Published in final edited form as:
J Ethnopharmacol. 2010 October 28; 132(1): 280–285. doi:10.1016/j.jep.2010.08.026.

Hydrophobic constituents and their potential anticancer activities from Devil's Club *Oplopanax horridus*

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Abstract

Ethnopharmacological relevance—Devil's Club *Oplopanax horridus* (Sm.) Miq. is a widely used folk medicine in the Pacific Northwest such as Alaska and British Columbia for treating a variety of ailments including arthritis, cold, fever, infections, diabetes and cancer.

Aim of the study—To investigate hydrophobic constituents and their potential anticancer activity from Devil's Club *O. horridus*.

Materials and methods—The root bark of *O. horridus* (Sm.) Miq was isolated by chromatographic techniques. Structures of isolated compounds were identified by spectroscopic methods and comparison with published data. The anti-proliferation of isolated hydrophobic constituents in human breast cancer MCF-7 cells, human colon cancer SW-480 and HCT-116 cells were tested. The potential mechanism of anti-proliferation was also investigated using cell cycle and apoptosis assays.

Results and discussion—Six compounds were isolated and structurally identified as 9,17-octadecadiene-12,14-diyne-1,11,16-triol, 1-acetate (**1**), oplopandiol acetate (**2**), falcarindiol (**3**), oplopandiol (**4**), *trans*-nerolidol (**5**) and *t*-cadinol (**6**). These compounds showed potential anticancer activities on human breast cancer and colon cancer cells, of which compound **3** possesses the strongest activity. Further cell cycle and apoptosis test by flow cytometry showed the polyacetylenes **1**–**4** induced HCT-116 cell arresting in G2/M phase and inhibited proliferation by the induction of apoptosis at both earlier and later stage.

Conclusion—These results provide promising baseline information for the potential use of *O. horridus* as well as some of the isolated compounds in the treatment of cancer.

Keywords

Devil's Club *Oplopanax horridus*; Hydrophobic constituents; Anticancer activities; Apoptosis; Colon HCT-116 cells; Falcarindiol

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1. Introduction

Oplopanax horridus (Sm.) Miq., also called Devil's Club belonging to the Araliaceae family (Artiukova et al., 2005; Xiang and Lowry, 2007), is a widely used folk medicine in the Pacific Northwest such as Alaska and British Columbia (Lantz et al., 2004; Schofield, 2000). The major effects of this plant are similar to those of well-known botanicals of the same family such as American ginseng (*Panax quinquefolius*) and Asian ginseng (*P. ginseng*) (Lin et al., 2008; Schofield, 2000; Wang et al., 2007). The root bark and berries of *O. horridus* have been used for treating a variety of ailments including arthritis, cold and fever, infections, diabetes, respiratory and gastrointestinal disorders, and cancer (Johnson, 2006; Moore, 1993; Schofield, 2000). Chemical investigations into *O. horridus* revealed that this plant consists of two major groups of constituents. One group is called sesquiterpenes such as equinopanacene (Kariyone and Morotomi, 1927) and oplopanone (Bloxton and Marderosian, 2002; Inui et al., 2007b). The other group is namely polyacetylenes or polyynes such as falcarindiol and oplopandiol (Kobaisy et al., 1997).

Pharmacological studies have shown that *O. horridus* extract and its constituents displayed hypoglycemic (Smith, 1983), antibacterial (Inui, 2008; Inui et al., 2007a), anti-fungal (Kobaisy et al., 1997) and anti-viral properties. Recently, the *O. horridus* extract was found to have potential anticancer activity on a number of cancer cell lines *in vitro* (Tai et al., 2009; Tai et al., 2006; Wang et al., 2009). Owing to unavailability of chemical entities, however, it is unclear which are bioactive compounds possessing inhibitory effects on cancer cells in this plant. In our previous works, the hydrophobic fraction of *O. horridus* was demonstrated to have strong anticancer activity on diverse cancer cells through a bioassay-guided fractionation technique. As an ongoing study, this work aims to systematically isolate hydrophobic constituents from the hydrophobic fraction of *O. horridus* by chromatographic techniques, and to evaluate the anticancer activities and related mechanisms of isolated compounds.

