days, the contents of the tubes were freeze-dried to afford 34.2 g of saponin mixts identical by TLC (yield = 4.7%). A sample of the mixt. (4.5 g) was dissolved in H_2O and subjected to ion exchange chro-

Table 3. ^{1}H and ^{13}C NMR data of the osidic moities of saponins **3** and **4** in CD_3OD and of derivatives **3a** and **4a** in $CDCl_3$ (ppm)

	3		**3a**		**4**		**4a**	
	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
β-D-glucose								
1	103.4	4.55 *m*	103.1	4.48 *d* (8)	104.5	4.36 *d* (7)	101.7	4.61 *d* (8)
2	82	3.53 *m*	74	3.8 *dd* (9, 8)	72	3.21 *t* (8)	71.3	5 *dd* (9.5, 8)
3	77.8	3.53 *m*	74.8	5.17 *t* (9)	77.7	3.3 *m*	72.5	5.2 *t* (9.5)
4	70.8	3.36 *t* (8)	68.5	4.93 *t* (9.5)	71	3.3 *m*	68.5	5.02 *t* (9.5)
5	78.4	3.39 *m*	71.7	3.67 *ddd* (9.5, 5, 2)	78.5	3.25 *m*	71.9	3.7 *ddd* (9, 5, 2.5)
6	62.3	3.72 *dd* (13, 5)	61.8	4.04 *dd* (12, 2)	62.1	3.68 *dd* (12, 5)	61.9	4.12 *dd* (12.5, 2.5)
		3.88 *dd* (13, 2)		4.24 *dd* (12, 5)		3.8 *dd* (12, 5)		4.21 *dd* (12.5, 5)
β-D-glucose (terminal)								
1	105	4.6 *d* (7.5)	99.6	4.57 *d* (7.5)				
2	71.5	3.27 *dd* (9, 8)	71.3	5.04 *dd* (9, 7.5)				
3	77.8	3.37 *m*	72	5.14 *m*				
4	71	3.37 *m*	68.6	5.1 *m*				
5	78.4	3.29 *m*	71.7	3.65 *m*				
6	62.3	3.69 *dd* (13, 3)	61.8	4.18 *dd* (12, 2.5)				
		3.8 *br d* (13)		4.31 *dd* (12, 5)				
β-D-fucose (ester)								
1	95	5.38 *d* (8)	93.5	5.45 *d* (8)	94	5.35 *d* (8)	93.4	5.57 *d* (8)
2	76.2	3.71 *br t* (9)	76.3	3.95 *dd* (9.5, 8)	76.1	3.67 *dd* (8, 7)	73.8	3.87 *dd* (9.5, 8)
3	74.3	3.89 *dd* (9, 3)	72.5	5.06 *dd* (9.5, 3)	74	3.86 *dd* (7, 3.5)	69.1	5.03 *dd* (9.5, 4)
4	75.4	5.1 *br t* (3)	70.7	5.21 *br d* (3)	75.2	5.09 *br d* (3.5)	70.3	5.21 *br d* (4)
5	71.5	3.87 *m*	69.5	3.87 *q* (6)	71.5	3.85 *q* (6)	69.5	3.82 *br q* (6.5)
6	16.7	1.19 *d* (6)	15.8	1.12 *d* (6)	16.7	1.08 *d* (6)	15.8	1.09 *d* (6)
α-L-rhamnose								
1	100.9	5.56 *br s*	98.9	5.14 *br s*	100.8	5.52 *d* (1)	99.2	5.06 *d* (1.5)
2	81.6	4.02 *dd* (3, 1)	77	3.88 *dd* (3, 1)	81	3.98 *dd* (3, 1)	77	3.78 *dd* (3, 2)
