

## Germacranolides from *Calea urticifolia*

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### Abstract

Four germacranolides, named calealactones A–C and 2,3-epoxycalalactone A, were isolated from the leaves of *Calea urticifolia* (Compositae) in addition to three known germacranolides. Their structures were established on the basis of spectroscopic analysis including by 2D NMR spectroscopy. Calealactone C and 2,3-epoxyjuanisulamin displayed potent toxicity to U937 cells.

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**Keywords:** Germacranolides; *Calea urticifolia*; Calealactones; U937 cell; Toxicity

### 1. Introduction

*Calea urticifolia* (Miller) DC. (Compositae) has been used in folk medicine as a tropical bactericide as well as for treatment for gastric ulcers in El Salvador, where it is known to “juanislama”. Seven germacranolides of calein D, 2,3-epoxycalein D (Castillo et al., 1980), juanisamin, 2,3-epoxyjuanisamin (Castillo et al., 1981), 3, 10-epoxy-9-(3-methylbutanoyl)-8-(2-methylpropenoyl)-15-hydroxy-1-oxo-germacra-2,4,11(13)-trien-12,6-olide, 3,10-epoxy-9-(3-methyl-2-butenoyl)-8-(2-methylpropenoyl)-15-hydroxy-1-oxo-germacra-2,4,11(13)-trien-12,6-olide and 3,10-epoxy-9-angeloyl-8-(2-methylpropenoyl)-15-hydroxy-1-oxo-germacra-2,4,11(13)-trien-12,6-olide, five heiangolides, eight isoeugenol derivative and a phloroglucinol derivative were previously isolated from this medicinal plant (Bohlmann and Jakupovic, 1979). Sesquiterpene lactones containing germacranolides dis-

played various biological properties such as cytotoxic, anti-tumorigenic, anti-bacterial and anti-fungal activities (Pieman, 1986). In this study, a chemical analysis of the leaves of *C. urticifolia*, afforded four new germacranolides (1–4) in addition to three known germacranolides (5–7). Their structural characterization and the results of cytotoxicity against U937 cells of the isolated germacranolides are described in this paper.

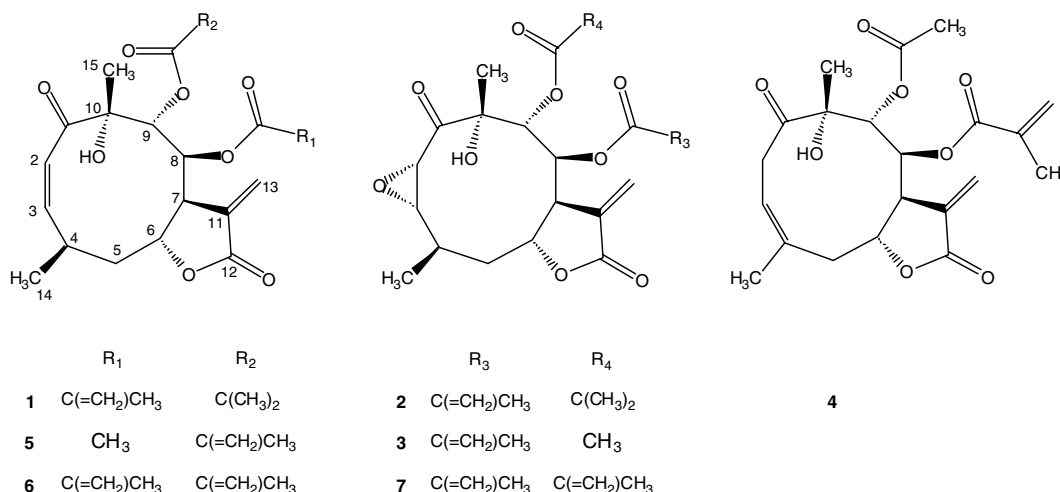
### 2. Results and discussion

By combination of chromatography on silica gel and preparative HPLC of an acetone extract of the leaves of *C. urticifolia*, seven germacranolides (1–7) were obtained.

