

Isojaponin C and other kauranoid diterpenoids from *Isodon japonicus*

Libin Yang^a, Shaojing Liu^a, Honglun Wang^b and Yourui Suo^{b*}

^a*Xi'an Medical University, Xian, Shaanxi 710021, P.R. China*

^b*Northwest Institute of Plateau Biology, Chinese Academy of Sciences, No. 59 Xiguan Street, Xining 810001, Qinghai, P.R. China*

A new *ent*-kaurane diterpenoid, 1 α ,6 β ,11 α -triacetoxy-7 α ,20-epoxy-*ent*-kaur-16-en-15-one, named as iso-japonin C, along with five known diterpenoids, lasiokaurinol, lasiokaurin, oridonin, and epinodosin were isolated from the leaves of *Isodon japonicus*. Their structures were elucidated on the basis of spectroscopic methods and from literature values.

Keywords: *Isodon japonicus*, *ent*-kaurane diterpenoid, iso-japonin C

The genus *Isodon* (Labiateae family) is a rich source of *ent*-kaurane diterpenoids, which have attracted considerable attention because of their diverse structures and bioactivities.^{1,2} *Isodon japonicus* (Burman f.) H. Hara is a traditional herb used in China and Japan.^{1,3} Phytochemical research of *I. japonicus* started in the 1950s. Most of the diterpenoids isolated from *I. japonicus* possess a range of biological activities, such as anti-bacterial and anti-cancer activities.⁴⁻⁷ Our research team have investigated the aerial parts of this species collected from the Qinling Mountain, Shanxi Province, and obtained two new compounds iso-japonins A and B.⁸ We have further explored this plant to search for new *ent*-kauranoids. As a result, a new *ent*-kauranoid iso-japonin C (**1**), along with four known diterpenoids lasiokaurinol (**2**), lasiokaurin (**3**), oridonin (**4**), epinodosin (**5**) were isolated (Fig. 1). In this paper, we describe the isolation and structure elucidation of compound **1**.

Compound **1** was obtained as white amorphous powder. It was assigned the molecular formula C₂₆H₃₄O₉ by HRESIMS

(*m/z* 513.2112 [M+Na]⁺). The ¹³C NMR spectra showed that there was a five-membered ketone conjugated with an *exo*-methylene group (δ_c 206.2, 153.8 and 110.1), a hemiketal quaternary carbon (δ_c 92.1), an oxygenated methine (δ_c 65.4), two methyls (δ_c 34.4 and 22.2), three methines (δ_c 46.9, 61.7 and 34.9), three quaternary carbons (δ_c 34.3, 48.3 and 42.7), and three oxygenated methines (δ_c 76.4, 75.7 and 75.6), suggesting **1** to be a 7 α ,20-epoxy-*ent*-kaur-16-en-15-one diterpenoid skeleton typically found in this plant.^{4,5,8} Further analysis of the ¹³C NMR and ¹H NMR spectrum of compound **1** revealed three acetoxy groups (δ_c 170.4, 21.8; 169.3, 20.83 and 169.7, 21.7). The position of one acetoxy was confirmed at C-1 (δ_c 76.4) by the HMBC correlation of H-1 (δ_H 5.41) with C-2 (δ_c 24.8), C-3 (δ_c 38.8), and C-20 (δ_c 65.4), and the other two acetoxy groups were substituted at C-6 (δ_c 75.7) and C-11 (δ_c 75.6) from the HMBC correlations of H-6 (δ_H 4.92) with C-5 (δ_c 46.9), C-7 (δ_c 92.1) and C-10 (δ_c 42.7); H-11 (δ_H 4.92) with C-12 (δ_c 40.9), C-9 (δ_c 61.7) and C-8 (δ_c 48.3), respectively (Fig. 2). The observed NOESY

