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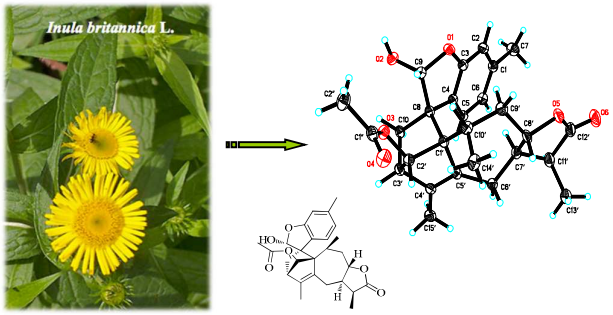

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ARTICLE TYPE

One new unusual sesterterpenoid and four new sesquiterpene dimers from *Inula britannica*

Xu-Feng Zhang,[⊥ab] Jie Ren,[⊥a] Xiang-Rong Cheng,[a] Hui-Zi Jin[*a] and Wei-Dong Zhang[*ac]

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One new unusual sesterterpenoid (1), four new sesquiterpene dimers (2−5) together with nine known sesquiterpenes (6−14) were isolated from the aerial parts of *Inula britannica*. The structures of the new compounds were elucidated by detailed spectroscopic analysis, including HR-ESIMS and 2D-NMR spectroscopic methods. In addition, compounds 1−8 and 10−11 were tested for their inhibitory effects against LPS-induced NO production in RAW264.7 macrophages.

Inula genus (Asteraceae) is an important genus of which there are more than 100 species distributed in Asia, Europe and Africa.[1] As one of the most popular traditional Chinese medicine (TCM) of this genus, *Inula britannica* has been reported to treat bronchitis, digestive disorders and inflammation.[2–3] Various bioactive secondary metabolites, such as sesquiterpene lactones, have been isolated from this species.[4–6] Our pursuit of biologically active sesquiterpenoids from *I. britannica* resulted in the isolation of one new unusual sesterterpenoid (**1**), four new sesquiterpene dimers (**2**−**5**), together with nine known sesquiterpenes (**6**–**14**). In this paper, we described the isolation and structure elucidation of these new sesquiterpene dimers. In addition, anti-inflammatory activities of these isolates against LPS-induced NO production in RAW 264.7 macrophages were also evaluated.

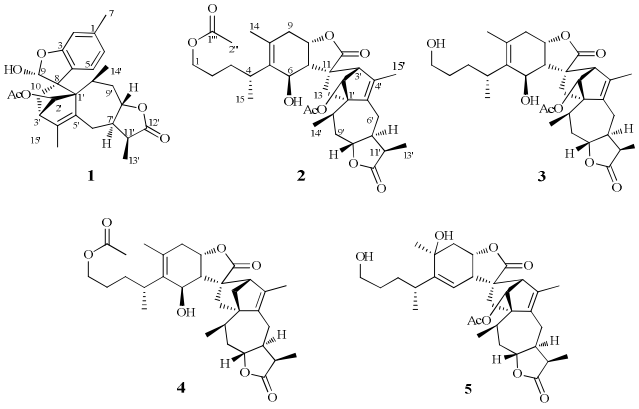

Fig. 1. Structures of compounds **1**–**5**

Dibritannilactone A (**1**) was obtained as orthorhombic crystals. Its molecular formula $C_{27}H_{32}O_6$ was established by HRESIMS peak at *m/z* 453.2292 $[M + H]^+$ (calcd for $C_{27}H_{33}O_6$, 453.2272), indicating twelve degrees of unsaturation. The IR spectrum showed bands characteristic of hydroxyl groups (3422 cm^{-1}), carbonyl groups (1765 and 1736 cm^{-1}) and olefinic bonds (1618 cm^{-1}). All the 27 carbon signals in ^{13}C NMR spectrum (Table 1) were classified by DEPT and HMQC experiments as five methyls, three methylenes, ten methines and nine quarternary carbons, from which typical signals of two ester carbonyls, eight olefinic carbons and three oxygen-bearing carbons were identified. The ^{13}C NMR spectrum also suggested the presence of an acetoxyl group (δ_C 170.3 and 21.2, δ_H 2.10), whose position was determined by HMBC experiment to be at C-2′ (Fig. 2). Besides,

