Pergamon

0031-9422(94)00565-6

Phytochemistry, Vol. 37, No. 6, pp. 1671–1677, 1994

0031-9422/94 $7.00 + 0.00

TRITERPENE SAPONINS FROM *MYRSINE PELLUCIDA*

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(*Received in revised form* 21 *June* 1994)

Key Word Index—*Myrsine pellucida*; Myrsinaceae; stem bark; triterpene saponins; *d*-quercitol; stigmasterol.

Abstract—Quercitol, five saponins and 3-*O*-(6′-*O*-palmitoyl) β-D-glucopyranosyl stigmasterol were isolated from the stem bark of *Myrsine pellucida*. These compounds are described for the first time in this plant and their structures were determined using a combination of ^{1}H and ^{13}C NMR, and mass spectroscopy. The two saponins are new compounds, 3-*O*-(α-L-rhamnopyranosyl (1→2) β-D-glucopyranosyl (1→4) α-L-arabinopyranosyl) cyclamiretin A and 3-*O*-(β-D-xylopyranosyl (1→2) β-D-glucopyranosyl (1→4) [β-D-glucopyranosyl (1→2)] α-L-arabinopyranosyl) cyclamiretin D.

INTRODUCTION

Myrsine pellucida (Ruiz and Pavon) Sprengel is a tree occurring wild in Bolivia and in other countries of the Andes. During ethnobotanical field work in the subtropical Chaco province of Bolivia, stem bark of *Myrsine pellucida* used as a fish poison by Chiriguano Indians was collected. We now report on the isolation and structural elucidation of five saponins, *d*-quercitol and a glycoside of stigmasterol from the ethanolic extract of the species.

RESULTS AND DISCUSSION

The stem bark of *M. pellucida* was defatted with petrol, then extracted with ethanol in a Soxhlet apparatus. A part of the ethanol extract was subjected to chromatographic purification on a silica gel column to yield *d*-quercitol **1** [1]. After dialysis against pure water, the ethanol extract deprived of quercitol, was purified by chromatography on a silica gel column and prep. TLC or reverse phase C_{18} column chromatography, to afford the known saponins saxifragolin B (**2**), primulanin (**3**) and ardisiacrispin B (**4**), the new saponins **5** and **6**, and **7**.

Compound **1** showed a molecular ion at *m/z* 164; the successive losses of three molecules of water at *m/z* 146, 128 and 110 suggested a cyclitol nature. The ^{1}H NMR spectrum displayed signals for seven protons, linked to six carbons according to a HMQC experiment. The CH_2 at δ34.7 was the only non-oxidized carbon, the others corresponded to five oxymethine carbons resonating between δ69 and 76. The COSY experiment showed correlations between the methine protons and confirmed a cyclic system by the observation of correlations between the geminal protons and two different oxymethine protons. The measurement of the coupling constants indicated four vicinal axial protons (H-6→H-1→H-2→H-3) followed by three vicinal equatorial protons (H-4→H-5→H-6). This established the structure of **1** as *d*-quercitol [2].

The main saponin **2** showed in the positive-ion FAB-mass spectrum a quasi-molecular peak at *m/z* 1083.5 $[M + Na]^+$ analysed as $C_{52}H_{84}O_{22}Na$. The negative-ion spectrum displayed a $[M - H]^-$ ion at *m/z* 1059.6. Negative fragment ions at *m/z* 927.6, 896.5 and 765.5 were attributed to the losses of a terminal pentose, a terminal hexose and a terminal hexose–pentose disaccharide, respectively. Structure of the triterpene, cyclamiretin A [3], was determined by ^{1}H and ^{13}C NMR using connectivities observed in COSY, HMQC and HMBC spectra. Most of the ^{13}C NMR signals of the triterpene were assigned through two and three bond ^{1}H–^{13}C couplings of the six angular methyls. The genin was thus identified as cyclamiretin A [4–6]. Several literature resonance assignments [6] were displaced following analysis of the HMQC and HMBC cross-peaks (Table 1).

The presence of a four sugar residue was deduced from the observation of four anomeric carbons at δ104.3, 104.7, 107.2 and 105.6 attached to protons at δ4.69 (*d*, $J = 7.5$ Hz), 4.52 (two doublets, $J = 7.8$ Hz) and 4.39 (*m*), respectively (HMQC). Identification of the sugars was done with the help of COSY and HOHAHA experiments, and allowed the identification of two β-D-glucoses, one β-D-xylose and a second pentose. Comparison of the chemical shifts of saponin **2** and saxifragifolin B [5, 6] showed superimposition of osidic resonances and consequently the second pentose was an α-L-arabinose (Table 1).