3	72.5	3.92 *dd* (9, 3)	72.7	4.94 *dd* (9.5, 2.5)	72.5	3.94 *dd* (9, 3.5)	72.8	4.95 *dd* (9, 3)
4	84.7	3.5 *t* (9)	76.6	3.57 *t* (9.5)	83.5	3.54 *dd* (11, 9)	77	3.54 *t* (9)
5	70	3.78 *m*	69.6	3.87 *dq* (9.5, 6)	69	3.77 *dq* (11, 6.5)	67.8	3.7 *m*
6	18.4	1.26 *d* (6)	17.9	1.27 *d* (6)	18.4	1.28 *d* (6.5)	18	1.27 *d* (6)
α-L-arabinose								
1	107.2	4.36 *d* (7)	102.1	4.35 *d* (7)	na	4.36 *d* (7)	101.3	4.37 *d* (5.5)
2	72.9	3.62 *dd* (9, 7)	69	5.18 *t* (7.5)	73	3.62 *dd* (9, 7)	69.2	5.13 *dd* (9, 5.5)
3	74.3	3.52 *dd* (9, 4)	71.3	5.07 *dd* (7.5, 4.5)	74.3	3.51 *dd* (9, 3.5)	69.1	5.07 *m*
4	69.8	3.78 *m*	67.1	5.25 *m*	69.8	3.78 *br d* (4)	66.6	5.24 *td* (5, 3)
5	67.2	3.52 *br d* (12)	62	3.57 *dd* (13, 2)	67.5	3.56 *br d* (13)	61.9	3.56 *dd* (12.5, 2.5)
		3.67 *br d* (12)		3.95 *dd* (13, 5)		3.87 *dd* (13, 3)		3.99 *dd* (12.5, 5)
β-D-xylose								
1	107.1	4.46 *d* (7)	101.2	4.57 *d* (7.5)	na	4.52 *d* (7.5)	101.6	4.59 *d* (7.5)
2	76.6	3.22 *t* (8)	71.1	4.85 *dd* (9, 7.5)	76	3.21 *dd* (8, 7.5)	71.4	4.85 *dd* (9, 7.5)
3	77.4	3.33 *m*	72.3	5.12 *t* (9)	77.7	3.3 *t* (8)	72.3	5.1 *t* (9)
4	71	3.54 *m*	69.1	4.98 *td* (9, 5)	71	3.48 *m*	69.3	4.95 *td* (9, 5)
5	67.3	3.18 *br t* (12)	62.4	3.29 *dd* (11.5, 9)	67.5	3.18 *t* (11.5)	62.5	3.28 *dd* (12, 9)
		3.85 *dd* (12, 3)		4.13 *dd* (11.5, 5)		3.85 *dd* (12, 5.5)		4.06 *dd* (12, 5)
nilic acid								
1	175.2		173.2		175		173.1	
2	46.7	2.78 *q'* (7)	45	2.76 *q'* (7)	47	2.78 *q'* (7)	45	2.76 *q'* (7)
3	72.5	5.16 *q'* (6.5)	70.9	5.1 *q'* (6)	na	5.16 *q'* (6.5)	71	5.13 *dq* (7, 6)
4	18.4	1.32 *d* (6.5)	17.7	1.26 *d* (5)	18.5	1.32 *d* (6)	17.9	1.26 *d* (6)
5	14	1.28 *d* (7)	13.8	1.16 *d* (6)	14.1	1.19 *d* (7)	13.7	1.27 *d* (7)
nilic acid (terminal)								
1	176		172.7		175		172.7	
2	49.4	2.46 *q'* (7)	45.2	2.67 *q'* (7)	na	2.45 *q'* (7)	45.2	2.67 *q'* (7.5)
3	70	3.91 *m*	71.4	5.06 *m*	70	3.92 *q'* (6.5)	71	5.1 *dq* (7, 6)
4	20.4	1.16 *d* (6)	17	1.22 *d* (5)	20.4	1.17 *d* (6.5)	16.9	1.23 *d* (6)
5	13.3	1.1 *d* (7)	12.9	1.17 *d* (6)	13.4	1.1 *d* (7)	12.9	1.16 *d* (7.5)

na: not assigned; signal was too weak.

