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MOLLUSCICIDAL SAPONINS FROM *SWARTZIA SIMPLEX*

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Key Word Index—*Swartzia simplex*; Leguminosae; triterpene saponins; schistosomiasis; molluscicidal activity.

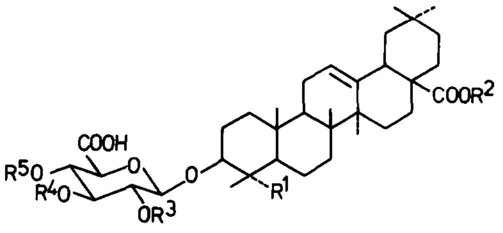
Abstract—One monodesmosidic saponin and six bidesmosidic saponins have been isolated from the methanolic extract of the leaves of *Swartzia simplex*. They were shown to be glucuronides of oleanolic acid, gypsogenin and gypsogenic acid by chemical and spectral means. The monodesmosidic saponins exhibit various molluscicidal activities against the schistosomiasis-transmitting snail *Biomphalaria glabrata*.

INTRODUCTION

Limited chemical investigation has been carried out on *Swartzia*, a genus of 125–150 species from tropical South America and Africa [1]. In the course of our systematic screening studies for molluscicidal constituents from plants, we have reported the isolation of saponins from the African tree *Swartzia madagascariensis* Desv. [2]. This plant was shown to be a potential candidate as a natural molluscicide through field trials carried out in Tanzania [3]. Continuing our examination of the genus *Swartzia*, we have observed that a methanolic extract of *Swartzia simplex* Spreng leaves exhibited an activity of 400 ppm within 24 hr against *Biomphalaria glabrata* snails. *Swartzia simplex* is a tree used in traditional medicine for treatment of inflammation of the liver [1] in South America. This paper describes the isolation and structure determination of saponins from *Swartzia simplex* leaves collected in Panama.

RESULTS AND DISCUSSION

Saponins **1**–**7** were isolated from the methanolic extract of *Swartzia simplex* leaves by different chromatographic techniques (see Experimental). On acid hydrolysis, saponins **1** and **3**–**6** afforded oleanolic acid as aglycone, identified by comparison with an authentic sample (TLC; D/CIMS). The sugars obtained from the saponin hydrolysates were identified (TLC) as rhamnose and glucuronic acid from **1**, glucose and glucuronic acid from **3**, glucose, rhamnose, and glucuronic acid from **4**, glucose, xylose, rhamnose and glucuronic acid from **6**.



Saponin	R_1	R_2	R_3	R_4	R_5	M.A.
1	Me	H	H	Rha	H	3
2	CHO	Glc	H	H	H	n.a
2a	CHO	Glc	H	H	H	25
3	Me	Glc	H	H	H	n.a
3a	Me	H	H	H	H	6
4	Me	Glc	H	Rha	H	n.a
4a	Me	H	H	Rha	H	3
5	Me	Glc	H	H	Glc	n.a
5a	Me	H	H	H	Glc	3
6	Me	Glc	Xyl	Rha	H	n.a
6a	Me	H	Xyl	Rha	H	25
6b	Me	H	Xyl	H	H	n.t
7	COOH	Glc	H	H	H	n.a.
7a	COOH	H	H	H	H	50

M.A., molluscicidal activity [mg/l]. Min. conc. required to kill 100% of the snails.
n.a, no activity.
n.t, not tested.

Acid hydrolysis of saponins **2** and **7** gave respectively gypsogenin and gypsogenic acid as aglycones, identified by comparison with authentic samples (TLC; D/CIMS). Glucose and glucuronic acid were identified (TLC) for both saponins **2** and **7**. Only saponin **1** remained unaffected by alkaline treatment whereas saponins **2**–**7** furnished the corresponding prosapogenins **2a**–**7a** and glucose. Thus, saponins **2**–**7** are bidesmosidic saponins with glucose esterified at position C-28 of the aglycone. All isolated monodesmosidic saponins were shown to be substituted at position C-3 by ^{13}C NMR spectroscopy. A downfield shift (*ca* 10 ppm) was discerned for this carbon in saponins **1**, **2a**–**7a** when compared with free oleanolic acid (Table 1) [4]. ^1H NMR spectroscopy showed that glucose, xylose, glucuronic acid have a β configuration whereas rhamnose has an α configuration.

