Inhibitory Effect of Human Breast Cancer Cell Proliferation *via* p21-Mediated G_1 Cell Cycle Arrest by Araliadiol Isolated from *Aralia cordata* Thunb.

Authors Wen-Ling Cheng[1], Ting-Yu Lin[1], Yen-Hsueh Tseng[1], Fang-Hua Chu[2], Pin-Ju Chueh[3], Yueh-Hsiung Kuo[4,5], Sheng-Yang Wang[1,5]

Affiliations The affiliations are listed at the end of the article

Key words
- araliadiol
- cytotoxicity
- cell cycle
- G_1 arrest
- *Aralia cordata*
- Araliaceae

Abstract

A new polyacetylenic compound, araliadiol, was isolated from the leaves of *Aralia cordata* Thunb. (Araliaceae). The structure of araliadiol was determined to be 3(*S*),8(*R*)-pentadeca-1,9(*Z*)-diene-4,6-diyne-3,8-diol by MS, NMR, IR, and UV spectroscopic analysis as well as Mosher ester reaction. Araliadiol displayed a significant inhibitory effect on the growth of a human breast adenocarcinoma cell line (MCF-7), with an IC_{50} value for cytotoxicity of 6.41 µg/mL. Cell cycle analysis revealed that the proportion of cells in the G_1 phase of the cell cycle increased in a dose-dependent manner (from 54.7% to 72.0%) after 48 h exposure to araliadiol at dosages ranging from 0 to 80 µM. The results suggest that araliadiol inhibits cell cycle progression of MCF-7 at the G_1-S transition. After treatment with araliadiol, phosphorylation of retinoblastoma protein (Rb) in MCF-7 cells was inhibited, accompanied by a decrease in the levels of cyclin D_3 and cyclin-dependent kinase 4 (cdk4) and an increase in the expression of $p21^{WAF-1/Cip1}$. However, the expression of phosphorylated p53 (Ser15) and Chk2 was not altered in MCF-7 cells. These findings indicate that araliadiol exhibits its growth-inhibitory effects on MCF-7 cells through downregulation of cdk4 and cyclin D_3, and upregulation of $p21^{WAF-1/Cip1}$ by a p53-independent mechanism.

Abbreviations

MCF-7:	human breast adenocarcinoma cell line
IC_{50}:	half maximal inhibitory concentration
cdk:	cyclin-dependent kinase
CKIs:	cdk inhibitors
MTT:	3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide
Rb:	retinoblastoma protein
MTPA:	α-methoxy-α-trifluoromethyl-phenylacetyl
DMAP:	dimethylaminopyridine
PI:	propidiumiodide

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Correspondence
Sheng-Yang Wang
Department of Forestry
National Chung-Hsing University
250 Kuo Kuang Road
Taichung 402
Taiwan
Phone: + 886 4 22 84 03 45-138
Fax: + 886 4 22 87 36 28
taiwanfir@dragon.nchu.edu.tw

Introduction

Aralia cordata Thunb. (Araliaceae) is a medicinal herb distributed at medium to high altitudes in the central range of Taiwan [1]. A number of polyacetylenes have been isolated from this family [2] and have been shown to possess cytotoxic [3,4] and anti-inflammatory activities [5,6]. In addition, some polyacetylenes such as panaxydol [7] and panaxytriol [8] have been demonstrated to exhibit anticancer activity by inducing G_1 and G_2/M cell cycle arrest, respectively.

The cell cycle is regulated by members of the cyclin family of proteins, which bind and activate members of the cyclin-dependent kinase (cdk) family to effect cell cycle progression. Progression of cells through the G_1-S-G_2-M cycle is followed by successive oscillations in the levels of cyclins D, E, A, and B [9]. Cyclin D and cyclin E-cdk complexes phosphorylate the Rb protein which plays an essential role in regulation of the passage through the G_1 phase into S. Regulation of cyclin-cdk complexes activity is controlled at three levels. First, expressions of cdks and cyclins are altered. Second, the cdk kinase activity is regulated by reversible phosphorylation. Finally, cdk inhibitors (CKIs), such as $p21^{WAF-1/Cip1}$ and $p27^{KIP}$, interact reversibly and prevent kinase activation [10].

In this study, we isolated a new polyacetylene, araliadiol, from *A. cordata*. We show that araliadiol has a potent cytotoxicity against human breast carcinoma cells (MCF-7 cells) and demonstrate that treatment of MCF-7 cells with aralia-

diol results in growth arrest that is mediated by $p21^{WAF-1/Cip1}$ and independent of p53. Hence, araliadiol has chemotherapeutic potential for suppressing tumor growth in p53-deficient malignancies.

