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Cytotoxic Constituents of *Saussurea lappa*

Jee Hyung Jung[1], Youngsoo Kim[2], Chong-Ock Lee[3], Sam Sik Kang[4], Jong-Hee Park[1] and Kwang Sik Im[1]

[1]College of Pharmacy, Pusan National University, Pusan 609-735, [2]College of Pharmacy, Chungbuk National University, Cheongju 360-763, [3]Korea Research Institute of Chemical Technology, Taejeon 305-606, and [4]Natural Product Research Institute, Seoul National University, Seoul 110-460, Korea

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The crude extract of *Saussurea lappa* displayed significant lethality to brine shrimp larvae. Investigation of the causative components by bioactivity-directed fractionation resulted in the isolation of three C_{17}-polyene alcohols. Based on various nmr spectral data, these compounds were identified as shikokiols which had been previously isolated from *Cirsium nipponicum* and/or *Centaurea aegyptica*. These C_{17}-polyene alcohols exhibited moderate cytotoxicities against the human tumor cell lines, A549, SK-OV-3, SK-MEL-2, XF498, and HCT15.

Key words : *Saussurea lappa*, Compositae, C_{17}-polyene alcohol, Cytotoxicity, Shikokiols, Brine shrimp

INTRODUCTION

Saussurea lappa is a perennial herb of which roots are used in Chinese medicine for symptoms such as lack of appetite, epigastric or abdominal pain, distention, nausea, and vomiting (Bensky and Gamble, 1986). Several sesquiterpene lactones had been identified as bioactive constituents of *S. lappa* (Taniguchi *et al.* 1995, Chen *et al.* 1995, Yoshikawa *et al.* 1993). In the process of bioactivity screening, significant cytotoxicity was detected in the crude extract of *S. lappa*. The bioactivity-directed fractionation, monitored by the brine shrimp lethality assay (Meyer *et al.*, 1982), lead to the isolation of three C_{17}-polyene alcohols which were not formerly reported from this genus. These C_{17}-polyene alcohols displayed moderate cytotoxicities against human tumor cell lines including A549 (lung cancer), SK-OV-3 (ovarian cancer), SK-MEL-5 (skin cancer), XF498 (CNS cancer), and HCT15 (colon cancer). The isolation, identification, and bioactivities of the C_{17}-polyene alcohols of *S. lappa* will be presented in this report.

MATERIALS AND METHODS

General experimental procedures

^{1}H nmr, ^{13}C nmr, COSY, HMQC experiments were performed at 500 MHz and 125 MHz with a Varian Unity 500 instrument using Varian standard pulse programs. Solutions in CD_3OD were used for all the nmr studies. Chemical shifts were reported relative to the residual solvent peaks (CD_3OD: ^{1}H δ 3.3, ^{13}C δ 49). The Europrep 60~60 (Knauer, 35~70 μ) was used for the reversed-phase flash column chromatography. An YMC-pack SIL (5 μm, 250×10 mm) column was used with an Alltech guard cartridge column for HPLC. HPLC was conducted on a Spectra-Physics isochrom pump equipped with UV detector (Spectra 100). The Silica Gel F_{254} (Merck) was used for TLC.

Extraction and isolation

Dried roots (1.7 kg) of *Saussurea lappa*, purchased from a commercial supplier, were chopped into small pieces and extracted three times with MeOH at room temp. The MeOH extract was evaporated to dryness and the resulting residue was partitioned between H_2O and CH_2Cl_2. The CH_2Cl_2 phase was evaporated *in vacuo*, then partitioned between 90% aq. MeOH and *n*-hexane. The aq. MeOH phase was evaporated *in vacuo* to yield 41.5 g of residue. A portion of this residue was chromatographed on a C-18 reversed-phase flash column eluting with solvent systems of MeOH:H_2O (2:1), MeOH:H_2O (3:1), MeOH:H_2O (10:1), followed by MeOH and EtOAc. A total of 9 fractions were obtained. Fraction #7 (F7, 323 mg) was subjected to a C-18 reversed-phase column chromatography eluting with an solvent systems of MeOH:H_2O (5:1), MeOH:H_2O (6:1), MeOH:H_2O (8:1), then MeOH. The resulting fraction #6 (F7-6, 44.7 mg) was subsequently subjected to normal phase HPLC with eluting solvent CH_2Cl_2 (YMC-pack SIL, 5 μm, 10×250 mm;

