SHORT REPORT

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Two New Sesquiterpenoids from *Kalimeris shimadae*

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Abstract: Five sesquiterpenoids were isolated from *Kalimeris shimadae*, of which compounds **1** and **2** were undescribed guaiane-type and eudesmane-type sesquiterpenoids, named kalshinoids G (**1**) and H (**2**). Their structures and relative configurations were elucidated based on HR-MS, NMR and chemical calculations. The inhibitory activity of those sesquiterpenes against nitric oxide (NO) production were also evaluated.

Keywords: *Kalimeris shimadae*; sesquiterpenoids; chemical calculations.

1. Plant Source

The whole of *Kalimeris shimadae* (Kitam.) Kitam were collected in Hefei, Anhui Province, People′s Republic of China, in July 2016, and was identified by Prof. Qing-Shan Yang of Anhui University of Chinese Medicine. A voucher specimen (NO. 20160701) was deposited at Anhui University of Chinese Medicine

2. Previous Studies

Kalimeris shimadae (Kitam.) Kitam. is a perennial herb of the genus *Kalimeris* (Asteraceae), widely distributed in the central, eastern, and southeastern regions of China [1]. It is mainly used in the folk to treat colds, fever, and sore throat, etc. And the tender seedlings of *Kalimeris* plants are often eaten as wild vegetables, called “Ma Lan Tou” in Chinese [2]. The plants of the genus *Kalimeris* are rich in phenols, flavonoids, anthraquinones and terpenoids [3], which have significant biological activities such as antibacterial, anti-inflammatory and analgesic, anti-tumor, antioxidant, procoagulant, hypolipidemic and antiviral [4]. Our group has been engaged in the research of the genus *Kalimeris* for a long time, and systematically studied the chemical constituents and pharmacological activities of

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K. shimadae [1,5,6], *K. indica* [7] and *K. integrifolia* [8]. As part of our systematic search for anti-inflammatory sesquiterpenoids of *Kalimeris* plants, two new sesquiterpenoids, kalshinoids G (**1**) and H (**2**), together with three known compounds were isolated from the 80% EtOH extract of the whole plant of *K. shimadae* (Figure 1)**,** and their anti-inflammatory activities were also evaluated based on the instructions of the literatures [1,9].

3. Present Study

The whole of *K. shimadae* (20 kg) was pulverized and extracted with 80% EtOH under reflux three times (2 h×3). The filtrate was concentrated under vacuum to give a crude residue (3.2 Kg).The crude residue were subjected to passage over a silica gel column, eluted with a gradient of CH_2Cl_2−MeOH (from 1:0 to 0:1, v/v), to yield eleven major fractions (L1−L11). Fr. L6 (40 g) was applied to ODS gel column (MeOH-H_2O from 20:80 to 100:0) to obtain twenty-six further fractions (L6a−L6z). L6b (47.5 g) was performed exposing to a silica gel column chromatography and eluted with petroleum ether-EtOAc (from 50:1 to 1:1, v/v) to yield nine fractions (Fr. L6b1-L6b9). Fr.L6b4 (1.8 g) was purified using Sephadex LH-20 (MeOH), followed by semi-preparative HPLC (MeCN−H_2O, 18:82-32:68, 10mL/min), to afford **4** (1.9 mg, 25min), **3** (27.1 mg, 27min) and **5** (24.5 mg, 28min). Fr.L6g (1.5g) was purified using Sephadex LH-20 (MeOH) to afford ten further fractions (L6g1-L6g10), Fr.L6g6 (124 mg) was purified by semi-preparative HPLC (MeCN-H_2O, 25:75-40:60, 10mL/min) to afford **2** (2.6 mg, 20 min). Compound **1** (1.7 mg, 29 min) was obtained by semi-preparative HPLC (MeOH-H_2O, 65:35) from Fr.L6g8 (69 mg).

