Triterpenoid Saponins from *Atriplex semibaccata*

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Four new triterpenoid saponins, 3-*O*-{[β-D-glucopyranosyl-(1→2)]-β-D-galactopyranosyl}-11α-methoxy-23-hydroxylongispinogenin, 3-*O*-{[β-D-glucopyranosyl-(1→2)]-β-D-galactopyranosyl}-11α-methoxy-23,29-dihydroxylongispinogenin, 3-*O*-{[β-D-glucopyranosyl-(1→2)]-β-D-galactopyranosyl}-29-hydroxysaikogenin F and 3-*O*-{[β-D-glucopyranosyl-(1→2)]-β-D-galactopyranosyl}-saikogenin F, have been isolated from *Atriplex semibaccata.* The structures were determined primarily by NMR spectroscopy. The assignment of NMR signals was performed by means of ^{1}H-^{1}H COSY, ROESY, HMQC and HMBC experiments.

Key words: Atriplex semibaccata, Triterpenoid Saponins, Chenopodiaceae

Introduction

Atriplex semibaccata R. Br. is an annual, green to greyish plant and belongs to the family of Chenopodiaceae (Zohary, 1966) which is known for the occurrence of saponins (Hostettmann and Marston, 1995). Therefore the objective of this work is the study of the saponin content of *A. semibaccata.* In this report we describe the isolation and structure elucidation of four new triterpenoid saponins.

Results and Discussion

The methanolic plant extract showed the Liebermann-Burchard- and Molisch-reaction indicating the presence of triterpenoid glycosides. The butanol extract of the whole plants of *A. semibaccata* was obtained as described in the experimental section. The crude saponin mixture was subjected to column chromatography on silica gel to be eluted successively with $CHCl_3$, $CHCl_3$-MeOH and $CHCl_3$-MeOH-H_2O with increasing amounts of MeOH and H_2O. Further purification by MPLC on RP-18 material, column chromatography on Sephadex LH-20 and semipreparative HPLC on RP-18 material yielded the saponins **1–4**.

The liquid secondary ion mass spectrum (LSI-MS) of **1** exhibited the $[M-1]^-$ at *m/z* 827. The fragment ion at *m/z* 665 $[M-1-162]^-$ showed the loss of a hexose moiety. The $[M-1]^-$ together with ^{1}H and ^{13}C NMR data allowed us to propose the molecular formula $C_{43}H_{72}O_{15}$.

The ^{1}H and ^{13}C NMR spectra of **1** (Fig. 1) showed the presence of 11α-methoxy-23-hydroxylongispinogenin as aglycone. Saikosaponin b3 (Matsuda *et al.*, 1997) and saikosaponin t (Liang *et al.*, 1998) possess the same aglycone. The signals of the axial and equatorial oriented protons of the aglycone were assigned by ROESY experiments. The ROESY cross peak of H-16ax/3H-27ax and the H-16 coupling constant $J_{15ax,16ax}$ = 11.5 Hz, confirmed the β-configuration of the 16-hydroxy group (Table I). The α-configuration of the 11-methoxy group was proved by the ROESY cross peaks H-11ax/3H-25ax and H-11ax/3H-26ax.

Two anomeric proton signals at δ 4.46 (J = 7.8 Hz) and 4.66 (J = 7.7 Hz) indicated the presence of two monosaccharide units which could be identified by ^{1}H and ^{13}C NMR data as galacto- and glucopyranose (Table II). The coupling constants of the H-3′ signal at δ 3.67 $J_{2'ax,3'ax}$ = 9.4 Hz, $J_{3'ax,4'eq}$ = 3.2 Hz and the ABX spin system at δ 3.71 (J = 6.1, 2.5 Hz, 2H-6′) and δ 3.49 (J = 6.1 Hz, H-5′) are characteristic for a galactopyranose. The coupling constants of the anomeric proton signals of the both monosaccharides J = 7.8 and 7.7 Hz are in agreement with a β-configuration. The linkage of galactose to the aglycone was determined by means of ROESY and HMBC spectra. The ROESY cross peak H-3 aglycone/H-1′ galactose and the HMBC cross peak C-3 aglycone/H-1′ galactose indicated the point of linkage to the sapogenin. The ROESY cross peak H-2′ galactose/H-1″ glucose and the HMBC cross

