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Steroidal saponins from the leaves of *Agave macroacantha*

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ABSTRACT

A new monodesmosidic spirostanol saponin, along with three known saponins was isolated from *Agave macroacantha* Zucc leaves. The structure of the new saponin was established as hecogenin-3-*O*-α-L-rhamnopyranosyl (1→4) β-D-xylopyranosyl (1→3)[β-D-glucopyranosyl (1→2)] β-D-glucopyranosyl (1→4) β-D-galactopyranoside. The ^{1}H and ^{13}C resonances of the four compounds were assigned using a combination of 1D and 2D NMR techniques including ^{1}H, ^{13}C, COSY, TOCSY, ROESY, HSQC and HMBC NMR and confirmed by mass spectrometry.

1. Introduction

The genus *Agave* belongs to family Agavaceae widely distributed in tropical and subtropical regions throughout the world [1]. *Agave* species possess both commercial importance as source of industrial fibers and medicinal importance as they are used in Chinese folk medicine in treatment of scabies, tumors, dysentery, and as insecticides [2]. Agavaceae is also known to be a rich source for steroidal sapogenins and saponins, the raw material for steroid hormones synthesis [3]. Several species of *Agave* were extensively investigated for the identification of steroidal saponins [4–6]. Saponins and hecogenin isolated from *A. americana* showed anti-inflammatory activity [7]. As part of series of phytochemical studies on plants growing in Egypt of medicinal value [8], we studied *Agave macroacantha* Zucc known as the Black-spined *Agave*. This is a very distinctive small to medium-sized *Agave* native to rocky ground in the Mexican state. A review of literature showed that *A. macroacantha* (*Syn*: *A. macracantha*) [9] was studied for steroidal sapogenins constituents [10] while, steroidal saponins were not investigated previously. This paper reports the isolation and structural elucidation of one new spirostanol saponin, along with three known steroidal saponins from *A. macroacantha* leaves.

2. Experimental

2.1. General

Optical rotations of the saponins were determined in MeOH with a Perkin-Elmer 241 automatic polarimeter and in H_2O with JASCO DIP-1000 digital polarimeter for the sugars. ^{1}H and ^{13}C NMR spectra were recorded in pyridine-d_5 on a Bruker Avance DRX-500 spectrometer operating at 500 MHz and 125 MHz, respectively, and 2D-NMR experiments were performed using standard Bruker microprograms. ESI-MS and high-resolution MS were recorded on Micromass Q-TOF micro instrument (Manchester, UK) with an electrospray source. The samples were introduced by infusion in a solution of MeOH (5 µl/min). The IR spectra were obtained with a JASCO FT/IR-5MP apparatus. TLC were carried out on pre-coated silica gel 60 F 254 (Merck) and spots were visualized by spraying with 50% H_2SO_4. Kieselgel 60 (63–200 µm, Merck) and Lichroprep RP-18 (40–63 µm, Merck) were used for column chromatography.

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Table 1
^{1}H and ^{13}C data for aglycone part of saponins **1**–**4** in C_5D_5N.

