Steroids 78 (2013) 1–7

Contents lists available at SciVerse ScienceDirect

Steroids

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

Cytotoxic steroidal saponins from *Ophiopogon japonicus*

Ning Li, Liang Zhang, Ke-Wu Zeng, Yuan Zhou, Jia-Yu Zhang, Yan-Yun Che, Peng-Fei Tu *

State Key Laboratory of Natural and Biomimetic Drugs, School of Pharmaceutical Sciences, Peking University Health Science Center, No. 38 Xueyuan Road, Beijing 100191, People's Republic of China

ARTICLE INFO

Article history:
Received 28 April 2012
Received in revised form 30 September 2012
Accepted 10 October 2012
Available online 2 November 2012

Keywords:
Ophiopogon japonicus
Steroidal saponins
Cytotoxic activity

ABSTRACT

Four new steroidal saponins, named ophiopogonin P–S (**1**–**4**), together with eleven known ones (**5**–**15**) were isolated from the tuberous roots of *Ophiopogon japonicus*. Their structures were elucidated by spectroscopic and chemical analysis. Compounds **2**–**15** were evaluated for their cytotoxic activity against five human tumor cell lines (HepG2, HLE, BEL7402, BEL7403 and Hela). Compounds **2**, **5**, **6**, 8 and **9** were cytotoxic for all cell lines tested. Compounds **7**, 11 and **15** showed selective cytotoxicity against some of the cell lines. The structure–activity relationship of these compounds was discussed.

1. Introduction

The genus *Ophiopogon* (Liliaceae) comprises approximately 50 species and some varieties distributed in East and South Asia. Thirty-three *Ophiopogon* species and four varieties can be found in China [1]. *Ophiopogon japonicus* (L.f.) Ker-Gawl is an evergreen perennial, widely distributed in mainland China, especially in Sichuan and Zhejiang provinces [2]. The tuberous roots of *O. japonicus* (known as Maidong) have been used in traditional Chinese medicine to cure acute and chronic inflammation and cardiovascular diseases for thousands of years [3]. Nowadays it is also clinically used in the alternative treatments for cancer. Previous phytochemical investigations have revealed that *O. japonicus* is rich in steroidal saponins [2–5,7–11]. As part of an ongoing effort to discover bioactive compounds from traditional Chinese medicines, the chemical constituents of the tuberous roots of *O. japonicus* were investigated, which led to the isolation of four new steroidal saponins, ophiopogonin P–S (**1**–**4**), along with eleven known ones (**5**–**15**) (Fig. 1). In this paper, the isolation and structural elucidation of the new compounds were described. Furthermore, compounds **2**–**15** were tested for their cytotoxic activity against five human tumor cell lines (HepG2, HLE, BEL7402, BEL7403 and Hela). Their structure–activity relationship was discussed.

2. Experimental

2.1. General methods

Optical rotations were measured with a Rudolph Research Analytical Autopol IV Automatic Polarimeter. IR spectra were recorded on a Thermo Nicolet Nexus 470 FT-IR spectrophotometer with KBr pellets. 1D and 2D NMR experiments were performed on Varian INOVA 500 and/or 600 spectrometers using pyridine-d_5 as solvent with tetramethylsilane (TMS) as internal reference, and chemical shifts were expressed in δ (ppm). HR-ESI-MS data were obtained on a Shimadzu LCMS-IT-TOF mass spectrometer. Column chromatography was performed over NKA macroporous resin (The Chemical Plant of Nankai University, Tianjin, PR China), silica gel (200–300 mesh, 10–40 μm, Qingdao Marine Chemical Inc., Qingdao, PR China) and octadecylsilanized silica gel (ODS) (40–63 μm, Merck). TLC was performed with glass precoated silica gel GF_{245} plates (Qingdao Marine Chemical Inc., Qingdao, PR China). Preparative high performance liquid chromatography (HPLC) was carried on a Waters 600 system equipped with an Alltech ELSD 2000 detector and an Agilent ZORBAX SB-C_{18} (250 mm × 10 mm i.d., 5 μm) column. GC analysis was performed on an Agilent HP6890 plus gas chromatograph instrument equipped with an H_2 flame ionization detector and an HP-5 (30 m × 0.32 mm × 0.25 μm) column.

