Fitoterapia 102 (2015) 198–202

Contents lists available at ScienceDirect

Fitoterapia

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

3-Keto umbilicagenin A and B, new sapogenins from *Allium umbilicatum* Boiss

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ARTICLE INFO

Article history:
Received 31 October 2014
Accepted in revised form 27 December 2014
Available online 8 January 2015

Keywords:
Allium
Steroidal sapogenins
3-Keto spirostanol
NMR
Cytotoxic activity

ABSTRACT

Two sapogenins, named 3-keto umbilicagenin A and B (**1** and **2**), possessing a novel chemical structure with a 3-keto group on the spirostane skeleton, have been isolated from *Allium umbilicatum* Boiss. Their chemical structure has been established through a combination of extensive spectroscopic analysis, mainly nuclear magnetic resonance and mass spectrometry, and chemical methods as (25*R*)-3-keto-spirostan-2α,5α,6β-triol (**1**) and (25*R*)-3-keto-spirostan-2α,5α-diol (**2**). The isolated compounds were tested for cytotoxic activity on J-774, murine monocyte/macrophage, and WEHI-164, murine fibrosarcoma cell lines.

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1. Introduction

Plants belonging to *Allium* genus have been used since ancient times both as a food and as medicine. In fact, these plants are rich source of phytonutrients considered an important element of the daily diet because of interesting pharmacological properties, such as cholesterol lowering, anti-hypertensive, antispasmodic, antibacterial, antiviral, and anticancer [1–4]. The history of natural and cultivated *Allium* species is well documented dating back to 3000 B.C. The major centre of *Allium* species diversity spreads from central Asia to the Mediterranean basin. Since 2000 B.C. these plants were introduced in Europe by the Phoenicians and consumed by Romans and Greeks, appreciating their taste and curative properties [5]. Epidemiological studies have consistently shown an inverse association between consumption of *Allium* vegetables and the risk of human diseases. Notably, a population-based case–control study showed that the consumption of *Allium* vegetables was associated to a reduced risk of prostate cancer [6].

In the ongoing investigation on the chemistry of *Allium* genus [7–11], it was analysed *Allium umbilicatum* Boiss. This species has been collected in Iran but it can be also found in Afghanistan and Pakistan at considerable altitude. It is a wild plant species that could be cultivated easily. It is a pretty little *Allium* sp. characterized by grassy leaves and rounded umbels packed with lilac-pink flowers on 15 cm. The flowers are a nice bright pink and are held in small, dense flowered, hemispherical or spherical umbels, with each little flower on a 1 cm pedice. The leaves of the plant are chopped into small pieces and added to flour to make a kind of bread called "Paratha". It is also used as general condiment [12]. The phytochemical analysis of *Allium umblicatum* afforded the isolation of two new sapogenins, named 3-keto umbilicagenin A and B (**1** and **2**, Chart 1). The compounds were tested for cytotoxic activity on J-774, murine monocyte/macrophage, and WEHI-164, murine fibrosarcoma, cell lines.

2. Experimental

2.1. General experimental procedures

Optical rotations were measured by a Jasco P-1010 polarimeter equipped with a sodium lamp (589 nm) and 10 cm microcell. ESI MS experiments were performed by

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http://dx.doi.org/10.1016/j.fitote.2014.12.016
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an Applied Biosystem API 2000 triple-quadrupole mass spectrometer. The spectra were recorded by infusion into the ESI source using methanol as solvent. GC analysis was performed by an Agilent Technologies 6890N Network gas 236 instrument. ^1H and ^{13}C NMR spectra were recorded by Bruker 500 (^1H at 500 MHz and ^{13}C at 125 MHz) and 400 (^1H at 400 MHz and ^{13}C at 100 MHz) spectrometers, using solvent signal for calibration (CD_3OD: δ_H 3.31, δ_C 49.0). The multiplicities of ^{13}C NMR resonances were determined by DEPT experiments. ^1H connectivities were determined by COSY. One-bond heteronuclear ^1H–^{13}C connectivities were determined with 2D Heteronuclear Single-Quantum Coherence (HSQC) pulse sequence with an interpulse delay set for $^1J_{CH}$ of 130 Hz while two and three bond heteronuclear ^1H–^{13}C connectivities were determined with 2D Heteronuclear Multiple Bond Correlation (HMBC) and optimized for $^{2-3}J_{CH}$ of 8 Hz. Nuclear Overhauser effect (NOE) measurements were performed by 2D ROESY experiments. Medium pressure liquid chromatography (MPLC) was performed by a BUCHI Gradient System C-605 apparatus using glass columns of LiChroprep RP-18 (25–40 μm) and C-660 BUCHI fraction collector. TLC was performed on SiO_2 plates with BuOH: CH_3CO_2H: H_2O 60:15:25 (BAW) as a mobile phase and cerium sulphate in 2 N H_2SO_4 as a reagent for visualizing the spots. HPLC was performed in isocratic mode by a Waters 515 apparatus equipped with a refractive index detector (Waters 2414) and using semipreparative Novapak C_{18} (7.8 × 300 mm i.d.) and analytical Novapak C_{18} (3.9 × 300 mm i.d.) columns.

