Fitoterapia 98 (2014) 192–198

Contents lists available at ScienceDirect

Fitoterapia

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

Cytotoxic and anti-inflammatory *ent*-kaurane diterpenoids from *Isodon wikstroemioides*

Hai-Yan Wu [a,b], Wei-Guang Wang [a], Hua-Yi Jiang [a,b], Xue Du [a], Xiao-Nian Li [a], Jian-Xin Pu [a,*], Han-Dong Sun [a,*]

[a] State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, PR China
[b] University of Chinese Academy of Sciences, Beijing 100049, PR China

ARTICLE INFO

Article history:
Received 27 June 2014
Accepted in revised form 4 August 2014
Available online 12 August 2014

Keywords:
Lamiaceae
Isodon wikstroemioides
ent-Kaurane diterpenoid
Cytotoxicity

ABSTRACT

Seven new *ent*-kaurane diterpenoids, isowikstroemins A–G (**1**–**7**), were isolated from EtOAc extracts of the aerial parts of *Isodon wikstroemioides*. Their structures were elucidated by extensive spectroscopic analysis. The isolates were evaluated for their cytotoxicity against five human tumor cell lines, and compounds **1–4** exhibited significant activity with IC_{50} values ranging from 0.9 to 7.0 μM. In addition, compounds **1**, **2**, **3**, **4**, and **7** exhibited inhibitory activity against nitric oxide (NO) production in LPS-activated RAW264.7 macrophages.

1. Introduction

The genus *Isodon* (Lamiaceae) includes about 150 species and is distributed all over the world [1,2]. The use of *Isodon* species in Chinese folk medicines has a long tradition [3]. Over the past 30 years, phytochemical investigation of this genus has isolated and elucidated a large number of diterpenoids including *ent*-kaurane-type, abietane-type, isopimarane-type, gibberellane-type, labdane-type, and clerodane-type [4]. Many obtained diterpenoids exhibited interesting biological properties, such as antitumor, anti-inflammatory, and antibacterial activities [5–7].

Isodon wikstroemioides (Hand.-Mazz.) H. Hara, a perennial herb, is primarily distributed in the northwestern regions of Yunnan Province and the western district of Sichuan Province in the People's Republic of China [8]. Previous phytochemical investigations of this plant have resulted in the isolation of 44 *ent*-kauranoids [9,10]. In our continuing work, seven new 7,20-epoxy-*ent*-kauranoids, isowikstroemins A–G (**1**–**7**), have been isolated from *I. wikstroemioides*. All of the isolates were evaluated for their cytotoxicity against the HL-60, SMMC-7721, A-549, MCF-7, and SW-480 human tumor cell lines, and tested for their ability to inhibit LPS-induced NO production in RAW264.7 macrophages. This paper reports the isolation, structure elucidation, and biological activities of these compounds.

2. Experimental

2.1. General experimental procedures

Melting points of the isolates were obtained on an XRC-1 apparatus and were uncorrected. Optical rotations were measured in MeOH with Horiba SEPA-300 and JASCO P-1020 polarimeters. UV spectra were recorded using a Shimadzu UV-2401A spectrophotometer. IR spectra were obtained on a Tenor 27 FT-IR spectrometer using KBr pellets. NMR spectra were

* Corresponding authors at: State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, Yunnan, PR China. Tel.: +86 871 65223251.
E-mail addresses: pujianxin@mail.kib.ac.cn (J.-X. Pu), hdsun@mail.kib.ac.cn (H.-D. Sun).