Sequencing of the sugar chain and linkage on cyclamiretin A were achieved by ROESY and HMBC experiments. Observation of an Overhauser effect between H-3

Table 1. ^{13}C NMR data for **2**–**6** (in CD_3OD) and **4a** (in $CDCl_3$)

C	2	3	5	6	4a	C	2	3	5	6	4a
1	37.8*	37.8*	37.8*	37.8	36.5*		arabinose				
2	28.4	27.2	27.1	27.1	25.8	1	105.6	107.4	107.1	105.6	101.6
3	91.3	90.8	90.9	91.3	89.0	2	79.5	74.0	74.3	79.5	75.0
4	40.6	40.3	40.3	40.0	39.2	3	74.2	74.5	73.2	74.3	71.0
5	56.8	56.9	56.8	57.0	55.2	4	80.2	81.3	78.8	80.1	68.5
6	18.7	18.7	18.7	19.3	17.4	5	65.8	66.8	66.2	65.7	61.9
7	35.1	35.1	35.1	34.0	33.5						
8	43.4	43.4	43.4	41.2	42.6		glucose (inner)				
9	51.3	54.0	54.0	44.0	49.9	1	104.7	105.2	105.0	104.8	101.6
10	40.2*	40.2*	40.0*	35.3	38.8*	2	85.0	86.0	79.5†	85.1	76.0
11	19.3	20.0	19.8	24.5	18.4	3	77.5	77.4	78.4†	77.6	74.1
12	33.2	33.1	33.2	124.7	31.5	4	70.9	71.0	71.6	71.0	68.5
13	88.1	88.2	88.2	144.5	86.1	5	78.0	77.7	77.8	78.8	71.8
14	45.3	45.3	45.6	42.8	49.9	6	63.3	62.5	62.7	63.3	61.9
15	37.0	37.0	37.0	35.2	45.3						
16	77.5	77.7	77.8	71.6	77.8		xylose		rhamnose	xylose	rhamnose
17	44.7	44.7	44.7	40.5	44.6	1	107.2	107.8	102.0	107.3	97.3
18	53.9	51.3	51.3	44.9	56.1	2	75.8	76.1	71.9	75.9	71.8
19	33.9	34.0	34.0	30.7	44.6	3	77.8	77.4	71.9	78.0	69.7
20	49.3	49.3	49.0	49.0	36.5	4	71.0	70.8	74.0	71.1	71.0
21	30.9	30.7	30.8	30.2	36.5‡	5	67.3	67.1	70.5	67.4	66.9
22	32.7	32.7	32.8	28.5	25.8‡	6			18.7		17.6
23	28.4	28.4	28.5	28.5	27.7						
24	16.7	16.8	16.8	16.3	16.0		glucose'				
25	16.7	16.8	16.8	16.9	15.9	1	104.3			104.4	101.2
26	18.8	18.8	18.0	16.9	17.6	2	76.0			76.0	71.3
27	20.2	20.1	20.1	27.9	21.1	3	77.8			77.9	72.6
28	78.4	78.4	78.4	62.8	75.0	4	71.9			72.0	68.2
29	24.3	24.3	24.3	24.5	28.2	5	77.8			77.9	71.8
30	209.7	206.5	209.0	209.5	71.8	6	62.5			62.6	61.9

*†Values may be interchanged in each column.
‡Not confirmed by HMBC or HMQC experiments.

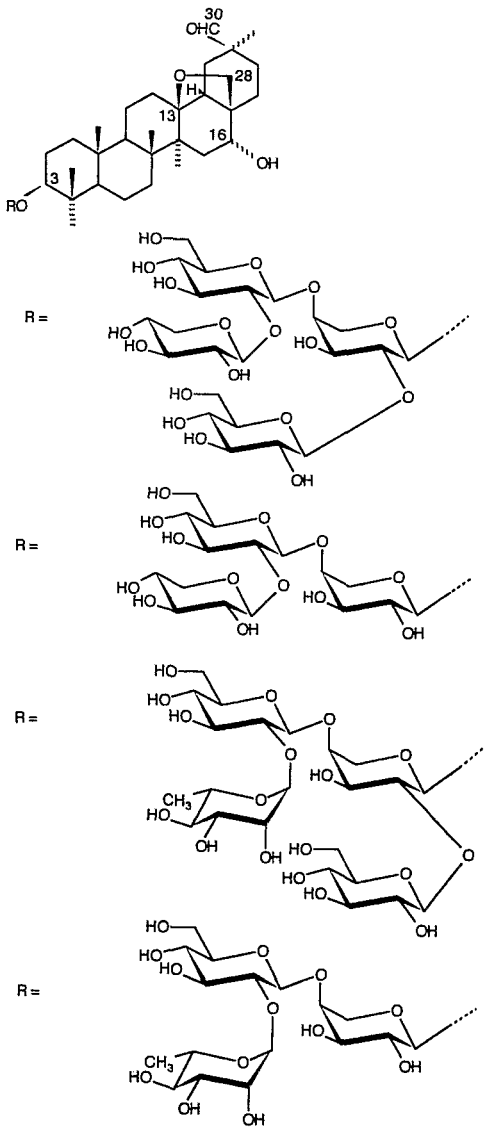
of the genin and H-1 of arabinose in the ROESY experiment, confirmed the attachment of the sugar chain on position C-3 of cyclamiretin A. In the HMBC spectrum, strong correlations between the superimposed anomeric protons at δ4.52 and the C-4 of arabinose, and between H-2 of the inner glucose and C-1 of the xylose, allowed linking xylose at position C-2 of the glucose and this glucose at position C-4 of arabinose. The terminal glucose was attached to position C-2 of arabinose by comparison of ^{13}C NMR data published for saxifragifolin B [5, 6] (Table 1).

All these observations confirm the identity of saponin **2** with saxifragifolin B or 3-*O*-(β-D-xylopyranosyl (1→2) β-D-glucopyranosyl (1→4) [β-D-glucopyranosyl (1→2)] α-L-arabinopyranosyl) cyclamiretin A. This saponin has been isolated from *Androsace saxifragifolia* [4], in the genus *Cyclamen* under the name of desglucocyclamin I [6], and in *Ardisia crispa* as ardisiacrispin A [7].