Materials and Methods

General experimental procedures

Optical rotation, UV, and IR data were acquired on a PerkinElmer 241 polarimeter, a Bio-Tek µQuant MQX200 ELISA reader, and a PerkinElmer Spectrum 100 FT-IR spectrometer, respectively. ^{1}H-NMR, ^{13}C-NMR, HSQC, HMBC, ^{1}H-^{1}H gCOSY, and DEPT spectra were obtained on a Varian Unity Inova-600 MHz spectrometer using $CDCl_3$ as the solvent. HREIMS data were determined with a Thermo/Finnigan Quest MAT 95XL Mass Spectrometer. HPLC was carried out over a Phenomenex column (5 µm, 250 × 10 mm, Phenomenex Co.) using a Shodex RI-101 detector. Open column chromatography was carried out over silica gel (4 × 30 cm; 60–80 mesh; Merck).

Plant material

The leaves of *A. cordata* were collected in July 2009 from Tsuifeng, Nantou County, Taiwan, and were identified by Dr. Yen-Hsueh Tseng (NCHU). The voucher specimen [TCF13524] was deposited in the herbarium of the same university.

Extraction and purification

Air-dried leaves of *A. cordata* (242 g) were extracted with EtOH (10 L) at ambient temperature and concentrated under vacuum to yield the EtOH extract (23.88 g). The EtOH extract was partitioned between EtOAc-H_2O to give the EtOAc-soluble fraction (13.25 g) and the H_2O-soluble fraction. The EtOAc-soluble fraction displayed potent cytotoxicity (IC_{50} = 27.3 µg/mL) and was further chromatographed over silica gel (4 × 30 cm; 60–80 mesh; Merck) eluted with *n*-hexane and a gradient of *n*-hexane-EtOAc (100 : 0; 98 : 2; 95 : 5; 92 : 8; 89 : 11; 86 : 14; 83 : 17; 80 : 20; 75 : 25; 70 : 30; 65 : 35; 60 : 40; 50 : 50; 40 : 60; 30 : 70; 20 : 80; 10 : 90; 0 : 100, each 2 L). The eluent was collected in constant volumes (each 500 mL), and combined into 19 fractions based on TLC properties. Fraction 7 (obtained with *n*-hexane : EtOAc = 89 : 11, amount = 5.3 g) displayed the strongest cytotoxicity (IC_{50} = 10.9 µg/mL) and was further separated by HPLC using a normal-phase column (250 × 10 mm, 5 µm, Phenomenex Co.) with a mixture of *n*-hexane : EtOAc = 75 : 25 as eluent at a flow rate of 2 mL/min to obtain compound araliadiol (0.25 g).

Araliadiol (**1**): yellow oil; $[\alpha]_D^{25}$ = 198.8° (*c* 0.89, $CHCl_3$); UV (MeOH) λ_{max} (log ε) = 231, 244, 257 nm; IR ν_{max} = 3306, 3012, 2954, 2924, 2855, 2231, 2150, 1644, 1456, 1378, 1301, 1265, 1117, 984, 931, 879, 783 cm^{-1}; ^{1}H NMR (600 MHz, $CDCl_3$): δ = 5.47 (1H, *d*, *J* = 16.8 Hz), 5.25 (1H, *d*, *J* = 10.2 Hz), 5.94 (1H, *ddd*, *J* = 5.4, 10.2, 16.8 Hz), 4.94 (1H, *d*, *J* = 5.4 Hz), 5.20 (1H, *d*, *J* = 8.4 Hz), 5.51 (1H, *dd*, *J* = 10.2, 8.4 Hz), 5.60 (1H, *ddd*, *J* = 7.8, 10.2 Hz), 2.10 (2H, *q*, *J* = 7.8 Hz), 1.38 (2H, *m*), 1.23–1.31 (4H, *m*), 0.89 (3H, *t*, *J* = 6.6 Hz); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 117.3 (C-1), 135.7 (C-2), 63.3 (C-3), 78.2 (C-4), 70.2 (C-5), 68.8 (C-6), 79.8 (C-7), 58.4 (C-8), 127.5 (C-9), 134.5 (C-10), 27.6 (C-11), 29.1 (C-12), 22.6 (C-13), 31.7 (C-14), 14.1(C-15); EIMS *m*/*z* (rel. int) = 232.2 (M^+), HREIMS: $C_{15}H_{20}O_2$ found 232.1470[M^+], calc.: 232.1463.

