Fitoterapia 113 (2016) 69–73

Contents lists available at ScienceDirect

Fitoterapia

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

Triterpenoids from the stems of *Tripterygium regelii*

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ARTICLE INFO

Article history:
Received 11 June 2016
Received in revised form 8 July 2016
Accepted 13 July 2016
Available online 15 July 2016

Chemical compounds studied in this article:
Celastrol (PubChem CID: 122,724)
triptocalline A (PubChem CID: 44,559,634)
polpunonic acid (PubChem CID: 343,427)
demethylzeylasteral (PubChem CID: 10,322,911)
wilforol A (PubChem CID: 10,096,097)
regelin D (PubChem CID: 129,520)
triptotriterpenic acid B (PubChem CID: 195,563)
wilforlide A (PubChem CID: 158,477)
wilforlide B (PubChem CID: 174,362)
triptocallic acid A (PubChem CID: 44,575,704)
regelinol (PubChem CID: 163,809)
regelin (PubChem CID: 163,808)
dulcioic acid (PubChem CID: 101,051,955)
tripterygic acid A (PubChem CID: 21,672,627)
demethylregelin (PubChem CID: 44,559,663)

Keywords:
Tripterygium regelii Triterpenoids Cytotoxicity

ABSTRACT

Three new triterpenoids, triregelolides A, B (**1**, **2**), and triregeloic acid (**3**), were isolated from the stems of *Tripterygium regelii* along with twenty known triterpene analogues (**4**–**23**). The structures of three new compounds were identified by analyzing their NMR spectroscopic and HRESIMS data. Compounds **4**, **7**, **8**, **10**, **13**, **14**, **17**, **21**–**23** were isolated from *T. regelii* for the first time. Compounds **3**, **5**, **6**, **8**, **9**, **10**, **14** and **16** showed inhibitory effects on the proliferation of human breast cancer cells MCF-7 by 24.1%, 69.6%, 72.8%, 21.6%, 23.1%, 43.3%, 25.5% and 23.5% ($p < 0.05$) at a concentration of 10 μM, respectively.

1. Introduction

Triterpenoids naturally occurring in the plant kingdom are one of the largest groups of natural products [1]. Until now, it has been reported that some of them exhibited a wide spectrum of biological activities, such as antitumor, antiviral, antidiabetic, anti-inflammatory, antimicrobial, hepatoprotective, cardiotonic, gastroprotective and analgesic effects, *etc.* [2–4]. More importantly, some triterpenoids or their derivatives are promising candidates or lead compounds for the development of future drugs due to their therapeutic potential [3,5,6].

The plants in *Tripterygium* genus of family Celastraceae are well known for a rich source of triterpenoids. Many triterpenes isolated from this genus, showed various promising bioactivities. Celastrol, a quinone methide triterpene isolated from *Tripterygium* plants [7–9], exhibited potent anticancer activity against a variety of human cancer cell lines [4], anti-inflammatory [10] and neuroprotective effects [10,11]. Recently, it has been reported to be used as a powerful anti-obesity agent [12]. In addition, celastrol, pristimerin, tingenone, iguesterin and dihydrocelastrol showed SARS-CoV $3CL^{pro}$ inhibitory activity [13].

Tripterygium regelii, which is distributed throughout northeast China, Korea and Japan [14], has been used as a folk medicine in China for the treatment of rheumatoid arthritis, jaundice, swelling, *etc.* [15]. A few previous studies [9,16–20] have shown that terpenoids were the principal constituents of *T. regelii*. Recently, we have reported the isolation of twelve new dihydro-β-agarofuran sesquiterpenoids from its stems [21]. As a part of our ongoing phytochemical investigation, three new triterpenoids and twenty known analogues were isolated and characterized from the stems of *T. regelii*. Herein, we reported the isolation and characterization of three novel triterpenoids (**1**–**3**) and

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http://dx.doi.org/10.1016/j.fitote.2016.07.006
0367-326X/

twenty known compounds (**4**–**23**), as well as a cytotoxic evaluation of nine selected triterpenes against human breast cancer MCF-7 cells.