Anomeric carbon atom signals indicated that all sugars were in a pyranose form [5]. Saponin **1** was submitted to

Table 1. ^{13}C NMR chemical shifts of the

		1	2	2a	3	3a	4
Glu	1	106.5	105.3	104.7	107.2	107.1	107.0
	2	76.1^{a}	75.2	75.2	75.5	75.6	76.0
	3	82.0	78.0^{a}	78.4^{a}	78.2^{a}	78.2^{a}	82.2
	4	72.8^{b}	73.3	74.0	73.6	73.5	72.8^{a}
	5	76.3^{a}	77.8^{a}	76.0^{a}	77.4^{a}	77.6^{a}	77.3
	6	175.5	172.5	172.3	173.5	172.9	173.8
Rha	1	102.2					102.8
	2	72.3					71.9
	3	72.6^{b}					72.6^{a}
	4	74.3					74.2^{b}
	5	69.5					69.8
	6				18.7		18.8
Glc(4′)	1						
	2						
	3						
	4						
	5						
	6						
Xyl	1						
	2						
	3						
	4						
	5						
Glc(28)	1		95.8		95.8		95.8
	2		74.2		74.0		74.1^{b}
	3		78.9		78.8		79.4
	4		71.3		71.0		71.0
	5		78.0^{a}		78.2^{a}		78.8
	6		62.4		62.2		62.2

Glu: β-D-glucuronopyranosyl, Rha: α-L-rhamnopyranosyl, Glc: β-D-
^{a,b,c,d,e}: Assignments in the vertical column with the same sign may be

fast atom bombardment MS (FAB-MS) (negative ion mode) in order to establish the sugar sequence. A quasi-molecular ion was observed at m/z 777 $[M-H]^-$, indicating a M_r of 778. Signals observed at m/z 631 $[M-H-146]^-$ and 455 $[M-H-322]^-$ corresponded to the successive elimination of one rhamnosyl and one glucuronic acid moiety; thus rhamnose is the terminal sugar. The interglycosidic linkage was shown to be rhamnosyl (1 → 3) glucuronic acid by ^{13}C NMR (Table 1) and GC of the permethylated alditol acetates of saponin **1**.

Saponin **1** is identical to [α-L-rhamnopyranosyl-(1 → 3)-β-D-glucuronopyranosyl-(1 → 3)]-3β-hydroxy-olean-12-en-28-oic acid which was previously isolated from *Swartzia madagascariensis* [2, 3].

FAB-MS of saponin **2** showed a quasi-molecular ion at m/z 807 $[M-H]^-$ indicating a M_r of 808. Signals at m/z 645 $[M-H-162]^-$ and m/z 631 $[M-H-176]^-$ indicated the elimination of one glucosyl and one glucuronic acid moiety. Basic hydrolysis of saponin **2** afforded prosapogenin **2a** and glucose. The ^{13}C NMR spectrum of **2** showed that glucose was linked at position C-28 of the aglycone. An upfield shift (4–5 ppm) is observed for carbon atom C-28 when the carboxylic group is esterified by a sugar [6]. Thus, **2** is β-D-glucopyranosyl[β-D-glucuronopyranosyl-(1 → 3)]-β-hydroxyolean-12-en-23-al-28-oate. FAB-MS (negative ion mode) of saponin **3** showed a quasi-molecular ion at m/z 793 $[M-H]^-$ indicating a M_r of 794. Signals observed at m/z 631 $[M-H-162]^-$ and m/z 455 $[M-H-338]^-$ corresponded to the elimination of one glucosyl and one glucuronic acid moiety. Basic hydrolysis of saponin **3** afforded the prosapogenin **3a** and glucose. Thus **3** is β-D-glucopyranosyl[β-D-glucuronopyranosyl-(1 → 3)]-3β-hydroxyolean-12-en-28-oate.

FAB-MS (negative ion mode) of saponin **4** showed a quasi-molecular ion at m/z 939 $[M-H]^-$. Signals observed at m/z 777 $[M-H-162]^-$, m/z 631 $[M-H-308]^-$ indicated an elimination of one glucosyl and one rhamnosyl moiety. The ^{13}C NMR spectrum gave evidence that oleanolic acid was substituted at position C(3) and C(28). Basic hydrolysis of saponin **4** afforded prosapogenin **4a** and glucose. Signals in the ^{13}C NMR spectrum of **4a** corresponded to those of saponin **1** (Table 1). Thus, **4** is the bidesmosidic saponin of **1** with a glucose unit esterified at position C-28 of oleanolic acid. Therefore, **4** is β-D-glucopyranosyl[α-L-rhamnoglycosidic moieties of *Swartzia simplex* saponins

