Steroids 77 (2012) 686–690

Contents lists available at SciVerse ScienceDirect

Steroids

journal homepage: www.elsevier.com/locate/steroids

Desmettianosides A and B, bisdesmosidic furostanol saponins with molluscicidal activity from *Yucca desmettiana*

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ARTICLE INFO

Article history:
Received 21 December 2011
Received in revised form 20 February 2012
Accepted 21 February 2012
Available online 3 March 2012

Keywords:
Yucca desmettiana
Desmettianosides A and B
Furostanol saponins
Structure elucidation
Molluscicidal activity
Biomphalaria alexandrina

ABSTRACT

Bioactivity-guided separation of the aqueous methanolic extract of *Yucca desmettiana* leaves, which in a preliminary screening exhibited significant molluscicidal activity, led to the isolation and structure elucidation of two new steroidal saponins (**1** and **2**). The structures of desmettianosides A and B, identified as bisdesmosidic furostanol glycosides with six and five sugar units, respectively, were established by detailed spectroscopic analyses of their NMR and MS data. Compounds **1** and **2** exhibited high molluscicidal activity against *Biomphalaria alexandrina* snails with LC_{100} values of 6 and 11 mg/L, respectively.

1. Introduction

Schistosomiasis (bilharziasis) is one of the most prevalent parasitic infections in tropical and sub-tropical parts of the world [1]. The disease is endemic in 76 developing countries in Africa, South America, the Middle East, and Asia and poses a significant socio-economic and public-health problem [2]. Despite the introduction of highly effective antischistosomal drug therapy in the 1980s, more than 207 million people are currently infected with schistosomiasis. Among them, 120 million are symptomatic and 20 million have severe debilitating diseases and hence place another 600 million at the risk of infection [3].

Aquatic snails of the genus *Biomphalaria* are the main intermediate hosts of the parasite *Schistosoma mansoni* that causes schistosomiasis in humans. Chemotherapy and eradication of the intermediate host snails by molluscicides are the two main approaches used in the control of schistosomiasis. Synthetic molluscicides, such as niclosamide which is the only commercially available molluscicide recommended by WHO, have been used extensively over the years. However, they are too expensive for the people in most countries where schistosomiasis is endemic and they are toxic to non-target organisms including humans [4,5]. This has given impetus to the search for inexpensive, effective and environmentally-friendly molluscicides readily available from plants endemic in rural areas with infested communities that need an alternative to the expensive, synthetic chemotherapeutic agents available so far [6]. During the last 70 years more than 1500 plant extracts have been screened for molluscicidal activity and recently a number of plant metabolites with noteworthy activity have been reported [7–14]. The snail *Biomphalaria alexandrina* is the most common intermediate host of *S. mansoni* in Egypt. During the last two decades various local plants have been evaluated for molluscicidal activity against *B. alexandrina* snails [15–20].

In a preliminary screening for the identification of plant natural products that could be used to control the vector of this disease, the aqueous suspensions of a number of Egyptian plants were evaluated for their molluscicidal activity against the snail *B. alexandrina.* The aqueous suspension of the leaves of *Yucca desmettiana* exhibited significant molluscicidal activity. Bioactivity-guided separation of the water–methanol extract of *Y. desmettiana* leaves led to the isolation and structure elucidation of two new steroidal saponins (**1** and **2**).

2. Experimental

2.1. General methods

Optical rotations were measured on a Perkin–Elmer model 341 polarimeter with a 1 dm cell. IR spectra were obtained on a Bruker Tensor 27 spectrometer. NMR spectra were recorded on Bruker AC

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0039-128X/$ - see front matter © 2012 Published by Elsevier Inc.
doi:10.1016/j.steroids.2012.02.014