http://dx.doi.org/10.1016/j.fitote.2014.08.012
0367-326X/

recorded on Bruker AM-400, DRX-500, and DRX-600 spectrometers using TMS as the internal standard. All chemical shifts (δ) are expressed in ppm relative to the solvent signals. HREIMS was performed on an API QSTAR TOF spectrometer. X-ray crystallographic data were collected on a Bruker APEX DUO diffractometer equipped with an APEX II CCD using Cu Kα radiation. Column chromatography (CC) was performed with silica gel (100–200 mesh and 200–300 mesh; Qingdao Marine Chemical, Inc., Qingdao, People's Republic of China), LiChroprep RP-18 gel (40–63 μm, Merck, Darmstadt, Germany), and MCI gel (75–150 μm, Mitsubishi Chemical Corporation, Tokyo, Japan). Thin-layer chromatography was performed on precoated TLC plates (200–250 μm thickness, silica gel 60 F_{254}, Qingdao Marine Chemical, Inc.), and spots were visualized by UV light (254 nm) or by spraying heated silica gel plates with 10% H_2SO_4 in EtOH. Preparative HPLC was performed on a Shimadzu LC-8A preparative liquid chromatograph with a Shimadzu PRC-ODS (K) column. Semi-preparative HPLC was performed on an Agilent 1100 liquid chromatograph with a ZORBAX SB-C_{18} (9.4 mm × 25 cm) column.

2.2. Plant material

The aerial parts of *I. wikstroemioides* were collected in the Ranwu District of Sichuan Province, People's Republic of China, in July 2011 and identified by Prof. Xi-Wen Li at the Kunming Institute of Botany. A voucher specimen (KIB 20110939) has been deposited in the Herbarium of the Kunming Institute of Botany, Chinese Academy of Sciences.

2.3. Extraction and isolation

The dried and powdered aerial parts of *I. wikstroemioides* (7.5 kg) were extracted with 70% aqueous acetone (14 L) three times (three days each time) at room temperature and filtered. The filtrate was concentrated under reduced pressure and then partitioned between EtOAc and H_2O. The EtOAc-soluble portion (380 g) was subjected to silica gel CC (100–200 mesh, 11 × 120 cm, 2 kg), eluted with $CHCl_3$/acetone (1:0–0:1 gradient system) that afforded fractions A–G. The fractions were then decolorized using MCI gel and eluted with 90:10 MeOH/H_2O.

Fraction C ($CHCl_3$/acetone, 8:2; 19 g), which was a brown gum, was subjected to RP-18 column chromatography (8 × 50 cm, MeOH/H_2O 27:73 to 60:40 gradient) to provide three fractions, C1–C3. Fraction C2 (15 g) was separated into five subfractions (C2-1–C2-5) using RP-18 CC (6 × 40 cm, MeOH/H_2O 25:75 to 40:60 gradient). C2-4 (9 g) was subjected to RP-18 CC (6 × 40 cm, CH_3CN/H_2O 35:65) to obtain **3** (4 g). C2-5 (5 g) was separated by preparative HPLC (6 × 29 cm, CH_3CN/H_2O 34:66) to afford 7 fractions (C2-5-1–C2-5-7). C2-5-6 (40 mg) was submitted to semi-preparative HPLC (5 μm, 9.4 × 250 mm, flow rate 3 ml/min, UV detection at λ_{max} = 210, 254, and 280 nm, eluted with CH_3CN/H_2O 40:60, t_R = 14 min) to yield **6** (4 mg). Compound **1** (2.4 mg) was isolated from fraction C2-5-7 (210 mg) by semi-preparative HPLC (MeOH/H_2O 60:40, t_R = 33 min). Fraction C3 (2 g) was separated by preparative HPLC (2.5 × 27 cm, CH_3CN/H_2O 34:66) to afford 17 fractions (C3-1–C3-17). C3-10 (105 mg) was submitted to semi-preparative HPLC (5 μm, 9.4 × 250 mm, flow rate 3 ml/min, UV detection at λ_{max} = 210, 254, and 280 nm, eluted with MeOH/CH_3CN/H_2O 15:30:55, t_R = 7.7 min) to yield **4** (22 mg). C3-11 (65 mg) was submitted to semi-preparative HPLC (5 μm, 9.4 × 250 mm, flow rate 3 ml/min, UV detection at λ_{max} = 210, 254, and 280 nm, eluted with MeOH/H_2O 65:35, t_R = 13.5 min) to yield **5** (11 mg).