4a	5	5a	6	6a	6b	7	7a
106.8	106.8	106.8	104.1[a]	104.7[a]	105.2	105.6	105.9
76.3[a]	75.0	75.0	78.4[b]	79.2[b]	83.6	75.3	75.6
82.0	76.5[a]	76.4[a]	84.2	85.1	77.8[a]	77.8[a]	78.1[a]
72.8[b]	82.7	82.6	72.1[c]	72.9[c]	73.3	73.5[b]	73.8
76.3[a]	74.9	74.8	77.8[b]	78.5[b]	78.1[a]	77.6[a]	77.7[a]
176.0	172.8	172.4	171.6	172.2	173.7	175.5	173.7
102.6			102.7	103.5			
72.6			71.8[c]	72.7[c]			
72.8[b]			71.0[d]	71.8			
74.5			73.3[e]	74.0			
69.8			69.7	71.3			
18.7			17.8	18.7			
	104.7	104.8					
	76.0[a]	75.8[a]					
	78.0[b]	78.1[b]					
	71.7[c]	71.6					
	78.4[b]	78.5[b]					
	62.6	62.7					
			104.5[a]	105.2[a]	106.9		
			75.0	75.8	76.5		
			76.9	77.6	77.8[a]		
			70.5[d]	70.5	71.1		
			66.5	67.2	67.5		
	95.8		95.1			95.8	
	74.2		73.5[e]			74.1[b]	
	79.1		78.2[b]			78.8	
	71.4[c]		70.6[d]			71.0	
	78.9		78.6[b]			79.4	
	62.5		61.6			62.2	

glucopyranosyl, Xyl: β-D-xylopyranosyl.
alternated although those given here are preferred.

pyranosyl-(1 → 3)-β-D-glucuronopyranosyl-(1 → 3)]-3β-hydroxyolean-12-en-28-oate.

FAB-MS (negative ion mode) of saponin **5** showed a quasi-molecular ion at *m/z* 955 $[M-H]^-$ indicating a M_r of 956. Signals observed at *m/z* 793 $[M-H-162]^-$, *m/z* 631 $[M-H-324]^-$ and *m/z* 455 $[M-H-500]^-$ showed a successive elimination of two glucosyl moieties and one glucuronic acid moiety. Basic hydrolysis of saponin **5** afforded prosapogenin **5a** and glucose. The ^{13}C NMR spectrum of **5a** showed that the interglycosidic linkage was glucosyl (1 → 4) glucuronic acid, as carbon atom C-4′ was downfield shifted (9 ppm) and carbon atoms C-3′ and C-5′ were upfield shifted (1.8 and 2.8 ppm). This was in accordance with the glycosylation rule previously established by Konishi *et al.* [7]. The substitution scheme was confirmed by GC/MS of the methylated alditol acetates obtained from saponin **5**. The procedure was conducted according to [8]. GC/MS analysis showed a 1,5-di-*O*-acetyl 2,3,4,6-tetra-*O*-methylhexitol corresponding to a terminal glucose. Substitution at position C-4′ on glucuronic acid was indicated by the presence in the EIMS of signals at *m/z* 117 and 161 obtained from the fragmentation of a 1,4,5,6-tetra-*O*-acetyl 2,3-di-*O*-methylhexitol [6]. Thus, **5** is β-D-glucopyranosyl[β-D-glucopyranosyl-(1 → 4)-β-D-glucuronopyranosyl-(1 → 3)]-3β-hydroxyolean-12-en-28-oate.