	Saponin **1**		Saponins **2**–**4**	
	* δ H *J* = Hz	δ C	δ H *J* = Hz	δ C
1	0.78 (td, 13.7, 3.5)	37.1	0.65 (m)	36.3
1	1.48 (dd, 9.0, 3.0)		1.25 (m)	
2 (2H)	1.62, 2.02 (m)	29.5	1.28, 1.98 (m)	29.3
3	3.96 (m)	76.7	4.00 (m)	75.0
4ax	1.43 (d, 11.5)	28.2	1.30 (m)	34.3
4eq	3.37 (brd, 12.6)		1.77 (brd, 13.1)	
5	1.23 (m)	50.5	0.84 (m)	44.1
6 (2H)	3.53 (td, 12.0, 5.0)	79.6	1.09 (m)	28.2
7 (2H)	7ax: 1.14 (dd, 11.5, 4.7)	41.0	1.60, 2.09 (m)	31.1
	7eq: 2.55 (dd, 12.2, 4.2)			
8	1.46 (m)	33.5	1.72 (dd, 10.5, 3.0)	34.0
9	0.50 (td, 11.5, 3.8)	53.4	1.34 (m)	55.6
10	–	36.3	–	35.9
11	1.10 (dd, 10.5, 2.8)	20.8	2.20 (dd, 13.9, 5.1)	37.7
11	1.32 (dd, 12.5, 3.0)		2.35 (t, 13.7)	
12	1.04 (m)	40.0	–	212.8
	1.66 (dd, 8.0, 3.5)			
13	–	40.9	–	55.1
14	1.02 (m)	56.0	0.88 (m)	55.2
15 (2H)	1.47, 2.00 (m)	31.7	1.58 (m)	31.3
16	4.53 (q, 6.0)	81.3	4.48 (m)	79.4
17	1.83 (dd, 8.7, 7.0)	62.1	2.75 (dd, 8.4, 7.0)	53.9
18	0.95 (s)	16.6	1.06 (s)	15.8
19	0.60 (s)	13.0	0.62 (s)	11.4
20	3.01 (dq, 7.0, 6.7)	35.4	1.90 (dq, 7.2, 6.5)	42.3
21	1.17 (d, 7.0)	14.4	1.35 (d, 6.8)	13.6
22	–	111.4	–	109.1
23eq	3.85 (m)	67.1	1.66 (m)	31.4
23 ax			1.70 (m)	
24 (2H)	24ax 1.77 (dd, 11.5, 9.0)	38.4	1.54 (m)	28.9
	24eq 2.09 (m)			
25	1.80 (m)	31.4	1.58 (m)	30.2
26 ax	3.44 (t, 10.7)	65.6	3.46 (t, 11.0)	66.6
26 eq	3.53 (dd, 10.3, 5.2)		3.58 (dd, 12.0, 3.5)	
27	0.72 (d, 6.1)	16.5	0.67 (d, 5.7)	17.0

* Δδ H and δ C varied in compounds **2**–**4** by ± 0.05 and ± 0.2 ppm, respectively.

2.2. Plant material

The leaves of *A. macroacantha* were collected at El-Orman Public Botanical Garden, Giza, Egypt, in June 2008. The plant was identified by Dr. Thérèse Labib, senior specialist of plant identification at El-Orman Public Botanical Garden. A voucher Herbarium specimen (H.M.G. 32, 2008) was deposited in the Herbal Medicinal Garden of Helwan University.

2.3. Extraction and isolation

The air dried powdered leaves of *A. macroacantha* (2 kg) was extracted twice with MeOH at room temperature to obtain a concentrated extract (420 g) after evaporation of solvent. The combined methanol extract was suspended in MeOH (300 ml) and precipitated by addition of a large excess of Me_2CO (2 L). The resulting precipitate was filtered and dried to give (30 g) of crude mixture. This mixture was passed through a porous polymer gel column (Mitsubishi Diaion HP-20), eluted with H_2O then MeOH:H_2O (50:50 and 75:25) and finally 100% MeOH. Saponins of fractions eluted with MeOH:H_2O (75:25) (2 g) were chromatographed on RP-18 column chromatography using a gradient of MeOH–H_2O (40:60 to 50:50) to give 180 frs. Frs. (132–138) were subjected to further purification on silica gel column eluted with $CHCl_3$: MeOH (80: 20) to give saponin **1** (15 mg). Saponins from gel HP-20 fractions eluted with 100% MeOH (700 mg) were subjected to RP-18 column chromatography using a gradient of MeOH–H_2O (70:30 to 80:20) to give 80 frs. Frs. (12–19) were chromatographed successively on silica gel column eluted with $CHCl_3$:MeOH (85: 15) then by preparative TLC eluted with $CHCl_3$–MeOH–H_2O (60:40:3) to yield saponins **2** (10 mg), **3** (8 mg) and **4** (10 mg).

