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New triterpenoids with diverse side-chains from the barks of *Melia Toosendan*

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ABSTRACT

Nine new euphane- and apotirucallane-type triterpenoids (Toosendines A–I; **1–9**), along with three known tirucallane-type compounds were isolated from the barks of *Melia toosendan*. Their structures were elucidated based on detailed spectroscopic analyses (HRESIMS, 1D/2D-NMR) and circular dichroism spectra. Results of bioactivities screening exhibited that compounds **1**, **4** and **5** showed remarkable NO inhibitory activities in LPS-activated RAW 264.7 macrophages, meanwhile, compounds **1** and **4** showed moderate cytotoxicities against U2OS human cancer cell line.

1. Introduction

Melia toosendan Sieb. et Zucc. (Meliaceae) is distributed in the southwest region of China (mainly in Sichuan and Yunnan Provinces). The fruits and barks of this plant are recorded in the "Chinese Pharmacopoeia" and have been used in traditional Chinese medicine for acesodyne and anthelmintic [1,2]. Euphane-, tirucallane-type triterpenoids and limonoids are the main constituents of its fruits and barks [3–7], which have been reported to possess a variety of biological properties such as cytotoxic, antifeedant, antibacterial, anti-inflammatory and analgesic activities [5,8–11]. Previous phytochemical and pharmacological studies mainly focused on *M. toosendan* fruits. To our knowledge, there have been few phytochemical investigations on its barks [3]. In this paper, we reported the isolation and structural elucidation of nine new euphane- and apotirucallane-type triterpenoids (**1–9**) (Fig. 1), together with three known tirucallane-type compounds from the barks of *M. toosendan*. Their structures were identified by spectroscopic methods including HRESIMS, 1D and 2D-NMR, and circular dichroism spectra. Moreover, all new compounds were evaluated in vitro for their potential biological activities, such as the anti-inflammatory and cytotoxic activities. Herein, we described the isolation, identification, and bioactivity screening of these new compounds.

2. Experimental

2.1. General experimental procedures

Optical rotations were measured on a JASCO P-1020 polarimeter (Jasco, Tokyo, Japan). ECD spectra were obtained on a JASCO J-810 spectropolarimeter (Jasco, Tokyo, Japan). UV and IR were recorded on a Shimadzu UV-2450 spectrophotometer (Shimadzu, Tokyo, Japan) and Bruker Tensor 27 spectrometer (Bruker, Karlsruhe, Germany), respectively. 1D and 2D NMR spectra were conducted on a Bruker AVIII-500 and AVIII-600 NMR instrument at 500 and 600 MHz (^{1}H), 125 MHz (^{13}C) and 150 MHz (^{13}C) in $CDCl_3$. HRESI mass spectra were acquired on an Agilent 6520B UPLC-Q-TOF mass spectrometer (Agilent Technologies, Santa Clara, CA, USA). Silica gel (Qingdao Haiyang Chemical Co., Ltd.), MCI gel (Mitsubishi Chemical Corp., Tokyo, Japan), MPLC (Beijing H&E Co., Ltd., Beijing, China), and RP-C_{18} (40–63 μm, FuJi) were used for column chromatography. Preparative

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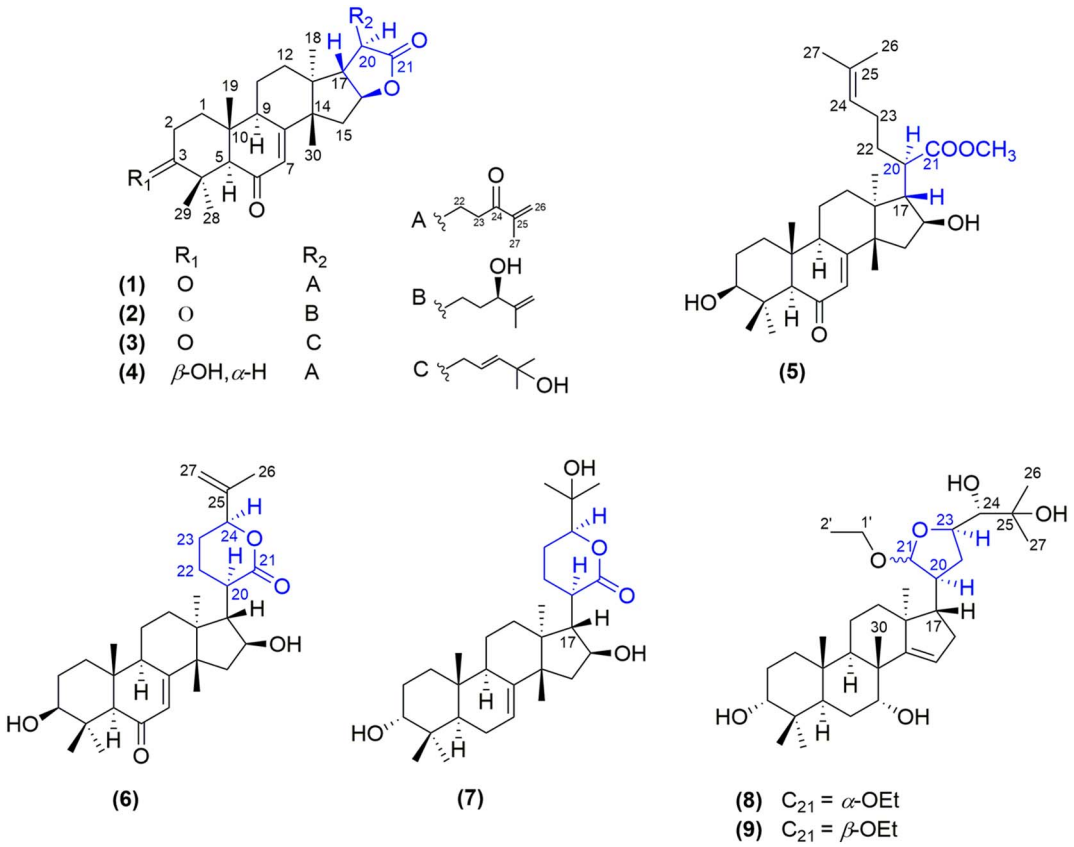

Fig. 1. Structure of compounds **1–9**.