FAB-MS (negative ion mode) of saponin **6** showed a quasi-molecular ion at *m/z* 1071 $[M-H]^-$ indicating a M_r of 1072. The signals at *m/z* 939 $[M-H-132]^-$, *m/z* 925 $[M-H-146]^-$, *m/z* 909 $[M-H-162]^-$, corresponded to the simultaneous elimination of one xylosyl, one rhamnosyl and one glucosyl moiety, respectively; therefore they are terminal sugars. Basic hydrolysis of saponin **6** afforded the prosapogenin **6a** and glucose. FAB-MS (negative ion mode) of **6a** showed a quasi-molecular ion at *m/z* 909 $[M-H]^-$. The signals at *m/z* 777 $[M-H-132]^-$ and *m/z* 763 $[M-H-146]^-$ corresponded to the successive elimination of one xylosyl and one rhamnosyl moiety. Additional signals for the glucuronic acid-oleanolic acid moiety and the oleanolic acid moiety were observed at *m/z* 631 $[M-H-278]^-$ and *m/z* 455 $[M-H-454]^-$. Thus xylose and rhamnose are linked to glucuronic acid. The ^{13}C NMR spectrum of **6** showed that sugars were attached to oleanolic acid at positions C-3 and C-28. Substitution of glucuronic acid was found to be at positions C-2′ and C-3′ since both carbon atoms were downfield shifted by 2.8 ppm and 6.0 ppm, respectively, when compared with unsubstituted glucuronic acid (Table 1). The substitution scheme of glucuronic acid was confirmed by GC/MS analysis of the methylated alditol acetates obtained from saponin **6**. The procedure was conducted according to [2]. Thus the characteristic fragments of a 2′,3′-disubstituted glucuronic acid were observed at m/z 261 and m/z 191 in the EIMS. Mild acid hydrolysis of prosapogenin **6a** afforded saponin **6b**. Acid hydrolysis of **6b**, gave oleanolic acid, glucuronic acid and xylose. The ^{13}C NMR spectrum of **6b** showed by comparison with literature data [6] that the interglycosidic linkage was xylosyl (1 → 2) glucuronic acid. Thus, **6** is β-D-glucopyranosyl [(β-D-xylopyranosyl (1 → 2)) (α-L-rhamnopyranosyl (1 → 3))-β-D-glucuronopyranosyl (1 → 3)]-3β-hydroxyolean-12-en-28-oate.

FAB-MS of saponin **7** showed a quasi-molecular ion at m/z 823 $[M-H]^-$ indicating a M_r of 824. Signals at m/z 661 $[M-H-162]^-$ and m/z 647 $[M-H-176]^-$ showed a simultaneous elimination of one glucosyl and one glucuronic acid moiety. Confirmation of the fragmentation scheme was obtained by FAB-MS/MS of the quasimolecular ion. In the FAB-MS of **7**, no peak was observed for the aglycone. Acid hydrolysis of the mass sample of **7** and direct introduction of the reaction mixture into the mass spectrometer showed a quasimolecular ion at m/z $[M-H]^-$ indicating a M_r of 486 for the aglycone. Basic hydrolysis of **7** afforded prosapogenin **7a** and glucose. The ^{13}C NMR spectrum of **7a** showed signals corresponding to three carboxylic groups (173.7, 180.3 and 180.6 ppm).

In order to establish the position of the esterified glucose, usual reactions were carried out according to [9]. This procedure included methylation, basic hydrolysis and bromination. It was observed that after methylation of **7** the alkaline hydrolysis led to several products. Thus, this method was not adequate for our needs. Methylation of saponins **3**, **3a**, **7a** showed by ^{1}H NMR that C-6′, C-23 and C-28 methyl ester groups presented different chemical shifts. Thus, by direct comparison with methylated saponin **7** the ^{1}H NMR spectrum showed that **7** had methyl ester groups in position C-6′ and C-23. Therefore, **7** is β-D-glucopyranosyl[β-D-glucuronopyranosyl(1 → 3)]-3β-hydroxyolean-12-en-23-oic-28-oate.

Saponins **1** and **5a** showed the highest molluscicidal activity (3 mg/l) of the isolated compounds against schistosomiasis-transmitting snails *Biomphalaria glabrata*. Saponins with disubstituted glucuronic acid as well as those with gypsogenin and gypsogenic acid as aglycone, had a lower activity; these observations are similar to those reported previously [2]. In accordance with general structure–activity relationships of other molluscicidal saponins [10], bidesmosidic saponins have no snail killing activity. Saponins **2**, **5**, **6**, **6a**, have not been reported previously whereas saponins **1**, **4** have been reported for example in *Zexmenia buphthalmiflora* (Compositae) [11], **2a** in *Saponaria officinalis* (Caryophyllaceae) [13], **3**, **3a** in *Panax japonicum* (Araliaceae) [12], **5a** in *Ladyginia bucharica* (Umbelliferae) [12] and **7**, **7a** in *Climacoptera transoxana* (Chenopodiaceae) [14].