2. Materials and methods

2.1. Plant material

The root bark of *O. horridus* (Sm.) Miq was purchased from Pacific Botanicals (Grants Pass, OR). The sample was identified by one of the authors, Chong-Zhi Wang, and a voucher specimen (no. 20080729-4) was deposited in the Tang Center for Herbal Medicine Research, the University of Chicago.

2.2. Chemicals and reagents

All solvents were of high-performance liquid chromatography (HPLC) grade (Fisher Scientific, Norcross, GA). Materials for column chromatography included Silica gel RP-18 (43–60 μm, EMD Chemicals Inc. Germany) from VWR, silica gel 60 (200–425 mesh, Aldrich; 40–60 μm, Acros Organics) from Fisher, TLC Silica gel 60 F254 (EMD Chemicals Inc., Darmstadt, Germany), RP-18 F254 (Merck, Darmstadt, Germany) aluminum sheets from Fisher, and Diaion Resin Styrenic adsorbent (HP20-1) from Sorbent Technologies (Atlanta, GA). Milli Q water was supplied by a water purification system (US Filter, Palm Desert, CA). Plastic materials were purchased from Falcon Labware (Franklin Lakes, NJ). Trypsin, Leibovitz's L-15 medium, fetal bovine serum (FBS), and penicillin/streptomycin solution (200×) were obtained from Mediatech, Inc. (Herndon, VA).

2.3. Extraction and isolation

Dried and ground root bark of *O. horridus* (2.4 kg) was extracted twice with 70% EtOH under refluxing at 90 °C, the first time with 1:6 solvent (*w:v*) for 4 h, and the second time

with 1:4 solvent for 2 H. The filtrate was then combined and evaporated at 60 °C under vacuo to afford a total extract. A 1.2 g of the total extract was dissolved in 3.6 mL of 90% MeOH by ultrasonic and vortexing, and the supernatant fluid was then filtered through a 0.2 μm (Millex-GN) Syringe Driven Filter Unit (Millipore Corporation, Bedford, MA). Semi-preparative HPLC system (Waters 2965, Milford, MA) consisting of a quaternary pump, an automatic injector, a photodiode array detector (Model 996), and Waters Millennium 32 software was directly used to isolate compounds **1–4**. The separation was carried out on an XTerra Prep RP18 column (150 × 10 mm i.d. 5 μ). A 90 μL sample was injected into the column and eluted at room temperature with methanol (A) and water (B) as the mobile phase at a constant flow rate of 3.0 mL/min. The gradient elution program was 65% A at 0–1 min, 65%–72% A at 1–5 min, 72% A at 5–18 min, 72%–85% A at 18–23 min, 85% A at 23–27 min, 85%–100% A at 27–29 min, and 100% A at 29–33 min. The detection wavelength was set at 208 nm. Purified compounds **1–4** with an amount of 18.2 mg, 4.8 mg, 7.8 mg and 7.4 mg respectively were obtained by manual collection.

The EtOH extract (503.2g) was submitted to Diaion HP-20 column chromatography eluted with a H_2O-EtOH gradient system (0, 30, 50, 70, and 100% EtOH) to produce fractions A-E 284.0, 38.5, 56.0, 75.0 and 53.5 g, respectively. A 53.1 g of fraction E was subjected to silica gel (60 Å, 200–425 mesh, Sigma-Aldrich, Inc., St Louis, USA) column (Buchi 70 × 230 cm glass with precolumn) eluted with hexane:acetone (100:0–100:50) to afford 7.02 g of Fraction E2. Fraction E2 was then subjected to silica gel column (Buchi 49×230 mm column with precolumn) eluted with Hexane:EtOAC (100:0–100:8) to provide 0.23 g of Fraction E2-4. This fraction was further submitted to silica gel column (Buchi 36×230 mm column with precolumn) eluted with CH_2Cl_2:Acetone (100:0–100:3) to provide oil 150.9 mg (0.006%). The obtained compound showed one spot on TLC and one peak in HPLC, but NMR and GC/MS analysis demonstrated that it is a mixture of compounds **5** and **6** with a ratio of approximately 6:1.

2.4. TLC, HPLC and NMR analysis

The purity of each isolated compound was tested by TLC and HPLC, and their structures were elucidated by NMR assay. TLC was carried out on silica gel 60 F254 or RP-18 F_{254} aluminium sheets, and spots were visualized by spraying the plates with 10% H_2SO_4 solution followed by heating. HPLC was performed on a Prodigy ODS column (250 mm × 3.2 mm × 5 μm) with acetonitrile and water as the mobile phase at 1.0 mL/min. $_1$H, $_{13}$C, and DEPT-135 NMR spectroscopy experiments were performed on a DMX 500 spectrometer (Bruker, Reinstetten, Germany). Deuterochloform was used as the solvent, and tetramethylsilane was used as the internal standard.