200, Bruker DRX 400 and Varian 600 spectrometers. Chemical shifts are given on a δ (ppm) scale using TMS as internal standard. The 2D experiments (HSQC, HMBC, COSY, TOCSY, NOESY) were performed using standard Bruker or Varian pulse sequences. High resolution mass spectra were recorded on a Thermo Scientific LTQ Orbitrap Velos. Column chromatography separations were performed with Kieselgel 60 (Merck). HPLC separations were conducted using a Waters 600 HPLC pump with Waters 600 LCD controller equipped with a RI Waters 410 detector and a Kromasil 100 C_{18} (MZ-Analysentechnik, 25 cm × 8 mm, 5 μm) column. TLC were performed with Kieselgel 60 F_{254} (Merck aluminum support plates) using solvent systems A ($BuOH/CH_3COOH/H_2O$, 4:1:5), B ($CHCl_3/(CH_3)_2CO$, 50:6), or C (CH_2Cl_2/MeOH/H_2O, 10:5:1) for development. Spots were detected after spraying with either 15% H_2SO_4 in MeOH reagent (for saponins and aglycones) or napthoresorcinol/H_3PO_4 reagent (for sugars) and heating at 100 °C for 1 min. GC–MS analyses were carried out using a Hewlett–Packard 5973-6890 system operating in electron ionization mode at 70 eV, equipped with a split–splitless injector and a HP-5 MS fused silica capillary column (30 m × 0.25 mm; film thickness 0.25 μm). The carrier gas was He at 2 mL/min. Injection was performed at 200 °C in a split ratio 1:10, while detection was performed at 250 °C. The oven temperature was 60 °C at the time of the injection, raised to 300 °C at 3 °C/min and subsequently held at 300 °C for 10 min. All chemicals and reagents used were of analytical grade and were purchased from Sigma Chemical Co. (St. Louis, MO, USA), Aldrich Chemical Co. (Steinehein, Germany) or BDH (Dorset, England).

2.2. Plant material

Leaf samples of *Y. desmettiana* were collected during April and May 2008 from El-Shrouk farm, Cairo-Alexandria desert road at 72 km north of Cairo. The botanical identification of the plant was authenticated by Dr. T. Labeb (Herbarium of Orman Garden, Horticulture Research Institute, Giza, Egypt). A voucher specimen of the plant is deposited at the Herbarium of the Department of Biochemistry, Faculty of Agriculture, Fayoum University. The leaves were cleaned, air-dried in the shade, and powdered by laboratory mill. Powdered material was maintained in an air-tight container at room temperature and protected from light until used.

2.3. Extraction and isolation

Dry, powdered leaves of *Y. desmettiana* (475 g) were exhaustively macerated with MeOH/H_2O (4:1) at room temperature. The extract was filtered through Whatman No. 1 filters and the filtrate was evaporated *in vacuo*. The residue (132 g) was defatted using chloroform and the remaining solid (130 g) was partitioned between BuOH and H_2O (1:1). The organic phase was separated and then precipitated by $(CH_3)_2CO$ to yield a mixture of saponins (8.14 g). A part of the saponin precipitate (6 g) was subjected to gravity column chromatography on silica gel, using $CHCl_3$/MeOH/H_2O (7:3:0.5 and 6:4:1, 600 mL each) as the mobile phase and 60 fractions (20 mL each) were obtained and analyzed by TLC using system A. The eluates were combined on the basis of similarity of the TLC profiles to afford five fractions (1–5). Fraction 5 (6:4:1, 1.75 g), which was the most active fraction, was subjected to solid phase extraction using C_{18} cartridges (Waters, Milford, MA, USA) and subsequently purified by reversed phase HPLC, using MeOH/H_2O (70:30) as eluent, to yield compounds **1** (382 mg) and **2** (84 mg).

2.3.1. Desmettianoside A (**1**)

White amorphous solid; $[\alpha]_D^{20}$ −15.8 (*c* 0.50, MeOH); IR (thin film) ν_{max} 3359, 2929, 1029 cm^{-1}; ^1H and ^{13}C NMR data, see Tables

Table 1
^1H and ^{13}C NMR data (C_5D_5N) of the aglycone moiety of compounds **1** and **2**.

