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Japonicones A–D, bioactive dimeric sesquiterpenes from *Inula japonica* Thunb.

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ABSTRACT

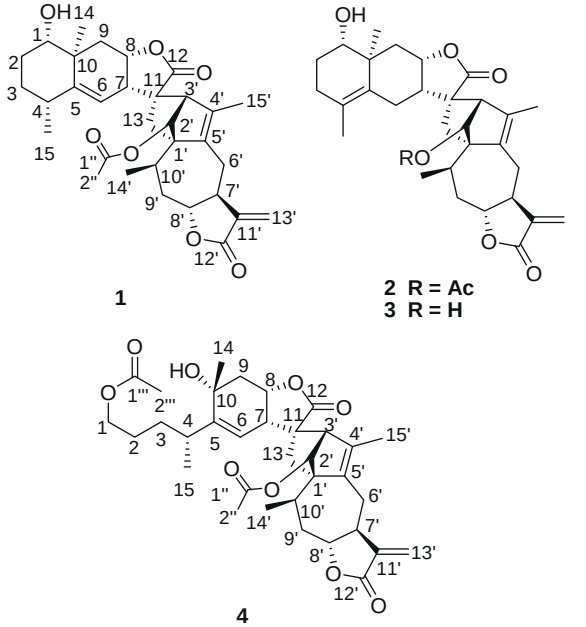
Four new dimeric sesquiterpene lactones japonicones A–D (**1**–**4**), comprised by eudesmane and guaiane sesquiterpenes, were isolated from the aerial part of *Inula japonica* Thunb. The structures and stereochemistry of **1**–**4** were elucidated by use of 2D NMR spectroscopic techniques, X-ray crystallography and modified Mosher method. Japonicone A (**1**) showed the most potent cytotoxicities against four tumor cell lines, A549, LOVO, CEM and MDA-MB-435.

Inula japonica Thunb., as a well known traditional herbal medicine, is widely distributed in China, Japan and Korea.[1,2] The dried roots and leaves of this plant have been used as Chinese folk medicine to treat knife wounds, furunculosis and cough.[3] The flowers always employed for peptic, relieving phlegm, detumescence, anti-inflammatory and vermifuge properties.[3] Modern pharmacological study also has exhibited its diversified effects, such as antidiabetes and hypolipidemia, anti-tumor, antifungus, antibacterial, hepatoprotective, and anti-hepatitis.[4–10]

The prior phytochemical studies on this plant mostly focused on sesquiterpene lactones due to their diverse biological activities, particularly in bactericidal, hepatoprotective, and anti-tumor effects, but the dimeric sesquiterpene lactones have never been reported.[11–15] In this paper, we described the isolation, identification and cytotoxicities of four new dimeric sesquiterpene lactones.

The dried aerial parts of *I. japonica* were powdered and extracted with ethanol and the extract was successively partitioned with petroleum ether, CH_2Cl_2, EtOAc, and *n*-BuOH, respectively. The CH_2Cl_2 fraction was chromatographed on a silica gel column (CH_2Cl_2-MeOH, 1:0 → 0:1) and then a Sephadex LH-20 column (CH_2Cl_2-MeOH 1:1), to a complex of mixture of sesquiterpene lactones. This mixture was subjected to preparative HPLC, resulting the purification of four new compounds, japonicones A (**1**, 173 mg), B (**2**, 18 mg), C (**3**, 12 mg), and D (**4**, 40 mg) (Scheme 1).

Japonicone A (**1**)[16] was obtained as a colorless lamellar crystal. Its molecular formula was determined to be $C_{32}H_{40}O_7$ on the basis of HRESIMS (*m/z* 559.2676, calcd for $C_{32}H_{40}O_7Na$, 559.2672), indicating 13° of unsaturation (Table 1). The ^{13}C and DEPT NMR spectra of **1** revealed 32 carbon signals including 5 methyls, 7 methylenes, 10 methines, and 10 quarternary carbons. The presence of an acet-



Scheme 1.