matography by using an Amberlite IRN 77 $[H]^+$ resin column. After freeze-drying, this sample was fractioned by vacuum liquid chromatography (VLC) with $CHCl_3$–MeOH (3:2). The saponin containing fr. (2.2 g) was chromatographed on reversed-phase RP-18 CC; the column was eluted with a gradient of MeOH–H_2O (from 3:2 to 4:1). Saponin **2** (isolated: 19 mg) was in fr. 3 and saponin **1** (36 mg) in frs 4 and 5 eluting with MeOH–H_2O (3:2); final purifications were performed by silica gel CC eluting with mixts of $CHCl_3$–MeOH–H_2O. Frs 28–42 eluted with MeOH–H_2O (7:3) contained saponins **3** (15 mg) and **4** (4 mg); these compounds were sepd by reversed-phase silica gel CC and prep. TLC in $CHCl_3$–MeOH–H_2O (10:6:1). The subsequent fr. from the VLC contained saponin **4** (7 mg) which was purified by a combination of silica gel CC and reversed-phase CC.

Acid hydrolysis of saponin mixture. Crude saponin (200 mg) was dissolved in 8 ml of a mixt. (1:1) of 6.5% aq. $HClO_4$ and H_2SO_4 0.02 N, and heated at 140° in a sealed tube for 2 hr. After cooling, the sapogenin ppt. was filtered, rinsed with H_2O and dried *in vacuo* over P_2O_5. The acid aq. layer was neutralized with KOH 0.5 M and freeze-dried. Sugars were identified with authentic samples as glucose, rhamnose, xylose, arabinose and fucose by TLC in 4-butanone–*iso*-PrOH–Me_2CO–H_2O (20:10:7:6). After prep. TLC of the sugar mixt. (100 mg) in this solvent and in CH_3COOEt–CH_3COOH–MeOH–H_2O (13:5:3:3), the optical rotation of each purified sugar was measured. The ppt. of sapogenins were purified by CC using a gradient of $CHCl_3$–MeOH (from 49:1 to 1:1); 9 mg of medicagenic acid and 2 mg of gypsogenic acid were obtained.

Medicagenic acid. EIMS *m/z*: 456 $[M\text{-}HCOOH]^+$, 248 $[RDA]^+$, 203 $[RDA\text{-}COOH]^+$; ^1H NMR ($CDCl_3+CD_3OD$): δ 0.83 (*s*, H-26), 0.9 (*s*, H-29), 0.95 (*s*, H-30), 1.16 (*s*, H-27), 1.26 (*s*, H-25), 1.3 (*s*, H-24), 2.86 (*dd*, $J = 14.5$, 3.5 Hz, H-18), 3.98 (*d*, $J = 3.5$ Hz, H-3), 4.06 (*m*, $W_{1/2} = 11$ Hz, H-2), 5.23 (*t*, $J = 3$ Hz, H-12); ^{13}C NMR signals superimposable to ± 1.7 ppm on those reported in ref [4].

Gypsogenic acid. Positive FABMS (+LiCl) *m/z*: 493 $[M+Li]^+$; ^1H NMR ($CDCl_3+CD_3OD$): δ 0.81 (*s*, H-26), 0.9 (*s*, H-29), 0.94 (*s*, H-30), 0.95 (*s*, H-25), 1.08 (*s*, H-27), 1.15 (*s*, H-24), 2.85 (*dd*, $J = 14.5$, 3.5 Hz, H-18), 3.98 (*br d*, $J = 7$ Hz, H-3), 5.23 (*t*, $J = 3$ Hz, H-12).

Basic hydrolysis of saponin mixture. The VLC fr. of saponins (130 mg) was refluxed for 3 hr in 10 ml of KOH 5% in dioxane–H_2O (1:1). After acidification with HCl 2 N until pH = 4, the reaction mixt. was extracted with Et_2O. The organic soln. was rinsed with H_2O, dried over Na_2SO_4 and evapd to give 6.8 mg of a residue. Purification by CC yielded 0.4 mg of nilic acid in the fr. eluting with MeOH $[\alpha]_D = +44°$ (MeOH; *c* 0.03).

