Fitoterapia 83 (2012) 1396–1401

Contents lists available at SciVerse ScienceDirect

Fitoterapia

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

Three new triterpenoid saponins from root of *Gardenia jasminoides* Ellis

Jing Wang, Jincai Lu, Chongning Lv, Tanye Xu, Lingyun Jia *

School of Traditional Chinese Materia Medica, Shenyang Pharmaceutical University, Box 79, Shenyang, Liaoning Province, 110016, PR China

ARTICLE INFO

Article history:
Received 3 June 2012
Accepted in revised form 29 June 2012
Available online 14 July 2012

Keywords:
Triterpenoid saponin
Roots of *Gardenia jasminoides*
Gardeniside A–C
Cytotoxic activity

ABSTRACT

Three new compounds (Gardeniside A–C), 11α,12α-epoxy-3β-[(*O*-β-D-glucuronopyranoside-6′-*O*-methly ester)oxy]olean-28,13-olide (1), siaresinolic acid 3-*O*-β-*D*-glucuronopyranoside-6′-*O*-methly ester (2), and 3-*O*-β-*D*- glucuronopyranoside-6′-*O*-methly ester-siaresinolic acid-28-*O*-β-*D*- glucopyranoside (3), with seven known compounds oleanolic acid 3-*O*-β-*D*- glucuronopyranoside-6′-*O*-methly ester (4), oleanolic acid 3-*O*-β-*D*- glucuropyranoside (5), hederagenin 3-*O*-β-*D*- glucuronopyranoside-6′-*O*- methly ester (6), chikusetsusaponin IVa methyl ester (7), chikusetsusaponin (8), chikusetsusaponin IVa butyl ester (9), siaresinolic acid 28-O-β-D-glucopyranosyl ester (10) were isolated from the root of *Gardenia jasminoides* Ellis. Six compounds (1, 4–7, and 9) showed cytotoxic activities against HeLa, A549, MCF-7 and A354-S2 cell lines.

1. Introduction

The genera *Gardenia* (Rubiaceae) has about 450–500 species, is mostly distributed in tropical and subtropical regions, and *Gardenia jasminoides* Ellis is widely distributed in the southeast provinces of China. Its flower, fruit, leaf and root are medicine and effect clearing heat and cooling blood. It is reported that *G. jasminoides* is very rich in flavonols, irridoids and triterpenoids [1]. Its fruit is the traditional Chinese medicine named "Zhizi". Its root is widely used for the treatment of curing icterohepatitis and nephritis edema in south provinces of China [2]. At present, there is very little chemical composition research on its root. We only know from literature that the root contained iridoids, oleanolic acid acetate and stigmasterol [3]. In order to clarify the material basis on pharmacological effects of the root, we study on chemical constituents of it. The present study was directed at investigating further chemical constituents of this herb medicine. The investigation resulted in isolation and structural elucidation of three new oleanane-type triterpene saponins, 1–3, and seven known compounds, 4–10, as well as their cytotoxic activities.

2. Experimental

2.1. General

Melting points were measured on a Yanaco-53 micromelting point apparatus and were uncorrected. Optical rotations were determined on a Perkin-Elmer 241MC automatic digital polarimeter. IR spectra were recorded using a Bruker IFS-55 IR spectrometer with KBr disks. NMR experiments were performed on Bruker 300 and 600 FT-NMR spectrometers. TMS was used as internal standard. Mass spectra were recorded on a Bruker Daltonic micrOTOF mass spectrometer. Silica GF_{254} for TLC, silica gel (200–300 mesh) for column chromatography were obtained from Qingdao Marine Chemical Company, China. RP-ODS (50 μm) was purchased from Fuji Silysia Chemical Ltd. RP-MDS (50 μm) was bought from Maidisheng Co., Ltd. (Beijing, China). The authentic sugar was bought from Aldrich. Reagents used were of analytical grade and purchased from Yuwang Group Co., Ltd. (Shandong, China).