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Table 1
^{1}H and ^{13}C NMR data of japonicones A–D (**1**–**4**)[a] (δ_H, *J* in Hz, δ_C)

No.	1		2		3		4	
	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C
1	3.28 dd (11.7, 3.8)	80.7	3.44 m	78.4	3.56 dd (11.8, 4.5)	71.2	4.07 m 3.91 m	64.3
2	1.82 m 1.62 m	26.0	1.66 m	26.8	1.76 m	27.0	1.46 m	26.7
3	1.65 m 1.57 m	29.7	2.11 m 2.01 m	31.6	2.05 m	30.7	1.42 m	34.4
4	2.45 m	38.2		126.6		126.7	2.50 m	32.5
5		149.5		130.3		130.6		150.6
6	5.37 d (3.0)	118.1	2.63 m 1.71 m	24.2	1.88 m	25.7	5.35 s	116.1
7	2.79 dd (5.5, 3.2)	42.2	2.14 m	43.6	2.73 m	44.7	2.79 dd (4.2, 2.2)	42.8
8	4.89 dd (5.3, 2.7)	75.3	4.67 br s	76.1	4.54 m	77.9	4.87 br s	75.2
9	2.59 dd (14.8, 3.3) 1.53 dd (15.1, 2.0)	39.8	2.63 m 1.47 m	40.0	2.38 m 1.42 m	37.5	2.57 dd (15.5, 3.6) 1.86 dd (15.4, 1.7)	39.3
10		38.5		38.4		39.1		67.4
11		56.8		58.1		57.0		58.1
12		178.8		179.6		185.8		178.0
13	2.00 m 1.88 m	36.4	1.95 m	36.7	2.41 dd (12.8, 3.5) 2.12 dd (14.7, 3.8)	35.9	2.01 m 1.96 m	36.1
14	1.19 s	21.6	1.08 s	18.8	1.03 s	20.6	1.33 s	27.1
15	1.11 d (7.6)	23.0	1.66 s	19.1	1.42 s	18.5	1.09 d (7.0)	22.8
1′		62.3		61.9		70.5		62.4
2′	4.60 s	81.9	4.56 s	81.9	3.49 d (10.9)	85.7	4.61 s	81.7
3′	2.86 d (1.4)	56.2	2.88 s	54.4	2.65 m	53.0	2.92 m	55.2
4′		134.2		133.9		135.4		133.7
5′		136.4		136.3		140.8		137.1
6′	3.03 d (15.5) 2.08 m	26.0	3.05 d (15.7) 2.10 m	26.0	2.90 m 2.35 m	25.9	3.01 d (15.7) 2.08 m	26.0
7′	2.82 s	45.3	2.82 t (10.5)	45.3	2.86 m	44.7	2.92 m	45.0
8′	4.21 ddd (12.4, 8.4, 3.2)	82.5	4.21 ddd (12.7, 8.9, 3.1)	82.5	4.27 ddd (12.5, 8.9, 4.0)	80.5	4.22 ddd (12.6, 8.9, 3.1)	82.4
9′	2.36 dt (13.1, 4.1) 2.00 m	36.1	2.36 dt (13.0, 4.0) 1.95 m	36.0	1.91 m	36.8	2.37 dt (13.0, 3.9) 2.01 m	35.9
10′	2.15 m	29.8	2.15 m	29.7	2.62 m	27.8	2.15 m	29.8
11′		139.4		139.4		140.3		139.7
12′		170.0		170.1		169.6		170.0
13′	6.22 d (3.3) 5.54 d (3.1)	119.5	6.23 d (3.2) 5.56 d (2.9)	119.5	6.22 d (3.3) 5.50 d (3.0)	119.0	6.22 d (3.3) 5.53 d (3.0)	119.2
14′	1.05 d (7.3)	17.0	1.05 d (7.3)	16.9	1.21 d (7.0)	18.7	1.06 d (7.3)	17.0
15′	1.67 d (1.5)	14.3	1.66 s	14.2	1.80 s	13.6	1.67 d (1.2)	14.2
1″		170.0		169.8	-OH 6.12 d (11.2)			170.0
2″	2.08 s	21.2	2.06 s	21.1			1.96 s	21.3
1‴								171.0
2‴							2.08 s	20.9

[a] Data recorded in $CDCl_3$ for 1–4 on a Bruker Avance-400 instrument.