Preparation of Mosher's esters

Absolute configuration of compound **1** at C-3 and C-8 was determined by the modified Mosher's method [11]. Briefly, compound **1** was dried under vacuum for 1 h, and then two portions (4 mg each) were dissolved in dry CH_2Cl_2 (2 mL) containing 2 mg of dimethylaminopyridine (Alfa Aesar). Eight equivalents of dicyclohexylcarbodiimide (Alfa Aesar) were added to each portion of araliadiol. Six equivalents of either the (*S*) or the (*R*) isomer of a-methoxy-a-trifluoromethylphenylacetyl chloride (Alfa Aesar) were added to a portion of araliadiol, resulting in the formation of the (*R*) and (*S*) Mosher's esters of araliadiol, respectively. The reaction mixtures were stirred for 24 h at room temperature, concentrated on a rotary evaporator and then redissolved in diethyl ether (3 mL). The reaction mixtures were partitioned between H_2O and ether, and extracts were concentrated and purified by HPLC. ^{1}H NMR spectrum was recorded, and differences in the signals of protons that neighbored the chiral centers noted. The bulk effect on chemical shifts induced by esterification with the chiral reagents was calculated using $\Delta\delta$ (= $\delta_S - \delta_R$), where δ_S and δ_R are the shifts (in ppm) of diagnostic protons neighboring the chiral centers in araliadiol of the (*S*) and (*R*) Mosher's esters, respectively. The absolute configuration of araliadiol was obtained by positioning the protons with positive $\Delta\delta$ values on the right side and those with negative $\Delta\delta$ values on the left side in the model (**Fig. 1 B**).

Cell culture and proliferation assay

MCF-7 (human breast adenocarcinoma, BCRC 60436) was purchased from BCRC (Bioresource Collection and Research Center), Food Industry Research, and Development Institute, Taiwan. MCF-7 cells were cultured in DMEM supplemented with 10% FBS, 1% penicillin–streptomycin, and 1 mM sodium pyruvate, and were maintained at 37 °C and 5% CO_2. All cells (1×10^3 per well) were seeded in 96-well plates and incubated for 24 h, and different dosages of extracts of araliadiol were added to each well in triplicate for 5 days. The cell viability was determined by the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay [12]. Plumbagin (Sigma) was used as positive control.

Cell cycle analysis

MCF-7 cells, seeded in a 60-mm dish (2×10^5 cells/dish), were treated with 20 µM, 40 µM, and 80 µM araliadiol for 48 h or with 40 µM araliadiol for 0, 6, 12, 18, 24, and 48 h. Subsequently, cells were trypsinized, collected with ice-cold PBS and resuspended in 1 mL PBS. Cells were then fixed by the addition of 3 mL ice-cold 95% ethanol at −20 °C overnight. The cell pellets were collected by centrifugation and rinsed with ice-cold PBS. Cells were stained with 1 mL of 50 µg/mL propidiumiodide (PI) in hypotonic buffer (0.5% Triton X-100 in PBS and 0.5 mg/mL RNase A) for 30 min. Fluorescence emitted from the PI–DNA complex was quantified after excitation of the fluorescent dye by flow cytometry (Cytomics FC 500, Beckman Coulter).

Protein expression analysis

The protein expression after treatment with araliadiol was determined by a Western blotting assay. Briefly, MCF-7 cells incubated in 100-mm culture dishes (1×10^6 cells per dish) and were treated with araliadiol at the dosages of 20 µM, 40 µM, and 80 µM for 48 h. 40 µg of total cell proteins were separated by 12% SDS–PAGE and transferred to a PVDF membrane. Detection was performed by immunostaining using specific primary antibodies and horse-