2.2. Plant material

Roots of *G. jasminoides* Ellis were bought from Fujian Province of China, in October 2008 and were identified by Prof. Qishi Sun (School of Traditional Chinese Materia Medica, Shenyang Pharmaceutical University, China). A voucher specimen was

* Corresponding author. Tel.: +86 24 23986500; fax: +86 24 23986500.
E-mail address: jialingyun2003@yahoo.com.cn (L. Jia).

0367-326X/$ – see front matter.
doi:10.1016/j.fitote.2012.07.004

deposited in the Herbarium of Shenyang Pharmaceutical University.

2.3. Extraction and isolation

Air-dried and powered root of *G. jasminoides* (15.0 kg) was refluxed with ethanol (70%, 10 L×3 times, 2 h every time). The combined alcohol extracts were concentrated under reduced pressure to yield a dark brown residue (1440 g), which was suspended in 15 L water and then extracted successively with petroleum ether (15 L), $CHCl_3$ (15 L), EtOAc (15 L), and *n*-BuOH (15 L). The EtOAc-soluble part (66 g) was chromatographed on 400 g silica gel column (Φ 8×120 cm), eluting with CH_2Cl_2 (5 L), CH_2Cl_2/CH_3OH (50:1, 6 L; 30:1, 5 L; 20:1, 6 L; 15:1, 5 L; 10:1, 6 L; 5:1, 4 L; 2:1, 3 L; 1:1, 5 L;) to obtain 214 fractions. The fractions were combined into 16 major fractions (B1–B16) based on TLC behaviors. Fraction B5 was applied to a silica gel column this being eluted with $CHCl_2/CH_3OH$ (25:1) and Sephadex LH-20 columns with $CHCl_3/CH_3OH$ (1:1) to give 4 (60 mg). The fraction B9 (2.8 g) was further subjected to silica gel column (Φ2.5×80 cm) eluting with $CHCl_2/CH_3OH$ (15:1) to obtain 60 subfractions and the sub-fractions were combined into 4 major subfractions (B9a–B9d). A crude compound (about 50 mg) was obtained from B9a, then was isolated in pure Gardeniside A (15 mg) by ODS column, eluting with MeOH/H_2O (8:2). The fraction B10 (20 g) was further subjected to silica gel column (Φ3.5×100 cm) eluting with $CHCl_3/CH_3OH$ (20:1) to obtain 110 subfractions and the sub-fractions were combined into 8 major subfractions (B10a–B10h). A crude compound (about 110 mg) was obtained from B10c, then was isolated in pure Gardeniside B (55 mg) by ODS column, eluting with MeOH/H_2O (8:2). Subfraction B10d was subjected to a silica gel column this being eluted with $CHCl_2/CH_3OH$ (15:1) and Sephadex LH-20 columns with $CHCl_3/CH_3OH$ (1:1) and ODS silica gel with MeOH/H_2O (8:2) to yield 5 (12 mg), 6 (7 mg), and 7 (65 mg). Fraction B12 was subjected to a silica gel column eluting with $CHCl_3/CH_3OH/H_2O$ (7:3:0.5) to give 8 (150 mg). The *n*-BuOH-soluble part (450 g) was chromatographed on 2.0 kg silica gel column (Φ 12×150 cm), eluting with EtOAc (4 L), EtOAc/CH_3OH (10:1, 8 L; 6:1, 12 L; 3:1, 14 L) to obtain 150 fractions. The fractions were combined into 8 major fractions (B1–B8) based on TLC behaviors. The fraction B1 (40 g) was further subjected to silica gel column (Φ5×100 cm) eluting with CH_2Cl_2/CH_3OH (20: 1–5: 1) to obtain 145 sub-fractions and the sub-fractions were combined into 10 major sub-fractions (B1a–B1j). Subfraction B1a was subjected to a silica gel column this being eluted with $CHCl_2/CH_3OH$ (15:1) and ODS silica gel with MeOH/H_2O (8:2) to give 9 (100 mg). A crude compound (about 150 mg) was obtained from B1b by MDS column, eluting with MeOH/H_2O (7:3), then was isolated in pure Gardeniside C (110 mg) by ODS column, eluting with MeOH/H_2O (7:3). Fraction B3 was applied to a silica gel column which was eluted with $CHCl_3/CH_3OH/H_2O$ (7:3:0.5) and ODS silica gel with MeOH/H_2O (8:2) to give 10 (20 mg) (Fig. 1).

