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Bioactive sesquiterpene polyol esters from the leaves of *Tripterygium wilfordii*

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ABSTRACT

Tripterygium wilfordii, a member of Celastraceae family, has been used as a traditional plant insecticide and a medicinal plant. Phytochemical investigation of the leaves of *T. wilfordii* has resulted in the isolation of eight sesquiterpene polyol esters triptersinines M–T (**1**–**8**) and one sesquiterpene pyridine alkaloid (**9**). The structures of the compounds were elucidated on the basis of spectroscopic data analyses, including UV, IR, MS, and NMR experiments. The inhibitory effects on nitric oxide production in LPS-induced macrophages of **1**–**9** were also evaluated.

1. Introduction

Tripterygium wilfordii Hook. F. is a well-known medicinal plant of Celastraceae family, distributed in the southern part of China. In the last forty years, the extract of the roots of *T. wilfordii* has been employed in the clinical treatment of rheumatoid arthritis, skin disorders, male-fertility control, and other inflammatory and autoimmune diseases [1–3]. It was reported that the extract of the leaves also exhibited bioactivities, such as anti-inflammatory [4,5]. However, few chemical investigations had been conducted on the leaves to expound the bioactive substance. In order to promote the utilization of *T. wilfordii*, we performed extensive chemical and bioactive investigations on the ethanolic extract of the leaves.

Our previous chemical studies on the EtOAc extract of *T. wilfordii* have obtained twelve dihydroagarofuran sesquiterpene polyol esters, triptersinines A–L, and eight sesquiterpene pyridine alkaloids [6]. In a continuing phytochemical study of the same extract, eight new dihydroagarofuran sesquiterpene polyol esters, triptersinines M–T (**1**–**8**), and one known sesquiterpene pyridine alkaloid were obtained (Fig. 1). We described herein the isolation and structural elucidation of the new compounds. The inhibitory effects on nitric oxide production in LPS-induced macrophages of **1**–**9** were also evaluated.

2. Experimental procedure

2.1. General

Optical rotations were measured on a JASCO P2000 automatic digital polarimeter. UV spectra were recorded on a JASCO V-650 spectrophotometer, and IR spectra were recorded on a Nicolet 5700 spectrometer using a FT-IR microscope transmission method. NMR spectra were acquired with

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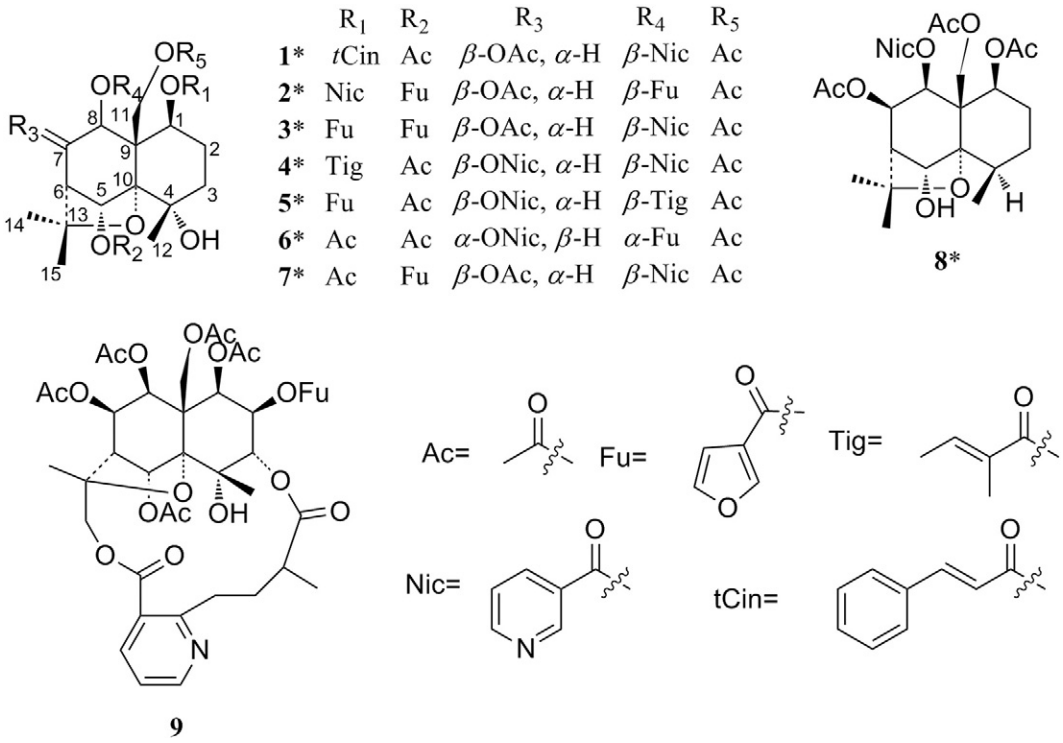

Fig. 1. The structures of **1–9** isolated from *T. wilfordii*.