Correspondence to: Jee Hyung Jung, College of Pharmacy, Pusan National University, Pusan 609-735, Korea

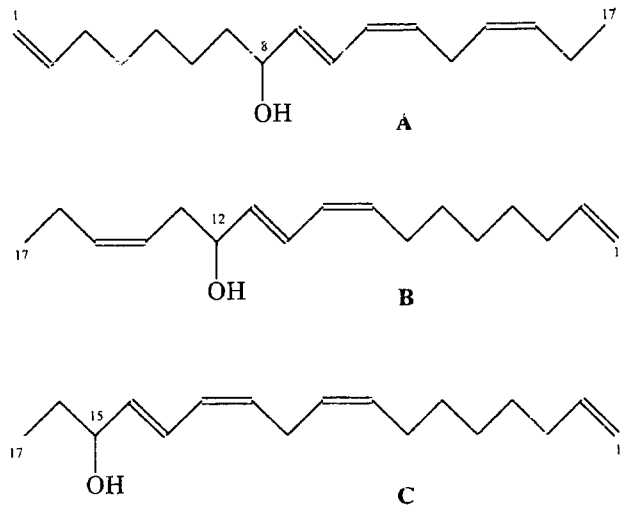

Fig. 1.

flow rate, 1.5 ml/min; UV 254 nm) to afford compounds **A** (2.7 mg), **B** (2.2 mg), and **C** (3.7 mg).

Compound A (shikokiol B): liquid; ^{1}H nmr (500 MHz, CD_3OD) δ 4.99 (1H, d, *J*=18.6 Hz, H-1), 4.93 (1H, d, *J*=10.2 Hz, H-1), 5.81 (1H, ddt, *J*=18.6, 10.2, 6.6 Hz, H-2), 2.05 (2H, m, H-3), 1.30~1.40 (6H, m, H-4, 5, 6), 1.46 (2H, m, H-7), 4.17 (1H, q, *J*=6.5 Hz, H-8), 5.69 (1H, dd, *J*=15.3, 6.5 Hz, H-9), 6.52 (1H, dd, *J*= 15.3, 11.2 Hz, H-10), 5.99 (1H, t, *J*=11.2 Hz, H-11), 5.42 (1H, m, H-12), 2.93 (2H, t, *J*=7.3 Hz, H-13), 5.32 (1H, m, H-14), 5.39 (1H, m, H-15), 2.09 (2H, m, H-16), 0.98 (3H, t, *J*=7.6 Hz, H-17).

Compound B: liquid; ^{1}H nmr (500 MHz, CD_3OD) δ 5.00 (1H, d, *J*=18.5 Hz, H-1), 4.94 (1H, d, *J*=10 Hz, H-1), 5.82 (1H, m, H-2), 2.04 (2H, m, H-3), 1.30~ 1.40 (6H, m, H-4, 5, 6), 2.18 (2H, m, H-7), 5.45 (1H, q, *J*=11 Hz, H-8), 5.96 (1H, t, *J*=11 Hz, H-9), 6.53 (1H, dd, *J*=15, 11 Hz, H-10), 5.70 (1H, dd, *J*=15, 6.5 Hz, H-11), 4.22 (1H, q, 6.5 Hz, H-12), 2.34 (2H, m, H-13), 5.37 (1H, m, H-14), 5.58 (1H, m, H-15), 2.06 (2H, m, H-16), 0.97 (3H, t, *J*=7.5 Hz, H-17).