2.4. Acid hydrolysis

A part of saponin mixture (30 mg) was refluxed with 5 ml of 2 N HCl at 100 °C for 5 h. After cooling, the reaction was extracted with $CHCl_3$ four times to remove aglycones. The acid aqueous layer was neutralised with 1 N KOH and evaporated. Four sugars were identified and compared with authentic samples by TLC using solvent MeCOEt–iso-PrOH–Me_2CO–H_2O (20:10:7:6) as glucose, xylose, galactose and rhamnose. The purification of sugars was achieved by prep. TLC using Kieselgel 60 plates which were eluted three times with solv., $CHCl_3$–MeOH–H_2O (70:30:1) to afford D-xylose (R_f 0.53, $[\alpha]^{21}{}_D$ + 48 to + 12; H_2O, 16 h), D-galactose (R_f 0.28, $[\alpha]^{21}{}_D$ + 55; H_2O), D-glucose (R_f 0.31, $[\alpha]^{21}{}_D$ + 50; H_2O and L-rhamnose (R_f 0.63, $[\alpha]^{21}{}_D$ + 8.5; H_2O).

2.5. Data of compounds

2.5.1. Saponin **1**

White amorphous powder; $[\alpha]^{21}{}_D$ − 15.2 (c 0.30, MeOH); ^{1}H and ^{13}C NMR of the aglycone and glycosidic part: see Tables 1 and 2. High-resolution ESI-MS$^-$ $[M+Cl]^-$: *m/z* 807.3945 (calc. 807.3934, $C_{39}H_{64}O_{15}Cl$).

2.5.2. Saponin **2**

White amorphous powder; $[\alpha]^{21}{}_D$ − 29.0 (c 0.24, MeOH); ^{1}H and ^{13}C NMR of the aglycone and glycosidic part: see Tables 1 and 2. ESI-MS$^-$ $[M-H]^-$: *m/z* 1047.8; ESI-MS$^+$ $[M+Na]^+$: *m/z* 1071.5.

2.5.3. Saponin **3**

White amorphous powder; $[\alpha]^{21}{}_D$ − 20.0 (c 0.11, MeOH); ^{1}H and ^{13}C NMR of the aglycone and glycosidic part: see Tables 1 and 2. ESI-MS$^-$ $[M-H]^-$: *m/z* 1179.7; ESI-MS$^+$ $[M+Na]^+$: *m/z* 1203.5.

2.5.4. Saponin **4**

White amorphous powder; $[\alpha]^{21}{}_D$ − 37.4 (c 0.20, MeOH); ^{1}H and ^{13}C NMR of the aglycone and glycosidic part: see Tables 1 and 2. High-resolution ESI-MS$^-$ $[M-H]^-$: *m/z* 1193.5610 (calc. 1193.5591, $C_{56}H_{89}O_{27}$); ESI-MS$^+$ $[M+Na]^+$: *m/z* 1217.5; ESI-MS$^-$: *m/z* 1193.9 $[M-H]^-$, 1047.8, 1031.7, 915.7, 885.6, 753.6, 687.5, 591.5.

3. Results and discussion

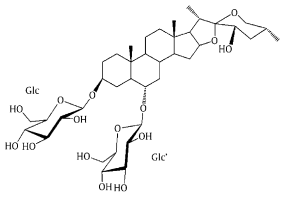
The leaves of *A. macroacantha* were extracted with MeOH to give a crude extract which was precipitated by acetone. The

Table 2
^{1}H and ^{13}C data for osidic part of saponins **1**–**4** in C_5D_5N.