2.2. Plant material

Samples of flowers of *A. umbilicatum* were collected on the Alburz' montains in “Challus-Karaj” road, Iran. The plant was identified by Prof. Shahin Zarre, Tehran, Iran as *A. umbilicatum* Boiss. A voucher specimen (N. 2755) is deposited at the Herbarium of Pharmacognosy, Department of Pharmacognosy, Isfahan University of Medical Sciences.

2.3. Extraction and isolation

The flowers were finely powdered by means of mill and the powder (706 g) was extracted at room temperature and in a four step extraction method with the following solvents at increasing polarity: hexane, chloroform, chloroform–methanol (9:1) and methanol. Each step was conducted for 1 day under stirring and was repeated four times using 2.5 L of solvent. The chloroform–methanol (9:1) extract, concentrated under vacuum (40.5 g), was fractionated by MPLC on a RP-18 column (36 × 460 mm) using a linear gradient solvent system from H_2O to MeOH. By TLC (SiO_2, BAW 60:15:25 *v*/*v*/*v*). Fractions eluted with H_2O-MeOH (60:40) (1.427 g) contained pure kaempferol-3-O-rutinoside. Fractions eluted with H_2O-MeOH (25:75) (1050 mg), further purified by HPLC on a semipreparative C_{18} column with the mobile phase H_2O-MeOH (65:35), afforded the new sapogenins **1** (17 mg, t_R = 24.8 min) and **2** (16 mg, t_R = 25 min).

2.4. Data of compounds **1** and **2**

12-Keto umbilicagenin A (**1**). (25*R*)-3-keto-spirostan-2α,5α,6β-triol: $[\alpha]_D^{25}$ −58.18 (*c* = 0.1 MeOH); HRESIMS (negative ion): found *m/z* 463.3157 [M + H]$^+$; calculated for $C_{27}H_{43}O_6$ *m/z* 463.3146; ^1H and ^{13}C NMR data, see Table 1.

12-Keto umbilicagenin B (**2**). (25*R*)-3-keto-spirostan-2α,5α-diol: $[\alpha]_D^{25}$ −70.59 (*c* = 0.1 MeOH); HRESIMS (negative ion): found *m/z* 447.6448 [M + H]$^+$; calculated for $C_{27}H_{43}O_5$ *m/z* 447.6446; ^1H and ^{13}C NMR data, see Tables 1 and 2.

2.5. Cells and culture

WEHI 164 cells (murine fibrosarcoma cell line) were maintained in adhesion on Petri dishes with Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% heat-inactivated foetal bovine serum (FBS), 25 mM HEPES, penicillin (100 U/mL) and streptomycin (100 μg/mL). J774 cells (murine monocyte/macrophage cell line) were grown in suspension culture, in Techne stirrer bottles, spun at 25 rpm and incubated at 37 °C in DMEM medium supplemented with 10% FBS, 25 mM Hepes, glutamine (2 mM), penicillin (100 U/mL) and streptomycin (100 μg/m).

2.6. Cytotoxicity assays

WEHI 164 and J774 cells were placed on 96-well microliter plates and allowed to adhere at 37 °C in 5% CO_2/95% air for 2 h. Then, the medium was replaced with 50 μL of fresh medium and 75 μL aliquot of 1.2 *v*/*v* serial dilution of each sapogenin was added and then the cells were incubated for 72 h. The cell viability was assessed through an MTT [3-(4,5-dimethyl-thiazol-2-yl)-2,5-phenyl-2H-tetrazolium bromide] conversion

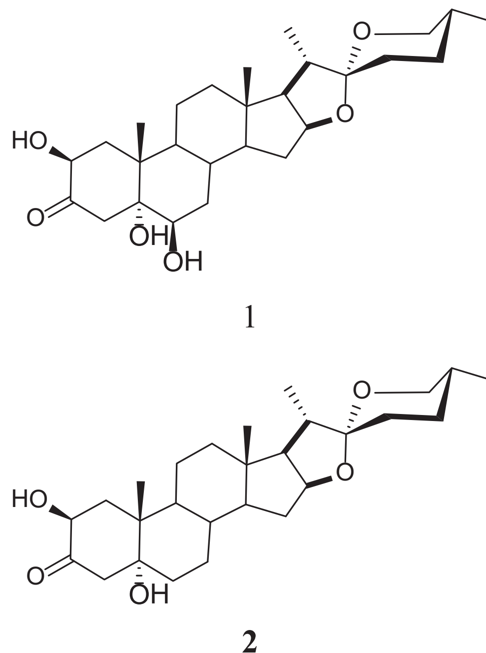

Chart 1. Chemical structure of 3-keto umbilicagenin A (**1**) and B (**2**) from *A. umbilicatum*.

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