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New unusual pregnane glycosides with antiproliferative activity from *Solenostemma argel*

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Abstract

Seven new 15-keto pregnane glycosides, namely Stemmosides E–K, were isolated from *Solenostemma argel*. Stemmosides E–J are characterized by the occurrence of an uncommon 14β proton configuration while stemmosides E and F possess in addition a rare enolic function in C-16. On the other hand, stemmosides G–J display an unusual C-17α side chain. Their structures were established by ESI-MS and NMR experiments. Moreover, the effect of these compounds on the VEGF-induced in Kaposi's sarcoma cell proliferation was tested. Results indicated that all the compounds reduced the cell proliferation in a dose dependent manner.

Keywords: *Solenostemma argel*; Pregnane glycosides; 15-Keto; *cis* CD junction; Cell proliferation; Kaposi's sarcoma

1. Introduction

Plants belonging to the family Asclepiadaceae are frequently used in traditional medicine and have been reported to be rich in steroidal glycosides [1,2]. *Solenostemma argel* Hayne (Asclepiadaceae) is an Egyptian wild perennial erect shrub growing in the eastern desert and along the Nile in South Egypt [3], whose leaves are commonly used in traditional medicine as a purgative, antipyretic, expectorant, antispasmodic and in cases of bile congestion [4]. Previous studies have reported the occurrence of monoterpene glycosides [5], pregnane derivatives [6] and pregnane glycosides including stemmosides A and B [5,7] and acylated phenolic glycosides in the leaves [8] while 14,15-secopregnane glycosides [9] and pregnane glycosides namely stemmosides C and D have been isolated from the pericarps [10].

In our on going research of new bioactive compounds of *S. argel*, here we report the occurrence of five new unusual 15-keto pregnane glycosides namely stemmosides E–I (**1**–**5**) from the leaves and two new 15-keto pregnane glycosides, stemmoside J and K (**6**, **7**), along with the known compound stemmoside A, from the pericarps. Their structures were elucidated by extensive spectroscopic methods including 1D- (^{1}H and ^{13}C) and 2D-NMR experiments (DQF-COSY, HSQC, HMBC, ROESY and HOHAHA) as well as ESI-MS analysis. Compounds **1**–**6** are characterized by the occurrence of an uncommon 14β proton configuration while compounds **1** and **2** possess in addition a rare enolic function in C-16. Apparently, *S. argel* is the only plant from which pregnanes possessing a 15-keto, *cis* CD ring junction have been isolated [10]. A few other naturally occurring steroids showing a 15-keto, *cis* CD ring junction have been isolated only from marine sponges [11–15]. On the other hand, to the author's knowledge compounds **3**–**6** along with stemmoside C and D are the only natural compounds which

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display an unusual C-17α side chain and no substitution in C-12.

In addition, since pregnanes and their glycosides have shown to possess antitumor and cytotoxic activities [16–19] and on the basis of the novelty of the structure features of stemmoside **1**–**8** we examined whether these compounds, along with the previously isolated stemmosides C and D [10], can be useful in the control of tumor cell proliferation. Kaposi's sarcoma (KS) cells were used as a model to test the anti-proliferative properties of these compounds. Unlike the more indolent endemic and sporadic form of KS, this disease is usually aggressive and unpredictable in individuals infected by HIV-1, and the neoplasm is the cause of significant morbidity and occasional mortality. KS is usually treated with cytostatic drugs, cytokine or radiotherapy to control cell proliferation [20], thus the identification of new molecules of pharmacological interest could be of clinical relevance for the development of novel therapeutic strategies.