Acetylation of saponin mixture. After purification of 3 g of crude saponin extract by VLC eluting with $CHCl_3$–MeOH–H_2O (12:8:1), the saponin fr. (1.4 g) was acetylated in 10 ml of CH_2Cl_2; 1 ml of Ac_2O, 2 ml of pyridine and 40 mg of DMAP were added to the suspension. The reaction was stirred at room temp. for 4 days and the resulting clear soln was washed with 10 ml of 10% aq. $CuSO_4$, followed by H_2O. The organic layer was dried over Na_2SO_4 and evapd to afford 1.6 g of an acetylated mixt. Purification was achieved by successive CC with a gradient of $CHCl_3$–hexane–MeOH (from 90:10:0 to 90:10:3) or of hexane–AcOEt (from 1:1 to 1:4). Final purifications were obtained by prep. TLC in $CHCl_3$–Me_2CO–MeOH (90:10:1) to afford the peracetylated saponin **2a** (8 mg), in $CHCl_3$–hexane–MeOH (90:10:3) to yield derivative **4a** (7 mg), in Et_2O–MeOH (49:1) to obtain **1a** (23 mg) and **3a** (6 mg) or in hexane–AcOEt (3:7) to obtain **2a** (7 mg) and **3a** (5 mg).

Saponin **1**. $[\alpha]_D = +7.2°$ (*c* 0.53; MeOH). Positive ion ESIMS *m/z*: 1635.6 $[M+K]^+$, 1619.6 $[M+Na]^+$, 1489.7 $[M+Na\text{-}ara]^+$, 1473.6 $[M+K\text{-}glc]^+$, 1457.6 $[M+Na\text{-}glc]^+$, 1295.6 $[M+Na\text{-}glc\text{-}glc]^+$. Positive ion LSIMS *m/z*: 1635.6 $[M+K]^+$, 1619.6 $[M+Na]^+$. ^1H NMR: δ 0.83 (*s*, H-26), 0.91 (*s*, H-29), 0.96 (*s*, H-30), 1.01 (*s*, H-25), 1.1 (*d*, $J = 10$ Hz, H-19), 1.15 (*s*, H-24 and H-27), 1.37 (H-5), 1.4 (H-2), 1.62 (H-9), 1.65 (H-19), 1.9 (H-11), 2.81 (*br d*, $J = 11$ Hz, H-18), 4.14 (H-3), 5.29 (*m*, $W_{1/2} = 9$ Hz, H-12).

Saponin **2**. $[\alpha]_D = -1.8°$ (*c* 0.49; MeOH). Positive ion ESIMS *m/z*: 1635.6 $[M+Na]^+$, 1503.4 $[M+Na\text{-}ara]^+$, 1473.7 $[M+Na\text{-}glc]^+$, 1421.6 $[M+Na\text{-}ara\text{-}ang\ ac.]^+$, 849.4 $[M+Na\text{-}ester\ chain]^+$, 809 $[ester\ chain+Na]^+$. Positive ion LSIMS *m/z*: 1635.6 $[M+Na]^+$. ^1H NMR: δ 0.82 (*s*, H-26), 0.88 (*s*, H-29), 0.93 (*s*, H-30), 1.12 (*s*, H-27), 1.12 (*dd*, $J = 9$, 6 Hz, H-19), 1.24 (H-1), 1.28 (*s*, H-25), 1.36 (*s*, H-24), 1.67 (*dd*, $J = 9$, 7 Hz, H-19), 1.96 (H-11), 2.1 (H-1), 2.81 (*ddd*, $J = 7$, 6, 4 Hz, H-18), 4.2 (*m*, $W_{1/2} = 11$ Hz, H-3), 4.3 (*m*, $W_{1/2} = 10$ Hz, H-2), 5.27 (*br t*, $W_{1/2} = 2.5$ Hz, H-12).