The structures of related saponins **3**–**6** were elucidated by NMR spectroscopy and in particular by the application of above 2D NMR techniques and by comparison of their data with those of the major saponin **2**.

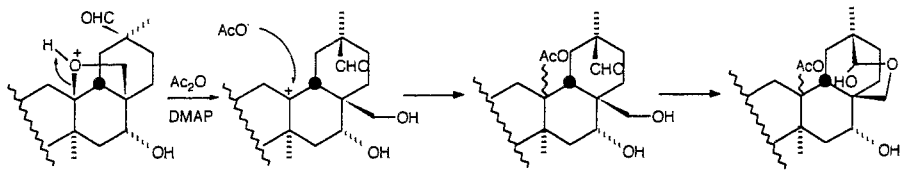
Saponin **3** was identified as primulanin from *Primula denticulata* [5]. A comparison of ^{13}C NMR data of **3** with those of **2** showed the same signals for the genin and indicated that the terminal glucose was absent from **3** (Table 1). The C-2 of arabinose was shifted upfield at δ74 ($\Delta\delta = -5.5$ ppm) suggesting that this position was not substituted by a sugar in saponin **3**; a small deshielding effect was observed on the anomeric carbon ($\Delta\delta = +1.8$ ppm). ROE effects were observed between H-1 of the inner glucose (δ4.49) and H-4 of arabinose (δ3.82), and between H-1 of arabinose (δ4.2) and H-3 of cyclamiretin A (δ3.13). On the basis of the above findings, the structure of **3** is concluded to be primulanin or 3-*O*-[β-D-xylopyranosyl (1→2) β-D-glucopyranosyl (1→4) α-L-arabinopyranosyl] cyclamiretin A.

Saponin **4** was in a mixture with saxifragifolin B (**2**) and separation of the compounds by silica gel chromatography was inefficient. The ^{1}H NMR spectrum of the mixture showed the four anomeric protons described in saponin **2**, and four supplementary doublets at δ5.27 ($J = 2$ Hz), 4.62 ($J = 7$ Hz), 4.58 ($J = 7$ Hz) and 4.52 (not resolved). The methyl singlets of the genin were not duplicated but broadened. One methyl doublet was observed at δ1.3 and was attributed to a 6-desoxyhexose. The structure of **4** was established unambiguously by analysis of the peracetylated derivative **4a**, which was



easily purified from the peracetylated derivative of saxifragifolin B by column chromatography. The spectra of peracetylated **4** was modified with time indicating the presence of an unstable compound which changed to one stable compound **4a**. One of the most remarkable signs of the rearrangement was the disappearance of the aldehyde signals in ^{1}H and ^{13}C NMR (Table 1). The integrity of the ABCD rings of cyclamiretin A was demonstrated by the long range couplings of the angular methyls which gave correlations with 11 of the 18 resonances of those rings. The C-16 was assigned by observing direct correlation with H-16 at δ4.03 in the HMQC spectrum. The rest of the ^{13}C resonances were identified by comparison with data for saponins **2**, **3** and **5**. The HMQC experiment showed a cross-peak between a singlet proton at δ4.07 and an oxymethine at δ71.8. These observations suggested the formation of an hemiketal function between the aldehydic C-30 and the hydroxymethylene C-28 in **4a**. COSY and HOHAHA experiments allowed identification of the four sugar units with one α-L-rhamnose, one α-L-arabinose and two β-D-glucoses. One of the glucoses had the H-2 signal upfield and was monosubstituted. Arabinose possessed shielded H-2 and H-4 at δ4.03 and 4.05, and was therefore disubstituted. The two remaining sugars were terminal, their positions C-2, C-3 and C-4 were acetylated and their α-protons resonated in the δ4.9–5.2 region. Sequencing was performed by means of a ROESY experiment. The anomeric proton of rhamnose (δ5.03) showed a ROE correlation with H-2 of the substituted glucose, the H-1 of which correlated with H-4 of arabinose. The terminal glucose showed an Overhauser effect with H-2 of arabinose. ROE correlation of H-3 of the genin with H-1 of arabinose, allowed determination of the four sugar chain on C-3. Compound **4a** is the peracetate of 3-*O*-(α-L-rhamnopyranosyl (1 → 2) β-D-glucopyranosyl (1 → 4) [β-D-glucopyranosyl (1 → 2)] α-L-arabinopyranosyl) 3β, 13β, 16α-trihydroxy 28β, 30β-epoxy-oleanane. The ^{1}H NMR spectrum of **4a** showed seven singlets of acetates from δ1.97 to 2.13 integrating for 12 methyls. Considering the 11 acetylated positions of the sugar units, only one acetate belonged to the genin. Position C-16, which was sterically hindered, was not acetylated and its ^{1}H chemical shift was unchanged. The acetate was therefore located at the C-13 position. Compound **4a** arises from the rearrangement of a 13-28 oxido compound with participation of C-29. Similar rearrangements were observed upon heating cyclamiretins A and D in chloroform in the presence of H_2SO_4 [6, 8, 9]. Scheme 1 gives a tentative explanation for the rearrangement. Saponin **4** is therefore ardisiacrispin B or 3-*O*-(α-L-rhamnopyranosyl (1 → 2) β-D-glucopyranosyl (1 → 4) [β-D-glucopyranosyl (1 → 2)] α-L-arabinopyranosyl) cyclamiretin A [7]. This known compound is a regio-isomer of a saponin recently isolated from *Ardisia japonica* [10].