Position	**1**			**2**		
	δ_C		δ_H (*J* in Hz)	δ_C		δ_H (*J* in Hz)
1	37.5	t	1.52 m, 0.80 m	37.5	t	1.51 m, 0.79 m
2	30.2	t	2.07 m, 1.64 m	30.3	t	2.04 m, 1.64 m
3	77.7	d	3.93 m	77.7	d	3.91 m
4	35.1	t	1.80 m, 1.38 m	35.2	t	1.80 m, 1.34 m
5	44.9	d	0.91 m	45.0	d	0.89 m
6	29.2	t	1.14 m	29.3	t	1.12 m
7	32.7	t	1.53 m, 0.81 m	32.7	t	1.52 m, 0.79 m
8	35.5	d	1.40 m	35.5	d	1.39 m
9	54.7	d	0.50 m	54.7	d	0.50 m
10	36.1	s		36.1	s	
11	21.5	t	1.39 m, 1.18 m	21.5	t	1.38 m, 1.18 m
12	40.3	t	1.66 m, 1.01 m	40.3	t	1.64 m, 1.01 m
13	41.4	s		41.4	s	
14	56.6	d	0.99 m	56.7	d	0.98 m
15	32.4	t	1.97 m, 1.37 m	32.4	t	1.98 m, 1.36 m
16	81.6	d	4.47 m	81.7	d	4.47 m
17	64.6	d	1.76 m	64.7	d	1.75 m
18	16.8	q	0.81 s	16.9	q	0.81 s
19	12.6	q	0.66 s	12.6	q	0.65 s
20	40.8	d	2.24 m	40.9	d	2.21 m
21	16.6	q	1.21 d (6.8)	16.7	q	1.21 d (6.8)
22	112.9	s		112.8	s	
23	31.1	t	2.01 m, 1.77 m	31.2	t	2.01 m, 1.75 m
24	28.5	t	1.82 m, 1.35 m	28.5	t	1.82 m, 1.35 m
25	34.5	d	1.92 m	34.5	d	1.90 m
26	75.4	t	3.98 m, 3.62 m	75.5	t	3.98 m, 3.61 m
27	17.4	q	1.02 d (6.6)	17.5	q	1.02 d (6.6)
OMe	47.6	q	3.28 s	47.4	q	3.28 s

Table 2
^1H and ^{13}C NMR data (C_5D_5N) of the sugar moiety of compounds **1** and **2**.

Position	**1**			**2**		
	δ_C		δ_H (*J* in Hz)	δ_C		δ_H (*J* in Hz)
1′	105.2	d	4.86 d (7.8)	105.3	d	4.87 d (7.7)
2′	75.3	d	4.05 m	75.5	d	4.06 m
3′	78.9	d	4.26 m	79.0	d	4.28 m
4′	72.0	d	4.24 m	72.1	d	4.26 m
5′	78.9	d	3.98 m	79.0	d	3.99 m
6′	63.1	t	4.58 m, 4.41 m	63.2	t	4.59 m, 4.42 m
1″	102.7	d	4.89 d (7.5)	102.7	d	4.90 d (7.6)
2″	73.4	d	4.42 m	73.5	d	4.45 m
3″	75.7	d	4.13 m	75.9	d	4.13 m
4″	80.2	d	4.60 m	80.5	d	4.61 m
5″	75.6	d	4.04 m	75.7	d	4.04 m
6″	60.9	t	4.69 m, 4.24 m	60.9	t	4.72 m, 4.26 m
1‴	105.1	d	5.15 d (7.7)	105.2	d	5.17 d (7.8)
2‴	81.1	d	4.36 m	81.6	d	4.42 m
3‴	88.7	d	4.18 m	88.8	d	4.23 m
4‴	71.0	d	3.84 m	71.1	d	3.86 m
5‴	77.8	d	3.85 m	77.9	d	3.89 m
6‴	63.3	t	4.49 m, 4.02 m	63.3	t	4.52 m, 4.04 m
1⁗	104.7	d	5.23 d (7.8)	104.8	d	5.33 d (7.9)
2⁗	75.6	d	4.05 m	75.6	d	4.08 m
3⁗	78.9	d	4.17 m	78.8	d	4.21 m
4⁗	71.7	d	4.13 m	71.9	d	4.17 m
5⁗	78.3	d	4.03 m	78.2	d	4.03 m
6⁗	62.6	t	4.56 m, 4.26 m	62.6	t	4.57 m, 4.29 m
1⁗′	104.3	d	5.58 d (6.3)	105.4	d	5.60 d (7.7)
2⁗′	75.3	d	4.07 m	75.5	d	4.09 m
3⁗′	87.1	d	4.08 m	78.9	d	4.14 m
4⁗′	69.3	d	4.09 m	71.8	d	4.27 m
5⁗′	78.8	d	3.84 m	78.2	d	3.88 m
6⁗′	62.3	t	4.48 m, 4.30 m	62.6	t	4.55 m, 4.39 m
1⁗″	106.3	d	5.11 d (7.6)			
2⁗″	75.5	d	3.94 m			
3⁗″	78.4	d	4.07 m			
4⁗″	71.8	d	4.12 m			
5⁗″	67.3	t	4.21 m, 3.54 t (10.6)			