Saponin **3**. $[\alpha]_D = +13°$ (*c* 0.19; MeOH). Positive ion ESIMS *m/z*: 1605.7 $[M+Na]^+$, 1443.6 $[M+Na\text{-}glc]^+$, 1355.6 $[M+Na\text{-}ara\text{-}nil\ ac.]^+$, 849.4 $[M+Na\text{-}ester\ chain]^+$, 779.3 $[ester\ chain+Na]^+$. Positive ion LSIMS *m/z*: 1621.9 $[M+K]^+$, 1605.9 $[M+Na]^+$, 1583.8 $[M+H]^+$. ^1H NMR: δ 0.79 (*s*, H-26), 0.9 (*s*, H-29), 0.94 (*s*, H-30), 1.14 (*s*, H-27), 1.15 (*d*, $J = 10$ Hz, H-19), 1.25 (H-1), 1.26 (*s*, H-25), 1.36 (*s*, H-24), 1.7 (*t*, $J = 13$ Hz, H-19), 1.93 (H-11), 2 (H-11), 2.1 (*br d*, $J = 14$ Hz, H-1), 2.84 (*dd*, $J = 10$, 3 Hz, H-18), 4.18 (*m*, $W_{1/2} = 9$ Hz, H-3), 4.32 (*m*, $W_{1/2} = 12$ Hz, H-2), 5.29 (*m*, $W_{1/2} = 8$ Hz, H-12).

Saponin **4**. $[\alpha]_D = -12°$ (*c* 0.05; MeOH). Positive ion ESIMS *m/z*: 1475.7 $[M+K]^+$, 1459.7 $[M+Na]^+$, 779.4 $[ester\ chain+Na]^+$, 703.4 $[M+Na\text{-}ester\ chain]^+$. Positive ion LSIMS *m/z*: 1475.6 $[M+K]^+$, 1459.6 $[M+Na]^+$. ^1H NMR: δ 0.8 (*s*, H-26), 0.87 (*s*, H-29), 0.96 (*s*, H-30), 1.07 (H-19), 1.27 (*br d*, $J = 14$ Hz, H-1), 1.29 (*s*, H-27), 1.38 (*s*, H-24 and H-25), 1.42 (*dd*, $J = 11$, 2 Hz, H-15), 1.65 (H-11), 1.69 (*dd*, $J = 11$, 4 Hz, H-15), 1.97 (H-11), 2.14 (*dd*, $J = 14$, 2 Hz, H-1), 2.29 (*t*, $J = 14$ Hz, H-19), 2.95 (*dd*, $J = 14$, 3.5 Hz,

H-18), 4.1 (*br d*, $J = 4$ Hz, H-3), 4.31 (H-2), 4.49 (*m*, $W_{1/2} = 6.5$ Hz, H-16), 5.36 (H-12).

Derivative **1a**. Positive FABMS *m/z*: 2375.8 $[M+Na]^+$, 2277.8 [M+Na-ang ac.]$^+$, 2119.6 [M+Na-ara]$^+$, 619.2 [Glc → glc]$^+$, 475.1 [Ara → xyl]$^+$, 331.1 [Glc]$^+$, 299.1 [Ara → ang ac.]$^+$, 259.1 [Ara]$^+$. Positive FABMS (+LiCl) *m/z*: 2365.8 [M+2Li-H]$^+$, 2359.7 [M+Li]$^+$, 2267.7 [M+2Li-H-ang ac.]$^+$, 2107.9 [M+2Li-H-ara]$^+$, 299.2 [Ara → ang ac.]$^+$, 259.1 [Ara]$^+$. ^{1}H NMR: δ 0.77 (*s*, H-26), 0.9 (*s*, H-29), 0.91 (*s*, H-30), 0.96 (*s*, H-25), 1.12 (*s*, H-27), 1.16 (H-19), 1.2 (*s*, H-24), 1.36 (H-5), 1.56 (H-9), 1.62 (H-19), 1.9 (H-11), 1.82 (*br d*, $J = 10$ Hz, H-2), 2.8 (*dd*, $J = 13$, 4 Hz, H-18), 4.05 (*dd*, $J = 12$, 5.5 Hz, H-3), 5.32 (*br t*, $J = 3$ Hz, H-12).