Compound C (shikokiol C): liquid; ^{1}H nmr (500 MHz, CD_3OD) δ 5.00 (1H, d, *J*=18.5 Hz, H-1), 4.94 (1H, d, *J*=10 Hz), 5.82 (1H, ddt, *J*=18.5, 10, 6.5 Hz, H-2), 2.05 (2H, m, H-3), 1.30~1.40 (6H, m, H-4, 5, 6), 2.05 (2H, m, H-7), 5.41 (1H, m, H-8), 5.35 (1H, m, H-9), 2.94 (1H, t, *J*=7.5 Hz, H-10), 5.41 (1H, m, H-11), 6.00 (1H, t, 11 Hz, H-12), 6.53 (2H, dd, *J*=15, 11 Hz, H-13), 5.69 (1H, dd, *J*=15, 6.5 Hz, H-14), 4.11 (1H, q, *J*=6.5 Hz, H-15), 1.60 (2H, m, H-16), 0.93 (3H, t, *J*=7.5 Hz, H-17).

Biological evaluations

The extracts, fractions, and isolated compounds were routinely evaluated for lethality to brine shrimp larvae. The brine shrimp assay was performed in house. *In vitro* cytotoxicities were determined at the Korea Institute of Chemical Technology following the protocols established by the National Cancer Institute, U.S.A. (Giard *et al.*, 1973). Five cancer cell lines, A549 (lung cancer), SK-OV-3 (ovarian cancer), SK-MEL-2 (skin cancer), XF498 (CNS cancer), and HCT15 (colon cancer) were employed for the measurement of cytotoxicities.

RESULTS AND DISCUSSION

Each solvent partition of *S. lappa* was tested for lethality to brine shrimp larvae. The activity was most concentrated in the 90% MeOH layer (LD_{50}: 28 μg/ml). Sequential fractionation of the 90 % MeOH partition lead to an active fraction #7 (F7, LD_{50}: 4 μg/ml). F7 was subsequently subjected to reversed-phase column chromatography to afford a more active fraction #6 (F7-6, LD_{50}: 1 μg/ml). Finally F7-6 was subjected to normal phase HPLC to afford compounds **A-C** as the causative constituents for brine shrimp lethality. However, the final lethality of each compound to brine shrimp larvae could not be quantitated due to the paucity of material.

In the ^{13}C nmr spectrum of compound **A**, seventeen carbon signals were observed. They were composed of eight olefinic carbons (δ 139.0, 136.3, 132.4, 130.9, 127.7, 126.5, 125.5, 114.2), one oxygenated carbon (δ 72.9), seven methylene carbons (δ 37.2, 33.7, 28.9, 28.8, 25.9, 25.3, 20.6), and one methyl carbon (δ 14.2). These carbons were correlated to the corresponding protons by a HMQC experiment.

According to the ^{1}H nmr and COSY spectra of com-

Table I. ^{13}C nmr data of compounds **A-C***

position**	Compound A (shikokiol B)	Compound B	Compound C (shikokiol C)
1	114.2 (t)	114.2	114.2
2	139.0 (d)	139.0	139.0
3	33.7 (t)	33.7	33.7
4	28.9 (t)	28.8	28.8
5	28.8 (t)	28.8	29.4
6	25.9 (t)	29.5	30.1
7	37.2 (t)	27.7	27.2
8	72.9 (d)	132.9	130.6
9	136.3 (d)	127.6	127.1
10	125.5 (d)	125.8	26.0
11	127.7 (d)	134.9	130.7
12	130.9 (d)	72.1	127.7
13	25.3 (t)	35.3	125.7
14	126.5 (d)	123.7	135.9
15	132.4 (d)	135.2	74.1
16	20.6 (t)	20.8	30.1
17	14.2 (q)	14.2	9.7

*^{13}C nmr spectra were measured at 125 MHz. Chemical shifts were reported relative to the residual solvent peaks (CD_3OD: δ 3.3, δ 49). Multiplicities in parentheses.

**Numbering of carbon positions was conformed to that of Takaishi *et al.* (1991) for convenience.

Table II. Cytotoxicities of compounds **A-C** against human tumor cell lines*

cell line** \ Compound	Compound A	Compound B	Compound C	doxorubicin	cisplatin
A549	>20	15.7	>20	0.2	0.6
SK-OV-3	18.1	13.7	13.9	0.04	0.3
SK-MEL-2	13.1	11.4	10.4	0.2	0.6
XF498	>20	10.3	11.2	0.1	0.5
HCT15	9.7	12.8	11.0	1.0	1.5

*Cytotoxicities were expressed as ED_{50} values in mg/ml concentration. Doxorubicin and cisplatin were tested as positive controls.