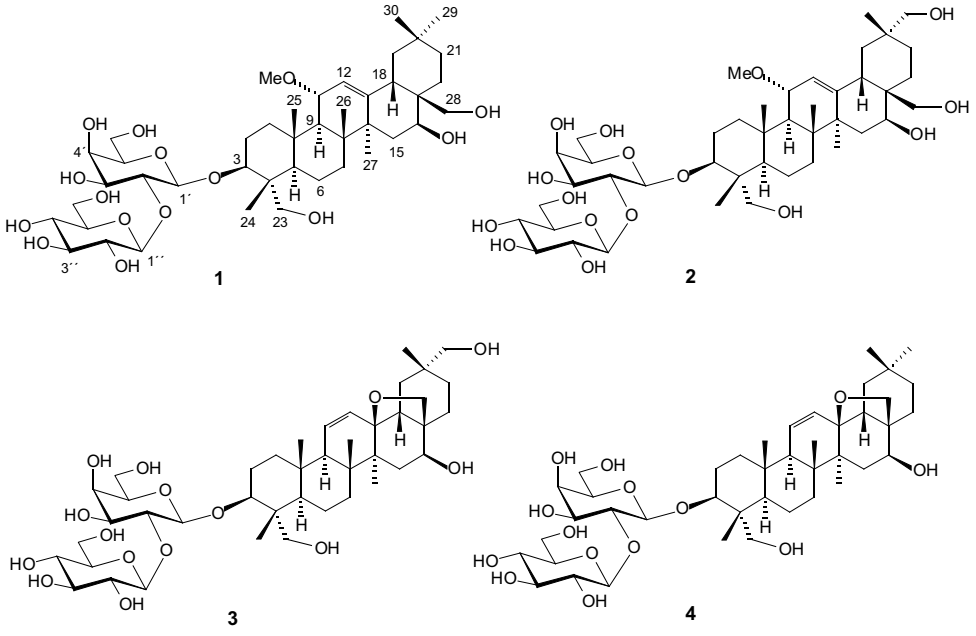

Fig. 1. Triterpenoid saponins from *Atriplex semibaccata*, 3-*O*-{[*β*-D-glucopyranosyl-(1→2)]-*β*-D-galactopyranosyl}-11α-methoxy-23-hydroxylongispinogenin (**1**), 3-*O*-{[*β*-D-glucopyranosyl-(1→2)]-*β*-D-galactopyranosyl}-11α-methoxy-23,29-dihydroxylongispinogenin (**2**), 3-*O*-{[*β*-D-glucopyranosyl-(1→2)]-*β*-D-galactopyranosyl}-29-hydroxysaikogenin F (**3**), 3-*O*-{[*β*-D-glucopyranosyl-(1→2)]-*β*-D-galactopyranosyl}-saikogenin F (**4**).

peak C-2′ galactose/H-1′′ glucose prove the interglycosidic linkage.

The liquid secondary ion mass spectrum of **2** (Fig. 1) exhibited the $[M\text{-}1]^-$ ion at *m/z* 843. This together with ^1H and ^{13}C NMR data led to the molecular formula $C_{43}H_{72}O_{16}$ which contains an additional oxygen atom compared with **1**. The 29-hydroxymethyl group of **2** is responsible for the downfield shift of the C-20 signal ($\Delta\delta$ = + 5.2) and the upfield shifts of the C-19 ($\Delta\delta$ = – 5.7), C-21 ($\Delta\delta$ = – 5.7) and C-30 signals ($\Delta\delta$ = – 4.5) in comparison with **1**. The ROESY cross peak between the axial methyl group 3H-30 and the axial proton H-18 proves the equatorial position of the 29-hydroxymethyl moiety in **2**. According to ^1H and ^{13}C NMR data the same disaccharide moiety as in **1** is bound in position 3 of 11α-methoxy-23,29-dihydroxylongispinogenin.