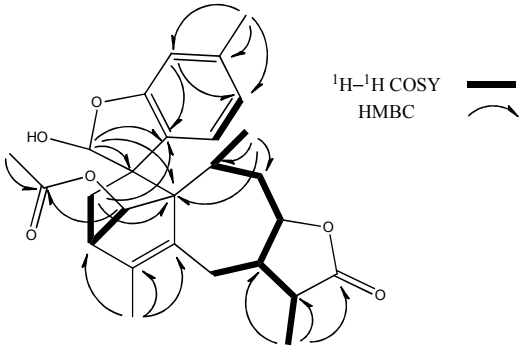

Fig. 2. Key ^{1}H–^{1}H COSY and HMBC correlations of **1**

detailed analysis of 1D and 2D NMR data of the remaining 25 carbon signals indicated that they were assigned to two units, one sesquiterpene unit (A) and one monoterpene unit (B). The ^{1}H–^{1}H COSY spectrum of unit A showed the following correlations: H-2′/H-3′, H_2-6′/H-7′/H-8′/H_2-9′/H-10′/H_3-14′ and H-7′/H-11′/H_3-13′. In addition, HMBC correlations from H_3-13′ to C-7′, C-11′ and C-12′, H_3-14′ to C-1′, C-9′ and C-10′, H_3-15′ to C-1′, C-3′, C-4′ and C-5′ suggested the presence of a partial structure of sesquiterpene unit. The remaining signals of **1** were assigned to a methyl (C-10), a 1,3,4-trisubstituted aromatic ring, a methylene (C-9), an oxygenated methine (C-8) and a quarternary carbon (C-

7). In the HMBC spectrum, correlations between H_3-10/C-1, C-2 and C-6, H-6/C-2, C-4, C-5 and C-10, H-3/C-1, C-5 and C-7, H-2/C-4 and C-10 were observed. These correlations suggested the existence of another partial structure of monoterpene unit. The connecting positions of the two units were established according to the following key correlations: in ^{1}H–^{1}H COSY spectrum, H-9 correlated to H-3′; in HMBC spectrum, correlations between H-8/C-7 and C-1′, H_2-9/C-1′, C-3′, C-4′, C-4 and C-7, H-3′/C-7, C-1′ and C-5′, which disclosed a new hexatomic ring (–C-7–C-9–C-3′–C-4′–C-5′–C-1′–). Therefore, the planar structure of **1** was constructed as shown in Fig. 2.

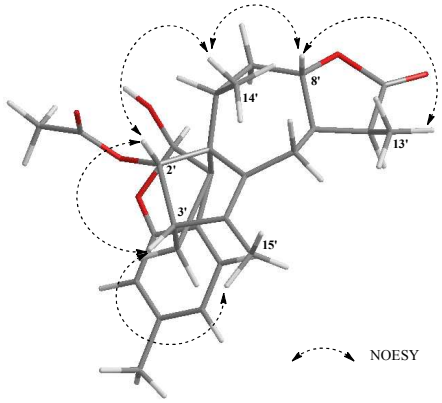

Fig. 3. Key NOESY correlations of compound **1**

The stereochemistry of **1** was further confirmed by detailed analysis of NOESY spectra and an X-ray diffraction study (Figs. 3 and 4). In the NOESY spectrum, the key correlations of H-13′/H-8′/H-14′ and H-2′ and H-3′/H-15′ were in good agreement with the X-ray diffraction study. The absolute configuration was determined by X-ray crystallographic analysis (Fig. 4). All relevant chiral centers in **1** were assigned as 8*R*,2′*S*,7′*R*,8′*S*,10′*S*,11′*S*. Hence, compound **1** was given the name (8*R*,2′*S*,7′*R*,8′*S*,10′*S*,11′*S*)-dibritannilactone A.