2.2. Plant material

The tuberous roots of *O. japonicus* were purchased in February 2009 from Ya'an Sanjiu Pharmaceutical Co. Ltd., Sichuang Province, PR China, and authenticated by one of the authors (Peng-Fei Tu). A

* Corresponding author. Tel./fax: +86 10 82802750.
E-mail address: pengfeitu@vip.163.com (P.-F. Tu).

0039-128X/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.steroids.2012.10.001

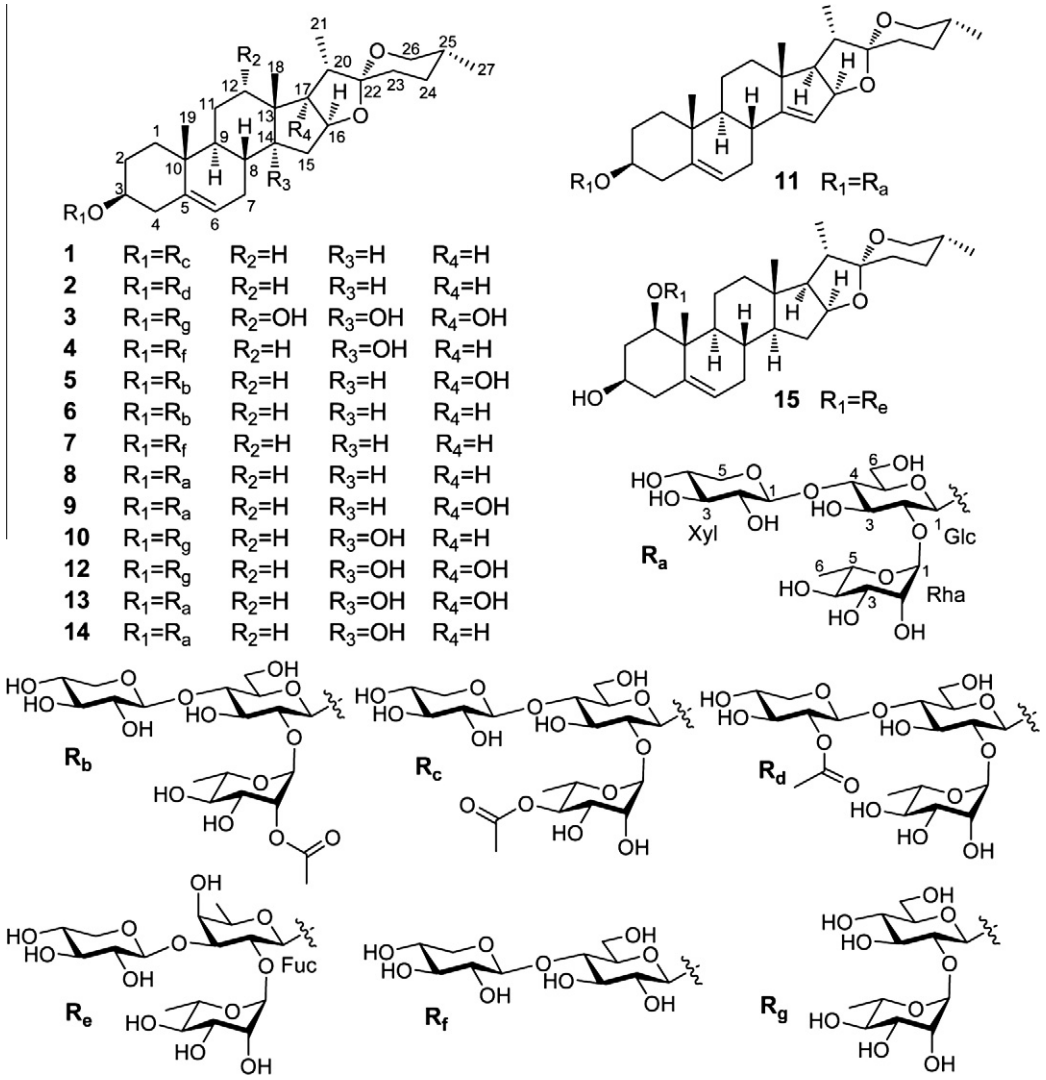

Fig. 1. The structures of compounds **1**–**15** isolated from *O. japonicus*.

