Fitoterapia 83 (2012) 343–347

Contents lists available at SciVerse ScienceDirect

Fitoterapia

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

Four new neolignans from the leaves of *Tripterygium wilfordii*

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ARTICLE INFO

Article history:
Received 20 July 2011
Received in revised form 18 November 2011
Accepted 25 November 2011
Available online 4 December 2011

Keywords:
Tripterygium wilfordii
Neolignan
Anti-inflammatory activity

ABSTRACT

Four new neolignans, wilfordiols A–D (1–4), together with five known compounds (5–9), were isolated from an aqueous extract of the dried leaves of *Tripterygium wilfordii*. Their structures were determined by spectroscopic methods, including 1D and 2D NMR, HRESIMS, and CD experiments. The anti-inflammation activities of compounds 1–9 were evaluated by the inhibitory effect on NO production, *in vitro*.

1. Introduction

Tripterygium wilfordii Hook f. (Celastraceae) is widespread in southern China. The root of *T. wilfordii* has been used as a traditional medicine for the treatment of rheumatoid arthritis, skin disorders, male-fertility control, and other inflammatory autoimmune diseases [1]. Previous investigations revealed that the root of *T. wilfordii* mainly contained triterpenoids, diterpenoids, and sesquiterpene alkaloids [2]. Some of them exhibited interesting pharmacological activities including anti-inflammatory, antitumor, antifertility and insecticidal [3]. However, few studies have been performed on the chemical constituents and bioactivities of the leaves of *T. wilfordii*, compared with its roots. As a part of our search for bioactive constituents from *T. wilfordii*, the aqueous extract of the leaves of *T. wilfordii* has been investigated. In this paper, we described herein the isolation, structure elucidation and biological assays of four new neolignans, named as wilfordiols A–D (1–4) (Fig. 1).

2. Experimental procedure

2.1. General

CD spectra were measured on a Jasco J-815 CD spectrophotometer. UV spectra were recorded in MeOH on a Jasco V650 spectrophotometer. IR spectra were measured on an IMPACT-400 FT-IR spectrophotometer (Thermo Nicolet Corporation, America). NMR spectra were recorded on an INOVA-500 spectrometer with tetramethylsilane (TMS) as internal standard. ESI-MS experiments were performed on an Agilent 1100 Series LC/MSD Trap-SL mass spectrometer. HRESIMS data were measured by an Agilent Technologies 6520 Accurate Mass Q-Tof LC/MS spectrometer. TLC was performed on precoated silica gel GF_{254} plates (Qingdao Haiyang Chemical Co. Ltd.). Column chromatography was carried out with silica gel 100–200 and 200–300 (Qingdao Haiyang Chemical Co. Ltd.) and Sephadex LH-20 (Pharmacia, Sweden).

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doi:10.1016/j.fitote.2011.11.013