1 and 2; HRESIMS *m/z* 1413.6563 $[M+Na]^+$ (calcd. for $C_{63}H_{106}O_{33}Na$ 1413.6509).

*2.3.2. Desmettianoside B (**2**)*

White amorphous solid; $[\alpha]_D^{20}$ −19.4 (*c* 0.50, MeOH); IR (thin film) ν_{max} 3365, 2932, 1031 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS *m/z* 1281.6143 $[M+Na]^+$ (calcd. for $C_{58}H_{98}O_{29}Na$ 1281.6086).

2.4. Acid hydrolysis of compounds 1 and 2

Compounds **1** and **2** (2 mg) were separately heated with 10% aqueous HCl (2 mL) in a sealed tube at 100 °C for 4 h. The aglycone was extracted with diethyl ether and analyzed by TLC and comparison of its R_f value with that of an authentic sample using system B. The aqueous layer was neutralized with *N*,*N*-dioctylamine (10% in $CHCl_3$). After evaporation of the solvent *in vacuo*, the sugars were initially identified by TLC and comparison of their R_f values with those of authentic samples using system C. In order to confirm the nature of the sugar residue, the latter was heated with pyridine (800 μL) and *N*-(trimethylsilyl)imidazole (200 μL) in a sealed tube at 60 °C for 1 h. The reaction was quenched by the addition of H_2O (3 mL) and cyclohexane (3 mL) and the mixture was partitioned between the aqueous and the organic layer. After evaporation of the organic layer *in vacuo*, the residue was analyzed by GC–MS and comparison of the retention times of the TMSi derivatives of the monosaccharides with those of authentic trimethylsilylated sugars. Thus, the sugar residues of compounds **1** and **2** were identified as D-galactose, D-glucose and D-xylose in a ratio 1:4:1 and D-galactose and D-glucose in a ratio 1:4, respectively.

2.5. Assessment of molluscicidal activity

The snails *B. alexandrina* (Ehrenberg, shell diameter 6–8 mm) were obtained from Theodore Bilharz Research Institute and were adapted to laboratory conditions 1 week before being used in the toxicity tests. The molluscicidal activity was evaluated according to the method described by WHO [21]. Aqueous suspensions of fractions and isolated metabolites were freshly prepared in dechlorinated water at concentrations ranging from 5 to 200 mg/L. For each concentration, three replicates of 10 snails/L were used. The exposure times were 24 and 48 h followed by a 24 h recovery period and subsequently the snail mortality was recorded. Statistical analysis of the data was carried out according to Litchfield and Wilcoxon [22].