Kalshinoid G (**1**): colorless oil; $[\alpha]_D^{20}$ +18.4 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 206 (4.02), 285 (2.74) nm; ^{1}H and ^{13}C NMR data, see Table 1; HRESIMS *m/z* 287.1044 $[M + K]^+$ (calcd. for $C_{15}H_{20}O_3K$, 287.1044).

Kalshinoid H (**2**): colorless oil; $[\alpha]_D^{24}$ -85.6 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 200 (2.80) nm; ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 205 (-10.1) nm; ^{1}H and ^{13}C NMR data, see Table 1; HRESIMS *m/z* 277.1772 $[M + Na]^+$ (calcd. for $C_{15}H_{26}O_3Na$, 277.1774).

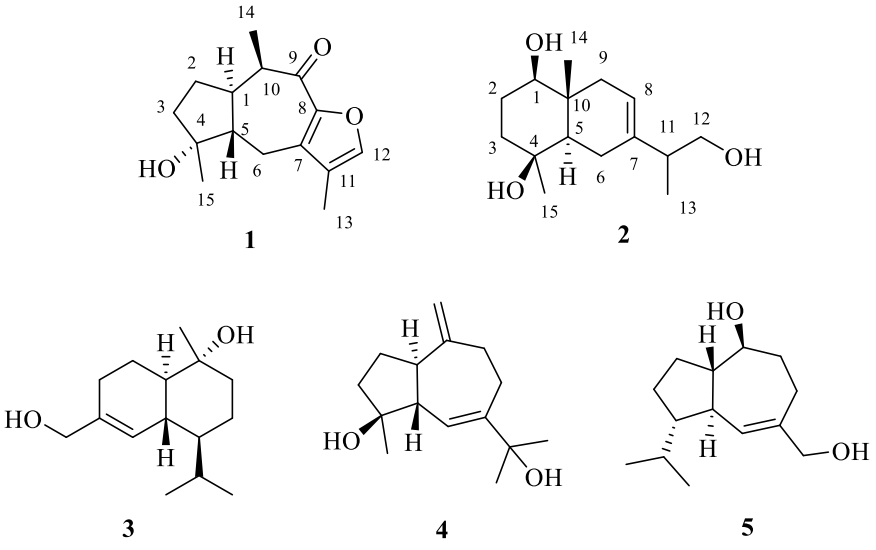

Figure 1. Structures of compounds **1-5**

Kalshinoid G (**1**) was obtained as a colorless oil and its molecular formula was determined to be $C_{15}H_{20}O_3$ on the basis of its HRESIMS data (*m/z* 287.1044 $[M + K]^+$, calcd for $C_{15}H_{20}O_3K$, 287.1044). The ^{1}H and ^{13}C NMR spectra of **1** displayed the presence of a *α,β*-unsaturated ketone carbonyl (δ_C 193.3), two double bonds (δ_H 7.38, s; δ_C 147.3, 144.0, 134.5, 122.9), a oxygenated

quaternary carbon (δ_C 81.5), and three methyl groups (δ_H 1.98, s; 1.40, s; 1.23, d, J = 7.4 Hz) (Table 1). In HMBC spectrum, the correlations from the uncommon olefinic proton (δ_H 7.38, s) to C-7 (δ_C 134.5), C-8 (δ_C 147.4), and C-11 (δ_C 122.9) indicated that the presence of a trisubstituted furan ring in **1**. Furthermore, one of the substituents was proved to be methyl by the HMBC correlations of Me-13 (δ_H 1.98, s) to C-7, C-11, and C-12 (δ_C 144.0). The above structural fragments suggests that **1** is a guaiane-type sesquiterpenoid with a structure similar to that of chlomultin A [10]. The main difference is that compound **1** is missing a carbonyl group and a pair of double bond signals. The ^{1}H-^{1}H COSY cross-peaks of H-1 (δ_H 2.62, d, J = 17.6 Hz)/ H-5 (δ_H 2.03, m)/ H-6 (δ_H 2.65, d, J = 17.9 Hz, 2.71, dd, J =17.7, 4.4 Hz) and the HMBC correlations of H-6 to C-4 (δ_C 81.5), C-7, and C-8, and Me-15 (δ_H 1.40, s) to C-4 and C-5 (δ_C 45.3) indicates that C-6 is a methylene group instead of a carbonyl group and there is a hydroxyl substitution at C-4 in **1** (Figure 2).