Derivative **2a**. Positive ion LSIMS *m/z*: 2391.8 $[M+Na]^+$, 2369.8 $[M+H]^+$, 2351.8 $[M+H-H_2O]^+$, 2135.5 [M+Na-ara]$^+$, 1249.2 [ester chain]$^+$, 619.1 [Glc → glc]$^+$, 529.1 [Rha → ara → ang. ac.]$^+$, 489.1 [Rha → ara]$^+$, 475.12 [Ara (or Xyl) → rha]$^+$, 331.0 [Glc]$^+$, 299.0 [Ara → ang ac.]$^+$, 259.1 [Ara]$^+$. ^{1}H NMR: δ 0.8 (*s*, H-26), 0.9 (*s*, H-29), 0.91 (*s*, H-30), 1.12 (*s*, H-27), 1.2 (*s*, H-19), 1.22 (H-1), 1.25 (*s*, H-25), 1.3 (H-24), 1.7 (H-19), 1.91 (H-11), 2.03 (H-11), 2.21 (H-1), 2.81 (*dd*, $J = 13$, 4.5 Hz, H-18), 4.08 (H-3), 4.25 (H-2), 5.4 (H-12).

Derivative **3a**. Positive FABMS *m/z*: 2277.8 $[M+Na]^+$, 705.4 [Ara → rha → xyl]$^+$, 619.2 [Glc → glc]$^+$, 489.2 [Ara (or Xyl) → rha]$^+$, 331.0 [Glc]$^+$, 250.0 [Ara (or Nil ac. → nil ac.)]$^+$. Positive FABMS (+LiCl) *m/z*: 2269.7 [M+2Li-H]$^+$, 2263.2 [M+Li]$^+$. ^{1}H NMR: δ 0.81 (*s*, H-26), 0.93 (*s*, H-29), 0.95 (*s*, H-30), 1.14 (*s*, H-27), 1.1 (*s*, H-19), 1.2 (H-1), 1.28 (*s*, H-25), 1.46 (*s*, H-24), 1.7 (*t*, $J = 13$ Hz, H-19), 1.9 (H-11), 2.03 (H-11), 2.18 (*d*, $J = 11.5$ Hz, H-1), 2.78 (*br d*, $J = 13$ Hz, H-18), 4.11 (*d*, $J = 2.5$ Hz, H-3), 4.24 (*dd*, $J = 5$, 2.5 Hz, H-2), 5.3 (*br t*, $J = 3$ Hz, H-12).

Derivative **4a**. Positive ion LSIMS *m/z*: 2047.5 $[M+Na]^+$, 705.1 [Ara → rha → xyl]$^+$, 619.2 [Glc → glc]$^+$, 331.0 [Glc]$^+$, 259.0 [Ara (or Nil ac. → nil ac.)]$^+$. ^{1}H NMR: δ 0.75 (*s*, H-26), 0.94 (*s*, H-29), 1.02 (*s*, H-30), 1.19 (H-19), 1.24 (H-1), 1.24 (H-27), 1.29 (*s*, H-25), 1.35 (*s*, H-24), 1.45 (*br d*, $J = 12$ Hz, H-15), 1.83 (*br d*, $J = 12$ Hz, H-15), 1.94 (H-11), 2.01 (H-11), 2.14 (H-19), 2.2 (*d*, $J = 10$ Hz, H-1), 3.03 (*dd*, $J = 14$, 4 Hz, H-18), 4.09 (*d*, $J = 3.5$ Hz, H-3), 4.25 (H-2), 5.44 (H-12), 5.6 (*m*, $W_{1/2} = 9$ Hz, H-16).

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