Fitoterapia 109 (2016) 261–265

Contents lists available at ScienceDirect

Fitoterapia

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

A new hexacyclic triterpene acid from the roots of *Euscaphis japonica* and its inhibitory activity on triglyceride accumulation

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ARTICLE INFO

Article history:
Received 14 December 2015
Received in revised form 25 January 2016
Accepted 28 January 2016
Available online 29 January 2016

Keywords:
Euscaphis japonica
Triterpene acid
HepG2
Triglyceride accumulation

ABSTRACT

A new taraxerene-type hexacyclic triterpene acid named (12R,13S)-3-methoxy-12,13-cyclo-taraxerene-2,14-diene-1-one-28-oic acid (**1**), together with a known compound 3,7-dihydroxy-5-octanolide (**2**), was isolated from the roots of *Euscaphis japonica*. The structure of new compound **1** was elucidated on the basis of NMR, HR-ESIMS and X-ray diffraction analysis. It showed promising inhibitory activity on oleic acid induced triglyceride accumulation on HepG2 cells.

1. Introduction

Euscaphis japonica is a deciduous small arbor or shrub that is mainly distributed in the south of the Yangtze River in China, Japan and Korea; the leaves, flowers, fruits and roots have been used as a traditional Chinese medicine for the relieve rheumatism, detumescence and analgesia properties, respectively [1,2]. Previous pharmacological research reported that the methanol extract of *E. japonica* possesses anti-inflammatory [3], anti-hepatic fibrotic activity [4] and downregulating lip genesis properties [5]. A variety of compounds, such as flavonoid glucosides [6], triterpenes [7,8], lactones [9], ellagitannin and tetraketide [10] have been reported from the acrial part of this plant. To date, however, there have been no reports focused on the roots of this herb. Therefore, we attempted to investigate the active constituents and a new hexacyclic triterpene acid named (12R,13S)-3-methoxy-12,13-cyclo-taraxerene-2,14-diene-1-one-28-oic acid (**1**), together with a known compound 3,7-dihydroxy-5-octanolide (**2**) (Fig. 1), was isolated from the roots of this herb. Triterpene compounds showed multiple pharmacological activities, such as anti-hepatic fibrotic activity [4] and inhibitory activity on triglyceride accumulation [11]. Based on the exact lipid-lowering effects as selective antagonistic properties on LXRa of *E. japonic*a [5], the lipid-decreasing effect of the new compound **1** in HepG2 cells was evaluated. Herein, we reported the isolation and structure elucidation of the new compound **1**. In addition, it exhibited promising inhibitory activity on oleic acid induced triglyceride accumulation in HepG2 cells.

2. Experimental

2.1. General experimental procedures

Melting points were measured using an X-5 micro melting point apparatus. Optical rotations were determined with an autopol IV-T automatic polarimeter. UV spectra were measured on a Macy UV-1500 ultraviolet and visible spectrophotometer. IR spectra were recorded on a perkin elmer spectrum one as KBr pellets and the absorption frequencies are expressed in reciprocal centimeters (cm^{-1}).1D and 2D NMR spectra were performed on a Bruker ultrashield™ 400 plus spectrometer at 400 MHz (^{1}H NMR) and 100 MHz (^{13}CNMR) with TMS (^{1}H NMR) as the internal reference. ESI-MS spectra were performed on LC–MS 8040 (Shimadzu, Japan) and High-resolution electrospray ionization mass spectra (HR-ESIMS) were carried out in the negative ion mode with a Thermo Fisher LC-LTQ-Orbitrap XL spectrometer. The X-ray diffraction experiment was collected on a Bruker SMART APEX CCD X-ray diffractometer using graph itemonochromated Cu Kα radiation. Column chromatography (CC) was performed using silica gel (100–200 and 200–300 mesh; Qingdao Marine Chemical Inc., China). TLC was carried out on precoated silica gel GF254 plates. Spots were visualized under UV light (254 or 356 nm) or by spraying with 10% H_2SO_4 in 95% EtOH followed by heating. All solvents used in column chromatography