**A549 (lung cancer), SK-OV-3 (ovarian cancer), SK-MEL-5 (skin cancer), XF498 (CNS cancer), HCT15 (colon cancer).

pound A, the signals at δ 4.99 (1H, d, *J*=18.6 Hz), δ 4.93 (1H, d, *J*=10.2 Hz), and δ 5.81 (1H, ddt, *J*=18.6, 10.2, 6.6 Hz) were attributable to the terminal vinyl group attached to methylene. The signals at δ 5.69 (1H, dd, *J*=15.3, 6.5 Hz) and δ 6.52 (1H, dd, *J*=15.3, 11.2 Hz) suggested one olefinic system with an *E* configuration (J_{trans}=15.3 Hz). The signals at δ 5.99 (1H, t, *J*=11.2 Hz) and δ 5.42 (1H, m), and the signals at δ 5.32 (1H, m) and δ 5.39 (1H, m) comprised of a diene system with one methylene unit (^{13}C: δ 25.3, ^{1}H: δ 2.93) in between. The configuration of diene unit should be *Z* according to the chemical shift of C-13 (δ 25.3) (Gunstone *et al.*, 1977, Jung *et al.*, 1996). One (δ 5.69) of the trans olefinic protons showed coupling with a proton at δ 4.17 (a proton attached to an oxygenated carbon at δ 72.9). The other one (δ 6.52) of this trans olefinic protons was coupled to the olefinic proton (δ 5.99) of the diene unit. The other end (δ 5.39) of this diene unit was coupled to methylene protons (δ 2.09) of the terminal ethyl group. The proton at δ 4.17 was coupled to another methylene protons (δ 1.46). The terminal vinyl protons showed coupling with methylene protons at δ 2.05 which showed coupling with methylene protons at δ 1.30~1.40. The gross structure of compound **A** was determined based on the analysis of nmr spectral data. But the absolute stereochemistry at C-8 was not determined.

The nmr spectral patterns of compounds **B** and **C** were similar to that of compound **A** implying the presence of analogous functional groups. The structure of compounds **B** and **C** were deduced to be polyene alcohols based on the analyses of various nmr spectral data including ^{1}H nmr, ^{13}C nmr, COSY, and HMQC data.

Compounds **A** and **C** were found identical to shikokiol B and C, respectively, which were previously reported from *Cirsium nipponicum* (Takaishi *et al.*, 1991). Compound **B** was identical to the compound previously isolated from *Cirsium nipponicum* (Takaishi *et al.*, 1991) and *Centaurea aegyptica* (Dahmy *et al.*, 1985). Compounds **A-C** were further assayed for cytotoxicities against the human tumor cell lines A549, SK-OV-3, SK-MEL-2, XF498, and HCT15. Moderate cytotoxicities were observed for these compounds (Table II).

These C_{17}-polyene alcohols has been previously isolated from *Cirsium nipponicum* and/or Centaurea aegyptica (Takaishi *et al.*, 1991, Dahmy *et al.*, 1985). However, this was the first time that the above C_{17}-polyene alcohols were isolated from the plant of the genus *Saussurea*. There have been several reports on C_{17}-polyacetylenes and C_{17}-polyolefines from the members of the Compositae (Yano, 1980, Kawazu *et al.*, 1980, Bohlmann and Abraham, 1981, Takaish *et al.*, 1990) or *Panax ginseng* (Shim *et al.*, 1983, Shim *et al.*, 1987, Fujimoto and Satoh, 1987, Fujimoto and Satoh, 1988). Nematicidal activities (Kawazu *et al.*, 1980) and cytotoxicities to KB cell (Takaishi *et al.*, 1990) were attributed to the C_{17}-polyacetylenes of *Cirsium japonicum*. C_{17}-Polyacetylenes of *Panax ginseng* displayed interesting cytotoxicities against L1210 (Fujimoto and Satoh, 1988) and sarcoma cells (Fujimoto and Satoh, 1987). However, reports on C_{17}-polyene alcohols are uncommon and bioactivities of these compounds were not fully investigated. Only 5-lipoxygenase inhibiting activity of shikokiol A has been reported (Takaishi *et al.*, 1991).

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