Table 1 ^{1}H NMR (600 MHz) and ^{13}C NMR (150 MHz) data of compounds **1** and **2** (J in Hz)

no.	**1**[a]		**2**[b]	
	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C
1	2.62, d (J = 17.6 Hz)	41.0	3.21, dd (J = 11.7, 3.6 Hz)	79.8
2α	1.48, m	27.2	1.45, m	27.8
2β	2.14, m		1.90, m	
3a	1.68, m	41.4	1.48, m	40.5
3b	1.77, ddd (J = 12.0, 8.0, 1.9 Hz)		1.69, dt (J = 7.8, 3.6 Hz)	
4		81.5		70.4
5	2.03, m	45.3	1.23, dd (J = 11.9, 5.0 Hz)	47.4
6α	2.65, d (J = 17.9 Hz)	24.3	2.04, m	24.0
6β	2.71, dd (J = 17.7, 4.4 Hz)		2.16, m	
7		134.5		139.8
8		147.3	5.30, d (J = 5.4 Hz)	119.6
9α		193.3	1.77, br d (J = 17.3 Hz)	41.7
9β			2.09, m	
10	2.84, ddd (J = 14.7, 7.3, 3.0 Hz)	48.2		38.6
11		122.9	2.21, m	44.5
12a	7.38, s	144.0	3.38, dd (J = 10.2, 7.5 Hz)	66.6
12b			3.54, dd (J = 10.2, 6.5 Hz)	
13	1.98, s	8.4	1.02, d (J = 7.0 Hz)	16.8
14	1.23, d (J = 7.4 Hz)	13.5	1.00, s	12.1
15	1.40, s	25.9	1.14, s	30.1

[a]measured in $CDCl_3$; [b] measured in acetone-d_6

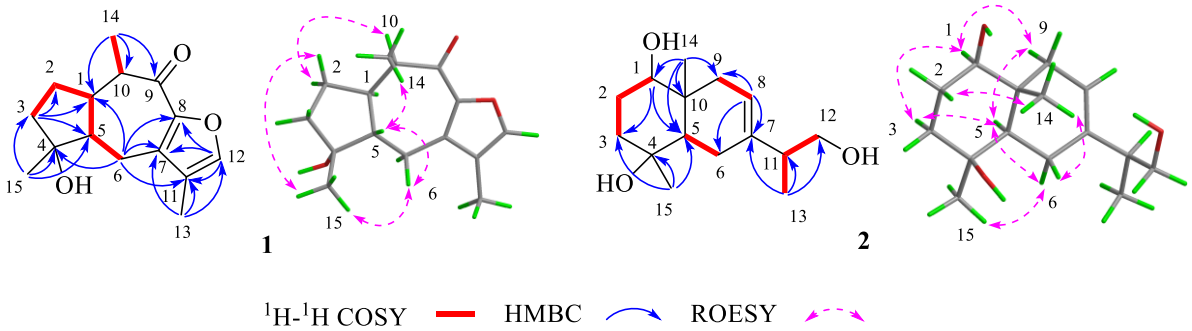

Figure 2. Key 2D NMR correlations of compounds **1** and **2**

In ROESY spectrum, the cross-peaks of Me-14/H-5/H-6/Me-15/H-2β suggested that these protons were on the same side, assigned as β-oriented. Similarly, H-10 was established as α-oriented

by the cross-peak of H-2*α*/H-10. Since the relative configuration of H-1 cannot be determined by ROESY spectrum, the NMR date of two possible epimers of **1** (1*R**-**1** and 1*S**-**1**) were calculated by the density functional theory (DFT). The calculated results were analyzed by DP4+, and the relative configuration of compound **1** was finally established as 1*R**,4*R**,5*S**,10*R** (Figure 3). Thus, the relative configuration of **1** was determined by ROESY spectrum and NMR calculations.