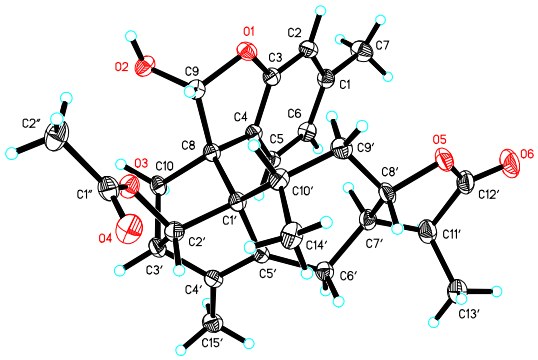

Fig. 4. Single-crystal X-ray structure (copper radiation) of **1**

Dibritannilactone B (**2**) was obtained as white amorphous powder. Its molecular formula $C_{34}H_{46}O_9$ was established from its HRESIMS peak at *m/z* 599.3187 $[M + H]^+$ (calcd for $C_{34}H_{47}O_9$, 599.3215), accounting for twelve degrees of unsaturation. The IR spectrum showed the presence of hydroxyl groups (3440 cm^{-1}), carbonyl groups (1750 cm^{-1}) and olefinic bonds (1635 cm^{-1}). The ^{13}C NMR and DEPT spectroscopic data of **2** showed great similarity to those of a known sesquiterpene dimer, inulanolide A[7] except for the α-methylene lactone functionality (Table 1). The absence of the $\Delta^{11,13}$ exocyclic methylene group was confirmed by the upfield shifts of C-11′ and C-13′ and the downfield shift of C-12 in **2** compared with those of inulanolide A. NOESY correlations (Fig. S1) of H-13′/H-8′ and H-14′ were observed. Other observed NOEs correlations suggested that **2** shared the same relative configuration with inulanolide A.

Dibritannilactone C (**3**) was obtained as white amorphous powder. Its molecular formula $C_{32}H_{44}O_8$ was established from its HRESIMS peak at *m/z* 579.2719 $[M + Na]^+$ (calcd for $C_{32}H_{45}O_8Na$, 579.2723), accounting for eleven degrees of unsaturation. The IR spectrum showed the presence of hydroxyl groups (3439 cm^{-1}), carbonyl groups (1762 cm^{-1}) and olefinic bonds (1630 cm^{-1}). The ^{1}H and ^{13}C NMR spectra of **3** were all comparable to those of **2** except for the presence of an hydroxyl group instead of the acetoxyl group which was attached to C-1 in **2** (Tables S1 and S2).

Table 1. ^{1}H and ^{13}C NMR data for compounds **1** and **2**

No.	1[a]		2[b]	
	δ_H	δ_C	δ_H	δ_C
1		139.9 s	4.00 m; 3.95 m	65.6 t
2	6.65 s	111.7 d	1.55 m; 1.35 m	28.2 t
3		158.1 s	1.33 m; 1.09 m	33.4 t
4		131.0 s	2.73 m	35.4 d
5	6.48 d (7.6)	125.2 d		138.1 s
6	6.61 d (7.6)	121.7 d	4.23 s	64.7 d
7	2.25 s	21.5 q	2.74 m	52.9 d
8		59.7 s	5.07 m	78.5 d
9	6.25 s	104.0 d	2.46 m; 2.42 m	35.1 t
10	2.86 dd (12.8, 4.1); 1.55 m	35.5 t		131.3s
11				56.5 s
12				181.3 s
13			2.08 m; 1.88 m	37.9 t
14			1.74 s	20.7 q
15			1.13 d (7.0)	20.0 q
1′		68.5 s		63.9 s
2′	4.58 s	84.7 d	4.52 brs	83.6 d
3′	2.75 m	47.3 d	2.93 d (1.2)	59.3 d
4′		139.2 s		134.4 s
5′		135.1 s		138.9 s
6′	2.48 brd (16.0)	24.5 t	2.72 m; 2.15 m	25.5 t
7′	0.58 m	41.8 d	2.41 m	44.3 d

8′	4.10 dt (11.5, 3.3)	80.8 d	4.57 dt (11.4, 3.6)	82.9 t
9′	2.00 m; 1.50 m	35.6 t	2.33 m; 1.88 m	37.3 t
10′	2.55 m	26.6 d	2.17 m	31.1 d
11′	2.20 m	39.9 d	2.20 m	41.8 d
12′		179.6 s		182.4 s
13′	1.05 d (7.8)	9.7 q	1.19 d (7.8)	10.1 q
14′	0.97 d (7.1)	19.5 q	1.06 d (7.3)	17.3 q
15′	1.88 s	13.4 q	1.58 d (1.0)	14.3 q
1″		170.3 s		172.2 s
2″	2.11 s	21.2 q	2.12 s	21.3 q
1‴				172.7 s
2‴			1.96 s	20.9 q

[a] Measured at 400 and 100 MHz respectively in $CDCl_3$;

[b] Measured at 400 and 100 MHz respectively in CD_3OD;

The ^1H and ^{13}C NMR spectra of dibritannilactone D (**4**) were also comparable to those of **2** except for the absence of the acetoxyl group which was attached to C-2′ in **2** (Tables S1 and S2).