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http://dx.doi.org/10.1016/j.fitote.2016.01.016
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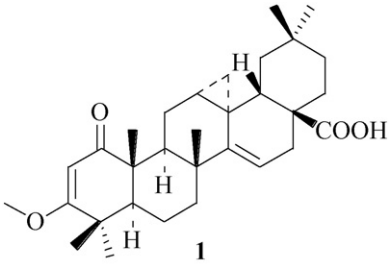

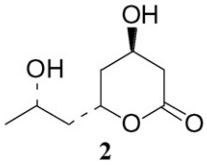

Fig. 1. Structures of **1** and **2.**

and HPLC were of analytical grade (Sinopharm Chemical Reagent Co., Ltd) and chromatographic grade (Fisher Scientific, New Jersey, USA), respectively.

2.2. Plant material

The experimental material was collected from Hunan province, in China. Original plant was authenticated by Doctor Zuning Ning who majors in plant classification, South China botanical garden, Chinese academy of sciences. A voucher specimen (No. 2012092005) has been deposited at Hubei key laboratory of bio-technology of Traditional Chinese Medicine.

2.3. Extraction and isolation

The dried roots of *E. japonica* (41 kg) were extracted with 95% alcohol at room temperature and evaporated to yield a residue (1177 g).The residue was suspended in H_2O and partitioned with PE, EtOAc and n-BuOH, successively. The PE extract (298 g) was fractionated on silica gel column (200–300 mesh) eluting with PE/EtOAc (40:1, 20:1, 10:1, 5:1, 2:1 and 1:1) to afford six fractions(A–F). Fraction A (10 g) was subjected to silica gel CC eluting with PE/EtOAC (60:1 → 10:1) to give five subfractions (A1–A5). The crystals of subfraction A1 were filtered and recrystallized to obtain compound **1** (42 mg). Fraction D was subjected to silica gel CC eluting with PE/EtOAC (50:1 → 40:1) to give five subfractions (D1–D5). D3 was purified by HPLC eluting with MeOH in H_2O from 30% to 70% to obtain compound **2** (20 mg).

*Compound **1***: colorless needles; mp: 201–203 °C; $[\alpha]_D^{20}$ + 60.8 (c 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 206.2 (4.12) and 249 (4.12) nm; IR ν max 3392, 3069, 2940, 2858, 1724, 1665, 1613, 1454, 1339, 1196, 1169, 1140, 832,738 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) spectral data are listed in Table 1; ESI-MS: m/z 481 $[M + H]^+$; 479 $[M - H]^-$; HR-ESIMS: m/z 479.3149 $[M - H]^-$ (calcd for $C_{31}H_{44}O_4$, 479.3167 $[M - H]^-$).

*Compound **2***: yellowish powder, mp: 329–331 °C; ^1H NMR (400 MHz, $CDCl_3$) δ: 4.86 (1H, br. s, H-5), 4.34 (1H, br. s, H-3), 3.91 (1H, m, H-7), 2.87 (1H, d, J = 13.2 Hz, H-2α), 2.76 (1 H, d, J = 13.2 Hz, 3.6 Hz, H-2β), 2.02 (1 H, d, J = 10.6 Hz, H-4α), 1.99 (1 H, d, J = 10.6 Hz, H-4β), 1.90 (1 H, d, J = 9.6 Hz, H-6α), 1.53 (1 H, dd, J = 9.6 Hz, 8.28 Hz, H-6β), 1.20 (3 H, d, J = 4.1 Hz, H-8); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 169.9 (C-1), 36.6 (C-2), 66.1 (C-3), 29.7 (C-4), 73.2 (C-5), 38.6 (C-6), 62.1 (C-7), 21.5 (C-8).