2.5. Chemical structure of compounds 1–6

Compound **1**: oil (18.2 mg, 0.37% dry weight); $[\alpha]_D^{25}$, +173.6°($CHCl_3$, c 0.14); UVλ_{max} (acetonitrile/H_2O) 210.0, 233.5 246.4, 260.5 nm (ε 271.9, 1.9, 1.8, 1.4). ^{1}H NMR (500 MHz, $CDCl_3$, TMS as internal standard): δ 1.29 (8H, m, H-3, 4, 5, 6), 1.37 (2H, m, H-7), 1.62 (2H, m, H-2), 2.05 (3H, s, COCH3), 2.11 (dq, 1.0, 7.5Hz), 4.06 (2H t, 7.5 Hz, H-1), 4.94 (1H, brd, 5.0 Hz, H-16), 5.21 (d, 8.0 Hz, H-10), 5.26 (1H, d, 10.5 Hz, H-18a), 5.49 (1H, dd, 17.0, 10.5, H-18b), 5.53 (1H, dd, 17.5, 8.5 Hz, H-10), 5.61 (1H, dt, 10.5, 7.5, H-9), 5.92 (1H, ddd, 17.0, 10.0, 1.5 Hz, H-17). ^{13}C NMR (125MHz, CDCl3, TMS as internal standard): δ 21.04 (COCH_3), 25.79 (C-2), 27.57^c (C-3), 28.55^c (C-5), 28.92^c (C-4), 28.92^c (C-6), 29.09^d (C-8), 29.19^d (C-7), 58.57 (C-11), 63.42 (C-16), 64.70 (C-1), 68.73 (C-13), 70.17 (C-14), 78.37 (C-15), 79.77 (C-12), 117.25 (C-18), 127.82 (C-10), 134.45 (C-9), 135.84 (C-17), 171.48 (C=O).

Compound **2**: oil (4.8 mg, 0.098% dry weight); $[\alpha]_D^{25}$, +154.5°($CHCl_3$, c 0.04); λ_{max} (acetonitrile/ H_2O) 203.0, 232.3, 245.2, 259.3 nm (ε 79.6, 1.3, 1.2, 1.1). ^1H NMR (500 MHz, $CDCl_3$, TMS as internal standard): 1.03 (3 H, t, 7.5 Hz, H-18), 1.36 (8H, m, H-3, H-4, H-5, H-6), (m, H-7), 1.62 (m, H-2), 1.76 (2H, m, H-17), 2.06 (s, COCH3), 2.1 (q, 7.5Hz, H-8), 4.07 (2H, t, 7.0 Hz, H-1), 4.39 (t, 6.5 Hz, H-16), 5.21 (d, 8.5 Hz, H-11), 5.53 (t, 9.5 Hz, H-9), 5.62 (dt, 7.5, 2.0 Hz, H-8). ^{13}C NMR (125MHz, $CDCl_3$, TMS as internal standard): δ 9.32 (C-18), 21.05 (COCH_3), 25.80 (C-2), 27.58[c] (C-8), 28.56 (C-6), 28.94[c] (C-5), 29.11[c] (C-4), 29.12[c] (C-3), 29.21[d] (C-7), 30.62 (C-17), 58.60 (C-11), 64.02 (C-16), 64.69 (C-1), 68.86 (C-13), 68.90 (C-14),79.11 (C-15), 80.82 (C-12), 127.90 (C-10), 134.40 (C-9), 171.44 (C=O).