3. Results and discussion

The aqueous suspensions of a number of Egyptian plants belonging to 56 families were evaluated for their molluscicidal activity against *B. alexandrina* snails. Among them, the aqueous suspension of *Y. desmettiana* exerted significant molluscicidal activity with LC_{50} values of 68 ± 2.1 and 55 ± 1.8 mg/L and LC_{90} values of 96 ± 2.4 and 83 ± 2.2 mg/L for an exposure time of 24 and 48 h, respectively, and was therefore selected for phytochemical analysis and isolation of its bioactive constituents. Bioactivity-guided separation of the aqueous methanolic extract of the dried leaves of *Y. desmettiana* resulted in the isolation of compounds **1** and **2** (Fig. 1). Both compounds showed positive reactions to Liebermann–Burchard and Ehrlich reagents indicating that they were furostanol saponins [23].

Desmettianoside A (**1**) was isolated as white amorphous solid and displayed in the HRESIMS spectrum a quasi-molecular ion peak at *m/z* 1413.6563 which was consistent for $[M+Na]^+$ and corresponded to the molecular formula $C_{63}H_{106}O_{33}$. The ^{13}C NMR spectrum displayed 63 carbon signals, 28 of which were assigned

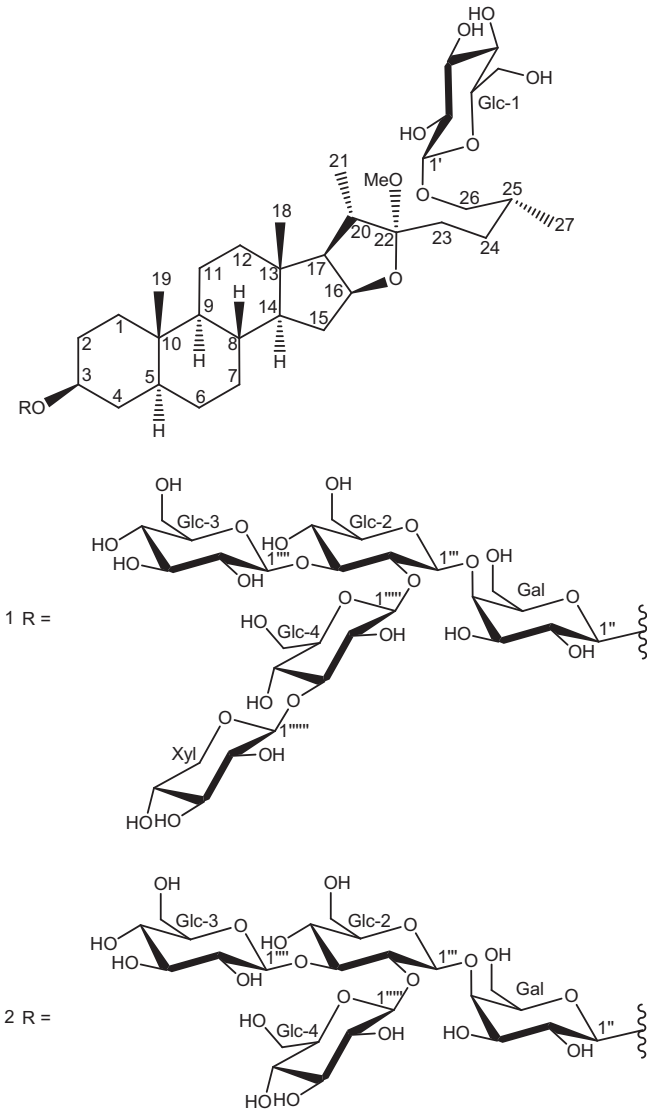

Fig. 1. Structures of compounds **1** and **2**.

to the steroidal aglycone, while the remaining 35 were due to six sugar units.