voucher specimen (No. OJ200902) was deposited in the Herbarium of Peking University Modern Research Center for Traditional Chinese Medicine, Beijing, PR China.

2.3. Extraction and isolation

The air-dried and powdered tuberous roots of *O. japonicus* (50 kg) were extracted twice (300 L, 2 h and 200 L, 1 h) with 75% EtOH under reflux. The combined extract was concentrated to 60 L under reduced pressure. Then the concentrated extract was subjected to an NKA macroporous resin (50 kg) column, eluting with H_2O (700 L), 40% EtOH (100 L), 80% EtOH (200 L), and 95% EtOH (300 L), respectively. After evaporation under reduced pressure, the 80% EtOH fraction (60 g) was subjected to a silica gel column [$CHCl_3$–MeOH (1:0 → 1:1)] to give eight fractions (A–H). Fraction E (18 g) was chromatographed on silica gel eluting with $CHCl_3$–MeOH–H_2O (7:1:0.1 → 2:1:0.1) to obtain three subfractions (E_1–E_3). Subfraction E_1 (6 g) was further chromatographed over ODS column [MeOH–H_2O (7:3 → 8:2 → 6:1)] and preparative HPLC [MeOH–H_2O (73:27 → 82:18)] to provide **1** (10 mg), **2** (10 mg), **5** (20 mg), **6** (20 mg) and **7** (20 mg). Subfraction E_2 (7 g) was separated by repeated ODS column chromatography [MeOH–H_2O (7:3 → 8:2 → 6:1)], followed by preparative HPLC [MeOH–H_2O (73:27 → 82:18)] to yield **3** (15 mg), **4** (15 mg), **8** (300 mg), **9** (90 mg), **10** (100 mg), **11** (10 mg) and **12** (80 mg). Compounds **13** (90 mg) and **14** (80 mg) were obtained from subfraction E_3 (5 g) by silica gel column chromatography [$CHCl_3$–MeOH–H_2O (7:1:0.1 → 3:1:0.1)] and preparative HPLC [MeOH–H_2O (81:19)]. Fraction F (5 g) was separated by silica gel column [$CHCl_3$–MeOH–H_2O (4:1:0.1 → 2:1:0.1)] to afford **15** (700 mg).

2.3.1. Ophiopogonin P (**1**)

10 mg; amorphous powder; $[\alpha]_D^{15}$ −175.4° (*c* 0.05, MeOH); IR (KBr) ν_{max} (cm^{-1}): 3444, 2934, 1726, 1633, 1456, 1373, 1244, 1158, 1134, 1049, 982, 918, 899, 868 (intensity: 899 > 918); HR-ESI-MS *m/z*: 941.4762 $[M+HCOO]^-$ (calcd. for $C_{47}H_{73}O_{19}$ 941.4752); ^1H NMR (500 MHz) data see Table 1; ^{13}C NMR (125 MHz) data see Table 2.

2.3.2. Ophiopogonin Q (**2**)

10 mg; amorphous powder; $[\alpha]_D^{15}$ -89.2° (*c* 0.05, MeOH); IR (KBr) ν_{max} (cm^{-1}): 3434, 2933, 1736, 1641, 1457, 1376, 1245, 1161, 1129, 1046, 983, 918, 898, 868 (intensity: 898 > 918); HR-ESI-MS *m/z*: 941.4756 $[M+HCOO]^-$ (calcd. for $C_{47}H_{73}O_{19}$ 941.4752); ^1H NMR (500 MHz) data see Table 1; ^{13}C NMR (125 MHz) data see Table 2.