Compound **3:** oil (7.8 mg, 0.16%) dry weight); $[\alpha]_D^{25}$, +196.5°($CHCl_3$, c 0.07); UVλ_{max} (acetonitrile/ H_2O) 208.9, 233.5, 246.4, 260.5 nm (ε 206.2, 1.5, 1.3,). ^1H NMR (500 MHz, $CDCl_3$, TMS as internal standard): δ 0.90 (3H, t, 7.0, H-17), 1.30 (8H, m, H-13, H-14, H-15, H-16), 1.40 (2H, m H-12), 2.11 (2H, q, 7.0, H-11), 4.96 (1 br d, 5.0 Hz, H-3), 5.22 (1H, d, 8.5Hz, H-8), 5.28 (1H, d, 10.0 Hz, H-1a), 5.49 (1H, dd, 11.5 Hz, H-9), 5.52 (1H, dd, 11.5, 8.5 Hz, H-9), 5.63 (1H, dt, 10.5, 7.5, H-1), 5.95 (1H, ddd, 11.0, 6.0, 1.0, H-2). ^{13}C NMR (125MHz, $CDCl_3$, TMS as internal standard): δ 14.09 (C-17), 22.63 (C-15), 27.69 (C-11), 29.10 (C-12), 29.15 (C-14), 29.27(C-13), 31.79 (C-16), 58.60 (C-8), 63.49 (C-3), 68.70 (C-6), 70.29 (C-5), 78.22 (C-4), 79.85 (C-7), 117.35(C-1), 127.64 (C10), 134.71 (C-9), 135.78 (C-2).

Compound **4**: oil (7.4 mg, 0.15% dry weight); $[\alpha]_D^{25}$, +204.8°($CHCl_3$, c 0.06); UVλ_{max} (acetonitrile/ H_2O) 204.2, 232.3, 245.2, 259.3 nm (ε 102.8, 1.3, 1.3, 1.2). ^1H NMR (500 MHz, $CDCl_3$, TMS as internal standard): δ 0.88 (3H, t, 7.0 HZ, H-17), 1.01 (3H, t, 7.5Hz, H-1), 1.28 (8H, m, H-13, H-14, H-15, H-16), 1.38 (2H, m, H-12), 1.73 (2H, m, H-2), 2.09 (dq,1.0, 7.0 Hz, H-11), 4.38 (1H, t, 6.5Hz, H-3), 5.20 (brd, 8.5Hz, H-8), 5.52 (t, 8.5, H-10), 5.61 (dt, 11.0, 7.5, H-9). ^{13}C NMR (125MHz, $CDCl_3$, TMS as internal standard): δ 9.28 (C-1), 14.09 (C-17), 22.64 (C-15), 27.68 (C-11), 29.10 (C-12), 29.15 (C-14), 29.27 (C-13), 30.61 (C-2), 31.79 (C-16), 58.60 (C-8), 64.06 (C-3), 68.85 (C-6), 68.96 (C-5), 79.18 (C-4), 80.68 (C-7), 127.73 (C10), 134.62 (C-9).

Compound **5**: UVλ_{max} (acetonitrile/H_2O) 211.2 nm (ε 233.9). ^1H NMR (500 MHz, $CDCl_3$, TMS as internal standard): δ 1.35 (3H, s, H-15), 1.58 (2H, m, H4), 1.59 (6H, s, H-13, H-14), 1.67 (3H, d, 2Hz, H-12), 1.96–2.07 (6H, m, H-5, H-8, H-9), 5.07 (1H, dd, 10.5, 1.5Hz, H-1a), 5.08 (1H, m, H-10), 5.14 (1H, tq, 7.1 Hz, H-6), 5.21 (1H, dd, 17.5, 1.5Hz, H-1b), 5.92 (1H, dd, 17.5, 10.5 Hz, H-1a). ^{13}C NMR (125MHz, $CDCl_3$, TMS as internal standard): δ 16.01 (C-14), 17.68 (C-13), 22.74 (C-5), 25.70 (C-12), 26.66 (C-9), 27.87 (C-15), 39.72 (C-8), 42.09 (C-4), 73.47 (C-3), 111.66 (C-1), 124.26 (C-10), 124.27 (C-6), 131.39 (C-11), 135.52 (C-7), 145.10 (C-2).