In the aglycone part of the molecule, the ^{13}C NMR spectrum and DEPT experiments revealed the presence of five methyls, 11 methylenes, nine methines, and three quaternary carbons. Among them, obvious were one oxygenated methyl at δ 47.6, one oxygenated methylene at δ 75.4 (C-26), two oxygenated methines at δ 77.7 (C-3) and 81.6 (C-16), and a bis-oxygenated quaternary carbon at δ 112.9 (C-22), characteristic for 22-methoxy-furostanol skeleton [24,25]. This observation was further supported by the ^1H NMR spectrum, which included signals for two angular methyl groups at δ 0.81 (Me-18) and 0.66 (Me-19), two secondary methyl groups at δ 1.02 (Me-27) and 1.21 (Me-21), and a methoxyl group at δ 3.28. The HMBC spectrum of metabolite **1** showed correlations between the methyl at δ 0.81 (Me-18) and the carbons at δ 40.3 (C-12), 41.4 (C-13), 56.6 (C-14), and 64.6 (C-17), the methyl at δ 0.66 (Me-19) and the carbons at δ 36.1 (C-10), 37.5 (C-1), 44.9 (C-5), and 54.7 (C-9), the methyl at δ 1.02 (Me-27) and the carbons at δ 28.5 (C-24), 34.5 (C-25) and 75.4 (C-26), the methyl at δ 1.21 (Me-21) and the carbons at δ 40.8 (C-20), 64.6 (C-17) and 112.9 (C-22), and the methyl at δ 3.28 (OMe) and the carbon at δ 112.9 (C-22). These correlations, in combination with the cross-peaks

observed in the COSY spectrum of **1** were consistent with a 22-methoxy-furostanol aglycone (Table 1).

The 3β and 5α configurations were determined on the basis of the ^{13}C NMR chemical shifts observed in rings A and B, which are quite distinct in the case of 5α and 5β isomers [26,27]. The configuration of the methoxyl group at C-22 was assigned as α due to the observed NOE correlation between the methoxy protons at δ 3.28 and H-16 at δ 4.47 [28]. The stereochemistry at C-25 was deduced as *R* on the basis of the chemical shifts difference for the geminal protons at H_2-26 ($\Delta\delta_{ab} = \delta_a - \delta_b = 0.36$ ppm). It has been reported that $\Delta\delta_{ab} > 0.57$ ppm is expected in 25*S* configuration, whereas for 25*R* configuration $\Delta\delta_{ab} < 0.48$ ppm is observed [29]. On the basis of the above-mentioned data the aglycone was identified as (25*R*)-22α-methoxy-5α-furostan-3β,26-diol [30].

The presence of six β-monosaccharides in the molecule was deduced from the ^1H and ^{13}C NMR data of compound **1**, which included six doublet signals at δ 4.86, 4.89, 5.11, 5.15, 5.23, and 5.58 with J = 6.3–7.8 Hz ascribed to six anomeric protons with their corresponding carbons resonating at δ 105.2, 102.7, 106.3, 105.1, 104.7, and 104.3 (Table 2). The fact that 35 signals observed in the ^{13}C NMR spectrum of **1** were assigned to the sugar moiety suggested that the latter consisted of one pentose and five hexose units. Acid hydrolysis of **1** yielded an artifactual aglycone, which was determined to be tigogenin (the corresponding cyclic spirostanol aglycone) by TLC analysis, and the sugar units, which were identified as D-galactose, D-glucose, and D-xylose by TLC and GC–MS analyses.

