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Symmetric and asymmetric *ent*-kaurane dimers isolated from *Isodon japonicus*

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Abstract

Bisjaponins A (**1**) and B (**2**), two new dimeric *ent*-kaurane diterpenoids connected with a rare four-membered carbon ring, which was formed by [2+2] reaction, were isolated from the aerial parts of *Isodon japonicus*. Their structures were elucidated by the analysis of spectroscopic evidence including extensive 2D NMR and MS data. Both the compounds were inactive for their cytotoxicity against human tumor cell lines, K562 and HepG2.
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Keywords: Dimer; *ent*-Kaurane; Four-membered carbon ring; [2+2] cycloaddition; *Isodon japonicus*

The genus *Isodon* has attracted much attention for being a prolific source of new and bioactive diterpenoids.[1–3] *Isodon japonicus* (Burman f.) H. Hara has been used as folk medicine for the treatment of antibacterial, anti-inflammation, and anthelmintic in China and Japan since ancient time. It has a name of 'enmei-so', which means a grass effective for the prolongation of human life.[1,3] Moreover, it is the first *Isodon* species that was taken phytochemical study in 1910.[4] Up to now, over 30 *ent*-kaurane diterpenoids have been isolated from the aerial parts of *I. japonicus*, which were collected from different places. Most of the diterpenoids are new compounds and indicate various biological activities.[5] Aimed at finding potentially bioactive and new diterpenoids, we investigated *I. japonicus* collected in Qinling Mountain, Shanxi Province, the People's Republic of China, and isolated 20 *ent*-kaurane diterpenoids.[6] Recently, epinodosin,[5i] oridonin,[7] taihangjaponicain A,[8] lushanrubescensin J,[9] as well as two new dimers, bisjaponins A (**1**) and B (**2**), were isolated from *I. japonicus* by our group. Among them, taihangjaponicain A was a dimer formed through C–17–C–17′ bond, and lushanrubescensin J formed through a six-membered dihydropyran ring, noteworthily, the two new dimmers, bisjaponins A (**1**) and B (**2**), were all formed through a rare four-membered carbon ring. This paper deals with the isolation and structure elucidation of the two new dimers.

Bisjaponin A (**1**)[10] was isolated as a white powder. The HRESIMS exhibited a $[M-H]^-$ ion at *m*/*z* 723.3367, corresponding to a molecular formula of $C_{40}H_{52}O_{12}$, requiring 15 degrees of unsaturation. In addition, an ion peak at *m*/*z* 361 $[M/2-H]^-$ in the FABMS was observed. Thus, compound **1** was presumed as a symmetric dimer. IR absorption bands at 3422, 1751, and 1717 cm^{-1} suggested the presence of hydroxyl, ketone, and lactone carbonyl groups. On the basis of the ^{13}C NMR (Table 1), DEPT and HSQC

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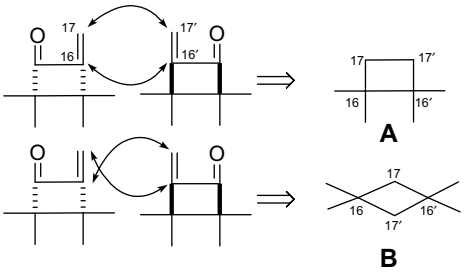
Table 1
^{1}H and ^{13}C NMR data of **1** (pyridine- d_5, δ in ppm, J in Hz)

No.	δ_H (mult)	δ_C (mult)	HMBC (H→C)
1,1′β	4.84 (dd 6.8, 10.4)	77.1 (d)	2,2′, 5,5′, 9,9′, 20,20′
2,2′	1.84 (2H m)	23.9 (t)	
3,3′	1.27 (2H m)	37.0 (t)	4,4′
4,4′		31.6 (s)	
5,5′β	3.1 (s)	54.1 (d)	4,4′, 6,6′, 9,9′, 10,10′, 18,18′, 19,19′, 20,20′
6,6′α	5.7 (s)	102.3 (d)	4,4′, 5,5′, 10,10′, 20,20′
7,7′		171.7 (s)	
8,8′		57.9 (s)	
9,9′α	2.69[a]	54.1 (d)	1,1′, 8,8′, 10,10′, 11,11′, 14,14′, 20,20′
10,10′		51.0 (s)	
11,11′β	4.41 (m)	62.9 (d)	9,9′, 10,10′
12,12′α	1.51 (m)	34.4 (t)	11,11′, 14,14′, 16,16′
12,12′β	2.51 (m)		9,9′
13,13′β	3.20 (m)	33.8 (d)	8,8′, 11,11′, 12,12′, 14,14′, 16,16′
14,14′α	2.44 (m)	32.2 (t)	7,7′, 9,9′, 12,12′, 13,13′
14,14′β	2.69[a]		8,8′, 9,9′, 12,12′, 13,13′, 16,16′
15,15′		215.6 (s)	
16,16′		63.6 (s)	
17,17′	2.04 (m)	23.4 (t)	13,13′, 15,15′, 16,16′
18,18′	0.94 (3H s)	33.0 (q)	3,3′, 4,4′, 5,5′, 19,19′
19,19′	0.94 (3H s)	23.2 (q)	3,3′, 4,4′, 5,5′, 18,18′
20,20′a	4.23 (d 9.2)	73.8 (t)	1,1′, 5,5′, 6,6′, 9,9′
20,20′b	4.35 (d 9.2)		5,5′, 6,6′, 9,9′, 10,10′