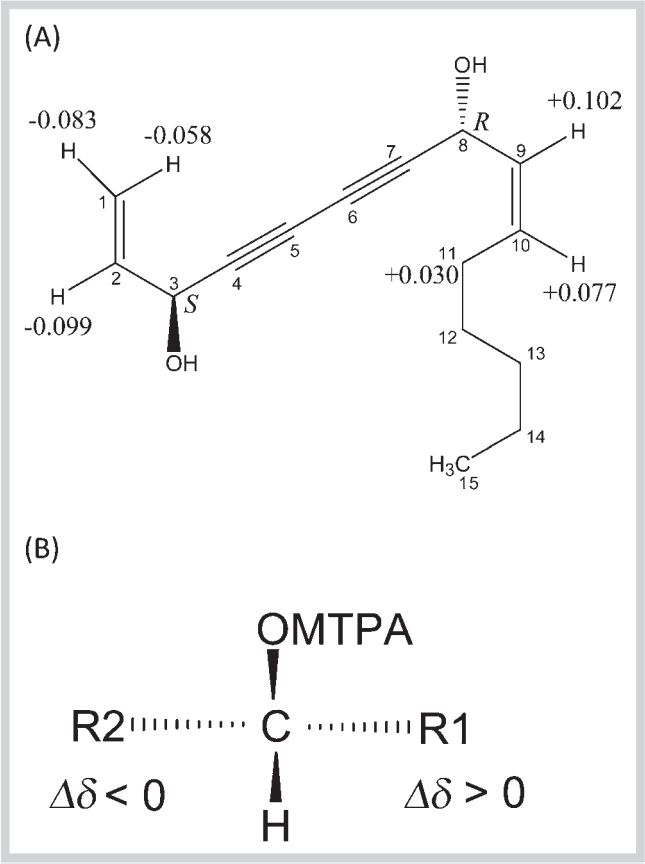

Fig. 1 A and **B** **A** Structure of araliadiol depicting its 3-(*S*),8-(*R*) absolute stereochemistry. The numbers represent $\Delta\delta$, where $\Delta\delta = \delta_S - \delta_R$. δ_S and δ_R are the chemical shift values for a particular group of the (*S*) and (*R*) Mosher's esters of araliadiol. **B** Model used to determine the absolute configurations of secondary alcohols.

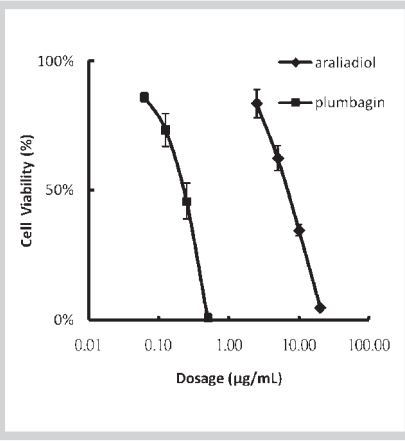

Fig. 2 Cytotoxic activity of araliadiol and plumbagin in MCF-7 cells. Cells were treated with various concentrations (2.5–20 μg/mL) of araliadiol for 5 days. The data are presented in terms of proportional viability (%) by comparing the araliadiol- and plumbagin-treated groups with untreated cells, the viability of which was assumed to be 100 %.

radish peroxidase-conjugated anti-IgG antibody. The proteins were detected by chemiluminescence (ECL, Pierce Biotechnology, Inc.). The following antibodies were used for the Western blots: rabbit polyclonal antibodies to $p27^{Kip1}$, cdk2, phospho(Ser807/811)-Rb, and Chk2; mouse polyclonal antibodies to cdk6, cdk4, cyclin D1, cyclin D3, cyclin E, $p21^{Waf1/Cip1}$, phospho(Ser15)-p53, and *β*-actin; goat anti-rabbit immunoglobulin G (IgG)-horseradish peroxidase-conjugate, and horse anti-mouse immunoglobulin G (IgG)-horseradish peroxidase-conjugate. Antibodies were used at working dilutions of 1 : 1000 with the exception of antibodies to *β*-actin, for which a working dilution of 1 : 10 000 was employed.

Statistical analysis

Data are expressed as means ± SD. Statistical comparisons of the results were made using analysis of variance (ANOVA). Significant differences (* $p < 0.05$) between the control (untreated) and treated cells were analyzed by Dunnett's test.