The ^1H and ^{13}C NMR spectra of **3** (Fig. 1) showed the presence of 29-hydroxysaikogenin F as aglycone and the same disaccharide moiety as in **1** and **2**. The HMBC cross peak C-3 aglycone/H-1′ galactose indicated the point of linkage to the sapogenin. The $[M\text{-}1]^-$ ion of **3** at *m/z* 811 showed a mass difference of 16 compared with the the $[M\text{-}1]^-$ ion of **4** at *m/z* 795. The ^1H and ^{13}C NMR spectra of **4** (Fig. 1) indicated that the 29-methyl group is not oxidized as in **3** and that the saponin **4** possesses the same disaccharide group as in **1**–**3**. The aglycone of triterpenoid glycoside **4** is the same as in clinoposaponin XII which has been isolated before (Miyase and Matsushima, 1997) from the aerial parts of *Clinopodium chinense* var. *parviflorum*. *3-O-{[β-D-Glucopyranosyl-(1→2)]-β-D-galactopyranosyl}-saikogenin E has been obtained previously from Corchorus acutangulus* and bears instead of the hydroxymethyl group of the saponin **4** a methyl moiety in position 23 (Mahato and Pal, 1987).

The saponins **1** and **2** have similar structures as saikosaponin b3 and bupleuroside IX which show hepatoprotective properties. The 11-oxygen function, the 16*β*-hydroxy group and the 3-*O*-diglycoside moiety were found to be essential for their activity (Matsuda *et al.*, 1997). Songarosaponin C has been isolated from *Verbascum songaricum*, contains the same aglycone as triterpenoid glycoside **4** and possesses a high immunosuppressive activity (Bernhardt *et al.*, 2001).

Table I. ^{1}H and ^{13}C NMR spectral data for the aglycones of saponins **1–4** in CD_3OD.

C	**1** ^{1}H ax/eq	**2** ^{1}H ax/eq	**3** ^{1}H ax/eq	**4** ^{1}H ax/eq	**1** ^{13}C	**2** ^{13}C	**3** ^{13}C	**4** ^{13}C
1	1.21/1.83	1.22/1.84	0.92/1.82	0.92/1.83	40.8	40.8	39.2	39.2
2	1.76/1.93	1.77/1.94	1.81/1.99	1.82/2.01	26.7	26.7	26.4	26.4
3	3.64	3.64	3.64	3.64	83.7	83.7	83.7	83.7
4					44.6	44.5	44.3	44.3
5	1.26	1.25	1.17	1.19	48.3	48.4	48.0	48.0
6	1.42/1.56	1.41/1.57	1.54	1.54	18.9	18.9	18.2	18.2
7	1.67/1.28	1.67/1.28	1.54/1.20	1.54/1.22	33.8	33.8	32.1	32.3
8					44.4	44.4	43.0	42.9
9	1.73	1.73	1.89	1.88	52.8	52.9	54.0	53.9
10					38.8	38.8	37.0	37.0
11	3.87	3.86	5.94, 10.3 Hz	5.94, 10.3 Hz	77.5	77.2	134.3	134.3
12	5.42	5.43	5.38, 10.3, 2.8 Hz	5.37, 10.3, 2.6 Hz	123.1	123.2	130.5	130.5
13					149.1	149.1	85.8	85.7
14					44.6	44.7	46.4	46.5
15	1.76/1.40	1.76/1.40	1.59/1.42	1.58/1.44	36.6	36.6	36.0	35.9
16	4.26, *dd*, 11.5, 5.0 Hz	4.26, *dd*, 11.8, 5.1 Hz	4.16, *dd*, 9.9, 5.8 Hz	4.16, *dd*, 9.8, 5.7 Hz	67.2	67.3	65.4	65.4
17					41.6	41.8	47.8	47.6
18	2.28	2.28	1.81	1.79	44.4	43.7	52.3	53.1
19	1.72/1.13	1.81/1.08	1.86/1.22	1.78/1.28	47.6	41.9	32.8	38.5
20					31.7	36.9	37.6	32.1
21	1.45/1.21	1.52/1.16	1.56/1.12	1.45/1.22	34.7	29.0	29.6	35.2
22	1.45/2.12	1.50/2.13	1.28/2.08	1.26/2.07	25.7	24.9	25.5	26.1
23	3.29/3.77	3.29/3.76	3.27/3.77	3.28/3.77	64.8	64.8	64.6	64.6
24	0.75	0.75	0.71	0.71	13.3	12.4	12.7	12.7
25	1.11	1.10	0.94	0.94	18.0	18.0	18.8	18.8
26	1.05	1.06	1.08	1.08	18.7	18.7	20.2	20.2
27	1.31	1.33	1.05	1.03	26.6	26.6	21.2	21.2
28	3.27/3.81	3.27/3.81	3.05/3.88	3.04/3.88	68.3	68.3	73.3	73.4
29	0.91	3.19/3.23	3.23	0.97	33.6	74.5	74.6	33.9
30	0.95	0.94	0.89	0.91	24.2	19.7	19.7	24.1
OMe	3.22	3.22			54.4	54.4		

C = carbon atoms of the aglycones.