The ^{13}C NMR of saponin **5** showed the same signals for the genin part as in saponins **2** and **3** (Table 1). The presence of cyclamiretin A was confirmed by the observation of the characteristic pattern of cross-peak correlations from the six methyl protons in the HMBC. The ^{1}H NMR spectrum of **5** displayed signals for three anomeric doublets at δ4.26 (J = 6 Hz), 4.56 (J = 7.3 Hz) and 5.26 (J = 1.9 Hz). These protons were linked to three anomeric carbons at δ107.1, 105 and 102, respectively, in view of the HMQC experiment. Values of the couplings taken from the 1D ^{1}H NMR spectrum or their magnitudes evaluated from the shape of the cross-peaks in the HOHAHA spectrum indicated the presence of one β-D-glucose, one α-L-arabinose and one α-L-rhamnose. In the ROESY spectrum, through-space interactions across the glycosidic bond were observed between H-1 of arabinose and H-3 of the genin, and between H-1 of glucose and H-4 of arabinose. The H-1 of rhamnose gave a correlation at δ3.44, with a multiplet due to the overlap of H-2 and H-3 of glucose. These two protons were linked to two carbons at δ78.4 and 79.5. In the HMBC spectrum, the correlation between H-5 of glucose and the shielded carbon corresponded to a $^3J_{H\text{-}C}$ coupling with the C-3 of this sugar. The anomeric proton of rhamnose displayed a correlation with the second carbon at δ79.5 therefore attributed to

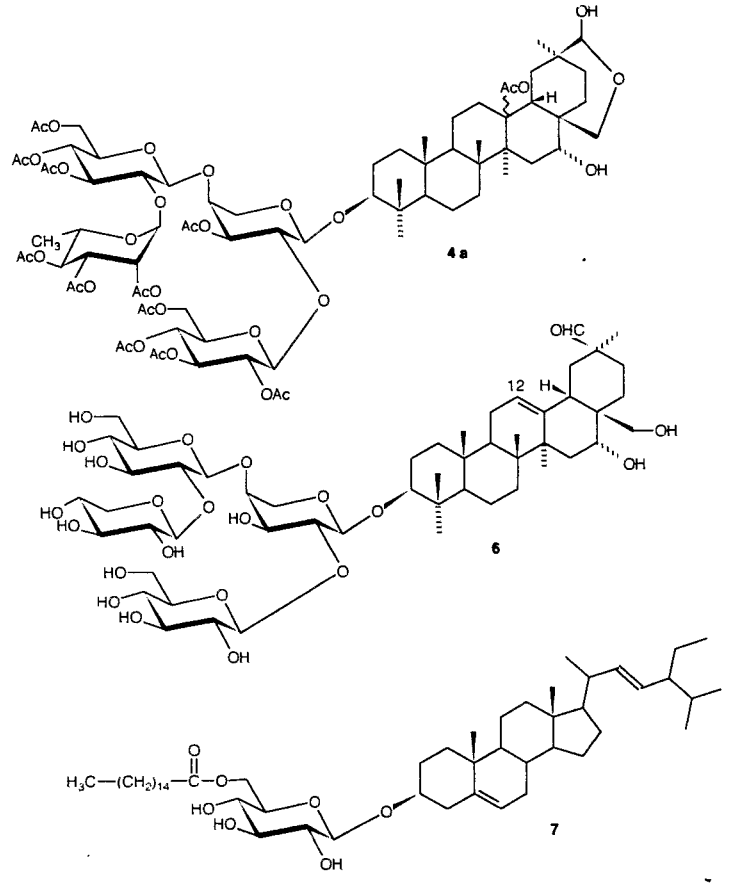


Scheme 1

the C-2 of glucose. These ^{13}C NMR chemical shift values were comparable with those found in the literature for a glucose substituted at position C-2 by a rhamnose [11]. Thus, saponin **5** has the novel structure of 3-*O*-(α-L-rhamnopyranosyl (1→2) β-D-glucopyranosyl (1→4) α-L-arabinopyranosyl) cyclamiretin A.

Saponin **6** was shown to possess the same sugar chain as saxifragifolin B (**2**) by comparison of its ^1H and ^{13}C NMR with those of **2** (Table 1). The major differences concerned the genin part. The ^{13}C NMR spectrum of **6** exhibited a trisubstituted ethylenic system with a quaternary sp^2 carbon at δ144.5 and a methine at δ124.7 attached to a proton at δ5.34. This proton was coupled with high field signals located at *ca* δ1.95 in the COSY spectrum. The oxycarbon C-28 was found at δ62.8 in saponin **6** instead of δ78.4 in saponin **2**. This shielding gave evidence for a terminal hydroxymethylene group on position C-17. Other carbons of the genin were assigned by comparison of chemical shifts with those described for cyclamiretin D [8]. Some differences were due to solvent effects and to the attached sugar chain in **6**. The C-3 resonated at δ91.3 in saponin **6** instead of δ77.8 in cyclamiretin D, suggesting that the four sugar chain was linked thereon. Saponin **6** is thus 3-*O*-(β-D-xylopyranosyl (1→2) β-D-glucopyranosyl (1→4) [β-D-glucopyranosyl (1→2)] α-L-arabinopyranosyl) cyclamiretin D. Cyclamiretin D has been reported to be an artefactual sapogenin formed by acid-catalysed rearrangement of cyclamiretin A [9]. Saponin **6** might thus be formed from saponin **2**, but analysis of the ^1H NMR spectrum of the mixture of saponins before purification showed a multiplet corresponding to ethylenic protons at δ5.4, thus favouring the natural origin of **6**.