Results and Discussion

To investigate the cytotoxicticity of *A. cordata*, we examined the effect of ethanolic extracts against cell proliferation in MCF-7 cells by using the standard MTT method [12]. Data showing the inhibitory effects of the extract and derived fractions on the growth of MCF-7 cells are presented in the "Extraction and Purification" section. A yellow oil compound (**1**) was isolated from the strongest cytotoxicity subfraction (fraction 7). The HREIMS spectrum for compound **1** gave an ion peak [M^+] at 232.1470, suggesting a molecular formula of $C_{15}H_{20}O_2$. The UV absorptions at 231, 245, and 257 nm were similar to published data for diacetylenes [13], whereas the IR spectrum had absorptions corresponding to hydroxyl groups (3360 cm^{-1}), double bonds (1644 cm^{-1}), and triple bonds (2231 and 2150 cm^{-1}), respectively. In the ^1H NMR spectrum, terminal olefin protons at δ_H 5.94 (H-2), 5.47, and 5.25 (H-1), another olefinic signal at δ_H 5.60 (H-10), 5.51 (H-9), two oxymethines at δ_H 5.20 (H-8) and at δ_H 4.94 (H-3), besides methylene protons at δ_H 2.10 (H-11), 1.38 (H-12), and 1.23–1.31 (H-13, H-14) and methyl protons at δ_H 0.89 (H-15) were observed (**Table 1**). The configuration of the two olefin protons (H-9 and H-10) was assigned as a *Z* configuration based on their coupling constants ($J_{9,10}$ = 10.2 Hz). Furthermore, analyses of the ^1H, ^{13}C NMR, and ^1H–^1H COSY spectra permitted establishment of the sequence C-8–C-15 of the molecule, which was characterized by an alcohol function (C-8), a *cis* double bond (C-9 and C-10), four methylenes (C-11–C-14), and a methyl group (C-15), respectively. Further NMR spectroscopic analysis resulted in the determination of the remaining part of the molecule, namely a terminal olefin (C-1 and C-2) and a hydroxy group (C-3). In the ^{13}C NMR spectrum, four quaternary carbon signals at δ_c 79.8 (C-7), 78.2 (C-4), 70.2 (C-5), and 68.8 (C-6) were characteristically observed (**Table 1**). Furthermore, long-range correlations of H-2 to C-4, H-3 to C-4, H-3 to C-4 and C-5, H-8 to C-6 and C-7, and H-9 to C-7 suggested that C-1–C-3 and C-8–C-15 were linked by a conjugated diyne moiety (C-4–C-7). Therefore, the structure of compound **1** was elucidated as heptadeca-1,9(*Z*)-diene-4,6-diyne-3,8-diol, and named araliadiol. In order to determine the absolute configurations at C-3 and C-8, a modified Mosher's method [11] was applied. Araliadiol was treated with (*R*)- and (*S*)-α-methoxy-α-trifluoromethylphenylacetyl chloride (MTPA-Cl) in the presence of 4-dimethylaminopyridine (DMAP) and dicyclohexylcarbodiimide to give the di-(*S*)-and di-(*R*)-MTPA esters respectively. In the ^1H NMR spectrum of the di-(*S*)-MTPA ester, the proton signals assigned to H-1, and H-2 were observed at a higher field than those of the di-(*R*)-MTPA ester, and H-9, H-10, and H-11 were observed at a lower field than those of the di-(*R*)-MTPA ester, indicating that C-3 and C-8 had *S*- and *R*-configurations respectively (**Fig. 1**).

As shown in **Fig. 2**, when MCF-7 cells were treated with araliadiol at dosages of 2.5–20 μg/mL, a dose-dependent decrease of cell viability was observed. The IC_{50} value of araliadiol against

Table 1 ^{1}H-NMR and ^{13}C-NMR spectral data for araliadiol (**1**) and its *S*- and *R*-MTPA mosher esters.

Carbon number	^{1}H(J_{H-H} in Hz)	^{13}C	δS-ester	δR-ester	$\Delta\delta$ ($=\delta_S-\delta_R$)
1	5.47 (1H, *d*, 16.8) 5.25 (1H, *d*, 10.3)	117.3	5.512 5.361	5.595 5.491	− 0.083 − 0.058
2	5.94 (1H, *ddd*, 5.4, 10.2, 16.8)	135.7	5.812	5.911	− 0.099
3	4.94 (1H, *d*, 5.4)	63.3			
4		78.2			
5		70.2			
6		68.8			
7		79.8			
8	5.20 (1H, *d*, 8.4)	58.4			
9	5.51 (1H, *dd*, 10.2, 8.4)	127.5	5.543	5.441	+ 0.102
10	5.60 (1H, *ddd*, 7.8, 10.2)	134.5	5.762	5.685	+ 0.077
11	2.10 (2H, *q*, 7.8)	27.6			
12	1.38 (2H, *m*)	29.1			
13	1.23–1.31 (4H, *m*)	22.6			
14		31.7			
15	0.89 (3H, *t*, 6.6)	14.1			

Table 2 Cell cycle distribution of MCF-7 cells treated with araliadiol.