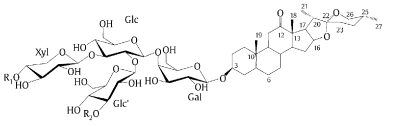
	1		2		3		4	
	δH	δC	δH	δC	δH	δC	δH	δC
Gal								
1			4.86 (d, 7.6)	102.0	4.86 (d, 7.5)	98.8	4.85 (d, 7.8)	102.1
2			4.40 (dd, 9.0, 7.2)	72.8	4.39 (dd, 9.0, 7.0)	72.8	4.40 (dd, 9.5, 7.5)	72.8
3			4.13 (m)	75.2	4.12 (m)	75.1	4.12 (m)	75.2
4			4.59 (d, 2.8)	79.6	4.59 (d, 2.8)	77.9	4.58 (d, 2.7)	79.5
5			3.88 (m)	76.8	3.99 (m)	75.0	4.03 (m)	75.7
6			4.22 (m)	60.4	4.24 (m)	60.5	4.23 (m)	60.5
6			4.68 (m)		4.66 (m)		4.66 (m)	
Glc								
1	5.12 (d, 7.8)	101.3	5.18 (d, 7.9)	104.9	5.17 (d, 8.0)	104.5	5.14 (d, 7.7)	104.7
2	4.06 (t, 8.7)	75.1	4.41 (t, 8.6)	81.0	4.37 (t, 8.8)	80.4	4.39 (m)	81.0
3	4.31 (t, 8.9)	78.2	4.14 (t, 8.9)	86.3	4.10 (t, 8.7)	86.3	4.14 (t, 8.8)	86.0
4	4.25 (dd, 9.0, 8.8)	71.3	3.81 (dd, 8.9, 8.0)	70.1	3.79 (t, 9.0)	70.0	3.79 (t, 9.4)	70.1
5	3.83 (m)	77.8	3.86 (m)	77.2	3.85 (m)	77.2	3.84 (m)	76.8
6	4.32 (dd, 11.4, 5.1)	62.3	4.04 (dd, 12.8, 5.2)	62.6	4.05 (m)	62.6	4.03 (dd, 12.0, 5.0)	63.5
6	4.43 (dd, 11.8, 2.2)		4.52 (m)		4.51 (m)		4.51 (m)	
Glc′								
1	4.86 (d, 7.8)	105.9	5.57 (d, 7.6)	104.6	5.59 (d, 7.0)	102.1	5.57 (d, 7.7)	104.4
2	4.04 (t, 8.8)	75.4	4.07 (dd, 9.3, 8.5)	75.9	4.07 (m)	74.8	4.08 (t, 8.5)	75.8
3	4.27 (t, 8.8)	78.1	4.13 (t, 9.5)	77.4	4.08 (m)	86.5	4.12 (dd, 9.0, 8.0)	77.3
4	4.23 (t, 8.6)	71.4	4.21 (m)	70.7	4.03 (m)	68.8	4.21 (t, 8.9)	70.7
5	3.95 (m)	77.7	3.93 (m)	78.4	3.89 (m)	77.9	3.94 (m)	78.4
6	4.39 (dd, 11.6, 5.1)	62.7	4.36 (dd, 11.5, 5.5)	62.1	4.29 (m)	61.8	4.36 (m)	62.6
6	4.52 (dd, 11.8, 3.4)		4.58 (brd, 11.0)		4.47 (brd, 10.7)		4.57 (m)	
Xyl								
1			5.22 (d, 7.8)	104.7	5.14 (d, 7.8)	104.5	5.22 (d, 7.6)	104.4
2			3.96 (dd, 9.3, 8.0)	74.7	3.95 (t, 8.5)	75.1	3.93 (t, 8.8)	74.9
3			4.08 (m)	78.2	4.05 (m)	77.5	4.05 (m)	75.7
4			4.12 (m)	70.4	4.09 (m)	70.4	4.07 (m)	75.7
5 ax			3.67 (t, 10.5)	67.0	3.64 (dd, 11.8, 10.0)	66.8	3.43 (m)	63.8
5 eq			4.22 (dd, 11.0, 4.8)		4.21 (dd, 11.2, 5.6)		4.19 (dd, 11.5, 5.1)	
					Xyl′		rha	
1					5.10 (d, 7.4)	105.8	5.45 (brs)	99.4
2					3.92 (t, 9.0)	75.1	4.49 (dd, 3.0, 1.7)	72.1
3					4.08 (dd, 9.0, 8.8)	78.0	4.51 (dd, 9.5, 3.3)	72.1
4					4.09 (m)	70.4	4.31 (t, 9.5)	73.6
5ax/ 5					3.56 (dd, 12.0, 10.5)	66.7	4.84 (dq, 9.7, 6.2)	69.6
5eq/6					4.20 (dd, 11.0, 5.4)		1.64 (d, 6.2)	18.3