Besides saponins, chromatography of the dialysed ethanolic extract yielded an acylated phytosterol glucoside **7**. The ^1H and ^{13}C NMR spectra of **7** displayed signals for three units: a C_{29}-sterol, an hexose and a fatty acid. The EI mass spectrum showed no molecular peak, but frag-



ments due to a stigmasterol unit. The ^{1}H NMR spectrum exhibited signals for six methyls: two were singlets at δ0.7 and 1.01, three were doublets at 0.86 (J=6 Hz), 0.88 (J=8 Hz) and 1.02 (J=7 Hz), and one was a triplet at 0.82 (J=6.2 Hz). An ethylenic proton at δ5.37 coupled to high field protons in the COSY spectrum revealed a trisubstituted double bond; a *trans*-disubstituted double bond was also present with two double doublets at δ5.03 and 5.17 (J=15 and 8.4 Hz). These signals belonged to stigmasterol and this was confirmed by the ^{13}C NMR assignments of **7** which were in agreement with those described in the literature [12]. The +7.7 ppm deshielding observed for C-3 allowed placement of the sugar unit at this position. The hexose unit was determinated as a β-D-glucose according to analysis of the seven spin system. The signals at 4.28 (*d*, J=11.7 Hz) and 4.48 (*dd*, J=11.7, 4.5 Hz), which corresponded to the H-6 methylene group, were shifted by +0.6 ppm with regard to values observed for a free terminal glucose, thus suggesting esterification of this position. The positive mode FAB-mass spectrum of **7** showed a pseudo-molecular peak $[M+Na]^+$ at *m/z* 835.4 and at *m/z* 819.4 $[M+Li-H]^+$ after addition of LiCl, and a molecular formula of $C_{51}H_{88}O_7$ was deduced ($[M]^+$ at *m/z* 812). The negative FAB-mass spectrum of **7** gave no pseudo-molecular ion, but an intense fragment at *m/z* 255.1 assigned to a C_{16}-acid part. Confirmation of the presence of a palmitoyl ester was obtained by running a FAB-MIKE spectra from the pseudomolecular ions; fragments at *m/z* 440 and at *m/z* 424 [M − stigmasterol +Na or Li]$^+$ were observed, respectively. Thus, **7** is the 3-*O*-(6′-*O*-palmitoyl) β-D-glucopyranosyl stigmasterol. This compound is isolated here for the first time in a pure form; it has previously been described in mixture with other sterol glycosides [13].

The crude ethanolic extract from the stem bark of *Myrsine pellucida* has been tested against New World Leishmania *in vitro*. Its activity was 50 μg ml^{-1} on promastigote cells of *Leishmania brasiliensis*. Further studies are in progress to determine which class of the three types of extracted compounds is responsible for the activity.

EXPERIMENTAL

General. ^{1}H and ^{13}C NMR spectra were recorded at 300 and 75 MHz. 2D expts were performed using standard Bruker microprograms. Hardware modifications of the AC-300 spectrometer allowed acquisition of the ^{13}C–^{1}H correlations in the reverse mode. HOHAHA expt was done using the decoupling channel to generate the spinlock (238 msec) and ROESY expt in the same mode with a single long pulse (200 msec).

Plant material. The stem bark was collected in the Chaco province of Bolivia on 18 November, 1991. The plant was kindly authenticated by Dr S. Beck and a voucher specimen is deposited at the herbarium of San Andrès University in La Paz.

Extraction and isolation of compounds. Dried, powdered stem bark (1.3 kg) was extracted in a Soxhlet apparatus with 3.5 l of petrol, then with 3.5 l EtOH. After cooling, the ethanolic soln was evapd and yielded 221.7 g of extract. A sample (1 g) was chromatographed on a silica gel column (40 g) eluted with mixts of $CHCl_3$–MeOH–H_2O [90:10:0 (0.5 l); 85:15:0 (3 l); 60:40:0 (2 l); 60:40:5 (3 l)]. Frs 125–152 yielded **1** (200 mg).

A sample of crude extract (20 g) was dissolved in H_2O (200 ml) and dialysed against H_2O in seamless cellulose tubes. After 4 days, the content of the tubes was freeze-dried and gave 9.9 g of saponin mixt. Part of this saponin extract (3 g) was purified by chromatography on silica gel (120 g). The column was eluted with mixts of $CHCl_3$–MeOH [100:0 (2 l), 98:2 (1.5 l), 95:5 (2.5 l), 90:10 (6.5 l), 85:15 (8 l), 75:25 (6 l), 0:100 (1.5 l)]. Frs 29–38 were purified by prep. TLC in $CHCl_3$–MeOH (95:5) to obtain **7** (12 mg); frs 79–95 were purified by prep. TLC in $CHCl_3$–MeOH (7:3) to give **3** (17 mg); frs 114–122 were purified by prep. TLC in $CHCl_3$–MeOH–H_2O (70:30:1) to yield **5** (10 mg); frs 145–152 yielded **2** (196 mg); frs 155–160 contained **2** and **4**, and were acetylated; frs 214–270 were further chromatographed on reverse phase silica gel C_{18} eluted with MeOH–H_2O (50:50) and the collected frs were purified by prep. TLC in *n*-BuOH–AcOH–Et_2O–H_2O (90:60:30:15) to obtain **6** (25 mg).