Compounds 5, 6 and 7 were identified as calein D, juanisamin and 2,3-epoxyjuanisamin, respectively, which have been previously isolated from this plant (Castillo et al., 1981); their structures were established by comparison of MS, <sup>1</sup>H (Table 1) and <sup>13</sup>C (Table 2) NMR spectral data.

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Compound **1**, calealactone A, was isolated as colorless needles. Its empirical formula C<sub>23</sub>H<sub>30</sub>O<sub>8</sub> was determined by HR-MS at 434.1932 [M]<sup>+</sup> (requires 434.1941). In the EI-MS, three prominent fragment peaks at *m/z* 69 [CH<sub>3</sub>(H<sub>2</sub>C=)CCO]<sup>+</sup>, 71 [(CH<sub>3</sub>)<sub>2</sub>CHCO]<sup>+</sup> and 364 [M-C<sub>2</sub>H<sub>6</sub>C=C=O]<sup>+</sup> indicated the existence of 2-methylacryloyl and an isobutyryl groups. All signals in the <sup>1</sup>H and <sup>13</sup>C NMR spectra were assigned with the aid of DEPT, <sup>1</sup>H–<sup>1</sup>H COSY, HMQC and HMBC spectral analysis and by comparison of those with compounds **5** and **6**. Among the 23 carbons observed in the <sup>13</sup>C NMR spectrum, 15 carbons had similar chemical shift values to those of **5** and **6** (Table 2), and were assigned to the skeletal carbons of a germacranolide containing an α-methylene-γ-lactone moiety (C-1 to C-15). The carbon signals due to the two ester groups were observed at δ 166.6 (C-1'), 135.2 (C-2'), 127.6 (C-3') and 18.4 (C-4') for the 2-methylacryloyl, and at δ 176.8 (C-1''), 34.3 (C-2''), 19.0 (C-4'') and 19.3 (C-4'') for the isobutyryl group, respectively. All proton signals observed in the <sup>1</sup>H NMR spectrum were also found to have similar chemical shifts and *J* values to those of **5** and **6** (Table 2) except for the signals of two methyls (δ 1.01 and 1.08) and a methine (δ 2.46) group based on the isobutyrate group. The signals of H-8 and H-9 appeared at δ 5.58 as a singlet in CDCl<sub>3</sub>, owing to overlap of their centers in an AB-system, which made a designation of the positions of the isobutyryl and the 2-methylacryloyl groups impossible. When measured in benzene-*d*<sub>6</sub>, the signals were separated at δ 6.00 and 5.73 and could be assigned to H-8 and H-9 in the HMQC spectrum. On the other hand, H-8 and H-9 were correlated to each carbonyl carbon (δ 165.8 and δ 176.6) on the 2-methylacryloyl and the isobutyryl group elucidated in the HMBC spectrum. These results permitted the structure of calealactone A to be as **1**, which is a 8-(2-methylacryloyl)-9-isobutyryl derivative of calein D or juanisamin.

Compound **2**, 2,3-epoxycalactone A, was obtained as colorless needles. Its empirical formula C<sub>23</sub>H<sub>30</sub>O<sub>9</sub> was determined by HR-MS analysis (450.1881 [M]<sup>+</sup>, requires 450.1890). The fragment pattern of the EI-MS was similar to that of **1**. The <sup>1</sup>H NMR spectrum was also closely similar to that of **1**, but two olefinic signals based on H-2 and H-3 in **1** appeared at δ 4.27 (1H, *d*, *J* = 4.1 Hz) and δ 3.35 (1H, *dd*, *J* = 4.1 and 9.4 Hz) in **2**, the pattern of which corresponds to oxiranic protons, i.e. the olefinic moiety at C-2 and C-3 in **1** was concluded to be substituted with an epoxide in **2**. In the <sup>13</sup>C NMR spectrum, the olefinic carbon signals at C-2 and C-3 in **1** were observed at δ 55.7 (*d*, *J* = 180 Hz) and 62.7 (*brd d*, *J* = 184 Hz) in **2**, which supported further C-2 and C-3 were oxiranic carbons. The structure of **2** was concluded to be 2,3-epoxycalactone A.