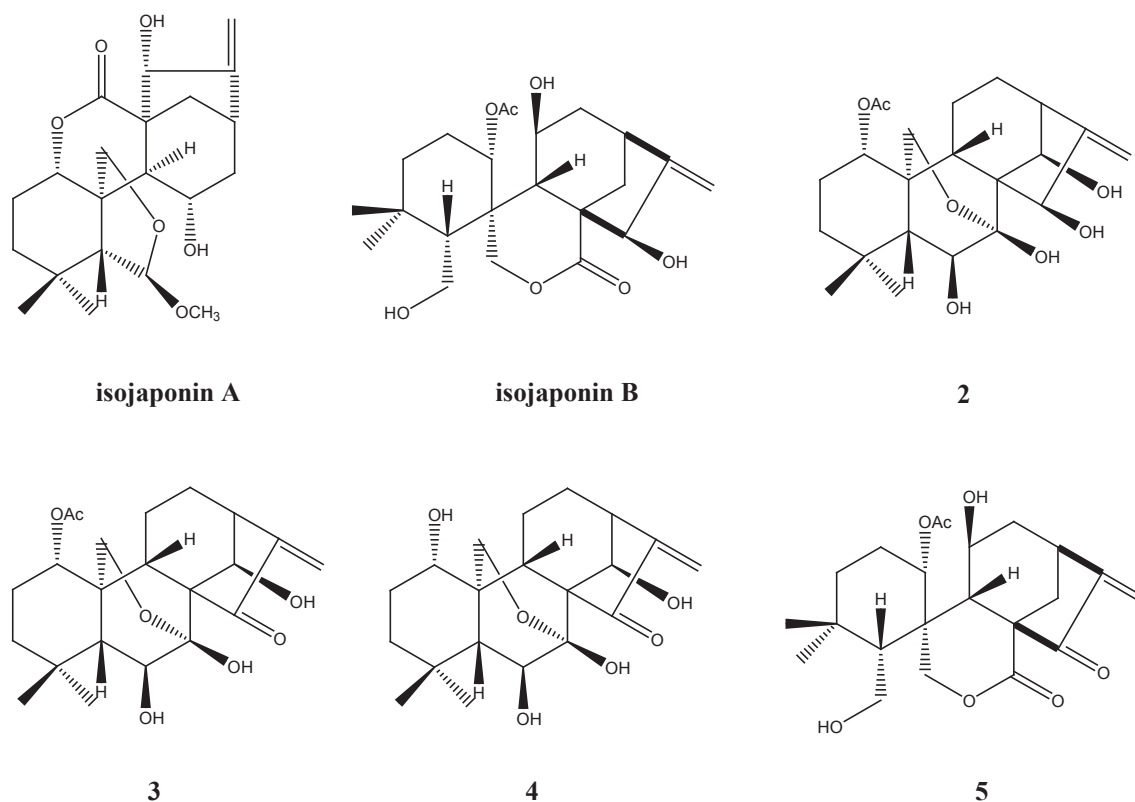


Fig. 1 Structures of compounds iso-japonins A, B and 2–5.

* Correspondent. E-mail: suo_yourui@163.com

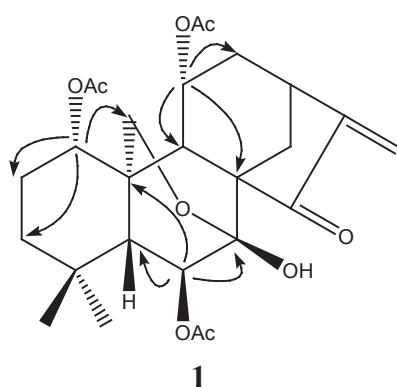


Fig. 2 The key HMBC (H→C) correlations of **1**.

correlations from H-1 to H-3 and 9 β , H-6 to H-20 and 19, and H-11 to H-9 β , 12 and 14 β showed that the H-1 was β -oriented, the H-6 was in α -orientation, and the H-11 was in β -orientation, respectively (Fig. 3). Thus, the structure of **1** was shown to be 1 α ,6 β ,11 α -triacetoxy-7 α ,20-epoxy-*ent*-kaur-16-en-15-one, and named isojaponin C.