Isolation of **4a**. The mixed frs 155–160 (200 mg) were suspended in CH_2Cl_2 (10 ml) and acetylated with an excess of Ac_2O (2 ml) and DMAP (40 mg). The resulting mixt. of acetylated saponins was chromatographed on silica gel (10 g). The column was eluted with mixts of $CHCl_3$–Et_2O (100:0, 99:1). Frs were collected and purified by prep. TLC in $CHCl_3$–Et_2O–MeOH (10:90:1) to obtain **4a** (25 mg).

Compound **1**. Mp 224° (MeOH); $[\alpha]_D$ +17° (MeOH; *c* 1). MS (rel. int.) *m/z*: 164 (18), 146 (10), 128 (80), 110 (32). ^{1}H NMR (CD_3OD): δ1.88 (H-6ax., *ddd*, J = 14, 11.5, 3 Hz), 2 (H-6eq., *dddd*, J=14, 4.8, 3.5, 0.9 Hz), 3.6 (H-2, *t*, J=9.2 Hz), 3.74 (H-3, *dd*, J=9.4, 3.1 Hz), 3.8 (H-1, *ddd*, J=11.2, 9, 4.9 Hz), 3.95 (H-4, *td*, J=3.2, 0.9 Hz), 4.04 (H-5, *q*, J=3.3 Hz); ^{13}C NMR (CD_3OD): δ34.7 (C-6), 69.8 (C-5), 70.1 (C-1), 72.4 (C-3), 73.6 (C-4), 75.7 (C-2).

Saponin **2**. Positive FAB-MS *m/z* (rel. int.): 1083.5 [M + Na]$^+$ (7); negative FAB-MS *m/z* (rel. int.): 1059.6 [M −H]$^-$ (55), 927.6 (20), 926.6 (13), 896.5 (8), 765.5 (18), 763.5 (10). ^{1}H NMR (CD_3OD): δ0.72 (H-5, *d*, J=5 Hz), 0.84 (H-24, *s*), 0.89 (H-25, *s*), 0.97 (H-29, *s*), 1.05 (H-23, *s*), 1.06 (H-18, *m*), 1.13 (H-26, *s*), 1.27 (H-27, *s*), 1.72 (H-2, *m*), 1.84 (H-2, *m*), 1.95 (H-19, *m*), 2.5 (H-19, *m*), 2.99 (H-28, *d*, J=7.7 Hz), 3.13 (H-3, *dd*, J=9.5, 4.5 Hz), 3.17 (glu′-4, *t*, J=9 Hz), 3.19 (glu′-2, *dd*, J=9, 7.5 Hz), 3.26 (xyl-2, *dd*, J=9, 7.5 Hz), 3.27 (glu′-5, *m*), 3.31 (glu-5, *m*), 3.33 (xyl-5, *dd*, J=11, 9 Hz), 3.35 (glu-4, *m*), 3.36 (xyl-3, *t*, J=9 Hz), 3.38 (glu′-3, *t*, J=9 Hz), 3.4 (glu-2, *dd*, J=9, 8 Hz), 3.48 (H-28, *d*, J=7.7 Hz), 3.53 (xyl-4, *td*, J=9, 5 Hz), 3.54 (ara-5, *d*, J=12.5 Hz), 3.56 (glu-3, *t*, J=9 Hz), 3.6 (glu′-6, *dd*, J=12.5, 6 Hz), 3.67 (glu-6, *dd*, J=12, 5.5 Hz), 3.79 (ara-2 and ara-3, *m*), 3.84 (glu′-6, *dd*, J=12, 2 Hz), 3.86 (glu-6, *dd*, 12, 2 Hz), 3.9 (ara-4, *brs*), 3.93 (H-16, *m*), 3.99 (xyl-5, *dd*, J=11, 5 Hz), 4.21 (ara-5, *dd*, J=12.5, 2.5 Hz), 4.39 (ara-1, *m*), 4.52 (glu-1 and xyl-1, *d*, J=7.8 Hz), 4.69 (glu′-1, *d*, J=7.5 Hz), 9.4 (H-30, *s*); ^{13}C NMR (CD_3OD): Table 1.