saponin mixture was obtained after removing of free sugars by passing the precipitate over a porous ion-exchange resin column. Purification of the saponin fraction was obtained after multiple separation processes by reversed-phase C_{18} column, silica gel column chromatography and finally by preparative TLC to afford saponins **1**–**4**.

Compounds **1**, **2** and **3** are known spirostanol steroidal saponins and their structures were elucidated as (25 *R*)-5α spirostane-3β, 6α, 23-triol 3,6-di-*O*-β-D-glucopyranoside, hecogenin-3-*O* β-D-glucopyranosyl-(1→2)-[β-D-xylopyranosyl-(1→3)]-β-D-glucopyranosyl (1→4) β-D-galactopyranoside and hecogenin-3-*O*-β-D- xylopyranosyl-(1→3)-β-D-glucopyranosyl-(1→2) [β-D-xylopyranosyl (1→3)]-β-D-glucopyranosyl (1→4)-β-D-galactopyranoside, respectively. Saponin **1** was previously isolated from *A. americana* and *A. cantala* [11,12], saponin **2** was identified in *A. americana* and *Hosta longipes* [11,13,14], while saponin **3** was not isolated previously from genus *Agave* but isolated once from *Polianthes tuberosa* [15].



Saponin 1



	R_1	R_2
Saponin 2	H	H
Saponin 3	H	Xyl'
Saponin 4	Rha	H

Saponin **4** exhibited on the negative mode HR-ESI-MS, a molecular ion peak $[M–H]^-$ at *m/z* 1193.5610, in accordance with an empirical molecular formula of $C_{56}H_{89}O_{27}$. The IR spectrum gave characteristic absorption bands at 981, 920, and 895 cm^{-1} (intensity: 920<895 cm^{-1}), which indicated the presence of (25 *R*)-spirostanol steroidal skeleton in the aglycone. The structure of the aglycone moiety of saponin **4** was identified as (25 *R*)-3 β-hydroxy-5α-spirost-12-one (hecogenin). The ^1H NMR spectrum showed signals for two tertiary methyl groups at δ 1.06 (s) and 0.62 (s) and two secondary methyl groups at δ 1.35 (d, *J*=6.8 Hz) and 0.67 (d, *J*=5.7 Hz) (Table 1). The HMBC correlations observed from these methyl groups and from the two quaternary carbons detected at δ 55.1 and 35.9 were consistent with hecogenin. The $^3J_{C-H}$ cross-peaks of $\delta_{C\,O}$ 212.8 with $\delta_{Me\text{-}18}$ 1.06, $\delta_{H\text{-}17}$ 2.75 confirmed the 12-one structure. The ROE correlations between Me-19/H-4 β (ax), Me-19/H-8 β (ax), Me-18/H-20, H-17/H-16, H-17/ Me-21 confirmed the A/B trans, C/D trans and D/E cis ring junctions, and the 20 *S* and 22 *R* configurations. The 25 *R* configuration was confirmed by the ROE correlation observed between H-26 ax at δ 3.46 (t, 11.0 Hz) and Me-27 at δ 0.67. The equatorial orientation of C-27 methyl was verified by the axial–axial coupling of H-26ax (δ 3.46) and H-25ax (δ 1.58) $J_{26ax,25ax}$=11.0 Hz in the ^1H NMR spectra and the downfield shift of C-27 (δ 17.0) as compared to the ^{13}C NMR chemical shift of (25 *S*)-spirostanes (δ 16.2±0.2) [16].