The sequence of the monosaccharides was determined by extensive analyses of the MS and NMR spectra of compound **1**. The ESIMS spectrum and MS–MS experiments indicated the nature and the sequence of the sugar units. In particular, **1** showed a quasi-molecular ion peak at *m/z* 1413 $[M+Na]^+$ and a fragment ion peak at *m/z* 1381 $[M+Na\text{-}CH_3OH]^+$. The fragment ion peaks at *m/z* 1281 $[M+Na\text{-}132]^+$ and 1251 $[M+Na\text{-}162]^+$ represented the glycosidic cleavage of a terminal pentose and a terminal hexose, respectively. Further fragment ion peaks were observed at *m/z* 1219 $[M+Na\text{-}CH_3OH\text{-}162]^+$, 1119 $[M+Na\text{-}132\text{-}162]^+$, 1087 $[M+Na\text{-}132\text{-}162\text{-}CH_3OH]^+$, 1057 $[M+Na\text{-}CH_3OH\text{-}162\text{-}162]^+$, 957 $[M+Na\text{-}132\text{-}162\text{-}162]^+$, and 925 $[M+Na\text{-}132\text{-}162\text{-}CH_3OH\text{-}162]^+$. Finally, fragment ion peaks were observed at *m/z* 509 $[162+162+162+Na]^+$, 641 $[162+162+162+132+Na]^+$, 671 $[162+162+162+162+Na]^+$, and 803 $[162+162+162+162+132+Na]^+$.

The ^1H NMR chemical shifts of the protons of the six sugar units were assigned by a combination of COSY and TOCSY cross-peaks starting from the anomeric proton of each sugar unit. The ^{13}C NMR chemical shifts of the corresponding carbons were assigned based on the HSQC spectrum, while HMBC correlations provided information on the glycosidic linkages. Specifically, the ^1H and ^{13}C NMR data were indicative of the presence of a 4-monosubstituted β-D-galactopyranosyl unit (Gal, δ_H 4.89; δ_C 102.7, 73.4, 75.7, 80.2, 75.6, 60.9), a 2,3-disubstituted β-D-glucopyranosyl unit (Glc-2, δ_H 5.15; δ_C 105.1, 81.1, 88.7, 71.0, 77.8, 63.3), a 3-monosubstituted β-D-glucopyranosyl unit (Glc-4, δ_H 5.58; δ_C 104.3, 75.3, 87.1, 69.3, 78.8, 62.3), two terminal β-D-glucopyranosyl units (Glc-1, δ_H 4.86; δ_C 105.2, 75.3, 78.9, 72.0, 78.9, 63.1 and Glc-3, δ_H 5.23; δ_C 104.7, 75.6, 78.9, 71.7, 78.3, 62.6), and a terminal β-D-xylopyranosyl unit (Xyl, δ_H 5.11; δ_C 106.3, 75.5, 78.4, 71.8, 67.3). In the HMBC spectrum of **1** correlations were observed between H-1′ of Glc-1 at δ 4.86 and C-26 of the aglycone at δ 75.4, H-1″ of Gal at δ 4.89 and C-3 of the aglycone at δ 77.7, H-1‴ of Glc-2 at δ 5.15 and C-4″ of Gal at δ 80.2, H-1⁗ of Glc-3 at δ 5.23 and C-3‴ of Glc-2 at δ 88.7, H-1⁗ of Glc-4 at δ 5.58 and C-2‴ of Glc-2 at δ 81.1, and H-1⁗ of Xyl at δ 5.11 and C-3⁗ of Glc-4 at δ 87.1.

Therefore, compound **1** was identified as (25*R*)-26-*O*-β-D-glucopyranosyl-22α-methoxy-5α-furostan-3β,26-diol 3-*O*-β-D-xylopyranosyl-(1→3)-β-D-glucopyranosyl-(1→2)-[β-D-glucopyranosyl-(1→3)]-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside and designated as desmettianoside A.