Compound **3**, calealactone B, ([M]<sup>+</sup> at *m/z* 422) was obtained as a white powder. In the EI-MS, the [M]<sup>+</sup> was observed at *m/z* 422 with the prominent fragments at *m/z* 380 and *m/z* 69 that indicated the existence of acetyl ([M]<sup>+</sup>-42) and a 2-methylacryloyl ([CH<sub>3</sub>(H<sub>2</sub>C=)CCO]<sup>+</sup>) groups. In the <sup>1</sup>H and <sup>13</sup>C NMR spectra, the signals were generally the same as those of **2**, except for signals corresponding to an acetyl, instead of an isobutyryl group. In the HMBC spectrum, a correlation was observed between H-8 and the carbonyl carbon of the 2-methylacryloyl group. These results indicated that calealactone B (**3**) was a 9-acetyl derivative of **2**.

Compound **4**, calealactone C, was obtained as colorless needles. Its empirical formula C<sub>21</sub>H<sub>26</sub>O<sub>8</sub> was determined by HR-MS: 406.1621 [M]<sup>+</sup>, requires 406.1627. In its EI-MS, the fragments showed the existence of acetyl (*m/z* 364; [M]<sup>+</sup>-42) and 2-methylacryloyl (*m/z* 336 and 69; [M]<sup>+</sup>-70) groups. Analysis of the <sup>1</sup>H and <sup>13</sup>C NMR, DEPT, HMQC, HMBC spectra also revealed the existence of acetyl and 2-methylacryloyl groups as well as an α-methylene-γ-lactone moiety. The main

Table 1  
<sup>1</sup>H NMR (400 MHz) spectral data in CDCl<sub>3</sub> for compounds 1–7

No.	1	1 <sup>a</sup>	2	3	4	5	6	7
2	5.95 ( <i>dd</i> , 120, 11.5, 1H)	4.95 ( <i>dd</i> , 11.8, 11.5, 1H)	4.27 ( <i>d</i> , 4.1, 1H)	4.21 ( <i>d</i> , 4.1, 1H)	3.08 ( <i>dd</i> , 16.0, 8.0, 1H)	6.50 ( <i>d</i> , 12.0, 1H)	6.03 ( <i>dd</i> , 12.0, 11.5, 1H)	4.30 ( <i>d</i> , 4.4, 1H)
3	6.54 ( <i>d</i> , 120, 1H)	6.02 ( <i>d</i> , 11.8, 1H)	3.35 ( <i>dd</i> , 9.4, 4.1, 1H)	3.30 ( <i>dd</i> , 9.4, 4.3, 1H)	3.60 ( <i>dd</i> , 16.0, 9.5, 1H) 5.92 (8.0, 9.5, 1H)	5.98 ( <i>dd</i> , 12.0, 12.0, 1H)	6.64 ( <i>d</i> , 12.0, 1H)	3.34 ( <i>dd</i> , 9.4, 4.3, 1H)
4	3.06 ( <i>m</i> , 1H)	2.80 ( <i>m</i> , 1H)	1.45 <sup>b</sup>	1.45 <sup>b</sup>		3.05 ( <i>m</i> , 1H)	3.13 ( <i>m</i> , 1H)	1.45 <sup>b</sup>
5	138 ( <i>m</i> , 1H)	0.95 ( <i>m</i> , 1H)	1.51 ( <i>m</i> , 1H)	1.46 ( <i>m</i> , 1H)	2.77 ( <i>dd</i> , 15.0, 4.0, 1H)	1.38 ( <i>m</i> , 1H)	1.45 ( <i>m</i> , 1H)	1.50 ( <i>m</i> , 1H)
	1.79 ( <i>m</i> , 1H)	1.25 ( <i>m</i> , 1H)	1.95 ( <i>m</i> , 1H)	1.90 ( <i>m</i> , 1H)	2.86 ( <i>dd</i> , 15.0, 3.6, 1H)	1.80 ( <i>m</i> , 1H)	1.83 ( <i>m</i> , 1H)	1.96 ( <i>m</i> , 1H)
6	4.55 ( <i>dd</i> , 11.7, 4.5, 1H)	4.38 ( <i>dd</i> , 11.7, 4.9, 1H)	4.86 ( <i>dd</i> , 12.1, 4.3, 1H)	4.80 ( <i>dd</i> , 12.2, 4.5, 1H)	4.95 ( <i>m</i> , 1H)	4.55 ( <i>dd</i> , 11.7, 4.9, 1H)	4.63 ( <i>dd</i> , 12.0, 4.9, 1H)	4.84 ( <i>dd</i> , 12.1, 4.5, 1H)
7	2.56 ( <i>s</i> , 1H)	2.48 ( <i>br s</i> , 1H)	2.39 ( <i>br s</i> , 1H)	2.34 ( <i>br s</i> , 1H)	2.68 ( <i>m</i> , 1H)	2.60 ( <i>s</i> , 1H)	2.66 ( <i>s</i> , 1H)	2.38 ( <i>br s</i> , 1H)
8	5.58 ( <i>s</i> , 1H)	6.00 ( <i>dd</i> , 9.8, 2.1, 1H)	5.72 ( <i>dd</i> , 9.9, 1.7, 1H)	5.66 ( <i>dd</i> , 10, 1.7, 1H)	5.90 ( <i>dd</i> , 10.5, 1.5, 1H)	5.55 ( <i>s</i> , 1H)	5.69 ( <i>s</i> , 1H)	5.75 ( <i>dd</i> , 9.7, 2.0, 1H)
9	5.58 ( <i>s</i> , 1H)	5.73 ( <i>d</i> , 9.8, 1H)	5.82 ( <i>d</i> , 9.9, 1H)	5.73 ( <i>d</i> , 10, 1H)	5.70 ( <i>d</i> , 8.1, 1H)	5.55 ( <i>s</i> , 1H)	5.69 ( <i>s</i> , 1H)	5.85 ( <i>d</i> , 12.0, 1H)
13	5.76 ( <i>d</i> , 1.2, 1H)	5.25 ( <i>d</i> , 1.5, 1H)	5.85 ( <i>d</i> , 1.0, 1H)	5.81 ( <i>m</i> , 1H)	5.72 ( <i>s</i> , 1H)	5.80 ( <i>d</i> , 1.1, 1H)	5.84 ( <i>d</i> , 1.2, 1H)	5.84 ( <i>s</i> , 1H)
	6.25 ( <i>br s</i> , 1H)	6.18 ( <i>d</i> , 1.0, 1H)	6.34 ( <i>s</i> , 1H)	6.31 ( <i>m</i> , 1H)	6.30 ( <i>d</i> , 3.4, 1H)	6.25 ( <i>d</i> , 11, 1H)	6.33 ( <i>s</i> , 1H)	6.34 ( <i>s</i> , 1H)
14	1.08 ( <i>d</i> , 7.1, 3H)	0.58 ( <i>d</i> , 6.3, 3H)	1.21 ( <i>d</i> , 6.1, 3H)	1.19 ( <i>d</i> , 7.1, 3H)	1.86 ( <i>s</i> , 3H)	1.05 ( <i>d</i> , 6.3, 3H)	1.15 ( <i>d</i> , 6.3, 3H)	1.23 ( <i>d</i> , 6.1, 3H)
15	1.25 ( <i>s</i> , 3H)	1.09 ( <i>s</i> , 3H)		1.45 ( <i>s</i> , 3H)	1.35 ( <i>s</i> , 3H)	1.30 ( <i>s</i> , 3H)	1.34 ( <i>s</i> , 3H)	1.46 ( <i>s</i> , 3H)
Acetyl								
2'						1.95 ( <i>s</i> , 3H)		
2-Methylacryloyl								
3'	5.47 ( <i>br s</i> , 1H)	5.19 ( <i>m</i> , 1H)	5.58 ( <i>m</i> , 1H)	5.56 ( <i>m</i> , 1H)	5.60 ( <i>m</i> , 1H)	5.45 ( <i>s</i> , 1H)	5.61 ( <i>br s</i> , 1H)	5.51 ( <i>m</i> , 1H)
	5.95 ( <i>s</i> , 1H)	6.23 ( <i>br s</i> , 1H)	6.05 ( <i>s</i> , 1H)	6.03 ( <i>m</i> , 1H)	6.00 ( <i>m</i> , 1H)	5.95 ( <i>s</i> , 1H)	6.13 ( <i>s</i> , 1H)	5.97 ( <i>s</i> , 1H)
4'	1.74 ( <i>s</i> , 3H)	1.83 ( <i>br s</i> , 3H)	1.83 ( <i>s</i> , 3H)	1.83 ( <i>br s</i> , 1H)	1.87 ( <i>s</i> , 3H)	1.78 ( <i>s</i> , 3H)	1.88 ( <i>s</i> , 3H)	1.78 ( <i>s</i> , 3H)
Acetyl								
2''				2.03 ( <i>s</i> , 3H)	2.06 ( <i>s</i> , 3H)			
2-Methylacryloyl								
3''							5.48 ( <i>br s</i> , 1H)	5.66 ( <i>m</i> , 1H)
							5.94 (1, 1H)	6.16 ( <i>s</i> , 1H)
4''							1.76 (1, 3H)	1.90 ( <i>s</i> , 3H)
iBu								
2'	2.46 ( <i>dq</i> , 7.1, 7.1, 1H)	2.34 ( <i>dq</i> , 7.1, 7.1, 1H)	2.57 ( <i>dq</i> , 7.1, 7.1, 1H)					
3''	1.01 ( <i>d</i> , 7.1, 3H)	0.98 ( <i>d</i> , 7.1, 3H)	1.10 ( <i>d</i> , 7.1, 3H)					
4''	1.08 ( <i>d</i> , 7.1, 3H)	0.98 ( <i>d</i> , 7.1, 3H)	1.16 ( <i>d</i> , 7.1, 3H)					
10-OH	4.04 ( <i>br s</i> , 1H)		4.05 ( <i>br s</i> , 1H)	3.89 ( <i>br s</i> , 1H)	4.28 ( <i>br s</i> , 1H)		4.13 ( <i>br s</i> , 1H)	

<sup>a</sup> Measured in benzene-*d*<sub>6</sub>.

<sup>b</sup> Assigned by <sup>1</sup>H–<sup>1</sup>H COSY spectrum.

Table 2  
<sup>13</sup>C (100 MHz) NMR spectral data in CDCl<sub>3</sub> for compounds 1–7

	1	1 <sup>a</sup>	2	3	4	5	6	7
C-1	205.1	204.9	206.0	206.0	210.7	204.7	204.9	206.1
2	148.4	148.2	55.7	55.6	36.0	148.2	148.1	55.7
3	125.7	125.5	62.7	62.8	121.5	125.3	125.4	62.8
4	28.6	28.3	25.9	26.0	136.6	28.3	28.3	26.0
5	40.6	40.5	38.7	38.8	35.9	40.2	40.3	38.8
6	76.4	76.2	74.7	74.6	76.6	76.3	76.4	74.7
7	41.7	41.9	40.9	40.9	42.0	41.2	41.2	40.9
8	74.3	75.1	73.8	73.9	67.0	74.4	74.3	73.8
9	73.2	73.9	70.8	71.5	72.5	73.8	74.0	71.7
10	79.7	79.8	79.8	79.7	80.3	79.2	79.4	79.9
11	134.9	135.6	134.2	134.3	134.1	134.5	134.6	134.3
12	169.1	168.6	168.0	168.3	168.1	168.7	168.8	168.4
13	127.0	126.0	126.8	126.8	124.4	126.6	126.5	126.7
14	20.0	19.6	18.5	18.6	25.4	19.7	19.7	18.6
15	23.9	23.8	24.4	24.5	22.3	23.4	23.6	24.6
1'	165.6	165.8	165.0	165.2	165.2	170.3	166.6	165.4
2'	135.2	135.9	134.8	134.8	135.1	20.3	134.7	134.8
3'	127.6	127.4	127.3	127.3	126.9		127.8	127.0
4'	18.4	18.4	18.0	18.1	18.1		18.0	18.0
1''	176.8	176.6	176.0	170.4	170.4	165.3	165.4	166.7
2''	34.3	34.4	33.9	20.2	20.4	134.9	134.9	134.5
3''	19.0	19.0	18.6			127.1	126.9	128.2
4''	19.3	19.3	18.8			18.0	18.1	18.0

<sup>a</sup> Measured in benzene-*d*<sub>6</sub>.

differences from **3** were the following three <sup>1</sup>H NMR signals: (1) H-2 [ $\delta$  3.08 (*dd*, *J* = 16.0, 8.0 Hz) and 3.60 (*dd*, *J* = 16.0, 9.5 Hz)] and H-3 [ $\delta$  5.92 (*dd*, *J* = 9.5 and 8.0 Hz)] which were part of an ABX system, (2) H-5 at  $\delta$  2.77 (*dd*, *J* = 15.0 and 4.0 Hz) and 2.86 (*dd*, *J* = 15.0 and 3.6 Hz), and (3) the methyl group at C-4 which was observed as a broad singlet at  $\delta$  1.86 (*br s*). These results were almost the same as for lobain A (Castillo et al., 1982) and indicated an olefinic moiety was located at C-3 and C-4. The positions of the acetyl and the 2-methylacryloyl groups were confirmed to be at C-8 ( $\delta$  67.0) and C-9 ( $\delta$  82.5), respectively, as the HMBC spectrum showed correlations between H-8 and a carbonyl carbon of an acetyl group, and H-9 and a carbonyl group of the 2-methylacryloyl group. Thus the structure of calealactone C was characterized as **4**.

Cytotoxic activity of the seven isolated germacranolides and parthenolide was assessed against human leukemia U937 cells. The viability results at concentra-

tion of 50% are shown in Table 3. Calealactone C (**4**) and 2,3-epoxyjuanisulamin (**7**) revealed more potent cytotoxic activity than parthenolide. The mechanism of cytotoxicity is now being investigated.

### 3. Experimental

#### 3.1. Plant material

Leaves of *C. urticifolia* (Miller) DC. were purchased at a medicinal herb market in San Salvador in 1998, and identified morphologically by one of the co-author, M. Inuma. A voucher specimen is kept at Gifu Pharmaceutical University (No. EL-091).

#### 3.2. Extraction and isolation

Air-dried leaves (170 g) of *C. urticifolia* were extracted with acetone 3 L for two times under conditions of reflux to obtain a crude extract (9.8 g). The latter was suspended in H<sub>2</sub>O (1 L) and extracted with CHCl<sub>3</sub> (3  $\times$  1 L), and the resulting concentrated CHCl<sub>3</sub> solubles (7.1 g) were subjected to silica CC eluted with a CHCl<sub>3</sub>–MeOH solvent system (ratio of 30:1 to 10:1 CHCl<sub>3</sub>:MeOH to give 10 fractions). Fraction 2 (2.1 g) was separated by reversed-phase Si gel CC eluted with MeOH–H<sub>2</sub>O (4:6 and 65:35, respectively). The MeOH–H<sub>2</sub>O (65:35) eluent was concentrated to give a syrup what was then further purified by reversed phase HPLC (Mightysil RP-18 250-20, Kanto Chemical Co., Inc., Japan); HPLC conditions: flow rate: 11.3 ml/min, detector: 210 nm, solvent: CH<sub>3</sub>CN–H<sub>2</sub>O (42:58), column oven temperature: 40 °C, to give compounds **1**–**7** as follows: **1**; 52 mg, **2**; 91 mg, **3**; 30 mg, **4**; 28 mg, **5**; 173 mg, **6**; 138 mg and **7**; 195 mg.

#### 3.3. Calealactone A (**1**)

Colorless needles; mp 98–100 °C; [ $\alpha$ ]<sub>D</sub><sup>20</sup> + 195.4 (CHCl<sub>3</sub>, *c*, 0.001); EI-MS *m/z* (rel. int.): 434 [*M*]<sup>+</sup> (21), 416 (16), 406 (6), 364 (59), 346 (58), 278 (83), 71 (99), 69 (100); HREIMS *m/z* 434.1932 (calcd for C<sub>23</sub>H<sub>30</sub>O<sub>8</sub>, 434.1941); For <sup>1</sup>H and <sup>13</sup>C NMR spectral data; see Tables 1 and 2, respectively.

#### 3.4. 2,3-Epoxycalealactone A (**2**)

Colorless needles; mp 99–101 °C; [ $\alpha$ ]<sub>D</sub><sup>20</sup> + 168.7 (CHCl<sub>3</sub>, *c*, 0.001); EI-MS *m/z* (rel. int.): 450 [*M*]<sup>+</sup> (24), 432 (6), 422 (18), 380 (12), 365 (22), 362 (18), 321 (14), 320 (13), 294 (26), 276 (42), 71 (99), 69 (100); HREIMS *m/z* 450.1881 (calcd for C<sub>23</sub>H<sub>30</sub>O<sub>9</sub>, 450.1890); For <sup>1</sup>H and <sup>13</sup>C NMR spectral data; see Tables 1 and 2, respectively.

Table 3  
 Cytotoxicity of sesquiterpenoid compounds against U937 cells

Compound	50% Viability ( $\mu$ M)
1	3.5
2	>5
3	>5
4	1.0
5	>5
6	3.0
7	1.8
Parthenolide	1.9

### 3.5. *Calealactone B* (3)

White powder;  $[\alpha]_D^{20} + 184.2$  (CHCl<sub>3</sub>, *c*, 0.001); EI-MS *m/z* (rel. int.): 422 [M]<sup>+</sup> (1), 380 (1), 224 (30), 206 (13), 164 (18), 69 (100), 44 (31); For <sup>1</sup>H and <sup>13</sup>C NMR spectral data; see [Tables 1 and 2](#), respectively.

### 3.6. *Calealactone C* (4)

Colorless needles; mp 170–172 °C;  $[\alpha]_D^{20} + 92.1$  (CHCl<sub>3</sub>, *c*, 0.001); EI-MS *m/z* (rel. int.): 406 [M]<sup>+</sup> (5), 378 (45), 364 (54), 336 (70), 292 (98), 69 (100); HREIMS *m/z* 406.1621 (calcd for C<sub>21</sub>H<sub>26</sub>O<sub>8</sub>, 406.1627); For <sup>1</sup>H and <sup>13</sup>C NMR data; see [Tables 1 and 2](#), respectively.

### 3.7. *Calein D* (5)

White powder;  $[\alpha]_D^{20} + 192.2$  (CHCl<sub>3</sub>, *c*, 0.001); EI-MS *m/z* (rel. int.): 406 [M]<sup>+</sup> (13), 64 (67), 346 (76), 337 (5), 278 (100), 69 (95); For <sup>1</sup>H and <sup>13</sup>C NMR spectral data; see [Tables 1 and 2](#), respectively.

### 3.8. *Juanislamin* (6)

White powder; EI-MS *m/z* (rel. int.): 432 [M] (2), 404 (3), 363 (1), 346 (4), 260 (7), 217 (7), 149 (22), 69 (100); For <sup>1</sup>H and <sup>13</sup>C NMR spectral data; see [Tables 1 and 2](#), respectively.

### 3.9. *2,3-Epoxyjuanislamin* (7)

White powder;  $[\alpha]_D^{20} + 154.0$  (CHCl<sub>3</sub>, *c*, 0.001); EI-MS *m/z* (rel. int.): 448 [M]<sup>+</sup> (1), 42 (3), 206 (10), 101 (33), 69 (100); For <sup>1</sup>H and <sup>13</sup>C NMR spectral data; see [Tables 1 and 2](#), respectively.

### 3.10. *Bioactivity*

U937 cells were purchased from Riken cell bank and maintained with RPMI medium containing 10% fetal bovine serum at 37 °C in 5% CO<sub>2</sub>. Cytotoxicity was assessed by the MTT method ([Mosmann, 1983](#)). Briefly U937 cells were plating on 96-well cell-culture plate at  $1.4 \times 10^4$  cells/well before 24 h adding test samples. MTT solution (10 μl) was added into each well and incubated at 37 °C for 4 h. 100 μl of isopropanol containing 0.04 N HCl was added to each well, and absorbance at 530 nm of each well was measured and viability was calculated. Parthenolide was purchased from Biomol Research Labs., Inc., USA.

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