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Chinese Journal of Natural Medicines 2015, **13**(5): 0383–0389
Chinese Journal of Natural Medicines

Six new cytotoxic and anti-inflammatory 11, 20-epoxy-*ent*-kaurane diterpenoids from *Isodon wikstroemioides*

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Available online 20 May 2015

[ABSTRACT] The present study was designed to determine the chemical constituents of EtOAc extracts of the aerial parts of *Isodon wikstroemioides*. Compounds **1**–**8** were isolated and purified by normal-phase silica gel and reversed-phase C_{18} silica gel column chromatography and HPLC. Their structures were elucidated by extensive spectroscopic methods. Most of them were evaluated for their *in vitro* cytotoxicity against human cancer HL-60, SMMC-7721, A-549, MCF-7, and SW-480 cells and their inhibitory activity against nitric oxide (NO) production in LPS-activated RAW264.7 macrophages. Among the eight 11, 20-epoxy-*ent*-kauranoids isolated, compounds **1**–**6** (isowikstroemins H–M) were new diterpenoids. Compounds **1**, **3**, and **7** exhibited significant cytotoxicity with IC_{50} values ranging from (0.84 ± 0.02) to (4.09 ± 0.34) μmol·L^{-1}, while compounds **4** and **5** showed selective cytotoxicity. In addition, compounds **1**, **3**, **4**, and **7** exhibited inhibitory activity against nitric oxide (NO) production in LPS-activated RAW264.7 macrophages. These results provide a basis for future development of these compounds as anti-cancer and anti-inflammatory agents.

[KEY WORDS] *Isodon wikstroemioides*; Isowikstroemins H–M; Cytotoxicity; Anti-inflammatory activity

[CLC Number] R284 **[Document code]** A **[Article ID]** 2095-6975(2015)05-0383-07

Introduction

Isodon is a cosmopolitan and important genus of the Lamiaceae family [1-2]. The *Isodon* species have long been used in traditional Chinese medicines [3]. The *ent*-kaurane diterpenoids, as the major secondary metabolites of this genus, have attracted considerable attention due to their diverse structures and interesting biological properties [4-7]. Over the past 30 years, more than 60 *Isodon* species have been investigated [8], and a large number of *ent*-kaurane diterpenoids have been isolated and characterized by our group [8].

Isodon wikstroemioides (Hand.–Mazz.) H. Hara (Lamiaceae), a perennial herb, is primarily distributed in the northwestern regions of Yunnan Province and the western Sichuan regions in China [9]. Previous studies on this herb have led to isolation of 7, 20-epoxy-*ent*-kauranoids [10-11], C-20-non- oxygenated-*ent*- kauranoids [12], and C-20-oxygenated-non-epoxy-*ent*-kauranoids [12]. In our continuing research with the aim at discovering new diterpenoids with diverse structures and bioactivities, six new 11, 20-epoxy-*ent*- kauranoids, isowikstroemins H–M (**1**–**6**), along with two known analogues, macrocalyxin B (**7**) [13] and pseudoirroratin A (**8**) [14], have been isolated from *I. wikstroemioides*. In the present report, the isolation and structure elucidation of these diterpenoids are described as alongside with the cytotoxicity evaluation against five human tumor cell lines and their inhibitory activity against LPS-induced NO production in RAW 264.7 macrophages.

Results and Discussion

A 70% aqueous acetone extract of the air-dried and powdered aerial parts of *I. wikstroemioides* (7.5 kg) was partitioned between EtOAc and H_2O. The EtOAc-soluble portion

[Received on] 07-Nov.-2014

[Research funding] This work was supported financially by the National Natural Science Foundation of China (Nos. 21322204 and 81172939), the NSFC-Joint Foundation of Yunnan Province (No. U1302223), the reservation-talent project of Yunnan Province (No. 2011CI043), and the West Light Foundation of the Chinese Academy of Sciences (PU JX).

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These authors have no conflict of interest to declare.

(380 g) was subjected to repeated column chromatography and semi-preparative HPLC to afford six new *ent*-kauranoids, isowikstroemins H–M (**1**–**6**), along with two known analogues, macrocalyxin B (**7**) and pseudoirroratin A (**8**) (Fig. 1).