Saponin **3**. ^{1}H NMR (CD_3OD): δ0.72 (H-5, *dd*, J=10, 2 Hz), 0.83 (H-24, *s*), 0.89 (H-25, *s*), 0.97 (H-29, *s*), 1.03 (H-18, *d*, J=3 Hz), 1.04 (H-23, *s*), 1.12 (H-26, *s*), 1.26 (H-27, *s*), 1.72 (H-2, *m*), 1.81 (H-2, *m*), 1.96 (H-19, *dd*, J=13, 2.5 Hz), 2.07 (H-15, *m*), 2.49 (H-19, *dd*, J=14, 13 Hz), 2.98 (H-28, *d*, J=7.6 Hz), 3.13 (H-3, *dd*, J=10, 4.5 Hz), 3.24 (xyl-2, *dd*, J=9, 7.5 Hz), 3.26 (xyl-5, *dd*, J=11.5, 9.5 Hz), 3.3 (glu-5, *m*), 3.34 (xyl-3, *t*, J=9 Hz), 3.35 (glu-4, *t*, J=9.2 Hz), 3.41 (glu-2, *dd*, J=9.5, 8 Hz), 3.44 (ara-2 and ara-3, *m*), 3.47 (H-28, *d*, J=8 Hz), 3.49 (xyl-4, *td*, J=9.2, 5 Hz), 3.52 (ara-5, *d*, J=9.5 Hz), 3.54 (glu-3, *t*, J=9.3 Hz), 3.66 (glu-6, *dd*, J=12, 5.5 Hz), 3.82 (ara-4, *brs*), 3.84 (glu-6, *dd*, J=12, 2.5 Hz), 3.92 (H-16, *m*, $W_{1/2}$=5 Hz), 3.98 (xyl-5, *dd*, J=11, 5 Hz), 4.18 (ara-5, *dd*, J=10, 2 Hz), 4.2 (ara-1, *brd*, J=5 Hz), 4.47 (xyl-1, *d*, J=7.6 Hz), 4.49 (glu-1, *d*, J=7.7 Hz), 9.4 (H-30, *s*); ^{13}C NMR (CD_3OD): Table 1.

Derivate saponin **4a**. $[\alpha]_D$ −17.2° ($CHCl_3$; *c* 0.96). ^{1}H NMR ($CDCl_3$): δ0.68 (H-5, *d*, J=7.8 Hz), 0.74 (H-24, *s*), 0.87 (H-25, *s*), 0.91 (H-23, *s*), 1.02 (H-27, *s*), 1.18 (rha-6, *d*, J=6.5 Hz), 1.22 (H-26, *s*), 1.24 (H-29, *s*), 3.03 (H-3, *dm*, J=10 Hz), 3.43 (H-28, *d*, J=9 Hz), 3.63 (ara-5, *brd*, J=10 Hz), 3.67 (glu'-5, *m*), 3.7 (glu-5, *m*), 3.73 (glu-2, *dd*, J=9, 8 Hz), 3.9 (H-28, *d*, J=9 Hz), 4.03 (H-16, *m*), 4.03 (ara-2, *brd*, J=5 Hz), 4.05 (ara-4, *m*), 4.05 (glu'-6, *dd*, J=12, 1.5 Hz), 4.07 (rha-5, *qd*, J=10, 6.5 Hz), 4.07 (H-30, *s*), 4.09 (ara-5, *brd*, J=10 Hz), 4.13 (glu-6, *dd*, J=12, 1.5 Hz), 4.21 (glu'-6, *dd*, J=12, 4.5 Hz), 4.26 (glu-6, *dd*, J=12, 5 Hz), 4.62 (ara-1, *m*, $W_{1/2}$=8 Hz), 4.49 (glu-1, *d*, J=7.5 Hz), 4.71 (glu'-1, *d*, J=7.9 Hz), 4.84 (ara-3, *dm*, J=5 Hz), 4.95 (glu-4, *t*, J=9.5 Hz), 4.97 (glu'-2, *t*, J=8.5 Hz), 5.03 (rha-1, rha-2 and rha-3, *m*), 5.04 (rha-4, *t*, J=10 Hz), 5.06 (glu'-4, *t*, J=9 Hz), 5.17 (glu'-3, *t*, J=9 Hz), 5.21 (glu-3, *t*, J=9 Hz); ^{13}C NMR ($CDCl_3$): Table 1.

Saponin **5**. $[\alpha]_D$ −10.9° (MeOH; *c* 0.23). ^{1}H NMR (CD_3OD): δ0.73 (H-5, *dd*, J=10, 2 Hz), 0.82 (H-24, *s*), 0.89 (H-25, *s*), 0.97 (H-29, *s*), 1.03 (H-23, *s*), 1.06 (H-18, *m*), 1.13 (H-26, *s*), 1.26 (rha-6, *d*, J=6.5 Hz), 1.27 (H-27, *s*), 1.95 (H-19, *dd*, J=12, 3 Hz), 2.5 (H-19, *dd*, J=14, 12 Hz), 2.97 (H-28, *d*, J=7.6 Hz), 3.12 (H-3, *dd*, J=11, 5 Hz), 3.25 (glu-5, *m*), 3.3 (glu-4, *m*), 3.35 (rha-4, *t*, J=9.5 Hz), 3.44 (glu-2 and glu-3, *m*), 3.47 (H-28, *d*, J=7.6 Hz), 3.52 (ara-5, *d*, J=12.1 Hz), 3.56 (ara-2 and ara-3, *m*), 3.65 (glu-6, *dd*, J=12, 5 Hz), 3.79 (rha-3, *dd*, J=9.5, 3.5 Hz), 3.84 (glu-6, *dd*, J=12, 2 Hz), 3.91 (rha-2, *dd*, J=3.5, 1.9 Hz), 3.92 (ara-4 and H-16, *m*), 4.06 (rha-5, *qd*, J=9.4, 6.5 Hz), 4.13 (ara-5, *dd*, J=12.2, 3.1 Hz), 4.26 (ara-1, *d*, J=6 Hz), 4.56 (glu-1, *d*, J=7.3 Hz), 5.26 (rha-1, *d*, J=1.9 Hz), 9.4 (H-30, *s*); ^{13}C NMR (CD_3OD): Table 1.