Experimental

General

Negative ion MS: MAT 8500 (Finnigan), matrix glycerol. NMR: 500.13 MHz (^{1}H) and 125.76 MHz (^{13}C), reverse probehead, δ in ppm, solvent CD_3OD, CD_3OD signals were used as int. standard (^{1}H: 3.30, ^{13}C: 49.0), temp. 290 K, HMQC: phase-sensitive using TPPI (Time Proportional Phase Increment), BIRD (Bilinear Rotation Decoupling) sequence, GARP decoupled, HMBC: using TPPI, delay to achieve long range couplings: 71 msec ($J_{C,H}$ = 14 Hz).

Liebermann-Burchard-reaction: To 5 mg of crude extract 1.5 ml of Ac_2O, 2 ml of $CHCl_3$ and 0.2 ml of H_2SO_4conc were added (Abisch and Reichstein, 1960). Molisch-reaction: A mixture of 5 mg of crude extract in 0.1 ml of H_2O, 1 drop of a 5% α-naphthol solution and 0.5 ml of HClconc was heated for 1 min (Laatsch, 1988).

CC: silica gel (0.063–0.2 mm); TLC: silica gel (0.25 and 1 mm precoated plates 60 F_{254}, Merck, 0.25 mm precoated plastic sheets SIL G/UV_{254} Macherey-Nagel, Düren, Germany), the spots were sprayed with 'triterpene reagent' (1% vanillin in 50% H_3PO_4), 'sugar reagent' (4% ethanolic aniline-4% ethanolic diphenylamine-H_3PO_4, 5:5:1 v/v/v) and phosphomolybdic acid reagent (Aldrich).

Isolation

A. semibaccata was collected in 1999 nearby Burg El-Arab Egypt and identified by Dr. M. Elgebaly from the National Research Centre (NRC) Cairo. A voucher specimen of the plant is deposited at the Herbarium of the NRC, Department

Table II. ^{1}H and ^{13}C NMR spectral data for the sugar moieties of saponins **1–4** in CD_3OD.

C	**1** ^{1}H	**2** ^{1}H	**3** ^{1}H	**4** ^{1}H	**1** ^{13}C	**2** ^{13}C	**3** ^{13}C	**4** ^{13}C
Gal								
1′	4.46,*d*, 7.8 Hz	4.45, *d*, 7.8 Hz	4.45, *d*, 7.8 Hz	4.45, *d*, 7.8 Hz	104.9	104.9	104.9	104.9
2′	3.83, *dd*, 7.8, 9.4 Hz	3.81, *dd*, 7.8, 9.4 Hz	3.81, *dd*, 7.8, 9.7 Hz	3.82, *dd*, 7.8, 9.6	79.6	79.6	79.7	79.6
3′	3.67, *dd*, 9.4, 3.2 Hz	3.67, *dd*, 9.4, 3.2 Hz	3.67, *dd*, 9.7, 3.4 Hz	3.67, *dd*, 9.6, 3.2 Hz	75.5	75.5	75.5	75.5
4′	3.84, *dd*, 3.2 Hz	3.83, *dd*, 3.2 Hz	3.82, *dd*, 3.4 Hz	3.84, *dd*, 3.2 Hz	70.4	70.7	70.3	70.3
5′	3.49, 6.1 Hz	3.49, 6.1 Hz	3.48, 6.1 Hz	3.49, 6.0 Hz	76.2	76.2	76.2	76.2
6′	3.71, 6.1, 2.5 Hz	3.72, 6.1, 2.5 Hz	3.71, 6.1 2.7 Hz	3.71, 6.0, 2.9 Hz	62.3	62.3	62.3	62.3
Glc								
1″	4.66, *d*,, 7.7 Hz	4.66, *d*, 7.7 Hz	4.66, *d*, 7,7 Hz	4.66, *d*, 7.7 Hz	104.6	104.6	104.6	104.6
2″	3.23, *dd*, 7.7, 8.9 Hz	3.23, *dd*, 7.7, 8.8 Hz	3.23, *dd*, 7.7, 8.9 Hz	3.23, *dd*, 7.7, 8.8 Hz	76.2	76.2	76.2	76.2
3″	3.37, *dd*, 8.9 Hz	3.36, *dd*, 8.8 Hz	3.35, *dd*, 8.9 Hz	3.36, *dd*, 8.8 Hz	77.9	77.5	77.9	77.9
4″	3.26, *dd*, 8.9 Hz	3.26, *dd*, 8.8 Hz	3.24, *dd*, 8.9 Hz	3.26, *dd*, 8.8 Hz	71.8	71.9	71.8	71.8
5″	3.26, m	3.26, m	3.25, m	3.26, m	78.3	78.6	78.3	78.3
6″	3.63, 11.9 Hz 3.84, 11.9 Hz	3.63, 11.9 Hz 3.83, 11.9 Hz	3.63, 11.9 Hz 3.82, 11.9 Hz	3.64, 11.9 Hz 3.84, 11.9 Hz	62.9	63.0	62.9	62.9