The ^{13}C NMR and DEPT spectroscopic data of **5** were similar to those of a known sesquiterpene dimer, japonicone I,[8] except for the α-methylene lactone functionality (Tables 1 and 2). The absence of the $\Delta^{11,13}$ exocyclic methylene group was confirmed by the upfield shifts of C-11′ and C-13′ and the downfield shift of C-12 in **5**, compared with those of japonicone I. NOESY (Fig. S2) correlations of H-13′/H-8′ and H-14′ were also observed. Other observed NOEs correlations suggested that **5** shared the same relative configuration with japonicone I.

The relative configuration of C-4 in compounds **2-5** could not be determined due to the rotatory nature of side chains. However, as one of the monomeric sesquiterpenoids composing compounds **2-5**, britannilactone and 1-acetoxy-6α-hydroxyeriolanolide[7] are abundant constituents in *Inula britannica.* And their absolute configurations were previously assigned since their single crystals were obtained. We have reasons to deduce that C-4 of compounds **2-5** possesses identical configurations to those of britannilactone and 1-acetoxy-6α-hydroxyeriolanolide because they probably were produced through same biosynthesis pathway. Nevertheless, the possibility of the existence of enantiomers cannot be excluded. Therefore, this is tentative assignment because of the absence of direct evidence.

Compounds **1–8** and **10–11** were tested for their inhibitory effects against LPS-induced NO production in RAW264.7 macrophages with aminoguanidine as positive control. As shown in Table 2, compounds **6**, **7** and **10** exhibited significant inhibitory activities with IC_{50} values of 1.63, 2.07 and 3.80 μM, respectively. Whereas, compound **1–5** and **8** showed moderate inhibitory effects with IC_{50} values ranged from 10.86 to 49.44 μM.

Table 2. Inhibitory effects of compounds **1**–**8** and **10**–**11** against LPS-induced NO production in RAW264.7 macrophages

Compounds	IC_{50} (μM)[a]
1	14.60
2	43.77
3	49.44
4	25.08
5	29.18
6	1.63
7	2.07
8	>50
10	3.80
11	10.86
aminoguanidine[b]	7.90

[a] Inhibitory effects of compounds **1–8** and **10–11** against LPS-induced NO production in RAW264.7 macrophages; [b] Positive control

In summary, dibritannilactones A–E (**1–5**), including one new unusual sesterterpenoid (**1**), four new sesquiterpene dimers (**2**−**5**) together with nine known ones (**6–14**) were obtained from aerial parts of *I. britannica*. By comparing physical and spectroscopic data with those reported in literatures, structures of known compounds were identified as 6α-(2-methybutyryloxy)-deacetylinulicin (**6**),[9] 14-hydroxyinulicin (**7**),[9] eupatolide (**8**),[10] 3β-hydroxyivangustin (**9**),[9] 3α-hydroxyivangustin (**10**),[9] desacetyl-β-cyclopyrethrosin (**11**),[11] bigelovin (**12**),[12] 8-epi-helenali (**13**),[12] and aromaticin (**14**).[13] Compounds **1–8** and **10–11** were tested for their inhibitory effects against LPS-induced NO production in RAW264.7 macrophages and the result displayed that **6**, **7** and **10** exhibited significant inhibitory activities with IC_{50} values of 1.63, 2.07 and 3.80 μM, respectively. **1–5** and **8** showed moderate inhibitory effects with IC_{50} values ranged from 10.86 to 49.44 μM.

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Notes and references

[a] *School of Pharmacy, Shanghai Jiao Tong University, Shanghai 200240, P. R. China. E-mail: kimhz@sjtu.edu.cn; Tel: +86-21-34205989; Fax: +86-21-34205989*

[b] *The First Affiliated Hospital of Zhengzhou University, Zhengzhou, 450000, P. R. China*

[c] *School of Pharmacy, Second Military Medical University, Shanghai 200433, P. R. China. E-mail: wdzhangy@hotmail.com*

⊥ These authors contributed equally to this work.

† Electronic Supplementary Information (ESI) available: 1D and 2D NMR, MS, IR spectra and data for **1**–**5**, crystallographic data for **1** (CCDC 1025166), and detailed experimental procedures. See DOI: 10.1039/b000000x/

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