2. Experimental

2.1. General

Optical rotations were measured on a Jasco DIP 1000 polarimeter. IR measurements were obtained on a Bruker IFS-48 spectrometer. Exact masses were measured by a Voyager DE mass spectrometer (Applied Biosystems, Foster City, CA, USA). Samples were analyzed by matrix assisted laser desorption ionization (MALDI) mass spectrometry. A mixture of analyte solution and α-ciano-4-hydroxycinnamic acid (Sigma) was applied to the metallic sample plate and dried. Mass calibration was performed with the ions from ACTH (fragment 18–39) at 2465.1989 Da and Angiotensin III at 931.5154 Da as internal standard. ESI-MS was performed on a Finnigan LC-Q Deca Ion Trap mass spectrometer scanned from 150 to 1200 Da. The mass spectral data was acquired and processed using Xcalibur software. Samples were dissolved in MeOH and infused in the ESI source by using a syringe pump at a flow rate of 3 μL/min. The capillary voltage was 5 V, the spray voltage 5 kV and the tube lens offset 50 V. The capillary temperature was 220 °C. NMR experiments were performed on a Bruker DRX-600 spectrometer at 300 K. All the 2D-NMR spectra were acquired in CD_3OD in the phase-sensitive mode with the transmitter set at the solvent resonance and TPPI (Time Proportional Phase Increment) used to achieve frequency discrimination in the ω_1 dimension. The standard pulse sequence and phase cycling were used for DQF-COSY, 2D-TOCSY, HSQC, HMBC and ROESY spectra. The spectra were acquired at 600 MHz. The NMR data were processed on a Silicon Graphic Indigo2 Workstation using UXNMR software. Column chromatography was performed over Sephadex LH-20 (Pharmacia), MPLC was carried out on a Büchi 688 chromatography pump and Büchi B-685 borosilicate glass column (230 mm × 26 mm). Silica gel 60 (0.040–0.063 mm; Carlo Erba) was used as column material. HPLC separations were carried out on a Waters 590 system equipped with a Waters R401 refractive index detector, a Waters XTerra Prep MSC_{18} column, and a U6K injector. TLC was performed on silica gel F254 (Merck) plates, and reagent grade chemicals (Carlo Erba) were used throughout.

2.2. Plant material

Fresh samples of *S. argel* leaves and pericarps were collected at Allaqi (south-east of Aswan, Egypt) in May 2002 and identified by one of the authors (A.I.H.).

2.3. Extraction and isolation

The dried pericarps (1.5 kg) extracted with EtOH 80% yielding 254 g of extract while the dried leaves (100 g) were extracted with EtOH 70% yielding 20 g of extract. Part of the leaves extract (2.5 × 2 g) was fractionated on Sephadex LH-20 (100 cm × 5 cm) using MeOH as the mobile phase. Sixty-seven fractions (8 mL) were obtained. The fractions containing pregnane glycosides (frs. 13–17, 1.0176 g) were chromatographed by MPLC on Si gel with a gradient (flow rate 3.0 mL/min) of chloroform/methanol (from 100:0 to 9:1, stepwise) as eluent to afford 1660 fractions (8 mL) monitored by TLC. Fractions 1133–1156 (48.5 mg) were chromatographed by RP-HPLC on a Waters (μ-Bondapak C_{18}) column (300 mm × 7.8 mm) using methanol/water (78:22) as mobile phase (flow rate 2.5 mL/min) to yield compound **4** (2.2 mg, t_R = 57.1 min), **5** (1.5 mg, t_R = 67.2 min) along with one fraction A (6.0 mg) that was finally purified by RP-HPLC on a Waters (μ-Bondapak C_{18}) column (300 mm × 7.8 mm) using methanol/water (75:25) as mobile phase (flow rate 2.5 mL/min) to yield compound **1** (2.6 mg, t_R = 54.1 min). Fractions 1157–1191 (66.6 mg) were chromatographed by RP-HPLC on a Waters (μ-Bondapak C_{18}) column (300 mm × 7.8 mm) using methanol/water (78:22) as mobile phase (flow rate 2.5 mL/min) to yield compound **3** (2.7 mg, t_R = 39.3 min) along with one fraction B (7.8 mg) that was finally purified by RP-HPLC on a Waters (μ-Bondapak C_{18}) column (300 mm × 7.8 mm) using methanol/water (75:25) as mobile phase (flow rate 2.5 mL/min) to yield compounds **2** (3.0 mg, t_R = 42.0 min) and **8** (1.3 mg, t_R = 38.0 min). Part of the pericarp extract (2.2 g) was fractionated on Sephadex LH-20 (100 cm × 5 cm) using MeOH as the mobile phase. Ninety-five fractions (8 mL) were obtained. The fractions containing pregnane glycosides (frs. 29–33, 375 mg) were chromatographed by HPLC (Refractive index detector), on a Waters (XTerra Prep MSC_{18}) column (300 mm × 7.8 mm) using methanol/water (74:26) as mobile phase (flow rate 2.5 mL/min) to yield compound **6** (2.6 mg, t_R = 52.0 min), and **7** (2.3 mg, t_R = 32.7 min).

2.3.1. Stemmoside E (*1*)

White amorphous powder; $[\alpha]_D^{24}$ −15.4° (*c* 0.2, MeOH); UV (MeOH) λ_{max} 244 nm; log ε 3.84; IR (KBr) ν_{max} 3451, 1719, 1650, 1070 cm^{-1}; ^1H NMR (CD_3OD, 600 MHz) agly-