Table 1
^1H and ^{13}C NMR spectral data of **1**.[a,b]

No.	δ_H (J in Hz)	δ_C	No.	δ_H (J in Hz)	δ_C
1		206.1	17		53.1
2	5.02 (s, 1H)	98.2	18	2.55 (dd, J = 9.2, 1.6, 1H)	34.7
3		178.4	19	0.65 (dd, J = 8.68, 2.0, 1H)	35.7
4		39.2		0.92 (overlapped, 1H)	
5	1.41 (overlapped, 1H)	51.0	20		29.0
6	1.62 (d, J = 8.36, 1H) 1.53 (dd, J = 8.36, 2.4, 1H)	18.8	21	1.09 (overlapped, 1H) 1.19 (overlapped, 1H)	33.8
7	1.83 (overlapped, 1H) 1.30 (dd, J = 8.0, 1H)	37.3	22	1.72, dt (J = 9.24, 2.24, 1H) 1.48, dd (J = 9.6, 2.12, 1H)	30.5
8		37.4	23	1.16 (s, 3H)	20.6
9	1.43, overlapped	38.7	24	1.13 (s, 3H)	28.9
10		47.2	25	1.22 (s, 3H)	17.4
11	2.60 (d, J = 8.28, 1H) 1.82 (dd, J = 8. 28, 2.0, 1H)	22.0	26	0.97 (s, 3H)	23.3
12	1.02	15.4	27	0.15, t (J = 3.16, 1H)	11.5
13		22.6		0.90–0.91 (overlapped, 1H)	
14		156.1	28		184.0
15	5.56, (dd, J = 4.68, 2.24, 1H)	118.2	29	0.91 (s, 3H)	32.2
16	2.37, (dd, J = 8.96, 4.68, 1H) 1.90 (dd, J = 8.96, 2.24, 1H)	31.8	30	0.89 (s, 3H)	29.1
			31	3.63 (s, 3H)	55.8

[a] 400 MHz for ^1H and 100 MHz for ^{13}C in $CDCl_3$.
[b] Overlapped signals were reported without designating multiplicity.

2.4. X-ray crystallographic analysis of compound *1*

Crystal data for compound **1**: $C_{31}H_{44}O_4$, M = 480.66, λ = 0.71073 Å at T = 296(2) K. Space group Orthorhombic, P2(1)2(1)2(1), a = 7.7859(10) Å, b = 16.427(2) Å, c = 20.665(3) Å, α = 90.00°, β = 90.00 (10)°, γ = 90.00°, V = 2642.9(6) Å3, Z = 4, Dx = 1.208 mg/m3, F (000) = 1048, Absorption coefficient 0.078 mm − 1, Full-matrix least-squares on F2 Refinement method, 23739 reflections collected to θ_{max} = 28.09°, 6442 independent reflections (R(int) = 0.0447). The final R1 values were 0.0443(I > 2σ (I)). The final wR2values were 0.1015 (I > 2σ (I)). The final R1 values were 0.0659 (all data). The final wR2 values were 0.1131 (all data). The goodness of fit on F2 was 1.028. Absolute structure parameter = −0.3 (11). CCDC-1445997 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre.

2.5. Cell culture

The human hepatocellular carcinoma cell line HepG2 was obtained from State Key Laboratory of Virology, Wuhan university and cultured in Dulbecco's Modified Eagle's medium (DMEM, GIBCO, USA.) supplemented with 10% fetal bovine serum (Biochrom AG, Germany), penicillin (100 U/mL) and streptomycin (100 U/mL) (Beijing Leagene company, China) at 37 °C in a humidified atmosphere with 5% CO_2. Compound **1** was dissolved in DMSO, diluted in DMEM and used to treat HepG2 cells. The final quantity of solvent did not exceed 0.1% of culture media for all experiments.

2.6. Cytotoxicity assay

Cellular viability was measured by MTT assay. HepG2 cells were grown in 96-well plate, after the cells reached confluence, the medium was removed and the cells were incubated with different concentrations of compound **1** (0, 1, 5, 25 and 50 μg/mL) for 24 h, 20 μL MTT (0.5 mg/mL) and 180 μL PBS were added and incubated for 4 h. The medium was discarded and 150 μL DMSO was added to dissolve the MTT-formazan complex. The absorbance was measured at 570 nm in a microplate reader (Molecular Devices, Mountain View, CA, USA). The percentage of cell survival was calculated compared to the untreated cells assumed to be 100% viable.