EXPERIMENTAL

General. Mps uncorr. TLC was carried out on silica gel precoated Al-sheets (Merck) with $CHCl_3$–MeOH–H_2O 63:35:5 and afforded the following R_f values for saponins **1**–**7**: 0.3, 0.26, 0.26, 0.23, 0.21, 0.11, 0.12. Detection with Godin reagent [15] showed a violet colour for oleanolic acid derivatives and a blue colour for gypsogenin and gypsogenic acid derivatives. For normal-phase CC silica gel 60, 40–63 μm (Merck) was used. Reversed-phase chromatography was performed on a Lobar LiChroprep RP-8 column (40–63 μm; i.d. 2.5 × 27 cm) (Merck) equipped with a Duramat 80 pump (Chemie und Filter). ^{13}C NMR spectra were measured on a Bruker AM-400 at 100.6 MHz and on a Varian X-L 400 at 101 MHz in (D5) pyridine. ^{1}H NMR spectra were measured at 360 MHz in C_5D_5N and $CDCl_3$. Before recording NMR spectra of glucuronides, the samples were desalted with Amberlite 200 (Fluka No 06437) in MeOH. δ's in the ^{13}C NMR of the aglycone for **1**–**7** corresponded to those previously described for oleanolic acid, gypsogenin and gypsogenic acid [4, 9] except for C(3) and C(28) when these carbons were substituted by sugars. FAB-MS (negative ion mode) were obtained on a ZAB-1S spectrometer. The target was bombarded with 5-keV Xe-atoms. Samples were suspended in thioglycerol. GC/MS spectra were obtained on a Dani 6500 apparatus using a 0.22 mm × 25 m fused silica column packed with SE 54, injection temp. 210°, column temp. 130°/3° min. and 5°/min up to 250° coupled with a Nermag R 3010 mass spectrometer. GC analyses were carried out on a Hewlett-Packard 5790 chromatograph using a 0.25 mm × 10 m capillary column SP-2330, injection temp. 240°, column temp. 170°/6 min and 5°/min up to 240°/6 min. Methylated alditol acetates were obtained as described previously [8].

Extraction and isolation. The dried leaves of *Swartzia simplex* (461 g) were collected in Panama. A voucher specimen was deposited at the herbarium of the University of Panama. The plant material was extracted with solvents of increasing polarity and afforded a petroleum extract (5 g), a $CHCl_3$ extract (13.6 g) and a MeOH extract (38.7 g). Only the methanolic extract exhibited molluscicidal activity at 400 ppm within 24 hr against the schistosomiasis-transmitting snail *Biomphalaria glabrata*. Thus, 3.5 g of the MeOH extract were separated on a silica gel column with $CHCl_3$–MeOH–H_2O (65:35:5) → (65:40:10). Separation was monitored by TLC and five fractions were obtained (I–V). The bioactive fraction II was purified by low pressure reversed-phase chromatography on RP-8 with MeOH–H_2O (7:3) and afforded 8 mg of saponin **1**. From fraction III saponins **2** (30 mg) and **3** (31 mg) were separated on a Lobar RP-8 column with MeOH–H_2O (8:2). Saponin **5** (34 mg) was obtained by CC on silica gel with Me_2CO–nPrOH–H_2O (35:35:5). Resulting fractions were further purified on a Lobar RP-8 column with MeOH–H_2O (55:45) and gave saponin **4** (9 mg). Fraction V was separated on a Lobar RP-8 column with MeOH–H_2O (6:4) and afforded saponin **6** (140 mg) and **7** (70 mg).

Acid hydrolysis. The saponin (2 mg) in 1 ml MeOH was refluxed in 10 ml of 4 N aq. HCl for 4 hr. The aglycone was extracted with AcOEt and identified by comparison with an authentic sample by TLC on silica gel with (i-Pr)$_2$O–Me_2CO (7:3) and D/CIMS. The aqueous layer was then adjusted to pH 6 with $NaHCO_3$. After evaporation to dryness, the sugars were extracted with pyridine from the residue and analysed by TLC on silica gel with AcOEt–MeOH–H_2O–AcOH (65:15:15:20); detection with p-anisidine phthalate.

Partial acid hydrolysis. The saponin (60 mg) was refluxed in 0.1 N aq. HCl (50 ml) for 5 hr. The mixture was extracted with BuOH (3 × 50 ml). The organic layer was washed with H_2O (3 × 50 ml), and the reaction products were purified on a silica gel column (1 × 30 cm) with $CHCl_3$–MeOH–H_2O (65:35:5).

Basic hydrolysis. The saponin (30 mg) was refluxed in 0.5 N aq. KOH (20 ml) for 2 hr. The mixture was adjusted to pH 6 with 1N aq. HCl and then extracted with BuOH (2 × 30 ml); the organic phase was washed with H_2O (3 × 30 ml).