Compound **6**: ^1H NMR (500 MHz, $CDCl_3$, TMS as internal standard): δ 0.80 (3H, d, 7.0Hz, H-14)), 0.91 (3H, d, 6.5 Hz, H-13), 1.01 (1H, tt, 11.3, 3.2 Hz, H-7a), 1.08 (1H, dt, 12.3, 1.9 Hz, H-1a), 1.22 (3H, s, H-15), 1.32 (1H, m, H-8β), 1.35 (1H, m, H-2β), 1.41 (1H, m, H-9a), 1.46 (1H, m, H-8a), 1.67 (3H, br s, H-11), 1.75 (1H, m, H-9β), 1.92 (1H, m, H-2a), 1.92–2.08 (2H, m, H-3a, H-3β), 1.95 (1H, m, H-6β), 5.55 (1H, br s, H-5). ^{13}C NMR (125MHz, $CDCl_3$, TMS as internal standard): δ 15.21 (C-14), 19.81 (C-8), 21.43 (C-13), 22.60 (C-2), 23.79 (C-11), 26.65 (C-12), 28.48 (C-15), 30.90 (C-3), 37.72 (C-6), 40.32 (C-9), 46.67 (C-7), 47.95 (C-1), 70.69 (C-10), 122.67 (C-5), 134.32 (C-4).

2.6. Anticancer assay

2.6.1. Cell culture—The human breast cancer cell lines MCF-7 (RPMI-1640), colon cancer cell lines HCT-116 (McCoy's 5A) and SW-480 (Leibovitz's L-15) were purchased from American Type Culture Collection (ATCC, Manassas, VA) and grown in the indicated media supplemented with 10 % FBS and 50 IU penicillin/streptomycin in a humidified atmosphere of 5 % CO_2 at 37 °C.

2.6.2. Cell proliferation analysis—Cells were seeded in a flat-bottomed 96-well plate with a multichannel pipet (1×10^4 cells/well). After 24 h, the medium was removed and 200 μL of fresh culture medium was added to each well. Various concentrations of extract and fractions were added to the wells. The final concentration of DMSO/EtOH tested groups was 0.1 or 0.5%. Controls were exposed to culture medium containing the same quantity of DMSO/EtOH without drugs. All experiments were performed at least three times. At the end of the drug exposure period (48 h), the medium was removed from all the wells and 100 μL of fresh medium plus 20 μL of CellTiter 96 aqueous solution was added to each well. CellTiter 96 aqueous solution is composed of a tetrazolium compound, 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium, an electron-coupling reagent (phenazine methosulfate), and buffer. When the solution contacts viable cells, it is bioreduced by dehydrogenase enzymes in metabolically active cells into a formazan product. The quantity of formazan product, measured by the amount of absorbance at 490 nm, is directly proportional to the number of living cells in culture. The plate was then incubated for 1 h in a humidified atmosphere at 37 °C; 60 μL of medium from each well was transferred to an ELISA 96-well plate, and the absorbance of the formazan product at 490 nm was measured. The blank was recorded by measuring the absorbance at 490 nm with wells containing medium but no cells. Results were expressed as percent of control (solvent vehicle set at 100%).

2.6.3. Cell cycle assay—Owing to the stability, availability, and validity, HCT-116 cells were selected for further cell cycle assay. Cells were seeded in 24-well tissue culture plates. On day 2, the medium was changed and the cells were treated with extract/fractions. The cells were incubated for 24 h before being harvested. The cells were fixed gently by adding 80% ethanol and placing them in a −20 °C freezer for 2 h. They were then treated with 0.25% Triton X-100 for 5 min in an ice bath. The cells were resuspended in 300 μL of PBS containing 40 μg/mL propidium iodide and 0.1 mg/mL RNase. Then the cells were incubated in a dark room for 20 min at room temperature, and analyzed with a FACScan flow cytometer (Becton Dickinson, Mountain View, CA) and FlowJo 7.1.0 software (Tree Star, Ashland, OR). For each measurement, at least 20,000 cells were counted.

2.6.4. Apoptosis assay—The HCT-116 cells were seeded in 24-well tissue culture plates. After culturing for 1 day, the medium was changed and the extract/fractions were added. After treatment for 48 h, the cells floating in the medium were collected. The adherent cells were detached with 0.05% trypsin. Then the culture medium containing 10% FBS (and floating cells) was added to inactivate the trypsin. After being pipetted gently, the cells were centrifuged for 5 min at 1500 g. The supernatant was removed and the cells were stained with annexin V-FITC and PI according to the manufacturer's instructions. Annexin V-FITC detects translocation of phosphatidylinositol from the inner to the outer cell membrane during early apoptosis, and PI can enter the cell in late apoptosis or necrosis (Cheng et al., 2008). Untreated cells were used as control for the double staining. The cells were analyzed immediately after staining using a FACScan flow cytometer and FlowJo 7.1.0 software. For each measurement, at least 20,000 cells were counted.