[a] Signals overlapped. Recorded at 400 MHz (^{1}H) and 100 MHz (^{13}C).

spectra of **1**, the 20 carbon resonances (C–5 and 9 were overlapped) were assigned to one ketone, one lactone carbonyl, one hemiacetal, two quaternary methyl groups, an oxymethylene carbon, three methines, and three quaternary carbons, which were the characteristic signals of a 6,7-*seco*-6,20-epoxy-*ent*-kaurane diterpenoid.[5g] Detailed comparison of the NMR data of **1** with those of epinodosin, which is a 6,7-*seco*-6,20-epoxy-*ent*-kaurane diterpenoid, indicated that NMR data of **1** were similar to those of epinodosin. Obviously, the differences between **1** and epinodosin were the chemical down-shift carbon C-15 ($\Delta\delta$ +14.1 ppm) and the up-shift carbon C-12 ($\Delta\delta$ −7.7 ppm) in **1**. In addition, the signals of the olefinic carbons of C-16 and 17 in epinodosin were replaced by a quaternary carbon (δ 63.6, s) and a methylene carbon (δ 23.4, t) in **1**. According to these changes together with 15 degrees of unsaturation, compound **1** was deduced to be a dimer of two epinodosin connected with a four-membered carbon



Scheme 1. The formation of the two possible four-membered carbon rings **A** and **B**.

ring. The HMBC (Fig. 2a) correlations of H-12,12′, 13,13′, and 14,14′ with C-16,16′, and of H-17,17′ with C-13,13′, 15,15′, and 16,16′ further confirmed the deduction.

The formation of the four-membered carbon ring could occur via two regioisomeric options, that is, cycloaddition to form a bond between C-17 and C-17′ (A in Scheme 1) or between C-17 and C-16′ (B in Scheme 1). A comparison of the chemical shifts between the compounds possessing similar moiety as structure A or B indicated that the chemical shift of C-17,17′ in structure A of about δ 25, and was much higher ($\Delta\delta$ about −10 ppm) than that in structure B.[11,12] According to the chemical shifts of C-17,17′ (δ 23.4, t), the structure of the four-membered carbon ring was confirmed to be structure A. On the other hand, the splitting pattern of H-17,17′ was multiple peaks rather than doublets, which also supported the conclusion.

Interestingly, cycloaddition between the two olefinic carbon bonds result in four possible configurations of C-16,16′ (C-16S^* and 16′R^*, C-16,16′S^*, C-16,16′R^*, and C-16R^* and 16′S^*). However, being a symmetric diterpenoid, compound **1** possesses a C_2 symmetric axis between the two epinodosin (Fig. 3a), which means that the relative configuration of C-16,16′ would be C-16,16′S^* or C-16,16′R^*, rather than C-16S^* and C-16′R^* or C-16R^* and C-16′S^*. On the basis of calculated lower energy conformation by means of the HF/6-31G(d) method, as implemented in the program package GAUSSIAN 03,[13] starting from preoptimized geometries generated by the MM2 force field in CHEM3D software overlaid with key correlations observed in the ROESY spectrum, if the relative configuration of C-16,16′ was S^*, the ROE correlations of H-17,17′ with H-12,12′α could not be observed (Fig. 3). However, the ROE correlations (Fig. 1) of H-17,17′ with H-12,12′

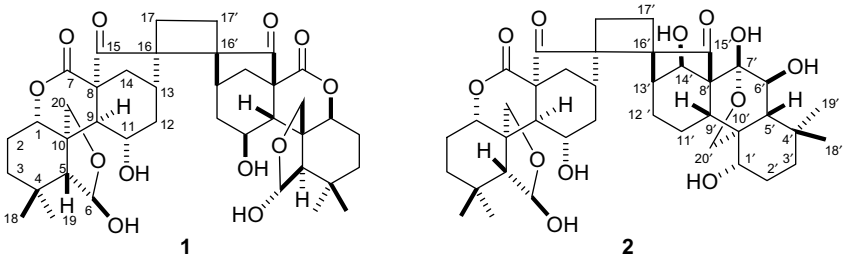


Fig. 1. Structures of **1** and **2**.

were clearly observed, which indicated that the relative configuration of C-16,16′ was R^*. Furthermore, the shortest interatomic distance of approximately 2.36 Å between H-17,17 and H-12,12′ further supported the assignment of C-16,16′R^* (Fig. 3). Finally, compound **1** was determined as bisjaponin A.