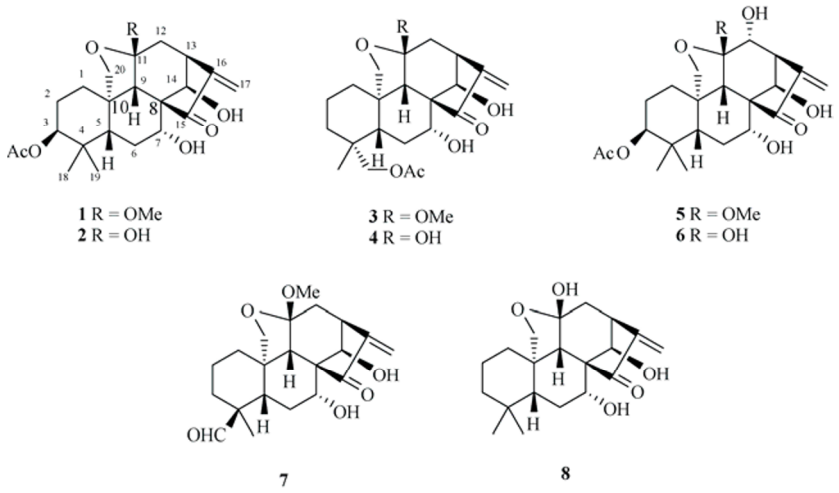

Fig. 1 Chemical structures of compounds 1–8

Isowikstroemin H (**1**) was obtained as white amorphous powder and gave a HREI-MS ion peak at *m/z* 420.214 7 ($[M]^+$, calcd 420.214 8), which corresponded to a molecular formula of $C_{23}H_{32}O_7$ with eight degrees of unsaturation. The IR spectrum indicated absorption bands for hydroxy group (3 425 cm^{-1}), carbonyl group (1 732 cm^{-1}), and double bond group (1 643 cm^{-1}). The ^1H NMR spectrum (Table 1) displayed characteristic signals of two methyls (δ_H 0.86 and 0.67), an acetyl (δ_H 2.01), and a methoxyl (δ_H 3.12), while its ^{13}C NMR and DEPT data (Table 2) exhibited 23 carbon resonances includ-

Table 1 ^1H NMR data of compounds 1–6 in pyridine-d_5 (*J* in Hz)

Position	1[a]	2[b]	3[a]	4[b]	5[b]	6[b]
1a	2.54, m	3.30, overlap	2.69, m	3.42, m	2.55, m	3.30, m
1b	1.49, m	1.66, overlap	1.01, overlap	1.15, m	1.51, m	1.67, overlap
2a	1.87, m	1.90, m	1.54, overlap	1.59, overlap	1.92, overlap	1.93, overlap
2b	1.68, m	1.69, overlap	1.37, m	1.37, m	1.72, m	1.71, overlap
3a	4.76, s	4.79, s	1.58, overlap	1.60, overlap	4.80, s	4.80, s
3b			0.97, overlap	1.00, overlap		
5	2.15, br d (12.4)	2.24, br d (12.2)	1.60, overlap	1.68, br d (12.5)	2.19, br d (12.2)	2.25, br d (12.1)
6a	2.07, m	2.12, overlap	2.24, m	2.30, overlap	2.08, overlap	2.11, d (12.1)
6b	1.93, d (12.4)	2.03, overlap	2.04, overlap	2.14, overlap	1.94, overlap	1.99, overlap
7	4.86, br d (12.0)	4.94, m	4.73, m	4.80, m	4.95, d (11.7)	4.97, dd (11.6, 2.9)
9	2.21, s	2.40, s	2.12, s	2.31, s	2.24, s	2.39, s
12a	2.89, dd (14.1, 9.1)	3.20, overlap	2.86, dd (14.1, 9.1)	3.18, dd (14.1, 9.0)	4.19, s	4.37, s
12b	1.76, d (14.1)	2.17, d (14.1)	1.75, d (14.1)	2.16, d (14.1)		
13	3.24, d (9.1)	3.28, overlap	3.22, d (9.1)	3.26, d (9.0)	3.58, s	3.66, s
14	5.24, s	5.46, s	5.21, s	5.42, s	5.30, s	5.47, s
17a	6.22, s	6.21, s	6.23, s	6.22, s	6.38, s	6.32, s
17b	5.43, s	5.38, s	5.43, s	5.38, s	5.62, s	5.53, s
18	0.86, s	0.89, s	0.93, s	0.96, s	0.88, s	0.89, s
19a	0.67, s	0.71, s	4.00, overlap	4.06, d (11.0)	0.69, s	0.70, s
19b			3.87, d (11.0)	3.95, d (11.0)		
20a	4.03, d (8.8)	4.22, d (8.6)	3.98, overlap	4.25, d (8.3)	4.24, d (8.6)	4.34, d (8.3)
20b	3.97, d (8.8)	4.12, d (8.6)		4.09, d (8.3)	4.07, d (8.6)	4.08, d (8.3)
MeO	3.12, s		3.10, s		3.45, s	
AcO-3	2.01, s	2.00, s			2.02, s	2.02, s
AcO-19			1.98, s	1.99, s		

[a] Recorded at 500 MHz. [b]Recorded at 400 MHz