2.6.5. Statistical analysis—Biological tests were conducted at least twice. Data are presented as mean ± standard error (SE). A one-way ANOVA determined whether the results had statistical significance. In some cases, Student's *t*-test was used for comparing two groups. The level of statistical significance was set at $P < 0.05$.

3. Results and discussion

3.1. Isolation of compounds 1–6

Spectral elucidation of isolated compounds (shown in Figure 1) using semi-preparative HPLC showed that compounds **1**–**4** are polyacetylenes (polyynes), namely 9,17-octadecadiene-12,14-diyne-1,11,16-triol, 1-acetate (compound **1**), oplopandiol acetate (compound **2**), falcarindiol (compound **3**), and oplopandiol (compound **4**). Compounds **1** and **2**, compounds **3** and **4** could be transformed from each other by hydrogenation or dehydrogenation.

It was very difficult to obtain the purified compounds by open column chromatography, but compounds **1**–**4** was shown to have a good HPLC isolation when using C_{18} column and acetonitril/H_2O or methanol/H_2O as the mobile phase. In the biogenesis (Minto and Blacklock, 2008), compounds **1**–**4** were hydroxylated and desaturated at position C-17 of 9-octadecene-12,14-diynoic acid. Deriving from this same key intermediate, one pathway to produce oplopandiol or falcarindiol is by decarboxylic reaction of the hydroxylated / hydroxylated and desaturated products; another pathway to produce oplopandiol acetate or 9,17-octadecadiene-12,14-diyne-1,11,16-triol, 1-acetate is by acylation of the hydroxyl reducing from carboxyl of the hydroxylated / hydroxylated and desaturated products (Minto and Blacklock, 2008).

Two sesquiterpenes *trans*-nerolidol (**5**) and *t*-cadinol (**6**) were also isolated from the hydrophobic fraction by repeated column chromatography. They can be biosynthesized from isoprenoid pathway. *Trans*-nerolidol is produced by the rearrangement of nerolidyl diphosphate (NPP), and *t*-cadinol is derived from the cyclization of NPP (Cane, 1999). These two compounds, mainly occurring in the hydrophobic fractions, are the main secondary metabolites of *O. horridus* root bark.

3.2. Cell proliferation and apoptosis analysis

Anti-proliferative test of the isolated compounds was firstly conducted on human breast cancer MCF-7 cells. As shown in Figure 2A, after treatment for 48 h, compound **3** decreased the proliferation by 70% at 30 μM and >95% at 100 μM. The compound **1** reduced the MCF-7 cells growth in a concentration-dependent manner, by 80% at 30 μM, by 60% at 100 μM, and >99% at 300 μM. Subsequently, the anti-proliferation of these compounds was tested on human colon cancer SW-480 (Figure 2B) and HCT-116 cells (Figure 2C). Results demonstrated that these compounds showed stronger anticancer potential on colon cancer cell lines than on breast cancer cell lines, and colon cancer SW-480 cells were the most susceptive to all compounds. Especially, compounds **3** and **4** decreased SW-480 cells proliferation by 75% at 10 μM, and compound **3** and **1** reduced HCT-116 cells proliferation by 90% at 10 μM and 75% at 10 μM, respectively.

Among these compounds, the 6:1 mixture of *trans*-nerolidol **5** and *t*-cadinol **6** had the lowest inhibitory effect on the tested cancer cell lines. A preliminary structural-activity relationship of polyacetylenic compounds **1**–**4** was considered. Compounds **1** with one extra ethylenic bond at the end showed higher activity compared to compound **2.** This view was supported by the observation in which compound **3** with one extra ethylenic bond also had higher activity compared to compound **4**. In addition, the unacylated polyacetyenes had higher

activity than that of the acylated ployacetylenes. In future studies, if more polyacetylenic compounds can be isolated, the structural-activity relationship should be better elucidated.