The structures of the known compound lasiokaurinol (**2**), lasiokaurin (**3**), oridonin (**4**), and epinodosin (**5**), were identified based on spectroscopic methods and literature data.^{9–12}

Experimental

Optical rotation was carried out on a PerkinElmer model 241 polarimeter. UV spectra was obtained using a Shimadzu UV-2401A. IR spectra was recorded as KBr pellets on a PerkinElmer 599B spectrophotometer. MS was determined on a Bruker Daltonics Apex III mass spectrometer. NMR spectra were measured on Bruker DRX-500 spectrometers with TMS as the internal standard and CDCl₃ as solvent. Silica gel (80–100 and 200–300 mesh), MCI (CHP20/P50, Mistubishi) and reversed-phase C-18 silica gel (60 mesh, Merck) were used for column chromatography. The silica gel GF254 for TLC (Qingdao Marine Chemical Co., China). Agilent 1220LC equipped with an Welchrom C-18 column (4.6×250 mm) was used for HPLC analysis and a semi-preparative Welchrom C-18 column (10×250 mm) was used in the sample preparation.

Plant material

The aerial parts of *I. japonicus* were collected in the Qinling Mountain, Shanxi Province, in July 2012, and identified by Prof. Yonghui Feng, Xi'an Medical University. A voucher specimen (No. Xiyi-2012002) was deposited in the Herbarium of Xi'an Medical University, China.

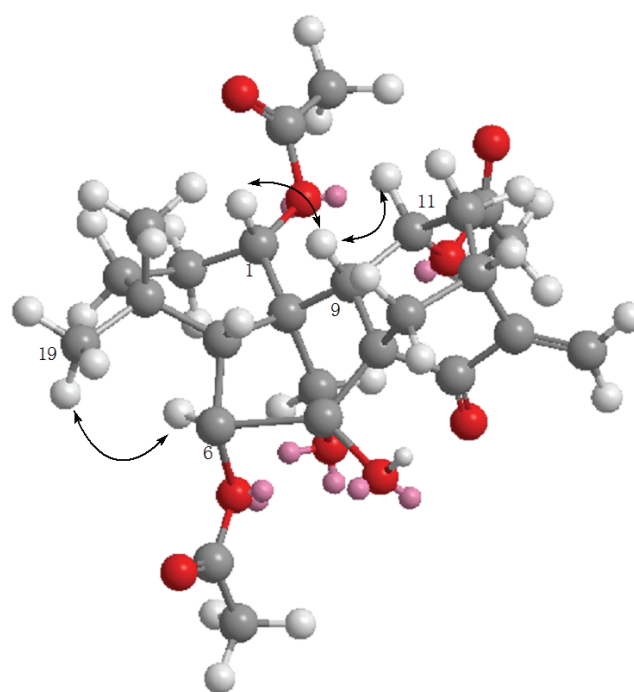


Fig. 3 Key ROESY (H↔H) correlations of **1**.

Extraction and isolation

Air-dried and powdered leaves of *I. japonicus* (0.8 kg) were extracted by 75% ethanol three times (every three days and 5 L) at room temperature. The extracts were concentrated *in vacuo* to afford a crude residue (210 g) which was suspended in water (600 mL) and subsequently partitioned successively with EtOAc to afford an EtOAc soluble fraction. The EtOAc fraction (82 g) was decolourised on MCI gel and eluted with 90% MeOH–H₂O to yield a pale yellow gum (61 g). The gum was subjected to column chromatography on silica gel using a step gradient elution of CHCl₃–Me₂CO (1:0–0:1). The fractions obtained from CHCl₃–Me₂CO (1:1) elution were combined and subjected to repeated column chromatography and further purified by preparative HPLC to yield pure compounds **1** (6 mg), **2** (11 mg), **3** (14 mg), **4** (4 mg) and **5** (3 mg).

1 α ,6 β ,11 α -Triacetox-7 α ,20-epoxy-*ent*-kaur-16-en-15-one (**1**): C₂₆H₃₄O₉, white amorphous powder; [α]_D¹⁹, –35.71° (C 0.84, CHCl₃); UV (CHCl₃): λ_{\max} (log ϵ) 236 (3.75) nm; IR_{max} (KBr, cm⁻¹): 3300, 1750, 1686, 1250; HRESIMS [M+Na]⁺ *m/z* 513.2112 (calcd for 513.2100) for C₂₆H₃₄O₉Na; ¹H and ¹³C NMR data see Table 1.