2. Experimental

2.1. General experimental procedures

Optical rotations and ultraviolet (UV) spectra were measured using a Rudolph Research Analytical Autopol I automatic polarimeter and a Beckman Coulter DU® 800 spectrophotometer (USA), respectively. HRMS spectra were performed on an Agilent 6230 electrospray ionization (ESI) time–of–flight (TOF) mass spectrometer (USA). Nuclear magnetic resonance (NMR) spectra were acquired with a Bruker Ascend 600 NMR spectrometer in $CDCl_3$ and pyridine-d_5 using tetramethylsilane (TMS) as an internal reference. Chemical shifts were given in δ (ppm), and coupling constants (J) were expressed in hertz (Hz). Preparative HPLC was carried out on a Waters liquid chromatography system equipped with 1525 Binary HPLC Pump and 2489 UV/ Visible detector using a Waters Xbridge Prep C_8 column (10 × 250 mm, 5 μm). Semi-preparative HPLC was conducted on an Agilent 1100 liquid chromatography system coupled with a quaternary pump and a diode array detector (DAD) using a Waters Xbridge Prep C_{18} column (10 × 250 mm, 5 μm). Silica gel (40–60 μm, Grace, USA) and Bondapak Waters ODS (40–63 μm, Waters, USA) were used for column chromatographies. Thin layer chromatography (TLC) used to monitor fractions was performed on precoated silica gel 60 F_{254} plates and TLC silica gel 60 RP-18 F_{254S} plates (200 μm thick, Merck KGaA, Germany). Spots on the TLC were visualized by UV light (254 nm) or heating after spraying with 5% H_2SO_4 in ethanol.

2.2. Plant material

The stems of *T. regelii* were collected in October 2012, from Changbai Mountain in Jilin province, People's Republic of China, and were identified by Dr. Liang Xu (Liaoning University of Traditional Chinese Medicine, Dalian, China). A voucher specimen (No. MUST — TR201210) has been deposited at State Key Laboratory of Quality Research in Chinese Medicine, Macau University of Science and Technology, Macau, China.

2.3. Extraction and isolation

The dried and ground stems of *T. regelii* (8.0 kg) were extracted with methanol (64 L × 3) under ultrasonic assistance at room temperature for 1 h. After evaporation of the solvent under reduced pressure, a dark brown residue was suspended in H_2O, and then sequentially partitioned with *n*-hexane, ethyl acetate (EtOAc) and *n*-butanol. The EtOAc-soluble extract (150.0 g) was subjected to column chromatography over silica gel eluting with PE–acetone (100:0–35:65, *v*/v) to yield thirteen fractions (Fr.1–Fr.13).

The fraction Fr.5 (5.0 g) was separated by a silica gel column using a gradient of n-hexane–EtOAc (100:0–50:50, *v*/v) to produce eight fractions (Fr.5–1 — Fr.5–8). The fraction Fr.5–7 (71.1 mg) was chromatographed over an ODS column with a gradient of CH_3OH — H_2O (60:40–100:0, *v*/v) to afford compound **8** (5.0 mg). The fraction Fr.7 (5.4 g) was subjected to an ODS column using a gradient system of CH_3OH — H_2O (50:50–100:0, *v*/v) to afford nine fractions (Fr.7–1 — Fr.7–9). The fraction Fr.7–5 (120.5 mg) was further separated by preparative HPLC using an isocratic solvent system of CH_3CN — H_2O (70:30, *v*/v) as mobile phase to yield compound **7** (1.5 mg). The fraction Fr.8 (5.0 g) was subjected to an ODS column with a gradient condition of CH_3OH — H_2O (50:50–100:0) to product nine fractions (Fr.8–1–Fr.8–9). The fraction Fr.8–3 (264.9 mg) was purified by semi-preparative HPLC using CH_3CN — H_2O (68:32, *v*/v) as mobile phase to give compound **6** (4.3 mg). The fraction Fr.8–4 (500.9 mg) was isolated by preparative HPLC using CH_3CN — H_2O (70:30, *v*/v) as mobile phase to give compounds **19** (1.5 mg), **20** (2.1 mg) and **12** (2.0 mg). The fraction Fr.8–6 (425.1 mg) was subjected to a silica gel column with a solvent system of PE — EtOAc (90:10–65:35, *v*/v), and purified using an ODS column with a gradient of MeOH — H_2O (70:30–100:0, *v*/v) to give compound **5** (60.0 mg). Compound **21** (28.3 mg) was obtained from fraction Fr.8–8 (80.5 mg) by using a silica gel column eluted sequentially with PE — EtOAc (90:10–60:40) solvent system. The fraction Fr.11 (5.5 g) was fractionated over an ODS column with a gradient system of CH_3OH — H_2O (40:60–90:10, *v*/v) to obtain fifteen fractions (Fr.11–1 — Fr.11–15). Fraction Fr.11–12 (261.0 mg) was separated by semi-preparative HPLC using CH_3CN — H_2O (60:40, *v*/v) as solvent system to furnish compound **11** (1.5 mg). The fraction Fr.11–13 (950.1 mg) was chromatographed on a silica gel column with a gradient of PE — EtOAc (70:30–0:100, *v*/v) to afford ten fractions (Fr.11–13-1 — Fr.11–13-10). The fraction Fr.11–13-1 (150.0 mg) was separated by semi-preparative HPLC using CH_3CN — H_2O (76:24, *v*/v) as solvent system to give compounds **1** (1.0 mg), **2** (0.6 mg) and **10** (30.0 mg). Compound **4** (2.0 mg) was purified by silica gel column using a gradient of $CHCl_3$ — CH_3OH (100:0–95:5, *v*/v) from fraction Fr.11–13-2 (100.0 mg). Then, the fraction Fr.11–13-6 (217.2 mg) was subjected to a silica gel column using a gradient elution of $CHCl_3$ — CH_3OH (100:0–90:10, *v*/v) to yield compound **13** (20.0 mg) and subfractions (Fr.11–13–6-1 — Fr.11–13–6-4). The subfractions Fr.11–13–6-2 and Fr.11–13–6-4 were isolated by semi-preparative HPLC using CH_3CN — H_2O (37:63 and 39:61, *v*/v, respectively) as mobile phase to afford compounds **18** (2.1 mg) and **23** (2.1 mg), respectively. The fraction Fr.11–13-9 (367.0 mg) was purified by semi-preparative HPLC using CH_3CN — H_2O (65:35, *v*/v) as eluting solvent to give compound **22** (2.0 mg). Compounds **14** (6.1 mg) and **17** (2.0 mg) were obtained by preparative HPLC using CH_3CN — H_2O (65:35, *v*/v) as mobile phase from fraction Fr.11–14 (200.0 mg). The fraction 11–15 (367.0 mg) was separated by a silica gel column using a PE-EtOAc (80:20–30:70 *v*/v) gradient solvent system to give compounds **15** (2.6 mg), **16** (9.3 mg), and six subfractions (Fr.11–15-3–Fr.11–15-8). Then, compounds **3** (3.0 mg) and **9** (5.0 mg) were isolated by an ODS columns with a gradient elution of CH_3OH — H_2O (40:60–100:0, *v*/v) from the subfractions Fr.11–15-7 (38.9 mg) and Fr.11–15-8 (40.0 mg), respectively.