In this study, we also explored the potential mechanism of anti-proliferation using cell cycle and apoptosis assays. Cell cycle test showed that compounds **1**–**4** induced HCT-116 cell arresting in G2/M phase as shown in Figure 3. Their arresting rates in G2/M phase, at the concentration of 10 μM, were 58.4, 26.1, 34.6, and 47.7%, respectively, while at the concentration of 20 μM, were 45.1, 27.1, 35.8, and 46.4%, respectively, while the control was 20.4%. The highest arrest percentage of HCT-116 cells treated with compound **1** at the concentration of 10 μM, was 2.5-fold of control. The least activity was observed by treatment with compound **2**, only increased 12.0% more than control. Treated with compounds **1**–**4** with 10 μM, cells in S-phase were 36.4, 36.2, 44.5, and 39.9%, respectively; with 20 μM, were 44.7, 38.2, 45.5, and 32.4%, respectively, while the control was 47.6%. These compounds did not show significant effect in the S-phase. However, the responses of the compounds on HCT-116 cell cycle were not concentration-dependent at the concentrations of 10 and 20 μM, and were not significantly correlated with the anti-proliferative effects. So their anti-proliferations may not be regarded as a result of the arrest effect in HCT-116 cell cycle.

Apoptosis test (Figure 4) indicated that compounds **1**–**4** had the strong induction to apoptosis of HCT-116 cell and dose-dependent responses. Among them, the inductive effect of compound **3** is the most significant. It inducted apoptosis 31.0% at the earlier stage and 40.2% at the later stage with a concentration of 10 μM, 33.5% earlier stage and 48.4% later stage with a concentration of 30 μM. Compound **4** also showed dose-dependent apoptotic induction activity. When the concentration of compound **4** increased from 10 to 30 μM, the apoptotic inductive effect increased from 11.9 to 44.4% at the early stage, from 21.2 % to 40.6 at the late stage. The inductive effects of compounds **1**–**4** were consistent with their anti-proliferative activities. So we regarded that the induction effect of polyacetylenes on the apoptosis contributed to their anti-proliferative effects on human colon cancer HCT-116 cells. Similar results were also observed using other colon cancer cell lines.

O. horridus belongs to the same family Araliaceae with ginsengs. *O. horridus* contains similar (C_{17}-) polyacetylenes as ginsengs (Moon et al., 2000; Rao et al., 1997; Yang et al., 2008). These types of polyacetylenic compounds are widely distributed in herbs of Araliaceae such as ginsengs and vegetables of Apiaceae, one sister family of Araliaceae, such as carrot (*Daucus carota* L) (Christensen and Brandt, 2006). The inhibition of cancer cell of ginseng partially (Kim et al., 2002; Matsunaga et al., 1990; Wang and Yuan, 2008) and of carrot wholly (Kobaek-Larsen et al., 2005; Susanne et al., 2003) resulted from the cytotoxic activities of the polyacetylenic compounds. The selective cytotoxic activity towards cancer cells indicates that these types of polyacetylenes and the herbs or vegetables that contain them may be valuable in the treatment and/or prevention of different types of cancer (Christensen and Brandt, 2006).

In this study, we isolated **4** polyacetylenes and **2** sesquiterpenes and evaluated their anti-cancer activities using selected human cancer lines. Our data suggested that constituents in *O. horridus*, especially polyacetylenes, may play an important role in human cancer chemoprevention.

4. Conclusion

Four polyacetylenic compounds, namely 9,17-octadecadiene-12,14-diyne-1,11,16-triol, 1-acetate (**1**), oplopandiol acetate (**2**), falcarindiol (**3**) and oplopandiol (**4**), and two sequisterpene compounds namely *trans*-nerolidol (**5**) and *t*-cadinol (**6**), were isolated and

elucidated from the root bark of *O. horridus*. All of these compounds presented some inhibitive effects on human breast cancer MCF-7 and colon cancer HCT-116 and SW-480 cell lines. Thus, the observed anti-cancer activities of *O. horridus* root bark are likely related to the hydrophobic compounds. Primary structure-activity analysis suggested that the observed inhibitions were influenced by the ethylenic bonds and acylations of polyacetylenes. The inductive effects on cancer cell apoptosis were more significant than the arrested effects on cell cycle. For the apoptosis, the late effect was much more significant than the early effect. These results provide promising baseline information for the potential use of *O. horridus* as well as some of the isolated compounds in the treatment of cancer.

Acknowledgments

This work was supported in part by the NIH/NCCAM grants AT003255 and AT004418.

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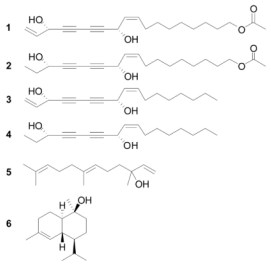

Figure 1.