Table 1 ¹H and ¹³C NMR data (in CDCl₃) for compound **1** (δ in ppm, *J* in Hz)

No.	δ_c^a	$\delta_H/J/Hz^b$	ROESY	No.	δ_c^a	$\delta_H/J/Hz^b$	ROESY
1 β	76.4 (d)	5.41(1H, dd, 10.0, 5.0)	H-3, 9 β	14 β		1.78 (1H, m)	H-11 β , 13
2 α	24.8 (t)	1.78 (1H, m)	H ₃ -19	15	206.2 (s)		
2 β		1.56 (1H, m)	H-1, 3	16	153.8 (s)		
3	38.8 (t)	1.51 (2H, m)	H-1,2	17a	110.1 (t)	6.04 (1H, s)	
4	34.3 (s)			17b		5.08 (1H, s)	
5 β	46.9 (d)	2.04 (1H, d, 6.0)	H-1, 9 β , H ₃ -18	18	34.4 (q)	1.47 (3H, s)	H-5 β
6 α	75.7 (d)	4.92 (1H, m)	H-20, H ₃ -19	19	22.2 (q)	1.12 (3H, s)	H-2 α , 6, 20
7	92.1 (s)			20a	65.4 (d)	4.85 (1H, d, 10.0)	H-6, H ₃ -19
8	48.3 (s)			20b		4.23 (1H, dd, 10.0, 2.0)	
9 β	61.7 (d)	2.33 (1H, d, 8.0)	H-5 β	OAc	170.4 (s)	2.18 (3H, s)	
10	42.7 (s)				169.3 (s)	1.98 (3H, s)	
11 β	75.6 (d)	4.92 (1H, m)	H-14 β , 12 α , 9 β		169.7 (s)	2.10 (3H, s)	
12 α	40.9 (d)	2.32 (1H, m)	H-11 α , 13		21.8 (q)		
12 β		1.56 (1H, m)			20.8 (q)		
13 α	34.9 (t)	2.76 (1H, m)	H-12 α , 14 α		21.7 (q)		
14 α	24.8 (t)	2.55 (1H, d, 10.0)	H-11 α , 13 α				

^aAt 125 MHz; ^bat 500 MHz.

Received 12 December 2013; accepted 7 January 2014
Paper 1302337 doi: 10.3184/174751914X13909296237361
Published online: 7 March 2014

References

- 1 H.D. Sun, S.X. Huang and Q.B. Han, *Nat. Prod. Rep.*, 2006, **23**, 693.
- 2 H.D. Sun, Y.L. Xu and B. Jiang, *Diterpenoids from isodon species*. Science Press, Beijing, 2001, 93.
- 3 E. Fujita and M. Node, In *Progress in the chemistry of organic natural products*, W. Herz, H. Grisebach, G.W. Kirby and C. Tamm, eds. Springer-Verlag, Vienna, 1984, 77.
- 4 J.X. Zhang, Q.B. Han, A.H. Zhao and H.D. Sun, *Fitoterapia*, 2003, **74**, 435.
- 5 S.S. Hong, S.A. Lee, X.H. Han, J.S. Wang, C. Lee, D. Lee, J.T. Hong, Y. Kim, H. Lee and B.Y. Wang. *J. Nat. Prod.*, 2008, **71**, 1055.
- 6 E. Fujita and M. Taoka, *Chem. Pharm. Bull.*, 1972, **20**, 1752.
- 7 E. Fujita, Y. Nagao, M. Node, K. Kaneko, S. Nakazawa and H. Kuroda, *Experientia*, 1976, **32**, 203.
- 8 L.B. Yang, S.X. Huang, L.M. Li, Y. Zhao, C. Lei, W.L. Xiao, J.X. Pu, Q.B. Han and H.D. Sun, *Helv. Chim. Acta*, 2007, **90**, 2375.
- 9 E. Fujita, M. Taoka and T. Fujita, *Chem. Pharm. Bull.*, 1974, **22**, 280.
- 10 E. Fujita, M. Taoka, *Chem. Pharm. Bull.*, 1972, **20**, 1752.
- 11 H.M. Liu, X.B. Yan, F. Kiuchi, *Chem. Pharm. Bull.*, 2000, **48**, 148.
- 12 I. Kubo, T. Kamikawa, T. Kubota, *Tetrahedron*, 1974, **30**, 615.

Copyright of Journal of Chemical Research is the property of Science Reviews 2000 Ltd. and its content may not be copied or emailed to multiple sites or posted to a listserv without the copyright holder's express written permission. However, users may print, download, or email articles for individual use.