HPLC was carried out on a Shimadzu LC-6AD instrument with a SPD-10A detector using a shim-pack reversed-phase C_{18} column (20 × 200 mm, 10 μm). Analytical HPLC was performed on an Agilent 1260 Series instrument with a DAD detector using a shim-pack VP-ODS column (250 × 4.6 mm, 5 μm). All solvents used were analytical grade (Jiangsu Hanbang Science and Technology. Co., Ltd.).

2.2. Plant material

The air-dried root and stem barks of *Melia toosendan* Sieb. et Zucc. (Meliaceae) were collected from Jiangyou, Sichuan province, People's Republic of China, in July 2015, and authenticated by Professor Mian Zhang, the Research Department of Pharmacognosy, China Pharmaceutical University. A voucher specimen (No. 2015-MMTA) has been deposited in the Department of Natural Medicinal Chemistry, China Pharmaceutical University.

2.3. Extraction and isolation

The air-dried barks (4.7 kg) were extracted by refluxing with 95% ethanol for three times. After removal of solvent under reduced pressure, the crude extract was suspended in H_2O and successively partitioned with petroleum ether and CH_2Cl_2. The CH_2Cl_2 fraction (105 g) was subjected to a silica gel column eluted with CH_2Cl_2-MeOH in a gradient (100:1 to 0:100, v/v) to afford seven fractions (Frs. A–G) after TLC and HPLC analysis. Fr. C (7.3 g) was chromatographed on a silica gel column eluted with a gradient of petroleum ether/acetone (10:1 to 2:1, v/v) to give three sub-fractions (Frs. C1-3). Fr. C2 (2.1 g) was separated on an ODS column using a gradient of MeOH/H_2O (20:80 to 100:0), to afford five sub-fractions (Frs. C2a–e). Fraction C2b (30.0 mg) was repeatedly purified using preparative HPLC on a C_{18} column (MeOH/H_2O, 30:70, 10 mL/min) to yield Indicalilacol B (2.7 mg). Fraction C2c (60.0 mg) was further purified by preparative HPLC with MeCN/H_2O (20:80,10 mL/min) as mobile phase to yield pure Mesendanin U (5.5 mg). Fr. D (6.2 g) was subjected to an MCI gel (MeOH-H_2O, 30:70 to 0:100, v/v) to yield five subfractions (Frs. D1-5). Fr. D2 (2.3 g) was run on an ODS column using a step gradient of MeOH-H_2O (40:60 to 100:0, v/v), to afford five subfractions (Frs. D2a–e). Fr. D2b (1.1 g) was separated by semi-preparative HPLC using MeOH-H_2O (40:60 v/v, 10 mL/min) as the mobile phase to give compounds **2** (1.0 mg) and **9** (2.2 mg). Fr. D2c (500 mg) was separated by semi-preparative HPLC, with 45% methanol in water, to yield compound **5** (1.1 mg) and a mixture of compounds **6** and **7**. This mixture was purified using CH_3CN-H_2O (35:65, v/v, 10 mL/min) as the mobile phase to give compounds **6** (0.8 mg) and **7** (2.6 mg). Separation of fraction E (9.8 g) via silica gel and Sephadex LH-20 gel columns successively produced six subfractions (Frs. E1-6). Fr. E3 (1.8 g) was subjected to an RP-C_{18} column using a gradient of MeOH–H_2O (50:50 to 100:0, v/v), to give three subfractions (Frs. E3a–c). Fr. E3b (80 mg) was puried by preparative HPLC (MeOH/H_2O 60:40, v/v) to yield compound **1** (1.4 mg). Using the same procedures, Frs. E3c (70 mg) yielded compounds **8** (6.7 mg), **3** (2.8 mg), Mesendanin S (2.0 mg) and **4** (0.8 mg).

2.3.1. Toosendine A (1)

White amorphous powder; $[\alpha]_D^{25}$ − 13.5 (*c* 0.14, MeOH); UV (MeOH) λ_{max} (log ε) 220 (3.67), 243 (3.66) nm; ECD (MeOH, $\Delta\varepsilon$) 209 (−14.460), 247 (+6.922) nm; IR (KBr) ν_{max} 3447, 2928, 1779, 1708, 1663, 1384 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; HRESIMS *m/z* 479.2808 $[M - H]^-$ (calcd. for $C_{30}H_{39}O_5$, 479.2803).

2.3.2. Toosendine B (2)

White amorphous powder; $[\alpha]_D^{25}$ − 8.7 (*c* 0.14, MeOH); UV (MeOH) λ_{max} (log ε) 245 (3.55) nm; ECD (MeOH, $\Delta\varepsilon$) 208 (−18.439), 244 (+12.633) nm; IR (KBr) ν_{max} 3447, 2926, 1780, 1713, 1665, 1386 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; HRESIMS *m/z* 481.2963