Saponin **6**. $[\alpha]_D$ −6.4° (MeOH; *c* 0.72). ^{1}H NMR (CD_3OD): δ0.84 (H-24, *s*), 0.89 (H-25, *s*), 0.98 (H-29, *s*), 1.07 (H-23 and H-26, *s*), 1.28 (H-27, *s*), 1.95 (H-19, *m*), 2.3 (H-19, *t*, J=13 Hz), 3.14 (H-3, *m*), 3.17 (glu'-4, *m*), 3.19 (glu'-2, *dd*, J=9, 7.8 Hz), 3.26 (xyl-2, *dd*, J=9.5, 7.5 Hz), 3.28 (glu'-5, *m*), 3.3 (glu-5, *m*), 3.3 (glu-4, *t*, J=9 Hz), 3.31 (xyl-5, *dd*, J=11, 9 Hz), 3.35 (xyl-3, *t*, J=8.8 Hz), 3.38 (glu'-3, *t*, J=9 Hz), 3.4 (glu-2, *dd*, J=9, 7.5 Hz), 3.51 (xyl-4, *m*), 3.52 (ara-5, *d*, J=12 Hz), 3.55 (glu-3, *t*, J=8.8 Hz), 3.6 (glu'-6, *d*, J=12 Hz), 3.66 (glu-6, *dd*, J=11.5, 5 Hz), 3.8 (ara-2 and ara-3, *m*), 3.83 (glu'-6, *dd*, J=12, 5 Hz), 3.85 (glu-6, *dd*, J=11, 5 Hz), 3.87 (ara-4, *m*), 3.99 (xyl-5, *dd*, J=11.2, 5.2 Hz), 4.02 (H-16, *m*), 4.2 (ara-5, *dd*, J=12.5, 3 Hz), 4.4 (ara-1, *m*), 4.5 (xyl-1, *d*, J=7.4 Hz), 4.51 (glu-1, *d*, J=7.7 Hz), 4.69 (glu'-1, *d*, J=7.6 Hz), 5.34 (H-12, *m*), 9.5 (H-30, *s*); ^{13}C NMR (CD_3OD): Table 1.

Compound **7**. $[\alpha]_D$ −27.3° (C_5H_5N; *c* 1). EIMS *m/z* (rel. int.): 412 (1), 397 (2), 394 (30), 351 (2), 300 (1), 273 (2), 271 (4), 255 (23), 213 (10), 55 (95); FAB-MS *m/z* (rel. int.): (positive mode) 176.1 (100), 597.4 (9.5), 835.4 (8); (negative mode) 255.1 (100), 410.9 (2); (LiCl, positive mode) 160.1 (100), 581.4 (14), 819.4 (13); FAB-MIKE (positive mode) *m/z*: 440.9, 649.2, 817.3; (+LiCl) 423.6, 634.1, 801.4. ^{1}H NMR ($CDCl_3$): δ0.7 (H-18, *s*), 0.82 (H-29, *t*, J=6.2 Hz), 0.86 (H-27, *d*, J=6 Hz), 0.88 (H-26, *d*, J=8 Hz), 0.89 (palmitate-18, *t*, J=6.6 Hz), 1.01 (H-19, *s*), 1.02 (H-21, *d*, J=7 Hz), 1.63 (H-1, *m*), 2.3 (H-2, *dm*, J=9 Hz), 2.36 (H-2, *t*, J=8 Hz), 3.36 (glu-2, *t*, J=8.1 Hz), 3.39 (glu-4, *t*, J=8.5 Hz), 3.44 (glu-5, *m*), 3.56 (H-3, *m*), 3.58 (glu-3, *t*, J=8.1 Hz), 4.28 (glu-6, *d*, J=11.7 Hz), 4.39 (glu-1, *d*, J=7.6 Hz), 4.48 (glu-6, *dd*, J=11.7, 4.5 Hz), 5.03 (H-23, *dd*, J=15, 8.4 Hz), 5.17 (H-22, *dd*, J=15, 8.4 Hz), 5.37 (H-6, *dd*, J=4.5, 3 Hz); ^{13}C NMR ($CDCl_3$): δ12.0 (C-29), 12.2 (C-18), 14.1 (palmitate-18), 19.0 (C-19), 19.3 (C-27), 21.1 (C-26), 21.2 (C-21), 22.7 (C-11), 24.9 (palmitate-3), 24.4 (C-15), 25.4 (C-28), 27.2 (palmitate-17), 28.9 (C-16), 29.3 (palmitate-4-15), 31.7 (C-2 and C-7), 31.9 (palmitate-16, C-8 and C-25), 34.2 (palmitate-2), 36.7 (C-10), 37.3 (C-1), 38.9 (C-12), 39.7 (C-13), 40.5 (C-20), 42.2 (C-4), 50.2 (C-9), 51.2 (C-24), 56 (C-17), 56.7 (C-14), 63.2 (glu-6), 70.1 (glu-4), 73.6 (glu-5), 74 (glu-2), 76 (glu-3), 79.5 (C-3), 101.2 (glu-1), 122.1 (C-6), 129 3 (C-23), 138.8 (C-22), 140.3 (C-5), 174.6 (palmitate-1).

Acknowledgements—The authors are grateful to Dr M. Becchi (SCA, CNRS) and to JEOL company for measurements of mass spectra.

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