C = carbon atoms of the sugar moieties, Gal = β-D-galactopyranose, Glc = β-D-glucopyranose.

of Chemotaxonomy. Dried powder of the whole plant of *A. semibaccata* (3.0 kg) was exhaustively extracted with 80% MeOH (15 l). After removal of the solvent by evaporation, the residue (150 g) was successively partitioned between H_2O and *n*-hexane, $CHCl_3$ and *n*-BuOH. The butanolic fr. was evaporated under red. pres. at 50 °C to obtain a crude saponin mixture (7.0 g). CC on silica gel eluting with $CHCl_3$-MeOH-H_2O with increasing amounts of MeOH and H_2O gave two frs. I (2.5 g) and II (3.0 g). I was further purified by means of MPLC on RP-18 eluting with MeOH-H_2O 67:33, Sephadex LH-20 eluting with MeOH, followed by semiprep. HPLC on RP-18 eluting with MeOH-H_2O 67:33 to give pure saponins **1** (3.5 mg), **2** (3.0 mg), **3** (3.0 mg) and **4** (2.5 mg).

Spectroscopic data

3-*O*-{[β-D-glucopyranosyl-(1→2)]-β-D-galactopyranosyl}-11α-methoxy-23-hydroxylongispinogenin (**1**): ($C_{43}H_{72}O_{15}$, M_r 828); amorphous powder. LSI-MS negative ion mode *m/z* (rel. int.): 827 $[M-H]^-$ (100), 665 $[M-H-Glc]^-$ (20). For ^{1}H NMR and ^{13}C NMR: see Tables I and II.

3-*O*-{[β-D-glucopyranosyl-(1→2)]-β-D-galactopyranosyl}-11α-methoxy-23,29-dihydroxylongispinogenin (**2**): ($C_{43}H_{72}O_{16}$, M_r 844); amorphous powder. LSI-MS negative ion mode *m/z* (rel. int.): 843 $[M-H]^-$ (100), 681 $[M-H-Glc]^-$ (21). For ^{1}H NMR and ^{13}C NMR: see Tables I and II.

3-*O*-{[β-D-glucopyranosyl-(1→2)]-β-D-galactopyranosyl}-29-hydroxysaikogenin F (**3**): ($C_{42}H_{68}O_{15}$, M_r 812); amorphous powder. LSI-MS negative ion mode *m/z* (rel. int.): 811 $[M-H]^-$ (100), 649 $[M-H-Glc]^-$ (20). For ^{1}H NMR and ^{13}C NMR: see Tables I and II.

3-*O*-{[β-D-glucopyranosyl-(1→2)]-β-D-galactopyranosyl}-saikogenin F (**4**): ($C_{42}H_{68}O_{14}$, M_r 796); amorphous powder. LSI-MS negative ion mode *m/z* (rel. int.): 795 $[M-H]^-$ (100), 633 $[M-H-Glc]^-$ (37). For ^{1}H NMR and ^{13}C NMR: see Tables I and II.

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