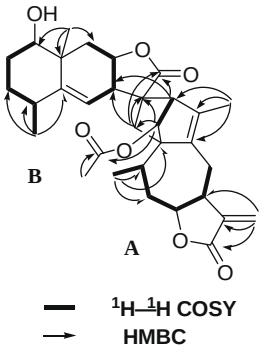

Figure 1. Selected 2D NMR correlations for japonicone A (**1**).

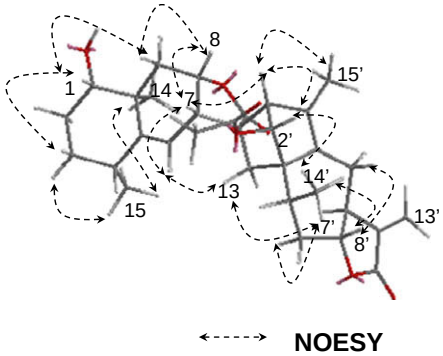

Figure 2. Selected NOESY correlations and relative stereochemistry for japonicone A (**1**).

oxy group was identified by the corresponding signals in the NMR spetrum: δ_C 170.0 and 21.2, δ_H 2.08, its position was determined by HMBC experiment (Fig. 1). The remaining 30 carbon signals belonged to two different sesquiterpene moieties. Compared with the spectral data of known sesquiterpenes isolated from this plant,[11,12] these signals were suggestive of the presence of an identical guaianolide skeleton moiety, named as moiety A (Table 1).

An olefinic carbons C-11′ (δ_C 139.4) and C-13′ (δ_C 119.5), and exocyclic olefinic protons H-13′a (δ_H 6.22) and H-13′b (δ_H 5.54) authenticated the existence of a characteristic α-methylene lac-

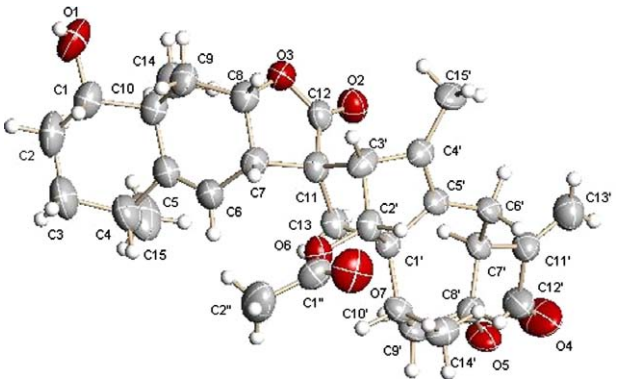

Figure 3. Single-crystal X-ray structure of japonicone A (**1**).

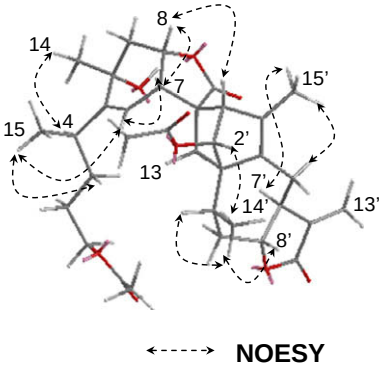

Figure 5. Selected NOESY correlations and relative stereochemistry for japonicone D (**4**).

tone functionality. ^{1}H–^{1}H COSY and HMBC correlations (Fig. 1) also established another moiety (moiety B), which was similar to eudesmane sesquiterpene 1β-hydroxyalantolactone.[12] Some other key COSY and HMBC correlations were also observed and depicted in Figure 1.