2.4. Spectroscopic data

Triregelolide A (**1**): white amorphous powder; $[\alpha]^{21}_{D}$ + 157.8 (*c* 0.50, MeOH); UV (MeOH) λ_{max} (log ε) 236 (3.65), 360 (3.88) nm; ^1H ($CDCl_3$, 600 MHz) and ^{13}C ($CDCl_3$, 150 MHz) NMR data, see Table 1; HRESIMS *m/z* 467.2812 $[M - H]^-$ (calcd for $C_{29}H_{39}O_5$, 467.2803).

Triregelolide B (**2**): white amorphous powder; $[\alpha]^{21}_{D}$ + 119.09 (*c* 0.50, MeOH); UV (MeOH) λ_{max} (log ε) 236 (3.24), 360 (3.42) nm; ^1H ($CDCl_3$, 600 MHz) and ^{13}C ($CDCl_3$, 150 MHz) NMR data, see Table 1; HRESIMS *m/z* 467.2810 $[M - H]^-$ (calcd for $C_{29}H_{39}O_5$, 467.2803).

Triregeloic acid (**3**): white amorphous powder; $[\alpha]^{21}_{D}$ — 12.27 (*c* 0.125, MeOH); UV (MeOH) λ_{max} (log ε) 255 (2.25) nm; ^1H (pyridine-d_5, 600 MHz) and ^{13}C (pyridine-d_5, 150 MHz) NMR data, see Table 1; HRESIMS *m/z* 471.3479 $[M - H]^-$ (calcd for $C_{30}H_{47}O_4$, 471.3480).

2.5. Cytotoxicity on human breast cancer cells MCF-7

Human breast cancer cell lines (MCF-7) were purchased from American Type Culture Collection. The cells were cultured in Dulbecco's modified Eagle medium-F12 medium (DMEM) supplemented with 10% fetal bovine serum (FBS, Invitrogen) and 1% penicillin-streptomycin (Sigma) at 37 °C in humidified atmosphere containing 5% CO_2. Exponentially growing cells were plated in a 96-well microplate at a density of 5×10^3 cells per well in 100 μL of culture medium and were allowed to adhere for 24 h before drug treatment. Then, the cells were treated with either fresh medium containing 0.1% DMSO or fresh medium containing 10 μM of triterpenes or paclitaxel (Taxol®), and incubated for another 24 h in a 5% CO_2 humidified atmosphere at 37 °C. A volume of 10 μL MTT saline solution (5 mg/mL) was added into each well for further 4 h of