Fraction D ($CHCl_3$/acetone, 7:3; 50 g), a brown gum, was subjected to silica gel CC (9 × 80 cm, 200–300 mesh, 1 kg), and eluted with $CHCl_3$/MeOH (80:1) to afford seven fractions (D1–D7). D4 (20 g) was applied to a silica gel column (5 × 60 cm, 200–300 mesh, 200 g) and eluted with $CHCl_3$/MeOH (80:1) to afford six fractions (D4-1–D4-6). D4-4 (14 g) was separated by preparative HPLC (6 × 29 cm, CH_3CN/H_2O 30:70) and then semi-preparative HPLC (9.4 × 250 mm, flow rate 3 ml/min, UV detection at λ_{max} = 210, 254, and 280 nm, eluted with CH_3CN/H_2O 33:67, t_R = 12.5 min) to yield **7** (3 mg). Compound **2** (29 mg) was obtained from fraction D4-5 (200 mg) by semi-preparative HPLC (9.4 × 250 mm, flow rate 3 ml/min, UV detection at λ_{max} = 210, 254, and 280 nm, eluted with CH_3CN/H_2O 22:78, t_R = 14 min).

2.4. Spectroscopic data

Isowikstroemin A (**1**)colorless needles (MeOH); mp 136–137 °C; $[\alpha]_D^{26}$: −60 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 231 (3.90), 196 (3.59) nm; IR (KBr) ν_{max} 3446, 2927, 1726, 1646, 1235, 1027 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; positive-ion ESIMS: *m/z* 427 $[M + Na]^+$ (100); positive-ion HREIMS $[M]^+$ *m/z* 404.2194 (calcd for $C_{23}H_{32}O_6$, 404.2199).

Isowikstroemin B (**2**)White amorphous powder; $[\alpha]_D^{26}$: −91 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 231 (3.86), 195 (3.55) nm; IR (KBr) ν_{max} 3440, 2933, 1726, 1644, 1237, 1031 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; positive-ion ESIMS: *m/z* 413 $[M + Na]^+$ (100); positive-ion HREIMS $[M]^+$ *m/z* 390.2049 (calcd for $C_{22}H_{30}O_6$, 390.2042).

Isowikstroemin C (**3**)White amorphous powder; $[\alpha]_D^{26}$: −51 (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 230 (3.91), 196 (3.61) nm; IR (KBr) ν_{max} 3432, 2926, 1722, 1646, 1269, 1056 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; positive-ion ESIMS: *m/z* 385 $[M + Na]^+$ (100); positive-ion HREIMS $[M]^+$ *m/z* 362.2076 (calcd for $C_{21}H_{30}O_5$, 362.2093).

Isowikstroemin D (**4**)White amorphous powder; $[\alpha]_D^{26}$: −68 (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 229 (3.86) nm; IR (KBr) ν_{max} 3418, 2945, 1727, 1650, 1256, 1094 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; positive-ion ESIMS: *m/z* 371 $[M + Na]^+$ (100); positive-ion HREIMS $[M]^+$ *m/z* 348.1938 (calcd for $C_{20}H_{28}O_5$, 348.1937).

Isowikstroemin E (**5**)White amorphous powder; $[\alpha]_D^{25}$: +4 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 203 (3.79) nm; IR (KBr) ν_{max} 3441, 2928, 1719, 1659, 1242, 1029 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; positive-ion ESIMS: *m/z* 429 $[M + Na]^+$ (100); positive-ion HREIMS $[M]^+$ *m/z* 406.2353 (calcd for $C_{23}H_{34}O_6$, 406.2355).

Isowikstroemin F (**6**)White amorphous powder; $[\alpha]_D^{26}$: −27 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 204 (3.80) nm; IR (KBr) ν_{max} 3438, 2933, 1718, 1630, 1246, 1030 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; positive-ion ESIMS: *m/z* 415 $[M + Na]^+$ (100); positive-ion HREIMS $[M]^+$ *m/z* 392.2185 (calcd for $C_{22}H_{32}O_6$, 392.2199).

Isowikstroemin G (**7**)White amorphous powder; $[\alpha]_D^{26}$: −31 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 204 (3.86) nm; IR (KBr)