Treatment	Percentage of cells in			
	G_1	S	G_2/M	SubG_1
Control	54.6 ± 0.7	18.2 ± 0.3	21.7 ± 1.1	1.1 ± 0.2
Araliadiol				
▸ 20 µM	68.2 ± 0.2*	12.7 ± 0.2*	14.6 ± 0.6*	1.1 ± 0.1
▸ 40 µM	75.2 ± 1.0*	9.3 ± 0.8*	12.1 ± 0.5*	1.3 ± 0.3
▸ 80 µM	72.0 ± 2.4*	11.4 ± 0.2*	13.2 ± 1.9*	1.3 ± 0.1
Plumbagin (positive control)				
4 µM	56.7 ± 2.0	12.3 ± 0.7*	11.2 ± 1.2*	17.3 ± 3.7*

* $P < 0.05$ versus control by analysis of variance with Dunnett's post hoc test

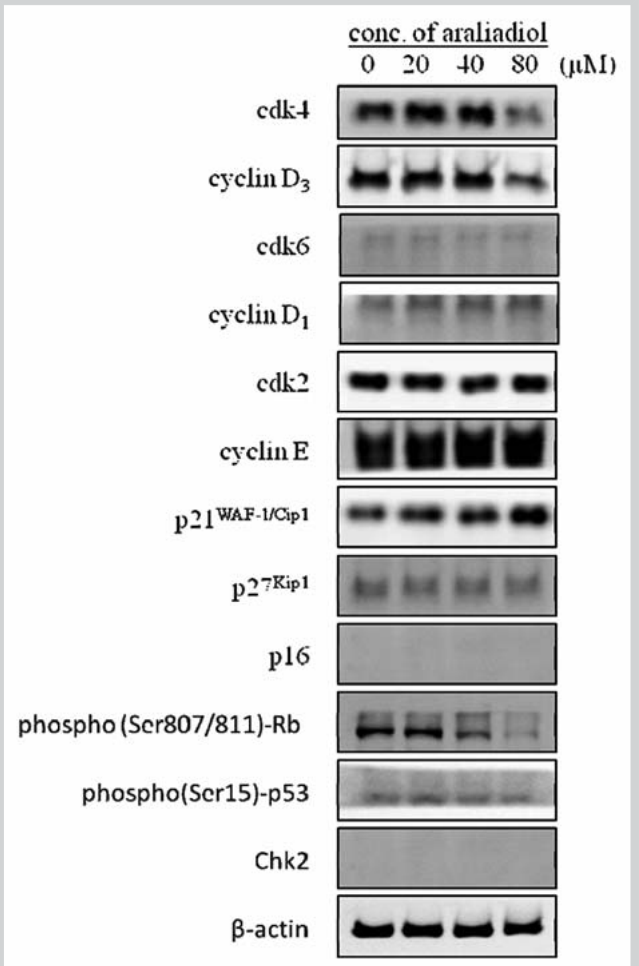

Fig. 3 The expression of cell cycle-related proteins in MCF-7 cells treated with various concentrations of araliadiol was determined by Western blotting.

MCF-7 cells was 6.41 µg/mL (27.6 µM). Although cytotoxicity of araliadiol was weaker than that of plumbagin (IC_{50} = 0.24 µg/mL) used as a positive control, comparison with the other polyacetylenic compounds, panaxytriol and dehydrofalcarindiol-8 acetate (IC_{50} values > 100 µM) [8, 14], revealed that the growth-inhibiting activity of araliadiol was potent. To examine the mechanism responsible for araliadiol-mediated cell growth inhibition, cell cycle distribution was evaluated using flow cytometric analysis. When MCF-7 cells were treated with 0, 20, 40, and 80 µM of araliadiol for 48 h, the percentage of G_1 cells was 54.6, 68.2, 75.2, and 72.0, respectively (**Table 2**). The results showed that treating cells with araliadiol caused a significant inhibition of cell cycle progression in MCF-7 cell lines at 48 h, resulting in a clear increase in the percentage of cells in the G_1 phase when compared with the control, which is consistent with G_1 arrest.

