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Triterpene saponins from the roots of *Ilex pubescens*

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ABSTRACT

Five new triterpene saponins, Ilexpublesnins N–R (**1**–**5**), along with seven known analogs were isolated from the root of *Ilex pubescens*. Their structures were elucidated on the basis of extensive spectroscopic analysis, including 1D and 2D NMR experiments. Ilexpublesnin N (**1**) possessed a rare 20-hydroxyursolic acid scaffold from natural resource. These compounds were evaluated *in vitro* for their cytotoxic effects on human cancer cell lines HepG2, HLE, BEL7402, BEL7403, BEL7405, MCF-7, HeLa. Among them, only compounds **5** and **10** showed cytotoxic potentiality against BEl-7403 and HEL cell lines [inhibition (%): 35.38 and 45.12, respectively].

1. Introduction

Ilex pubescens Hook. et Arn., under the Chinese name 'Mao-dong-qing', is an evergreen shrub belonging to Aquifoliaceae. Its roots are commonly used as Chinese herbal medicine for treatment of cardiovascular disease and hypercholestaemia in south China [1]. Previous phytochemical investigation on its roots and leaves led to the isolation of triterpene saponins [2–7], lignan glycosides [8], phenylethanols [9,10], and other minor compounds [11–13]. Pharmacological evaluation proved that *I. pubescens* extracts exhibited a variety of bioactivities, *i.e.*, to enlarge blood vessels, improve microcirculation, ease the blood pressure, inhibit platelet aggregation, prevent thrombosis, and reduce cardiac ischemia, as well as radiosensitization effect and so on [14,15].

As part of systematic research on seeking the bioactive constituents from *Ilex* species [16–24], a phytochemical investigation on the roots of *I. pubescens* was undertaken previously, which led to a revelation of the absolute structure of Ilexpublesnins C–M [25]. As an ongoing program toward the discovery of novel bioactive constituents, five new triterpene saponins, namely Ilexpublesnins N–R (**1**–**5**), and seven known ones (**6**–**12**) were isolated. This paper describes the isolation and structural elucidation of these compounds (Fig. 1). In addition, the cytotoxic evaluation for these compounds against the human cancer cell lines HepG2, HLE, BEL-7402, BEL-7403, BEL-7405, MCF-7, and HeLa is included.

2. Experimental

2.1. General procedures

HRESIMS were measured on a Bruker APEX IV FT_MS (7.0T) spectrometer in positive ion mode. Optical rotations were measured on an Autopol-IV polarimeter (RudoPH Research analytical). IR spectra were obtained using a NEXUS-470 FTIR (Nicolet) spectrometer. 1D and 2D NMR spectra were recorded on Bruker Avance DRX-400 and Vnmrs-500 spectrometers. Semi-preparative HPLC was performed on a Waters model 2487 (Agilent ODS column, 250 × 10 mm i.d., 5 μm) with an Alltech evaporative light

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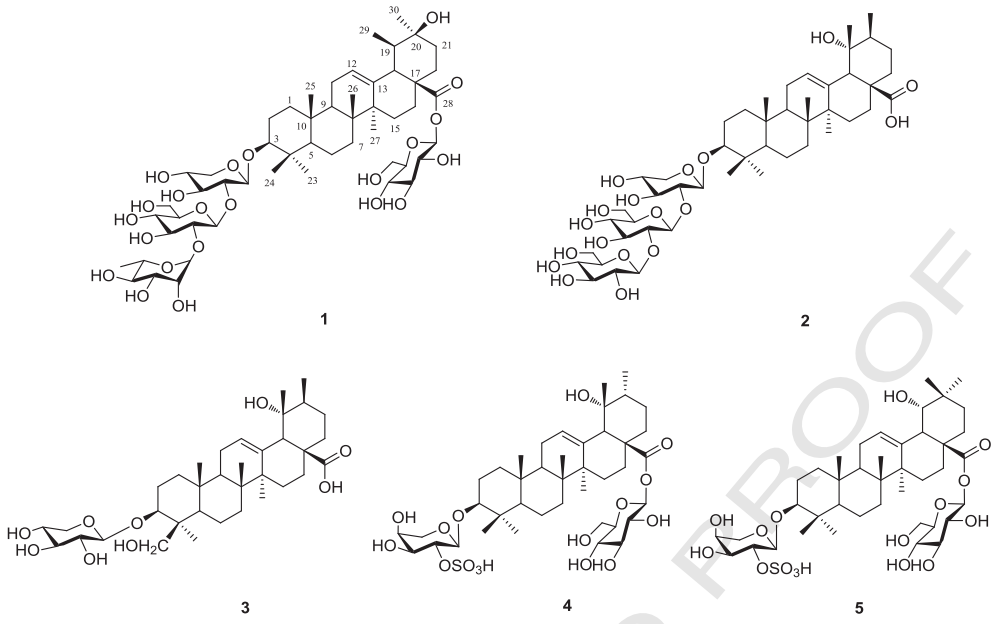