Bisjaponin B (**2**)[14] was obtained as a white powder. Its molecular formula was assigned as $C_{40}H_{54}O_{12}$ by the $[M-H]^-$ peak at *m/z* 725.3507 in the HRESIMS. Its ^{13}C NMR spectrum (Table 2) showed 40 carbon signals. Among them, 20 carbon resonances were assigned to the characteristic signals of a 6,7-*seco*-6,20-epoxy-*ent*-kaurane diterpenoid, including two methyl groups (C-18 and 19), three methines (C-5, 9, and 13), three unoxygenated quaternary carbons (C-4, 8, and 10), and a hemiacetal carbon C-6. The remaining 20 carbon resonances were the characteristic signals of a 7,20-epoxy-*ent*-kaurane diterpenoid,[2] containing two methyl groups (C-18′ and 19′), three methines (C-5′, 9′ and 13′), three unoxygenated quaternary carbons (C-4′, 8′ and 10′), and a hemiketal carbon C-7′. Thus, compound **2** was proposed to be a dimer comprised with a 6,7-*seco*-6,20-epoxy- and a 7,20-epoxy-*ent*-kaurane diterpenoid. Analysis and comparison of the 1D and 2D NMR data of **2** with those of epinodosin and oridonin revealed that the data of **2** were similar to those of epinodosin adding oridonin. The main differences were the olefinic carbon signals of C-16 (δ 151.1, s) and C-17 (δ 117.9, s) of epinodosin which were replaced by C-16 (δ 63.6, s) and C-17 (δ 27.1, t) in **2**, in addition, C-16 (δ 153.4, s) and C-17 (δ 119.1, t) of oridonin were replaced by C-16′ (δ 63.9, s) and C-17′ (δ 27.9, t) in **2** (Fig. 2b). Considering the differences mentioned above and the 15 unsaturated degrees of **2**, compound **2** should possess an additional four-membered carbon ring between epinodosin and oridonin.

Due to an asymmetric dimer, the correlations between H-17 and H-17′ in the COSY spectrum (Fig. 2b) could be observed obviously, thus, the structure of the four-membered carbon ring in **2** could be deduced to be structure A (Scheme 1) unanimously. The similar NMR data of the left part between **2** and **1**, in conjunction with the ROEs of H-17,17′ with H-12,12′ of **2**, suggested the similar relative configurations of C-16,16′ of **2** as **1**. Thus, the structure of **2** was unequivocally deduced.

To determine whether the dimers are natural products or isolation artefacts, epinodosin and epinodosin mixing with oridonin were put into several solutions (CH_3OH;

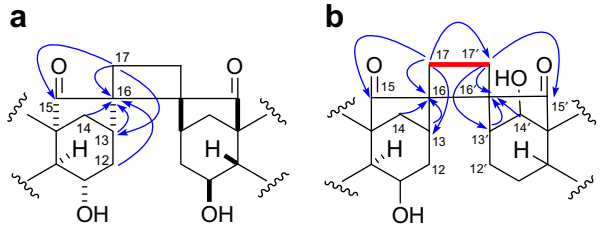


Fig. 2. (a) Key HMBC (H⟶C) correlations for **1**. (b) Key ^1H–^1H COSY (▬) and HMBC (H⟶C) correlations for **2**.

Table 2
^1H and ^{13}C NMR data for **2** (pyridine-d_5, δ in ppm, *J* in Hz)