VNS-600 and Mercury-400 spectrometers. HRESIMS spectra were collected on an Agilent 1100 series LC/MSD ion trap mass spectrometer. Preparative HPLC was conducted using a Shimadzu LC-6AD instrument with a SPD-20A detector and a YMC-Pack ODS-A column (250 × 20 mm, 5 μm). Column chromatography (CC) was performed with silica gel (200–300 mesh, Qingdao Marine Chemical Inc., Qingdao, China) and ODS (50 μm, YMC, Japan). TLC was carried out on glass precoated silica gel GF_{254} plates. Spots were visualized under UV lighter or by spraying with 10% sulfuric acid in EtOH followed by heating.

2.2. Plant materials

The leaves of *T. wilfordii* were collected in Taining, Fujian, China, in September 2009 and identified by Professor Lin Ma from the Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College. A voucher specimen (No. 20090034) is deposited at the herbarium of the Institute of Material Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, China.

2.3. Extraction and isolation

Air-dried leaves of *T. wilfordii* (50 kg) were extracted with 80% ethanol (400 L × 2 h × 3). After evaporation of ethanol in vacuo, the aqueous residue was diluted with water, and then partitioned with EtOAc (30 L × 3). The EtOAc extract (4000 g) was subjected to passage over polyamide by elution with water and 30%, 60%, and 95% EtOH–water in sequence to give fractions A_1 (478 g), A_2 (743 g), A_3 (828 g), and A_4 (1000 g). Fraction A_1 (478 g) was subjected to column chromatography on silica gel with $CHCl_3$–MeOH (1:0–10:1) to afford 10 fractions (B_1–B_{10}). Fraction B_7 (52 g) was separated by silica gel column (200–300 mesh) eluted with $CHCl_3$–MeOH (80:1–10:1) to afford 43 fractions (F_1–F_{43}). Subfraction F_{12} (2.8 g) was passed over a RP-18 column with MeOH–water (20–80%) and finally purified by preparative HPLC (detected at 210 nm, 8 mL/min) to give **1** (3.6 mg), **2** (5.0 mg), **3** (3.8 mg), **4** (2.1 mg), **5** (6.0 mg), **6** (2.5 mg), **7** (3.3 mg), **8** (4.1 mg), and **9** (8.4 mg).

2.3.1. Triptersinine M (1)

White amorphous powder; $[\alpha]_D^{25}$ −18.7 (*c* 0.12 CH_2Cl_2); UV (CH_2Cl_2) λ_{max} (log ε) 228 (2.61), 283 (2.77) nm; IR (microscope) ν_{max} 3413, 2925, 1733, 1370, 1285, 1236 cm^{-1}; ^1H NMR ($CDCl_3$, 600 MHz), see Table 1 and δ_H 9.22 (1H, brs), 8.45 (1H, d, J = 1.8 Hz), 8.33 (1H, d, J = 7.8 Hz), 7.17 (1H, m) (8-Nic), 7.33 (1H, t, J = 7.2 Hz), 7.27 (1H, d, J = 7.2 Hz), 7.03 (1H, d, J = 7.2 Hz), 7.22 (1H, d, J = 15.6 Hz), 5.80 (1H, d, J = 15.6 Hz) (1-*trans*-Cin), 2.07, 2.15, 2.34 (each 3H, s) (5, 7, 11-Ac); ^{13}C NMR ($CDCl_3$, 150 MHz), see Table 2 and δ_C 165.3, 145.2, 133.2, 130.7, 128.9, 127.8, 117.0 (1-*trans*-Cin), 163.3, 152.5, 149.8, 137.6, 126.0, 124.6 (8-Nic), 169.8, 170.1, 170.4, 20.9, 21.5, 21.6 (5, 7, 11-Ac); HRESIMS *m/z* 680.2702 (calcd for $C_{36}H_{42}NO_{12}$, 680.2702).

2.3.2. Triptersinine N (2)

White amorphous powder; $[\alpha]_D^{25}$ −33.3 (*c* 0.19 CH_2Cl_2); UV (CH_2Cl_2) λ_{max} (log ε) 230 (4.12) nm; IR (microscope) ν_{max} 3297, 2927, 1739, 1308, 1236 cm^{-1}; ^1H NMR (CD_3OD,