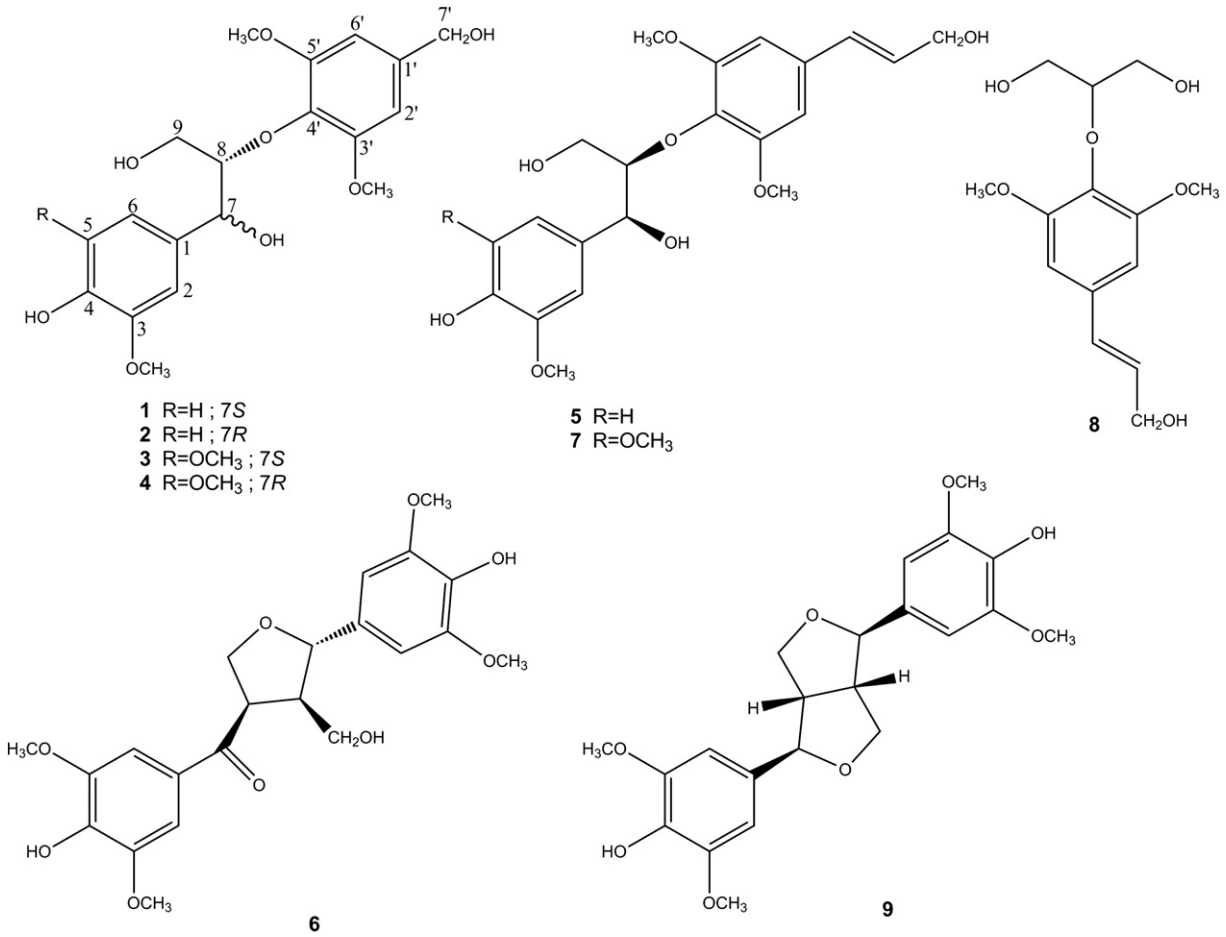

Fig. 1. Structures of compounds 1–9.

2.2. Plant material

The leaves of *Tripterygium wilfordii* were collected in Taining County, Fujian Province (2009), and identified by Prof. Lin Ma. A voucher specimen (Herbarium No. 20090034) was deposited at Institute of Materia Medica, Chinese Academy of Medical Sciences.

2.3. Extraction and isolation

Air-dried leaves of *Tripterygium wilfordii* Hook f. (8 kg) were powdered and refluxed by water for three times, then defatted with petroleum ether, and extracted with chloroform. The extraction was chromatographed on a silica gel, eluted with cyclohexane–ethyl acetate (50:1 to 5:1, v/v) to afford five fractions (A–E).

The Fr. B (55 g) was chromatographed on a silica gel column using a gradient of $CHCl_3$–MeOH (50:1 to 5:1, v/v) as eluent and gave 122 fractions (1–122). Fr. 21–26 (17.54 g) was further chromatographed again on a silica gel column eluting with a gradient of $CHCl_3$–MeOH (100:1 to 50:1, v/v) and yielded 9 fractions (21-1–21-9). Fraction 21–8 (2.6 g) was further isolated by Sephadex LH-20 (MeOH). Then fraction 21-8-6 (662 mg) was separated by HPLC (35% MeOH–H_2O for the first time, and then 15%–18% CH_3CN–H_2O) to obtained compounds 1 (10 mg), 2 (8 mg), 3 (7 mg), 4 (4 mg), 5 (37 mg), 6 (45 mg), 7 (4 mg), 8 (6 mg) and 9 (20 mg).

Compound 1: light yellow oil; $[\alpha]_D^{20}$ −2.9 (MeOH, *c* 0.10); UV (MeOH) λ_{max} (log ε) 204 (4.64), 233 (3.98), 279 (3.50) nm; CD (MeOH) 217 ($\Delta\varepsilon$ +1.08), 238 ($\Delta\varepsilon$ +0.38), 269 ($\Delta\varepsilon$ +0.29), 309 ($\Delta\varepsilon$ +0.27) nm; IR (KBr) ν_{max} 3409, 1594, 1516 and 825 cm^{-1}; ESI-MS: *m/z* 403 $[M+Na]^+$, HRESIMS: *m/z* 403.1361 $[M+Na]^+$ (calc. for $C_{19}H_{24}NaO_8$ 403.1363). ^1H NMR (500 MHz, CD_3OD) and ^{13}C NMR (125 MHz, CD_3OD); see Tables 1 and 2.

Compound 2: light yellow oil; $[\alpha]_D^{20}$ +1.9 (MeOH, *c* 0.10); UV (MeOH) λ_{max} (log ε) 204 (4.54), 230 (3.91), 279 (3.34) nm; CD (MeOH) 222 ($\Delta\varepsilon$ +0.73), 242 ($\Delta\varepsilon$ +0.26), 264 ($\Delta\varepsilon$ +0.19), 306 ($\Delta\varepsilon$ +0.17) nm; IR (KBr) ν_{max} 3366, 1594, 1515 and 826 cm^{-1}; ESI-MS: *m/z* 403 $[M+Na]^+$, HRESIMS: *m/z* 403.1368 $[M+Na]^+$ (calc. for $C_{19}H_{24}NaO_8$ 403.1363). ^1H NMR (500 MHz, CD_3OD) and ^{13}C NMR (125 MHz, CD_3OD); see Tables 1 and 2.

Compound 3: light yellow oil; $[\alpha]_D^{20}$ +15.6 (MeOH, *c* 0.10); UV (MeOH) λ_{max} (log ε) 207 (4.42), 229 (3.87), 242 (3.65), 273 (3.13) nm; CD (MeOH) 218 ($\Delta\varepsilon$ +2.10), 238 ($\Delta\varepsilon$ +0.47), 261 ($\Delta\varepsilon$ +0.71), 307 ($\Delta\varepsilon$ +0.35) nm; IR (KBr) ν_{max} 3403, 1594, 1511 and 836 cm^{-1}; ESI-MS: *m/z* 433 $[M+Na]^+$, HRESIMS: