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Cytotoxic Sesquiterpene Lactones from *Saussurea calcicola*

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Seven sesquiterpene lactones were isolated by the chromatographic separation of the MeOH extract of the aerial parts of *Saussurea calcicola* (Compositae). Their structures were determined spectroscopically to be cynaropicrin (**1**), arguerin B (**2**), cebellin F (**3**), 8α-hydroxy-11α,13-dihydrozaluzanin C (**4**), desacylcynaropicrin (**5**), 3β-hydroxy-8α-epoxymethylacriloiloxy-4(15),10(14),11(13)-trien-guaian-6,12-olide (**6**), and kandavanolide (**7**). Compounds **1** and **2** showed significant cytotoxicity against five cultured human tumor cell lines with ED_{50} values ranging from 0.23~1.72 μg/mL.

Key words: *Saussurea calcicola*, Compositae, Sesquiterpene lactone, Cytotoxicity

INTRODUCTION

Saussurea calcicola (Compositae) is a perennial herb that is mainly distributed in the mountains of South Korea. Sesquiterpenes, lignans and flavonoids have been reported from the genus *Saussurea* (Chhabra *et al.*, 1998; Cho *et al.*, 2000; Dai *et al.*, 2001; Matsuda *et al.*, 2000). *S. calcicola* is used in Chinese traditional medicine to treat epigastric or abdominal pain, distension and vomiting (Bensky and Gamble, 1986). However, there has been little or no research reported on this plant. As part of an ongoing investigation of the genus *Saussurea* of Korean Compositae plants, this study examined the constituents of *S. calcicola*. Seven sesquiterpene lactones were isolated from the hexane and CH_2Cl_2 soluble fractions of the MeOH extract. The compounds isolated were examined for their cytotoxicity against five human tumor cell lines *in vitro* using a SRB assay. This paper describes the isolation, structural characterization and cytotoxicity of the isolated compounds.

MATERIALS AND METHODS

General experimental procedure

Optical rotation: Jasco P-1020 Polarimeter. NMR: Bruker AMX 500 and Varian Unity Inova 500. IR: in $CHCl_3$, Nicolet model 205 FT-IR spectrophotometer. MS: VG70-VSEQ mass spectrometer. Column chromatography : Silica gel 60 (Merck, 70~230 mesh and 230~400 mesh), Lichroprep. RP-18 (Merck) and Sephadex LH-20. TLC: Merck precoated Si gel F_{254} plates and RP-18 F_{254s} plates. LPLC: Merck Lichroprep Lobar®-A Si 60 (240×10 mm).

Plant materials

The aerial parts of *S. calcicola* (Compositae) were collected at Gangwon province in August, 2001. A voucher specimen (SKK-01-015) was deposited at the herbarium of the College of Pharmacy in Sungkyunkwan University.

Cytotoxicity testing

The cytotoxicity of the compounds at several concentrations was examined using a Sulforhodamin B Bioassay (SRB) against the following five cultured human tumor cells: A549 (non small cell lung adenocarcinoma), SK-OV-3 (ovarian), SK-MEL-2 (skin melanoma), XF498 (CNS), and HCT15 (colon) (Skehan *et al.*, 1990).

Extraction, separation, and purification of compounds

The dried and chopped aerial parts of *Saussurea calcicola* (2 kg) were extracted five times with MeOH (10 L) at room temperature. The resulting methanol extract (200 g) was suspended in distilled water and partitioned successively to give *n*-hexane (30 g), CH_2Cl_2 (10 g), EtOAc (10 g), and BuOH (20 g). The *n*-hexane extract (30

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g) was chromatographed over a silica gel column using a gradient solvent system of *n*-hexane:EtOAc (5:1~0:1) to give 7 fractions (SC-1~SC-7). The fraction, SC-6 (2.5 g) was chromatographed over a silica gel column eluted with *n*-hexane:EtOAc (1:1) to give two subfractions (SC-61 and SC-62). Subfraction SC-62 (1.6 g) was purified with a Sephadex LH-20 (CH_2Cl_2:MeOH =1:1) and Lobar®-A column (*n*-hexane:EtOAc=1:1) to yield compounds **1** (20 mg) and **2** (10 mg). Fraction SC-7 (7.0 g) was chromatographed on a silica gel column eluted with $CHCl_3$:MeOH (10:1) to give four subfractions (SC-71~SC-74). Subfraction SC-71 (1.0 g) was purified in sequence using Sephadex LH-20 (CH_2Cl_2:MeOH=1:1) and RP Lobar®-A column (50% MeCN) to yield compound **3** (20 mg). Subfraction SC-73 (2 g) was repeatedly purified in sequence using a Sephadex LH-20 (CH_2Cl_2:MeOH=1:1) and Lobar®-A column (*n*-hexane:EtOAc=1:2) to yield compound **4** (10 mg). The CH_2Cl_2 extract (10 g) was chromatographed over a silica gel column using a CH_2Cl_2:MeOH solvent system (20:1) to give six fractions (SM-1~SM-6). Fraction SM-1 (2.4 g) was repeatedly chromatographed over a silica gel column eluted with CH_2Cl_2:MeOH (20:1~10:1) and purified with an RP Lobar®-A column (50% MeOH and 70% MeCN) to yield compounds **5** (12 mg) and **6** (8 mg). Fraction SM-2 (500 mg) was in turn purified in sequence using a Sephadex LH-20 (CH_2Cl_2:MeOH=1:1) and RP Lobar®-A column (50% MeOH) to yield compound **7** (6 mg).

Table I. ^{13}C-NMR spectral data of compounds **1~7** ($CDCl_3$, 125 MHz, δ ppm)

C	1	2	3	4	5	6	7
1	45.3	45.3	45.1	44.2	45.2	45.3	45.4
2	39.1	39.1	38.9	39.0	39.2	38.3	39.1
3	73.7	73.8	73.6	73.6	73.7	73.2	78.6
4	152.2	152.4	152.1	153.0	152.4	151.8	152.4
5	51.4	51.4	51.2	50.7	51.3	51.5	51.3
6	78.6	78.6	78.6	79.1	79.0	78.1	73.8
7	47.6	47.7	47.5	56.0	51.0	46.9	47.4
8	74.3	74.1	74.1	74.9	71.9	75.2	74.0
9	37.0	37.2	36.9	44.8	41.3	35.6	37.5
10	141.8	141.9	141.7	143.2	142.7	141.6	141.8
11	137.4	137.5	137.3	42.0	138.1	136.8	137.4
12	169.2	169.2	169.2	178.6	169.9	169.5	169.1
13	122.7	122.6	122.7	15.9	123.2	123.6	122.5
14	118.2	118.5	118.1	116.2	117.1	118.1	118.1
15	113.5	113.5	113.4	112.0	113.2	113.8	113.5
1'	165.4	166.4	166.2			174.8	170.1
2'	139.4	136.1	127.7			76.0	21.0
3'	126.6	126.6	141.9			68.1	
4'	62.1	18.3	59.7			21.6	
5'			12.7				

Cynaropicrin (1)

Colorless oil; $[\alpha]_D$ +150.7° (c 0.18, $CHCl_3$); IR ($CHCl_3$) ν_{max}^{neat} cm^{-1} ν = 3450, 3084, 2923, 1772, 1721, 1652 cm^{-1}; EIMS *m/z* (ret. Int.) : 346 (M^+, 5), 262 (12), 244 (36), 226 (23), 216 (13), 195 (15), 148 (25), 91 (49), 85 (100); ^{1}H-NMR ($CDCl_3$, 500 MHz) : δ 1.74 (1H, br. ddd, *J* = 7.5, 10.5, 13.5 Hz, H-2), 2.24 (1H, br. ddd, *J* = 7.5, 7.5, 13.5 Hz, H-2), 2.40 (1H, dd, *J* = 4.0, 14.5 Hz, H-9), 2.72 (1H, dd, *J* = 5.5, 14.5 Hz, H-9), 2.85 (1H, br.t, *J* = 9.0 Hz, H-5), 2.98 (1H, br. ddd, *J* = 7.5, 9.0, 10.5 Hz, H-1), 3.20 (1H, br. dddd, *J* = 3.0, 3.5, 9.0, 9.5 Hz, H-7), 4.27 (1H, dd, *J* = 9.0, 10.5 Hz, H-6), 4.39 (1H, br. s, H-4'), 4.56 (1H, br. t, *J* = 7.5 Hz, H-3), 4.94 (1H, br. s, H-14), 5.14 (1H, br. ddd, *J* = 4.0, 5.5, 9.5 Hz, H-8), 5.15 (1H, br. s, H-14), 5.37 (1H, br. d, *J* = 1.5 Hz, H-15), 5.49 (1H, br. d, *J* = 1.5 Hz, H-15), 5.63 (1H, d, *J* = 3.0 Hz, H-13), 5.97 (1H, br. s, H-3'), 6.24 (1H, d, *J* = 3.5 Hz, H-13), 6.34 (1H, br. s, H-3'); ^{13}C-NMR ($CDCl_3$, 125 MHz) : Table I.

Arguerin B (2)

Colorless oil; $[\alpha]_D$ +121.6° (c 0.14, $CHCl_3$); IR ($CHCl_3$) ν_{max}^{neat} cm^{-1} ν = 3507, 3080, 2931, 1770, 1725, and 1653 cm^{-1}; EIMS *m/z* (ret. Int.) : 330 (M^+, 23), 261 (6), 244 (75), 226 (54), 216 (34), 195 (27), 148 (38), 119 (32), 69 (100); ^{1}H-NMR ($CDCl_3$, 500 MHz) : δ 1.73 (1H, br. ddd, *J* = 7.5, 10.5, 13.5 Hz, H-2), 2.00 (3H, br. s, H-4'), 2.24 (1H, br. ddd, *J* = 7.5, 7.5, 13.5 Hz, H-2), 2.38 (1H, dd, *J* = 4.0, 14.5 Hz, H-9), 2.70 (1H, dd, *J* = 5.5, 14.5 Hz, H-9), 2.85 (1H, br. t, *J* = 9.0 Hz, H-5), 2.98 (1H, br. ddd, *J* = 7.5, 9.0, 10.5 Hz, H-1), 3.19 (1H, br. dddd, *J* = 3.0, 3.5, 9.0, 9.5 Hz, H-7), 4.25 (1H, dd, *J* = 9.0, 10.5 Hz, H-6), 4.56 (1H, br. t, *J* = 7.5 Hz, H-3), 4.94 (1H, br. s, H-14), 5.09 (1H, br. ddd, *J* = 4.0, 5.5, 9.5 Hz, H-8), 5.14 (1H, br. s, H-14), 5.37 (1H, br. d, *J* = 1.5 Hz, H-15), 5.50 (1H, br. d, *J* = 1.5 Hz, H-15), 5.61 (1H, d, *J* = 3.0 Hz, H-13), 5.68 (1H, br. s, H-3'), 6.19 (1H, br. s, H-3'), 6.22 (1H, d, *J* = 3.5 Hz, H-13); ^{13}C-NMR ($CDCl_3$, 125 MHz) : Table I.

Cebellin F (3)

Colorless oil; EIMS *m/z* : 360 $[M]^+$; ^{1}H-NMR ($CDCl_3$, 500 MHz) : δ 1.74 (1H, br. ddd, *J* = 7.5, 10.5, 13.5 Hz, H-2), 1.87 (3H, d, *J* = 1.5Hz, H-5'), 2.24 (1H, br. ddd, *J* = 7.5, 7.5, 13.5 Hz, H-2), 2.40 (1H, dd, *J* = 4.0, 14.5 Hz, H-9), 2.72 (1H, dd, *J* = 5.5, 14.5 Hz, H-9), 2.85 (1H, br. t, *J* = 9.0 Hz, H-5), 2.98 (1H, br. ddd, *J* = 7.5, 9.0, 10.5 Hz, H-1), 3.20 (1H, br. dddd, *J* = 3.0, 3.5, 9.0, 9.5 Hz, H-7), 4.27 (1H, dd, *J* = 9.0, 10.5 Hz, H-6), 4.39 (1H, br. d, *J* = 6.0 Hz, H-4'), 4.56 (1H, br. t, *J* = 7.5 Hz, H-3), 4.94 (1H, br. s, H-14), 5.14 (1H, br. ddd, *J* = 4.0, 5.5, 9.5 Hz, H-8), 5.15 (1H, br. s, H-14), 5.37 (1H, br. d, *J* = 1.5 Hz, H-15), 5.49 (1H, br. d, *J* = 1.5 Hz, H-15), 5.63 (1H, d, *J* = 3.0 Hz, H-13),

6.24 (1H, d, *J* = 3.5 Hz, H-13), 6.38 (1H, tq, *J* = 1.5, 6.0 Hz, H-3'); ^{13}C-NMR ($CDCl_3$, 125 MHz) : Table I.

8α-Hydroxy-11α,13-dihydrozaluzanin C (4)

Colorless oil; $[\alpha]_D$ +75.6° (c 0.08, $CHCl_3$); IR ($CHCl_3$) ν_{max}^{neat} cm^{-1} ν = 3401, 1775, 1605 cm^{-1}; EIMS *m/z* (ret. Int.) : 264 (M^+, 18), 246 (10), 228 (5), 173 (40), 155 (17), 145 (31), 131 (31), 109 (100), 105 (70); ^{1}H-NMR ($CDCl_3$, 500 MHz) : δ 1.29 (3H, d, *J* = 7.5 Hz, H-13), 1.69 (1H, br. ddd, *J* = 7.5, 10.0, 14.0 Hz, H-2), 2.20 (1H, dd, *J* = 8.5, 12.5 Hz, H-9), 2.30 (1H, br. ddd, *J* = 7.5, 7.5, 14.0 Hz, H-2), 2.37 (1H, br. ddd, *J* = 7.0, 9.5, 10.5 Hz, H-7), 2.71 (1H, dd, *J* = 5.5, 12.5 Hz, H-9), 2.87 (1H, dq, *J* = 7.0, 7.5Hz, H-11), 2.88 (1H, br. t, *J* = 9.0 Hz, H-5), 2.90 (1H, m, H-1), 3.79 (1H, m, H-8), 4.12 (1H, dd, *J* = 9.0, 10.0 Hz, H-6), 4.51 (1H, br. t, *J* = 7.5 Hz, H-3), 5.00 (1H, br. s, H-14), 5.08 (1H, br. s, H-14), 5.09 (1H, br. ddd, *J* = 4.0, 5.5, 9.5 Hz, H-8), 5.32 (1H, br. d, *J* = 1.5 Hz, H-15), 5.42 (1H, br. d, *J* = 1.5 Hz, H-15); ^{13}C-NMR ($CDCl_3$, 125 MHz) : Table I.

Desacylcynaropicrin (5)

Colorless oil; $[\alpha]_D$ +35.5° (c 0.10, $CHCl_3$); IR ($CHCl_3$) ν_{max}^{neat} cm^{-1} ν = 3413, 2925, 1751, 1160 cm^{-1}; EIMS *m/z* (ret. Int.) : 262 (M^+, 12), 244 (11), 226 (6), 119 (65), 105 (55), 91 (100), 69 (87); ^{1}H-NMR ($CDCl_3$, 500 MHz) : δ 1.73 (1H, br. ddd, *J* = 7.5, 10.5, 13.5 Hz, H-2), 2.23 (1H, m, H-2), 2.29 (1H, dd, *J* = 4.0, 14.5 Hz, H-9), 2.67 (1H, dd, *J* = 5.5, 14.5 Hz, H-9), 2.80 (1H, br. t, *J* = 9.0 Hz, H-5), 2.95 (1H, br. ddd, *J* = 7.5, 9.0, 10.5 Hz, H-1), 3.07 (1H, br. dddd, *J* = 3.0, 3.5, 9.0, 9.5 Hz, H-7), 3.96 (1H, br. ddd, *J* = 4.0, 5.5, 9.5 Hz, H-8), 4.15 (1H, dd, *J* = 9.0, 10.5 Hz, H-6), 4.55 (1H, br. t, *J* = 7.5 Hz, H-3), 4.97 (1H, br. s, H-14), 5.11 (1H, br. s, H-14), 5.33 (1H, br. d, *J* = 1.5 Hz, H-15), 5.46 (1H, br. d, *J* = 1.5 Hz, H-15), 6.15 (1H, d, *J* = 3.0 Hz, H-13), 6.25 (1H, d, *J* = 3.5 Hz, H-13); ^{13}C-NMR ($CDCl_3$, 125 MHz) : Table I.

3β-Hydroxy-8α-epoxymethylacriloiloxy-4(15),10(14),11(13)-trienguaian-6,12-olide (6)

Colorless oil; IR ($CHCl_3$) ν_{max}^{neat} cm^{-1} ν = 3504, 2930, 1755, 1654, 1270 cm^{-1}; EIMS *m/z* (ret. Int.) : 346 (M^+, 6), 262 (6), 244 (39), 226 (15); ^{1}H-NMR ($CDCl_3$, 500 MHz) : δ 1.34 (3H, br. s, H-4'), 1.70 (1H, br. ddd, *J* = 7.5, 10.5, 13.5 Hz, H-2), 2.18 (1H, br. ddd, *J* = 7.5, 7.5, 13.5 Hz, H-2), 2.35 (1H, dd, *J* = 4.0, 14.5 Hz, H-9), 2.68 (1H, dd, *J* = 5.5, 14.5 Hz, H-9), 2.81 (1H, br. t, *J* = 9.0 Hz, H-5), 2.91 (1H, br. ddd, *J* = 7.5, 9.0, 10.5 Hz, H-1), 3.16 (1H, br. dddd, *J* = 3.0, 3.5, 9.0, 9.5 Hz, H-7), 3.57 (1H, br. d, *J* = 11.5Hz, H-3'), 3.79 (1H, br. d, *J* = 11.5Hz, H-3'), 4.22 (1H, dd, *J* = 9.0, 10.5 Hz, H-6), 4.49 (1H, br. t, *J* = 7.5 Hz, H-3), 4.89 (1H, br. s, H-14), 5.05 (1H, br. ddd, *J* = 4.0, 5.5, 9.5 Hz, H-8), 5.10 (1H, br. s, H-14), 5.31 (1H, br. d, *J* = 1.5 Hz, H-15), 5.40 (1H, br. d, *J* = 1.5 Hz, H-15), 5.94 (1H, d, *J* = 3.0 Hz, H-13), 6.14 (1H, d, *J* = 3.5 Hz, H-13); ^{13}C-NMR ($CDCl_3$, 125 MHz) : Table I.

Kandavanolide (7)

Colorless oil; $[\alpha]_D$ +60.5° (c 0.20, $CHCl_3$); IR ($CHCl_3$) ν_{max}^{neat} cm^{-1} ν = 3510, 2912, 1765, 1717, 1652 cm^{-1}; EIMS *m/z* (ret. Int.) : 304 (M^+, 2), 279 (6), 262 (4), 244 (7), 226 (7), 197 (9), 166 (35), 149 (42), 124 (100), 95 (23), 69 (20); ^{1}H-NMR ($CDCl_3$, 500 MHz) : δ 1.73 (1H, br. ddd, *J* = 7.5, 10.5, 13.5 Hz, H-2), 2.15 (3H, s, H-2'), 2.23 (1H, br. ddd, *J* = 7.5, 7.5, 13.5 Hz, H-2), 2.35 (1H, dd, *J* = 4.0, 14.5 Hz, H-9), 2.70 (1H, dd, *J* = 5.5, 14.5 Hz, H-9), 2.84 (1H, m, H-5), 2.96 (1H, br. ddd, *J* = 7.5, 9.0, 10.5 Hz, H-1), 3.12 (1H, br. dddd, *J* = 3.0, 3.5, 9.0, 9.5 Hz, H-7), 4.20 (1H, dd, *J* = 9.0, 10.5 Hz, H-6), 4.55 (1H, br. dd, *J* = 7.5, 7.5 Hz, H-3), 4.95 (1H, br. s, H-14), 5.00 (1H, m, H-8), 5.14 (1H, br. s, H-14), 5.36 (1H, br. s, H-15), 5.49 (1H, br. s, H-15), 5.63 (1H, d, *J* = 3.0 Hz, H-13), 6.24 (1H, d, *J* = 3.5 Hz, H-13); ^{13}C-NMR ($CDCl_3$, 125 MHz) : Table I.

RESULTS AND DISCUSSION

Compound **1** was obtained as a colorless oil. The EIMS spectrum of **1** showed a molecular ion peak at *m/z* 346. The IR spectrum showed α,β-unsaturated lactone band at 1772 cm^{-1} and hydroxyl group at 3450 cm^{-1}. The ^{1}H-NMR spectrum showed the presence of a α-exomethylene-γ-lactone signals at δ 3.20 (1H, br. dddd, *J* = 3.0, 3.5, 9.0, 9.5 Hz), 4.27 (1H, dd, *J* = 9.0, 10.5 Hz), 5.63 (1H, d, *J* = 3.0 Hz), and 6.24 (1H, d, *J* = 3.5 Hz). In addition, the ^{1}H-NMR spectrum showed oxygenated methine protons at δ 4.56 (1H, br. t, *J* = 7.5 Hz), 5.14 (1H, br. ddd, *J* = 4.0, 5.5, 9.5 Hz), and four exomethylene protons at δ 4.94 (1H, br. s), 5.15 (1H, br. s), 5.37 (1H, br. d, *J* = 1.5 Hz), and 5.49 (1H, br. d, *J* = 1.5 Hz). The ^{1}H- and ^{13}C-NMR spectra signals at δ 4.39 (1H, br. s), 5.97 (1H, br. s), and 6.34 (1H, br. s) and δ 62.1, 126.6, and 139.4, respectively, indicated to the presence of 2-hydroxymethyl-2-propenoyl group. These spectral data suggested that **1** was a guaiane sesquiterpene lactone (Li *et al.*, 1989; Singhal *et al.*, 1982; Zdero *et al.*, 1991). Based on the data obtained and the reported chemical structures of sesquiterpene lactones from the genus *Saussurea* (Marco *et al.*, 1993; Marco *et al.*, 1994), the structure of **1** was determined to be 8-*O*-(2-hydroxymethyl-2-propenoyl)-3-hydroxy-4(15),10(14),11(13)-guaiatriene-12,6-olide (cynaropicrin) (Marco *et al.*, 1993; Rustaiyan *et al.*, 1981).

Compound **2** was obtained as a colorless oil. The EIMS spectrum of **2** showed a molecular ion peak at *m/z* 330. The IR, ^{1}H- and ^{13}C-NMR spectra of **2** were very similar to those of **1**. The major difference in the ^{13}C-NMR spectra was the absence of hydroxy signal (δ 62.1 in **1**) and the presence of methyl group (δ 18.3 in **2**). Based on the data

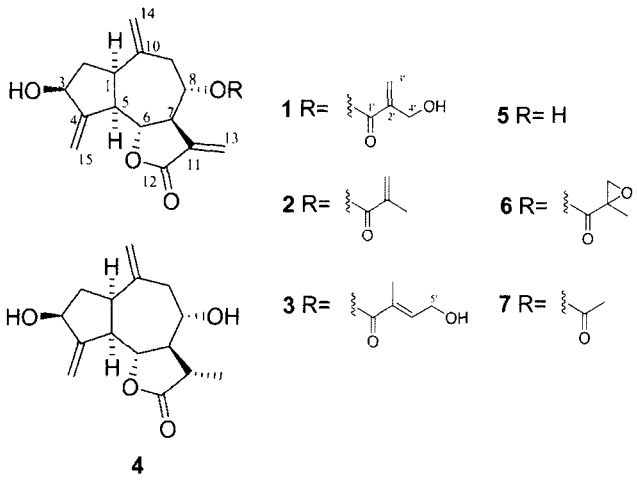

Fig. 1. Structures of compounds 1~7

obtained and the reported chemical structures of sesquiterpene lactones (Marco *et al.*, 1993; Rustaiyan *et al.*, 1981), the structure of **2** was determined to be arguerin B (Ha *et al.*, 2003).

Compound **3** was obtained as a colorless oil. The EIMS spectrum of **3** showed a molecular ion peak at *m/z* 360. The ^{1}H- and ^{13}C-NMR spectra of **3** were similar to those of **1**. The major difference in the ^{1}H-NMR spectra was the presence of methyl group at δ 1.87 (3H, d, J = 1.5 Hz), and an olefinic proton signal at δ 6.38 (1H, tq, J = 1.5, 6.0 Hz) in **3**, while compound **1** showed signals at δ 5.97 (1H, br. s) and 6.34 (1H, br. s). Based on the above mentioned data and the reported chemical structures of sesquiterpene lactones (Marco *et al.*, 1993; Youssef *et al.*, 1996), the structure of **3** was determined to be cebellin F (Helal *et al.*, 1997; Youssef *et al.*, 1996).

Compound **4** was obtained as a colorless oil. The EIMS spectrum of **4** showed a molecular ion peak at *m/z* 264. The IR spectrum showed the presence of α,β-unsaturated lactone group at 1775 cm^{-1} and the presence of hydroxyl group at 3401 cm^{-1}. The ^{1}H-NMR spectrum showed 6,7-lactone ring at δ 2.37 (1H, br. ddd, J = 7.0, 9.5, 10.5 Hz) and 4.12 (1H, dd, J = 9.0, 10.0 Hz), oxygenated methine protons at δ 3.79 (1H, m) and 4.51 (1H, br. t, J = 7.5 Hz), and four exomethylene protons at δ 5.00 (1H, br. s), 5.08 (1H, br. s), 5.32 (1H, br. d, J = 1.5 Hz), 5.42 (1H, br. d, J = 1.5 Hz). The ^{13}C-NMR spectrum showed 15 carbon signals, consisting of four olefinic carbon signals at δ 112.0, 116.2, 143.2, and 153.0, a carbonyl carbon signals at δ 178.6, and three oxygenated carbon signals at δ 73.6, 74.9, and 79.1. These spectral data suggested that **4** was a guaiane-type sesquiterpene lactone (Singhal *et al.*, 1982; Zdero *et al.*, 1991). Based on the data obtained and the reported chemical structures of sesquiterpene lactones (Marco *et al.*, 1993, 1994), the structure of **4** was determined to be 8α-hydroxy-11α,13-dihydrozaluzanin C (Bohlmann *et al.*, 1982; Marco *et al.*, 1994).

Compound **5** was obtained as a colorless oil. The EIMS spectrum of **5** showed a molecular ion peak at *m/z* 262. The IR, ^{1}H- and ^{13}C-NMR spectra of **5** were similar to those of **4**. The major difference in the NMR spectra was the absence of methyl signal (δ 15.9 in **4**) and the presence of exomethylene group (δ 123.2 and 138.1, in **5**). Based on the above mentioned data and the reported chemical structures of sesquiterpene lactones (Kisiel, 1983; Marco *et al.*, 1994), the structure of **5** was determined to be desacylcynaropicrin (Fernandez *et al.*, 1989; Rustaiyan *et al.*, 1981).

Compound **6** was obtained as a colorless oil. The EIMS spectrum of **6** showed a molecular ion peak at *m/z* 346. The IR spectrum showed the presence of α,β-unsaturated lactone group at 1755 cm^{-1} and the presence of hydroxyl group at 3504 cm^{-1}. The ^{1}H- and ^{13}C-NMR spectra of **6** were similar to those of **5**. The major difference in the NMR spectra was the presence of methyl epoxymethylacrylic acid moiety at δ 1.34 (3H, br. s), 3.57 (1H, br. d, J = 11.5 Hz), and 3.79 (1H, br. d, J = 11.5 Hz), two oxygenated carbon signals at δ 68.1 and 76.0, and an ester carbon signals at δ 174.8. Based on the data obtained and the reported chemical structures of sesquiterpene lactones (Fernandez *et al.*, 1989; Marco *et al.*, 1993), the structure of **6** was determined to be 3β-hydroxy-8α-epoxy-methylacriloiloxy-4(15),10(14),11(13)-trienguaian-6,12-olide (Fernandez *et al.*, 1987, 1989).

Compound **7** was obtained as a colorless oil. The EIMS spectrum of **7** showed a molecular ion peak at *m/z* 304 and a base peak at *m/z* 124. The IR, ^{1}H- and ^{13}C-NMR spectra of **7** were almost same with those of **5**. The main difference in the NMR spectra was the presence of acetyl group signals at δ 2.15 (3H, s) in the ^{1}H-NMR spectrum and δ 21.0 and 170.1 in the ^{13}C-NMR spectrum in **7**.

Table II. Cytotoxicity of compounds **1~7**

Compounds \Cancer Cell Lines	ED_{50} values [a]				
	A549	SK-OV-3	SK-MEL-2	XF498	HCT15
1	1.37	1.25	0.31	0.41	0.29
2	1.72	1.30	0.23	0.36	1.28
3	4.89	2.67	1.18	2.07	1.92
4	>30.0	>30.0	10.31	13.24	14.81
5	13.29	11.93	3.62	3.91	6.03
6	13.23	12.04	3.57	3.87	6.16
7	5.51	5.06	1.42	2.90	2.83
etoposide	1.47	2.68	0.08	2.64	2.16
doxorubicin	0.012	0.124	0.003	0.010	0.346

a) ED_{50} was defined as the concentration (μg/mL) that caused a 50% inhibition of cell growth *in vitro*.

Based on the above mentioned data and the reported chemical structures of sesquiterpene lactones (Singhal *et al.*, 1982), the structure of **7** was determined to be kandavanolide (Bohlmann *et al.*, 1982; Ha *et al.*, 2003).

Compounds (**1~7**) have not been reported from this plant.

The cytotoxicity of the compounds was tested by SRB (Sulforhodamin B) bioassay method against five cultured human tumor cells. Of the isolated compounds, compounds **1** and **2** showed non-specific significant cytotoxicity against five human tumor cell lines (**1**: 0.29~1.37 μg/mL and **2**: 0.23~1.72 μg/mL) (Table II).

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