Next, we performed Western blot analysis to examine the effects of araliadiol on the expression of G_1-specific cell cycle regulation proteins. When MCF-7 cells were exposed to different doses of araliadiol for 48 h, the levels of cyclin D_3 and cdk4, two major G_1 regulatory proteins, decreased significantly at high concentration (80 µM) treatment (**Fig. 3**). By contrast, protein levels of cdk6, cdk2, cyclin D_1 and cyclin E were found to be unaltered by araliadiol treatment. In addition, exposure of cells to araliadiol resulted in a dose-dependent increase in the levels of tumor suppressor [12] $p21^{Waf1/Cip1}$, a cyclin-dependent kinase inhibitor belonging to the Cip/Kip family ($p21^{Waf1/Cip1}$, $p27^{Kip1}$, and $p57^{Kip2}$), and there is no change in $p27^{Kip1}$ expression. Meanwhile, the p16, a cdk4/cdk6 inhibitor, was detected in MCF-7 cells, which is in agreement with the deletion of the MTS1 gene that encodes p16 [15, 16] in this cell line. The level of Rb (phosphorylation at Ser807/811), a major regulator of the G_1/S transition, was reduced in MCF-7 cells by araliadiol treatment. According to previous studies, Rb binds to and represses the transcription factor E2F during early and mid-G_1 phase [17], and can be phosphorylated by G_1 cyclin-cdk complexes in the late G_1 phase [18, 19]. Phosphorylated Rb releases E2F, permitting the transcription of the S-phase promoting gene [20, 21]. In araliadiol-treated cells, inhibition of cdk activity by a combination of cdk4 and cyclin D_3 downregulation and $p21^{waf1/Cip1}$ upregulation would therefore be expected to prevent changes in E2F-regulated gene expression following the failure of these cells to progress from G_1 into S.

Expression of $p21^{WAF-1/Cip1}$ can be regulated by p53-dependent or p53-independent mechanisms [22–25]. Previous studies indicated that G_1/S arrest could be achieved by p53, which is phosphorylated on Ser15 by ATM/ATR and on Ser20 by Chk2, through induction of $p21^{WAF-1/Cip1}$ transcription [26]. Because no altered expression of phosphorylated p53 (Ser15) and no expression of Chk2 were detected in MCF-7 cells under araliadiol treatment,

our data indicate that p21 is upregulated by a p53-independent mechanism.

Based on the results obtained in this study, we conclude that araliadiol, a new polyacetylene isolated from *A. cordata*, possesses a potent cytotoxicity activity against MCF-7 cells.

The antiproliferation effect of araliadiol on MCF-7 cells occurs through downregulation of cdk4 and cycline D_3 and upregulation of $p21^{WAF-1/Cip1}$ by a p53-independent mechanism. Further study is needed to evaluate its antitumor activity.

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Affiliations

[1] Department of Forestry, National Chung-Hsing University, Taichung, Taiwan
[2] School of Forestry and Resource Conservation, National Taiwan University, Taipei, Taiwan
[3] Institute of Biomedical Science, National Chung-Hsing University, Taichung, Taiwan
[4] Graduate Institute of Chinese Pharmaceutical Science, China Medical University, Taichung, Taiwan
[5] Agricultural Biotechnology Research Center, Academia Sinica, Taipei, Taiwan