Desmettianoside B (**2**) was isolated as white amorphous solid. Its molecular formula was assigned as $C_{58}H_{98}O_{29}$ on the basis of its HRESIMS spectrum, which exhibited a quasi-molecular ion peak at *m/z* 1281.6143 $[M+Na]^+$. The ^1H and ^{13}C NMR data of **2** included signals indicating the same aglycone as in metabolite **1** (Table 1). However, in contrast to **1**, examination of the ^1H NMR spectrum of **2** revealed the presence of five anomeric protons of β-monosaccharides resonating at δ 4.87, 4.90, 5.17, 5.33, and 5.60 with J = 7.6–7.9 Hz (Table 2). Acid hydrolysis of **2** yielded tigogenin as the artifactual aglycone and D-galactose and D-glucose as the sugar units. Analysis of the NMR and MS spectra suggested that the only difference between compounds **1** and **2** was the absence of the terminal β-D-xylopyranosyl unit. The ^{13}C NMR chemical shifts indicated the presence of one 4-monosubstituted β-D-galactopyranosyl unit (Gal), one 2,3-disubstituted β-D-glucopyranosyl unit (Glc-2), and three terminal β-D-glucopyranosyl units (Glc-1, Glc-3, and Glc-4). The connectivity of the sugar units was deduced from the HMBC correlations observed between H-1′ of Glc-1 at δ 4.87 and C-26 of the aglycone at δ 75.5, H-1″ of Gal at δ 4.90 and C-3 of the aglycone at δ 77.7, H-1‴ of Glc-2 at δ 5.17 and C-4″ of Gal at δ 80.5, H-1⁗ of Glc-3 at δ 5.33 and C-3‴ of Glc-2 at δ 88.8, and H-1⁗ of Glc-4 at δ 5.60 and C-2‴ of Glc-2 at δ 81.6. The nature and the sequence of the sugars were verified by the ion peaks observed in the ESIMS spectrum and MS–MS experiments of metabolite **2**. Specifically, it displayed a quasi-molecular ion peak at *m/z* 1281 $[M+Na]^+$ and fragment ion peaks at *m/z* 1249 $[M+Na\text{-}CH_3OH]^+$, 1119 $[M+Na\text{-}162]^+$, 1087 $[M+Na\text{-}CH_3OH\text{-}162]^+$, 957 $[M+Na\text{-}162\text{-}162]^+$, 925 $[M+Na\text{-}CH_3OH\text{-}162\text{-}162]^+$, 795 $[M+Na\text{-}162\text{-}162\text{-}162]^+$, and 763 $[M+Na\text{-}CH_3OH\text{-}162\text{-}162\text{-}162]^+$. In addition, fragment ion peaks at *m/z* 509 $[162+162+162+Na]^+$ and 671 $[162+162+162+162+Na]^+$ were observed.

Accordingly, the structure of compound **2** was elucidated as (25*R*)-26-*O*-β-D-glucopyranosyl-22α-methoxy-5α-furostan-3β,26-diol 3-*O*-β-D-glucopyranosyl-(1→2)-[β-D-glucopyranosyl-(1→3)]-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside and designated as desmettianoside B.

The natural origin of the aglycone part of compounds **1** and **2** through enzymatic processes in the living cells of the investigated organism cannot be unambiguously proposed, since they could potentially be artifacts formed from the corresponding 22-hydroxy-furostanols in the course of the extraction and isolation due to their possible interconversion in the presence of MeOH and H_2O [31].

Compounds **1** and **2** were evaluated for their molluscicidal activity against *B. alexandrina* snails and exhibited high levels of activity with LC_{100} values of 6 ± 0.6 and 11 ± 0.7 mg/L, respectively. The molluscicidal activity of these bisdesmosidic furostanol saponins is in accordance with data reported for other steroidal saponins. The molluscicidal LC_{100} values for different saponins isolated from other plant species varied considerably depending upon the nature of the sugar chain, the number of sugar residues, the sequence of the sugars, the interglycosidic linkages and the substitution patterns of the aglycone, as well as the species of snails [32]. Since desmettianosides A and B share the same aglycone moiety, it is assumed that the observed variation in their molluscicidal activity results from the difference in the nature and number of the sugar units.

Acknowledgements

The authors thank Fayoum University for a postdoctoral research studies scholarship awarded to Y.D., PNG Gerolymatos for a postdoctoral research studies scholarship awarded to E.I., and Mr. Ahmed Salah (Fayoum University, Egypt) for his assistance

in the preliminary screening of plants for the evaluation of their molluscicidal activity.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.steroids.2012.02.014.

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