Fig. 1. Structures of compounds **1**–**5**.

scattering detector (ELSD). GC analysis was carried out on an Agilent 6890N gas chromatograph, with an HP-5 capillary column (28 m × 0.32 mm) and an FID detector operated at 260 °C (column temp. 180 °C), 1.0 mL/min N_2 as carrier gas. Macroporous resin (HPD100) was purchased from Hebei Bao-En Biotech Ltd.. Thin-layer chromatography and column chromatography were performed using silica gel (Qingdao Haiyang Chemical Co. Ltd., GF_{254} or 200–300 mesh) and Sephadex LH-20 (Pharmacia Biotech Ltd.) and ODS (Merck & Co., Inc. USA). All the solvents were of analytical grade and were purchased from Beijing Chemical Company Ltd..

2.2. Plant material

The roots of *I. pubescens* were purchased from Guilin San-Jin Pharmaceutical Co. Ltd, and were originally collected from Guangxi province, China. The plants were identified by Prof. Peng-Fei Tu (one of the authors in this paper). A voucher specimen (No. 091005) is deposited in the Herbarium of Modern Research Center for Traditional Chinese Medicine (TCM), Peking University, Beijing.

2.3. Extraction and isolation

Dry crude materials (18 kg) were grinded and extracted with 70% EtOH at the temperature of 70 °C. After the retrieval of the ethanol, the residue suspended in water (50 L) was subjected to column chromatography (CC) on macroporous resin with an EtOH–H_2O gradient (30:70, 70:30, 90:10) to yield three fractions (Frs. 1–3). Fr. 2 (160 g) was chromatographed on silica gel (2.5 kg, 100 × 10 cm) with a gradient of $CHCl_3$–MeOH (40:1–1:1) elution to yield thirteen fractions (Frs. A–M).

Fr. A (11 g) was recrystallized with mixed solution of MeOH–$CHCl_3$ (1:1) yields compound **6** (8 g). Fr. C (1.5 g) was subjected to CC on silica gel (50 g, 15 × 2 cm) eluted with $CHCl_3$–MeOH (20:1) yields compound **10** (500 mg). Fr. G (25 g) was subjected to CC on silica gel (500 g, 50 × 4 cm) eluted with $CHCl_3$–MeOH (10:1, 5:1, 2:1) yields seven fractions (Fr. G-1–Fr. G-7). Fr. G-4 (1.2 g) was chromatographed on Sephadex LH-20 (50 × 3 cm) in MeOH afforded three fractions (Fr. G-4-1–Fr. G-4-3). Fr. G-4-3 (660 mg) was separated by semi-preparative HPLC (MeOH/H_2O 80:20, 2.0 mL/min) yields compounds **7** (5 mg), **8** (180 mg) and **11** (15 mg). Fr. I (3.6 g) was subjected to CC on silica gel (300 g, 50 × 3 cm) eluted with $CHCl_3$–MeOH (5:1, 2:1) yields two fractions (Fr. I-1–Fr. I-2). Fr. I-1 (2.5 g) was chromatographed on Sephadex LH-20 (50 × 3 cm) in MeOH, then subjected to RP-C_{18} CC (40 × 3 cm) eluted with MeOH–H_2O (70:30) and further purified by semi-preparative HPLC (MeOH/H_2O 56:44, 2.0 mL/min) yields compound **2** (10 mg). Fr. I-2 (400 mg) was chromatographed on Sephadex LH-20 (100 × 2 cm) in MeOH and then separated by semi-preparative HPLC (MeOH/H_2O 65:35, 2.0 mL/min) yields compound **3** (15 mg). Fr. J (18 g) was subjected to CC on silica gel (400 g, 50 × 4 cm) eluted with $CHCl_3$–MeOH–H_2O (60:30:10, low layer) yields three fractions (Fr. J-1–Fr. J-3). Fr. J-3 (2.8 g) was chromatographed on Sephadex LH-20 (50 × 3 cm) in MeOH afforded three fractions (Fr. J-3-1–Fr. J-3-3). Fr. J-3-3 (1.7 g) was subjected to RP-C_{18} CC (40 × 3 cm) eluted with MeOH–H_2O (70:30) and further purified by semi-preparative HPLC (MeOH/H_2O 41:59, 2.0 mL/min) yields compounds **4** (4 mg), **5** (5 mg) and **9** (60 mg). Fr. L (9 g) was chromatographed on RP-C_{18} CC (50 × 4 cm) using a gradient of MeOH–H_2O (20:80, 40:60, 60:40, 80:20) afforded five fractions (Fr. L-1–Fr. L-4). Compound **1** (10 mg) was obtained from Fr. L-3 (650 mg) by semi-preparative HPLC (MeOH/H_2O 53:47). Compound **12** (17 mg) was obtained from Fr. L-4 (1.6 g) by Sephadex LH-20 (50 × 3 cm) and then semi-preparative HPLC (MeOH/H_2O 65:35).

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