The connectivity of the two moieties was established by the key HMBC correlations: H-2′ correlated to C-11 and C-13; and H-3′ correlated to C-7 and C-12 (Fig. 1). These correlations suggested the presence of a bridged ring system, norbornene moiety, and their linkage mode is C-11–C-3′. The high chemical shift of the quaternary carbons C-1′ at δ_C 62.3 and C-11 at δ_C 56.8 also supported this moiety.

The relative stereochemistry of **1** was determined on the basis of the NOESY experiment and X-ray crystallography[17] (Figs. 2 and 3). The strong NOESY correlations of H-1/H-3b and H-9b, and H-8/H-9b and H-7 indicated that H-1, H-3b, H-7, H-8, and H-9b adopted the same orientation and were arbitrarily designated as the β-orientation. The NOESY correlations of H_3-15/H_3-14 and H-3a revealed that H-3a, Me-14 and Me-15 were α-orientation. In addition, the correlations of H-8′/ H-6′b, and H_3-14′ also suggested that H-6′b, H-8′ and Me-14′ were β-orientation. The NOESY correlation between H-7′ and H-9′a placed H-7′ at the α-configuration. The conformation of **1** in solution as established by NOESY spectrum was in good agreement with that in solid state as determined by X-ray study (Fig. 3). Crystallographic data for **1** have been deposited at the Cambridge Crystallographic Data Centre (deposition No. CCDC-688571). The absolute configuration of **1** was determined to be 1S, 4R, 7S, 8S, 10S, 1′R, 2′R, 3′S, 7′S, 8′R, and 10′R by modified Mosher method.[18,19]

Japonicone B (**2**)[20] was obtained as a white amorphous powder. Its molecular formula was determined to be $C_{32}H_{40}O_7$ on the basis of HRESIMS (*m/z* 559.2670, calcd for $C_{32}H_{40}O_7Na$, 559.2672), indicating 13 degrees of unsaturation. The analysis of the NMR spectra of **2** revealed that except an acetoxy group, the other 30 carbon signals belonged to two different sesquiterpene moieties. Compared with the spectroscopic data of **1**, **2** also has the guaianolide skeleton moiety (Table 1). The other moiety of **2** was determined by ^{1}H–^{1}H COSY and HMBC experiments. In fact, the main differences between **1** and **2** were the chemical shifts of C-4 at δ_C 38.2, C-5 at δ_C 149.5, and C-6 at 118.0 for **1**, in contrast to 126.6, 130.3, and 24.2 for **2**, respectively. There was a lack of vinyl doublet H-6 (δ_H 5.37), supported the positional change of a double bond. Therefore, the structure of **2** was established. Similar NOEs to those observed for **1** pointed to the same stereochemistry.

The spectral data of japonicone C (**3**)[21] indicated a close structural similarity to **2**. The HRESIMS indicated the molecule formula $C_{30}H_{38}O_6$ (*m/z* 517.2567, calcd for $C_{30}H_{38}O_6Na$, 517.2566). Compared the ^{1}H, ^{13}C, DEPT and HMBC spectral data with those of **2**, an acetoxy group (δ_C 169.8 and 21.1, δ_H 2.06) was absent, instead of a low field hydroxyl group at δ_H 6.12 appeared. The stereochemistry was also determined by comparison of NOEs between **2** and **3**.

Japonicone D (**4**)[22] was obtained as a white powder and shown a molecular formula $C_{34}H_{44}O_9$ established by HRESIMS (*m/z* 619.2880, calcd for $C_{34}H_{44}O_9Na$, 619.2883), indicating 13 degrees of unsaturation. The presence of two acetoxy groups was identified by the corresponding signals in the NMR spectrum: δ_C 171.0 and 20.9, δ_H 1.96; δ_C 170.0 and 21.3, δ_H 2.08, respectively. Their positions were determined by HMBC experiment (Fig. 4). Compared with the spectroscopic data of **1**, a guaianolide skeleton moiety was also appeared in the structure of **4** (Table 1). ^{1}H–^{1}H COSY and HMBC correlations (Fig. 4) also established another moiety, which was similar to acetylbritannilactone but another new eudesmane sesquiterpene.[12] The presence of a vinyl singlet: H-6 (δ_H 5.35) disclosed the positional changes of a double bond and a hydroxyl group. Some other key COSY and HMBC correlations were observed and depicted in Figure 4. Compared **4** with **1**, similar NOEs also helped us to determine its relative stereochemistry. The NOESY correlations of H-4/H_3-14 placed Me-14 at the β-configuration and OH-10 at the α-configuration (Fig. 5).