Chemical structures of 9,17-octadecadiene-12,14-diyne-1,11,16-triol, 1-acetate (compound 1), oplopandiol acetate (compound 2), falcarindiol (compound 3), oplopandiol (compound 4), trans-nerolidol (compound 5), and t-cadinol (compound 6) isolated from *O. horridus*.

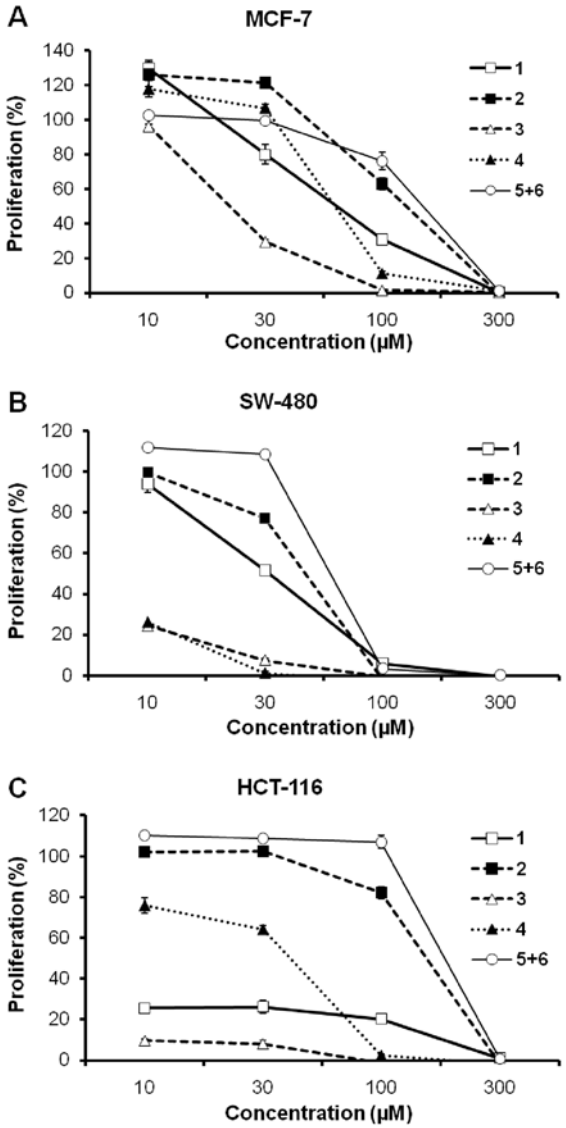

Figure 2.

Percentage of proliferation of (**A**) human breast cancer MCF-7 cells and (**B**) colon cancer SW-480 cells and (**C**) colon cancer HCT-116 cells treated for 48 h with the compounds **1**–**4**, **5** and **6** isolated from *O. horridus*.

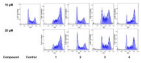

Figure 3.
Effects of compounds **1–4** from *O. horridus* on HCT-116 cell cycle. After treatment with these compounds for 24 h, the cells were stained with PI and assayed using flow cytometry.

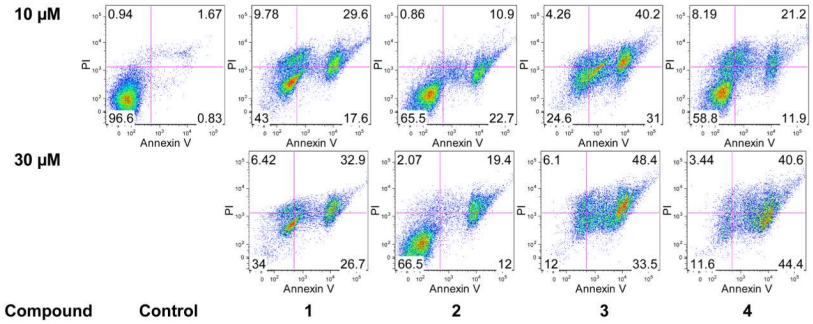

Figure 4.

Apoptosis assay using flow cytometry after annexin V-FITC/propidium iodide (PI) staining. HCT-116 cells were treated with compounds **1**–**4** for 48 h. Viable cells are in the lower left quadrant, early apoptotic cells are in the lower right quadrant, late apoptotic or necrotic cells are in the upper right quadrant and non-viable necrotic cells are in upper left quadrant.