References

1 *Lu FY.* Contributions to the dicotyledonous plants of Taiwan. Quart J Chin Forest 1976; 9: 127–143
2 *Minto RE, Blacklock BJ.* Biosynthesis and function of polyacetylenes and allied natural products. Prog Lipid Res 2008; 47: 233–306
3 *Matsunaga H, Katano M, Yamamoto H, Fujito H, Mori M, Takata K.* Cytotoxic activity of polyacetylene compounds in *Panax ginseng* C. A. Meyer. Chem Pharm Bull 1990; 38: 3480–3482
4 *Yang MC, Seo DS, Choi SU, Park YH, Lee KR.* Polyacetylenes from the roots of cultivated-wild ginseng and their cytotoxicity *in vitro*. Arch Pharm Res 2008; 31: 154–159
5 *Lee SW, Kim K, Rho MC, Chung MY, Kim YH, Lee S, Lee HS, Kim YK.* New polyacetylenes, DGAT inhibitors from the roots of *Panax ginseng*. Planta Med 2004; 70: 197–200
6 *Wang CN, Shiao YJ, Kuo YH, Chen CC, Lin YL.* Inducible nitric oxide synthase inhibitors from *Saposhnikovia divaricata* and *Panax quinquefolium*. Planta Med 2000; 66: 644–647
7 *Moon J, Yu SJ, Kim HS, Sohn J.* Induction of G_1 cell cycle arrest and $p27^{KIP1}$ increase by panaxydol isolated from *Panax ginseng*. Biochem Pharmacol 2000; 59: 1109–1116
8 *Kim JY, Lee KW, Kim SH, Wee JJ, Kim YS, Lee HJ.* Inhibitory effect of tumor cell proliferation and induction of G_2/M cell cycle arrest by panaxytriol. Planta Med 2002; 68: 119–122
9 *Morgan DO.* Principles of CDK regulation. Nature 1995; 374: 131–134
10 *Pavletich NP.* Mechanisms of cyclin-dependent kinase regulation: structures of Cdks, their cyclin activators, and Cip and INK4 inhibitors. J Mol Biol 1999; 287: 821–828
11 *Hoye TR, Jeffrey CS, Shao F.* Mosher ester analysis for the determination of absolute configuration of stereogenic (Chiral) carbinol carbons. Nat Protoc 2007; 2: 2451–2458
12 *Chang S, Wang S, Wu CL, Shiah SG, Kuo YH, Chang CJ.* Cytotoxicity of extractives from Taiwania cryptomerioides heartwood. Phytochemistry 2000; 55: 227–232
13 *Jung HJ, Min BS, Park JY, Kim YH, Lee HK, Bae KH.* Gymnasterkoreaynes A–F, cytotoxic polyacetylenes from *Gymnaster koraiensis*. J Nat Prod 2002; 65: 897–901
14 *Seo CS, Li G, Kim CH, Lee CS, Jahng Y, Chang HW, Son JK.* Cytotoxic and DNA topoisomerases I and II inhibitory constituents from the roots of *Aralia cordata*. Arch Pharm Res 2007; 30: 1404–1409
15 *Kamb A, Gruis NA, Weaver-Feldhaus J, Liu Q, Harshman K, Tavtigian SV, Stockert E, Day RS 3rd, Johnson BE, Skolnick MH.* A cell cycle regulator potentially involved in genesis of many tumor types. Science 1994; 264: 436–440
16 *Lukas J, Parry D, Aagaard L, Mann DJ, Bartkova J, Strauss M, Peters G, Bartek J.* Retinoblastoma-protein-dependent cell-cycle inhibition by the tumour suppressor p16. Nature 1995; 375: 503–506
17 *Dyson N.* The regulation of E2F by pRB-family proteins. Genes Dev 1998; 12: 2245–2262
18 *Kitagawa M, Higashi H, Jung HK, Suzuki-Takahashi I, Ikeda M, Tamai K, Kato J, Segawa K, Yoshida E, Nishimura S, Taya Y.* The consensus motif for phosphorylation by cyclin D1-Cdk4 is different from that for phosphorylation by cyclin A/E-Cdk2. EMBO J 1996; 15: 7060–7069
19 *Harbour JW, Luo RX, Dei Santi A, Postigo AA, Dean DC.* Cdk phosphorylation triggers sequential intramolecular interactions that progressively block Rb functions as cells move through G1. Cell 1999; 98: 859–869
20 *LaThangue NB.* DRTF1/E2F: an expanding family of heterodimeric transcription factors implicated in cell-cycle control. Trends Biochem Sci 1994; 19: 108–114
21 *Nevins JR.* E2F: a link between the Rb tumor suppressor protein and viral oncoproteins. Science 1992; 258: 424–429
22 *Dulić V, Kaufmann WK, Wilson S, Tlsty TD, Lees E, Harper JW, Elledge SJ, Reed SI.* p53-dependent inhibition of cyclin-dependent kinase activities in human fibroblasts during radiation-induced G1 arrest. Cell 1994; 76: 1013–1023
23 *el-Deiry WS, Tokino T, Velculescu VE, Levy DB, Parsons R, Trent JM, Lin D, Mercer WE, Kinzler KW, Vogelstein B.* WAF1, a potential mediator of p53 tumor suppression. Cell 1993; 75: 817–825
24 *Jiang H, Lin J, Su ZZ, Collart FR, Huberman E, Fisher PB.* Induction of differentiation in human promyelocytic HL-60 leukemia cells activates p21, WAF1/CIP1, expression in the absence of p53. Oncogene 1994; 9: 3397–3406
25 *Macleod KF, Sherry N, Hannon G, Beach D, Tokino T, Kinzler K, Vogelstein B, Jacks T.* p53-dependent and independent expression of p21 during cell growth, differentiation, and DNA damage. Genes Dev 1995; 9: 935–944
26 *Sancar A, Lindsey-Boltz LA, Unsal-Kaçmaz K, Linn S.* Molecular mechanisms of mammalian DNA repair and the DNA damage checkpoints. Annu Rev Biochem 2004; 73: 39–85