The anti-tumor activities of japonicones A–D (**1**–**4**) against four tumor cell lines, A549, LOVO, CEM and MDA-MB-435 were determined by MTT assay,[23] DOX (doxorubicin) was used as a positive control. Among them, japonicone A (**1**) showed the most potent cytotoxicities against these four tumor cell lines with IC_{50} values of 1.620, 0.256, 0.001, and 0.198 μg/mL, respectively, which was even stronger than DOX.

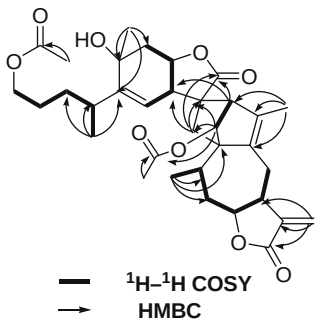

Figure 4. Selected 2D NMR correlations for japonicone D (**4**).

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2008.12.043.

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16. Japonicone A (**1**). White lamellar crystal, $[\alpha]_D^{20}$ +78° (*c* 0.71, $CHCl_3$); ^1H and ^{13}C NMR data, see Tables 1 and 2; IR (KBr) ν_{max} 3477, 2937, 1763, 1641, 1437, 1375, 1244, 1169, 1082, 1049, 1028, 982 cm^{-1}; ESIMS *m/z* 537 $[M+H]^+$, HRESIMS *m/z* 559.2676 (calcd for $C_{32}H_{40}O_7Na$, 559.2672).
17. Crystallographic data for japonicone A (**1**) have been deposited at the Cambridge Crystallographic Data Centre (deposition No. CCDC-688571). Copies of these data can be obtained free of charge via http://www.ccdc.cam.ac.uk/deposit.
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20. Japonicone B (**2**). White powder, $[\alpha]_D^{20}$ +60° (*c* 0.48, $CHCl_3$); ^1H and ^{13}C NMR data, see Tables 1 and 2; IR (KBr) ν_{max} 3435, 2939, 1767, 1732, 1664, 1439, 1375, 1240, 1163, 1032, 960, 731 cm^{-1}; ESIMS *m/z* 537 $[M+H]^+$, HRESIMS *m/z* 559.2670 (calcd for $C_{32}H_{40}O_7Na$, 559.2672).
21. Japonicone C (**3**). White powder, $[\alpha]_D^{20}$ −8° (*c* 0.26, $CHCl_3$); ^1H and ^{13}C NMR data, see Tables 1 and 2; IR (KBr) ν_{max} 3386, 2962, 1768, 1718, 1437, 1352, 1261, 1095, 1026, 800 cm^{-1}; ESIMS *m/z* 495 $[M+H]^+$, HRESIMS *m/z* 517.2567 (calcd for $C_{30}H_{38}O_6Na$, 517.2566).
22. Japonicone D (**4**). White powder, $[\alpha]_D^{20}$ +61° (*c* 0.55, $CHCl_3$); ^1H and ^{13}C NMR data, see Tables 1 and 2; IR (KBr) ν_{max} 3593, 3558, 3446, 2966, 2937, 1767, 1732, 1437, 1377, 1257, 1165, 1030, 802 cm^{-1}; ESIMS *m/z* 597 $[M+H]^+$, HRESIMS *m/z* 619.2880 (calcd for $C_{34}H_{44}O_9Na$, 619.2883).
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