[α-L-Rhamnopyranosyl-(1 → 3)-β-D-glucopyranosyl (1 → 3)]-3β-hydroxyolean-12-en-28-oic acid (**1**). Mp 260–275° (decomp). FAB-MS: *m/z* 777 $[M-H]^-$, *m/z* 631 $[M-H-146]^-$, *m/z* 455 $[M-H-322]^-$. Signals of the sugar moiety: see table. Acid hydrolysis of **1** afforded rhamnose, glucuronic acid and oleanolic acid.

β-D-Glucopyranosyl [β-D-glucuronopyranosyl (1 → 3)]-3-β-hydroxyolean-12-en-23-al-28-oate (**2**). Mp 220–235° (decomp). FAB-MS: *m/z* 807 $[M-H]^-$, *m/z* 645 $[M-H-162]^-$, *m/z* 631 $[M-H-176]^-$, *m/z* 469 $[M-H-338]^-$. Signals of the sugar moiety: see table. Acid hydrolysis of **2** afforded glucose, glucuronic acid and gypsogenin. Basic hydroysis of **2** gave prosapogenin **2a** and glucose.

β-D-Glucopyranosyl [β-D-glucuronopyranosyl (1 → 3)]-3-β-hydroxyolean-12-en-28-oate (**3**). Mp 215–228° (decomp). FAB-MS: *m/z* 793 $[M-H]^-$, *m/z* 631 $[M-H-162]^-$, *m/z* 455 $[M-H-338]^-$. Signals of the sugar moiety: Table 1. Acid hydrolysis of **3** afforded glucose, glucuronic acid and oleanolic acid. Basic hydrolysis of **3** gave prosapogenin **3a** and glucose.

β-D-Glucopyranosyl [α-L-rhamnopyranosyl-(1 → 3)-β-D-glucuronopyranosyl-(1 → 3)]-3β-hydroxyolean-12-en-28-oate (**4**). Mp 220–227° (decomp). FAB-MS: *m/z* 939 $[M-H]^-$, *m/z* 777 $[M-H-162]^-$, *m/z* 631 $[M-H-308]^-$, *m/z* 455 $[M-H-484]^-$. Signals of the sugar moiety: Table 1. Acid hydrolysis afforded glucose, rhamnose glucuronic acid and oleanolic acid. Basic hydrolysis gave prosapogenin **4a** and glucose.

β-D-Glucopyranosyl [β-D-glucopyranosyl-(1 → 4)-β-D-glucuronopyranosyl-(1 → 3)]-3β-hydroxyolean-12-en-28-oate (**5**). Mp 200–208° (decomp). FAB-MS: *m/z* 955 $[M-H]^-$, *m/z* 793 $[M-H-162]^-$, *m/z* 631 $[M-H-324]^-$, *m/z* 455 $[M-H-500]^-$. Signals of the sugar moiety: Table 1. Acid hydrolysis of **5** afforded glucose glucuronic acid and oleanolic acid. Basic hydrolysis gave prosapogenin **5a** and glucose.

β-D-Glucopyranosyl [(β-D-xylopyranosyl-(1 → 2)) (α-L-rhamnopyranosyl (1 → 3))-β-D-glucuronopyranosyl (1 → 3)]-3β-hydroxyolean-12-en-28-oate (**6**). Mp 248–258° (decomp). FAB-MS: *m/z* 1071 $[M-H]^-$, *m/z* 939 $[M-H-132]^-$, *m/z* 925 $[M-H-146]^-$, *m/z* 909 $[M-H-162]^-$, *m/z* 455 $[M-H-616]^-$. Signals of the sugar moiety: Table 1. Acid hydrolysis afforded xylose, rhamnose, glucose, glucuronic acid, and oleanolic acid. Basic hydrolysis gave prosapogenin **6a** and glucose. Mild acid hydrolysis afforded saponin **6b**.

β-D-Glucopyranosyl [β-D-glucuronopyranosyl (1 → 3)]-3β-hydroxyolean-12-en-23-oic-28-oate (**7**). Mp 230–245° (decomp). FAB-MS: *m/z* 823 $[M-H]^-$, *m/z* 661 $[M-H-162]^-$, *m/z* 647 $[M-H-176]^-$. Signals of the sugar moiety: see table. Acid hydrolysis afforded glucose, glucuronic acid, and gypsogenic acid. Basic hydrolysis gave prosapogenin **7a** and glucose.

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