2.4. Acid hydrolysis of compounds 1, 2 and 3

An aqueous solution (3 ml) of each sample (8 mg) was refluxed with 2 M HCl (3 ml) for 4 h. After neutralization with $NaHCO_3$ solution, the reaction mixture was extracted with $CHCl_3$ (3×6 mL). The org. phase was evaporated and subjected to PTLC using $CHCl_3-CH_3OH$ (9:1) as eluent to yield the aglycone. The water layer was concentrated and submitted to PTLC (EtOAc/CH_3OH/H_2O = 7:3:0.4) to yield the sugars which were identified by TLC (EtOAc/CH_3OH/H_2O = 7:3:0.4) with authentic samples and $[\alpha]_D$ [4] as following: D-glucose $[\alpha]_D^{25}$ +47.5° (*c* 0.12, H_2O). Spots were detected by spraying with EtOH–H_2SO_4–anisaldehyde (17:2:1) followed by heating.

2.5. Cytotoxicity assay [5]

2.5.1. Chemical reagents

5-Fluorouracil (5-FU) was purchased from Sigma-Aldrich (St. Louis, MO, USA). All the samples were dissolved in dimethyl sulfoxide (Me_2SO) and Me_2SO concentration in all cell cultures was kept below 0.1% which had no detectable effect on cell growth.

2.5.2. Cell culture

The human cervical cancer HeLa cells, human malignant melanoma A375-S2, human breast cancer MCF-7 and human lung carcinoma A549 cells were obtained from American Type Culture Collection (ATCC, Rockville, MD, USA). The four tumor cell lines were cultured in RPMI-1640 medium (GIBCO, Grand Island, NY, USA) containing 10% fetal bovine serum (FBS) (Yuanhengshengma Biological Reagent Institute, Beijing, China) and 0.03% *L*-glutamine (GIBCO) in 5% CO_2 at 37 °C. Cells in the exponential phase of growth were used in the experiments.

2.5.3. Cytotoxicity assay

All the cells were cultured at 5×10^3 cells/well in 96-well plates (NUNC, Roskilde, Denmark). The cells were incubated with or without different concentrations of samples for 48 h. Cell growth was measured with a plate reader by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method as previously described (Tecan, Austria).

2.6. Data of the compound

2.6.1. Gardeniside A

Gardeniside A was obtained as an amorphous solid (MeOH); mp 271–273 °C; $[\alpha]_D^{25}$ −0.025° (*c* 0.10, MeOH); IR (KBr): ν_{max} = 3429, 1775, 1630, 1050, 871 cm^{-1}; UVmax(MeOH): 255 nm; showed a quasi-molecular ion peak at m/z 695.3682 $[M+Cl]^-$ in the HR-FAB-MS, ^1H NMR data see Table 1 and ^{13}C NMR data see Table 2.

2.6.2. Gardeniside B

Gardeniside B was obtained as a white needle (MeOH); mp 290–292 °C; $[\alpha]_D^{25}$ −0.025° (*c* 0.10, MeOH); IR (KBr): ν_{max} = 3442, 1694, 1642, 1092, 595 cm^{-1}; UVmax(MeOH): 249 nm; HRESI-MS: *m/z* 685.3928 $[M+Na]^+$, ^1H NMR data see Table 1 and ^{13}C NMR data see Table 2.

2.6.3. Gardeniside C

Gardeniside **C** was obtained as a white needle (MeOH); mp 215–217 °C; $[\alpha]_D^{25}$ −0.025° (*c* 0.10, MeOH); IR (KBr): ν_{max} = 3422, 1690, 1645, 1070, 640 cm^{-1}; UVmax(MeOH):