The observation of five anomeric signals at δ_H 4.85 (d, *J*=7.8 Hz), 5.14 (d, *J*=7.7 Hz), 5.22 (d, *J*=7.6 Hz), 5.45 (brs) and 5.57 (d, *J*=7.7 Hz) in the ^1H NMR spectrum, respectively, linked to anomeric carbons in the HSQC spectrum at δ 102.1, 104.7, 104.4, 99.4 and 104.4, suggested that saponin **4** possesses five sugar moieties (Table 2). The nature of monosaccharides was identified from the acid hydrolysis as glucose, galactose, xylose and rhamnose; their absolute configuration was precised by measurement of their optical rotation after purification. Complete assignment of the glycosidic protons was achieved by analysis of the COSY and TOCSY experiments, while those of the corresponding glycosidic carbons were determined through the observation of the direct H–C correlations in the HSQC spectrum. The signals at δ 5.14 (d, *J*=7.7 Hz) and 5.57 (d, *J*=7.7 Hz) showed the typical spin system of β-D-glucopyranosyl moieties with their H-1 to H-5 in axial positions ($^3J_{H-H}$>7 Hz). The anomeric signal observed at δ 4.85 (d, *J*=7.8 Hz) was identified as β-D-galactopyranosyl from its characteristic equatorial H-4 appearing as a fine doublet ($J_{H\text{-}3\text{-}H\text{-}4}$=2.7 Hz). The fourth sugar unit δ 5.22 (d, *J*=7.6 Hz) contained six coupled protons and was identified as a β-D- xylopyranosyl unit like in saponin **2**. Its C-4 deshielded at 75.7 ppm instead of 70.4 ppm, attested that this position was substituted and induced a γ-effect on C-3 and C-5 upfield shifted by −2.5 and −3.2 ppm, respectively [17]. The large $^3J_{H\text{-}1,\ H\text{-}2}$ coupling constants (7.7±0.1 Hz) for those four sugar units indicated their β-anomeric configuration. The last anomeric proton signal at δ 5.45 (brs) was detected as α-L-rhamnopyranosyl unit by the observation of $^3J_{H-H}$ COSY correlations between the methyl doublet assigned to Me-C_6 at δ 1.64 (*J*=6.2 Hz) and H-5 at δ 4.84 (dq, *J*=9.7, 6.2 Hz) (Table 2). The axial–axial coupling constants H-3/H-4 (*J*=9.5 Hz) and H-4/H-5 (*J*=9.7 Hz), and the axial–equatorial H-2/H-3 (*J*=3.0 Hz) relationship in addition to the chemical shift of C-5 at δ_C 69.6 [18] (Table 2) led to the determination of L-rhamnose unit with α- configuration.

The negative ESI-MS spectra of saponin **4** showed two ions at *m/z* 1047.8 and 1031.7 due to the respective losses of terminal rhamnose and glucose. The ESI-MS-MS of $[M–H–rha]^-$ ion (1047.8 uma) yielded a first ion at 885.6 uma due to the loss of terminal glucose, and a second one at 915.7 uma indicating that the xylose bound the terminal rhamnose. A comparison of chemical shifts of carbons of the xylose unit between saponins **1**–**2** and saponin **4**, assumed that rhamnose was linked at position 4 of xylose ($\Delta\delta_{C\text{-}4}$=+5.3 ppm). The linkage of the sugar units and their sequencing were confirmed using the HMBC spectrum where long-range H–C correlations were observed between H-1 glc′ (δ 5.57) and C-2 glc (δ 81.0), H-1 rha (δ 5.45) and C-4 xyl (δ 75.7), H-1 xyl (δ 5.22) and C-3 glc (δ 86.0), H-1 glc (δ 5.14) and C-4 gal (δ 79.5), and H-1 gal (δ 4.85) and C-3 of the aglycone at (δ 75.0). ROEs observed across the glycosidic bonds confirmed the previous assignments of the HMBC spectrum. Thus, saponin **4** was deduced as hecogenin-3-*O*-α-L-rhamnopyranosyl (1→4) β-D-xylopyranosyl (1→3)[β-D-glucopyranosyl (1→2)] β-D-glucopyranosyl (1→4) β-D-galactopyranoside.

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