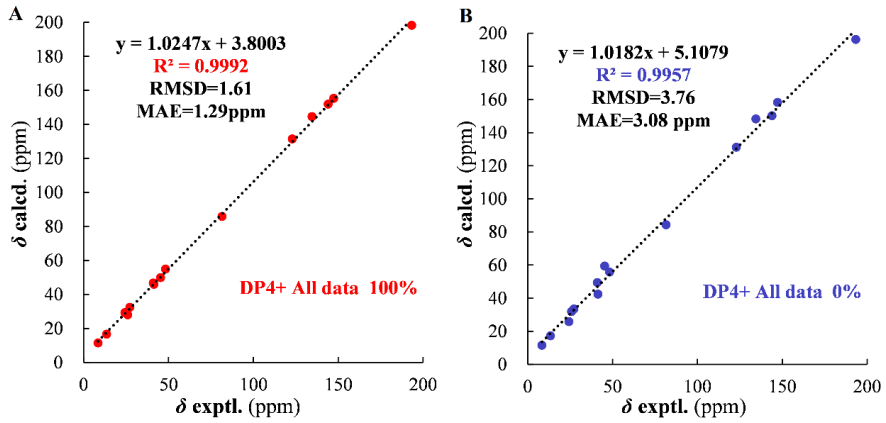

Figure 3. Correlations between experimental and calculated ^{13}C NMR chemical shifts of (1*R**)-**1** (**A**) and (1*S**)-**1** (**B**)

Kalshinoid H (**2**), a colorless oil, showed a quasimolecule peak at *m/z* 277.1772 $[M + Na]^+$ in the positive HRESIMS spectrum, corresponding to a molecular formula of $C_{15}H_{26}O_3$ with 3 double-bond equivalents. The ^{1}H and ^{13}C NMR data (Table 1) along with the HSQC spectrum showed the presence of two tertiary methyls (δ_H 1.14, s, 1.00, s; δ_C 30.1, 12.1), a secondary methyl (δ_H 1.02, d, J = 7.0 Hz; 16.8), five methylenes (one oxygenated, δ_H 3.38, dd, J = 10.2, 7.5 Hz, 3.54, dd, J = 10.2, 6.5 Hz, δ_C 66.6), four methines (one olefinic, δ_H 5.30, d, J = 5.4 Hz; δ_C 119.6, and one oxygenated, δ_H 3.21, dd, J = 11.7, 3.6 Hz; δ_C 79.8), and three quaternary carbons (one olefinic, δ_C 139.8, and one oxygenated, δ_C 70.4). The above data are very similar to those of known compound iwayoside C [11], a eucalyptane-type sesquiterpenoid glucoside, except that the absence of a glucosyl group in **2**. This can be determined by the significant upfield shifts of C-1 (δ_C 79.8, $\Delta\delta$ −7.1) and the HRESIMS data. The relative configuration of Me-14 was established as *β*-oriented by the ROESY correlation of H-6*β*/ H-14. Likewise, H-1, H-5, and H-15 were assigned as *α*-oriented by the observed cross-peaks of H-15/ H-6*α*/ H-5/ H-9*α*/ H-1 in ROESY spectrum (Figure 2). Therefore, the structure of **2** was identified as the aglycone of iwayoside C, named kalshinoid H.

Additionally, three known sesquiterpenoids were isolated and identified as 15-hydroxy-α-cadinol [12], 4*β*, 12-dihydroxyguaian-6, 10-diene [13], and 4-*epi*-isodauc-6-ene-10*β*,14-diol [14] by comparing their NMR data with references.

Based on our previous study on the anti-inflammatory sesquiterpenes in *K. shimadae* [1, 14], the inhibitory effects of the isolated sesquiterpenoids on NO release in LPS-stimulated RAW264.7 macrophage cells was evaluated. Unfortunately, the results indicated that none of these compounds have anti-inflammatory activity, showing IC_{50} values over 50 μM.

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Supporting Information

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