No.	δ_H (mult)	δ_C	No.	δ_H (mult)	δ_C
1β	4.81 (t 8.0)	76.6	1′β	3.6 (s)	73.0
2	1.83[a] (2H)	24.1	2′α	1.72[a]	30.5
			2′β	1.83[a]	
3	1.28 (2H m)	36.9	3′	1.31 (2H m)	39.3
4		31.6	4′		34.0
5β	3.16 (s)	54.0	5′β	1.53 (d 4.8)	60.0
6α	5.7 (d 5.2)	102.3	6′α	4.24[a]	74.9
7		171.6	7′		98.4
8		58.1	8′		62.3
9β	2.74[a]	53.0	9′α	2.04[a]	54.0
10		51.1	10′		41.7
11β	4.44[a]	63.2	11′β	1.93[a]	
			11′α	2.33 (m)	19.4
12α	1.72[a]	35.5	12′β	1.83[a]	23.5
12β	2.61 (m)		12′α	2.04[a]	
13α	2.92[a]	35.2	13′β	3.07 (d 9.2)	42.7
14α	2.42 (d 12.0)	32.5	14′α	5.18 (s)	74.9
14β	3.98 (m)				
15		217.9	15′		226.0
16		63.6	16′		63.9
17a	1.93[a]	27.1	17′a	1.93[a]	27.9
17b	2.74[a]		17′b	2.92[a]	
18	0.94 (3H s)	33.0	18′	1.28 (3H s)	33.0
19	0.94 (3H s)	23.2	19′	1.09 (3H s)	22.3
20a	4.24[a]	73.7	20′a	4.35 (d 8.8)	63.2
20b	4.44[a]		20′b	4.72 (d 8.8)	

[a] Signals overlapped. Recorded at 400 MHz (^1H) and 100 MHz (^{13}C).

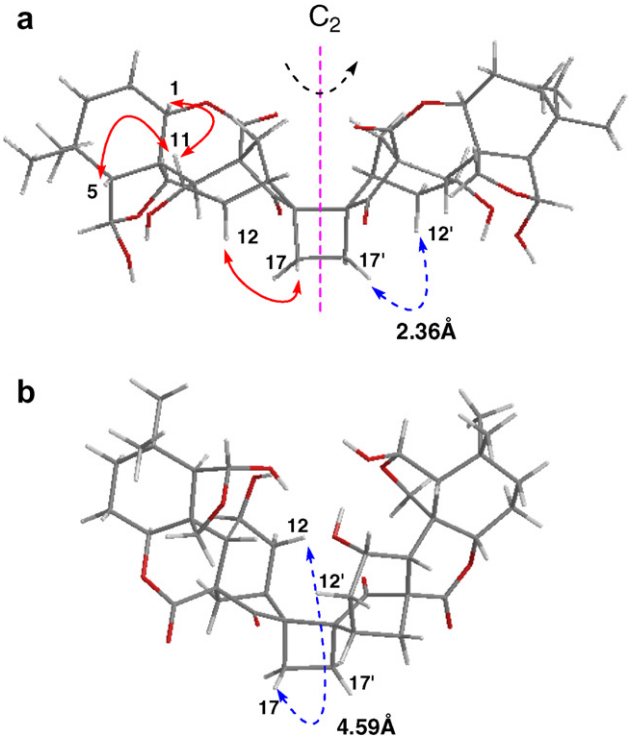


Fig. 3. (a) Key ROESY (H⟷H) correlations for **1**. (- - -) indicates C_2 symmetric axis of **1**. (b) The relative configuration of C-16,16′S^*. (⇠⇢) show the interatomic distance.

Me_2CO; $CHCl_3$), respectively, with little silica gel at room temperature and sunlight for four weeks. The reactions were monitored by HPLC with **1** and **2** as control. However, no changes were observed in any of the conditions, which proved that the two dimers are not isolation artefacts.

Several types of *ent*-kaurane diterpenoid dimers have been isolated from the genus *Isodon*. Most of them might be induced through Diels–Alderase mechanism.[8,9,15,16] Enzymatic Diels–Alderase reaction for the biosynthesis of natural products has been focused on for a long time, and some improvement has been achieved.[17,18] But the dimers formed by [2+2] cycloaddition reaction[19] were mediated spontaneously or mediated by enzyme in plants is still a mystery.[20]

All the compounds were tested for cytotoxicity against K562 and HepG2 cells using the sulforhodamine B (SRB) method as reported previously.[21] The four dimers were completely inactive against the tumor cell lines with IC_{50} values >100 μM. Only epinodosin and oridonin exhibited inhibitory activities against K562 with IC_{50} values of 7.4 μM, 18.6 μM, and against HepG2 of 1.4 μM, 0.7 μM, respectively. The results support our expectation that the removal of the α,β-unsaturated cyclopentanone system results in the loss of biological activities.[22]

Acknowledgements

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Supplementary data

Supplementary data (experimental procedure and full characterization of compounds **1** and **2**) associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2008.04.034.

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10. *Bisjaponin* A (**1**): White powder; $[\alpha]_D^{26}$ −3.6 (*c* 0.595 CH_3OH); IR (KBr) ν_{max} 3422, 2926, 1751, 1717, 1638, 1459, 1331, 1259, 1048 cm^{-1}; for ^1H and ^{13}C NMR data see Table 1; FABMS (negative) *m/z* 723 $[M-H]^-$; HRESIMS (*m/z*) calcd for $C_{40}H_{51}O_{12}$ 723.3380, found 723.3367 $[M-H]^-$.
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