CYTOTOXIC CONSTITUENTS FROM THE ROOT WOOD OF FORMOSAN *MICHELIA COMPRESSA*

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ABSTRACT

The methanolic extract of the root wood of *Michelia compressa* (Maxim.) Sargent showed cytotoxicity against MCF-7, NCI-H460 and SF-268 cancer cell lines. Bioassay-guided fractionation of the chloroform-soluble layer led to the isolation of costunolide and liriodenine with cytotoxic activities, along with the isolation of twenty-seven known compounds. The active costunolide existed as major constituent in this study.

Keywords: *Michelia compressa*, Magnoliaceae, root wood, cytotoxicity

INTRODUCTION

Michelia species have been used as folk medicine in the treatment of cancer[1]. In a screening program of Formosan plants for cytotoxic activity against MCF-7, NCI-H460 and SF-268 cancer cell lines, *Michelia compressa* (Maxim.) Sargent (*M. formosana* (Kaneh.) Masam. & Suzuki, *Magnolia compressa* Maxim.) (Magnoliaceae) was found to be one of the active species. *M. compressa* is an evergreen tree distributed in Japan, Ryukyu Island and Taiwan[2]. Its heart wood with high decay resistance against rots due to the presence of liriodenine[3]. The chemical investigation of leaves[4], bark[5], root bark[6], heart wood[7,8] stem[9], pericarp[10] have been studies, but the chemical and cytotoxic constituents of the root wood were still not conducted. Bioassay-guided fractionation of the CHCl₃-soluble layer of the root wood has led to the isolation of costunolide (**1**) and liriodenine (**11**) with cytotoxic activities, along with the isolation of twenty-five inactive known compounds. The isolation and the cytotoxic properties of the isolates are described herein.

EXPERIMENTAL

General experimental procedures

Melting points were determined with a YANACO micro-melting point apparatus and were uncorrected. IR spectra were taken on a Hitachi 260-30 spectrophotometer. UV spectra were obtained on a JASCO UV-240 spectrophotometer. EIMS spectra were recorded on a VG Biotech Quattro 5022 spectrometer. HREIMS were recorded on a JEOL JMX-HX 110 mass spectrometer. ¹H NMR and ¹³C NMR spectra were measured on a Varian Gemini 200, and Varian Unity Plus 400 spectrometers, and are given in parts per million (δ) downfield from internal TMS. Si gel 60 (Merck 70-230 mesh, 230-400 mesh) was used for column chromatography, and Si gel 60 F$_{254}$ (Merck) for TLC.

Plant material

The root wood of *M. compressa* was collected from Lai-I, Pingtung County, Taiwan, in July, 2004, and identified by Prof. I. S. Chen. A voucher specimen (Chen 5443) was deposited in the herbarium of the College of Pharmacy, Kaohsiung University, Kaohsiung, Taiwan.

Cytotoxicity assay

Human MCF-7 cells (breast cancer), NCI-H460 cells (lung cancer), and SF-268 cells (central nervous cancer) were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum and nonessential amino acid (Life Technologies, Inc.) and maintained at 37ºC in a humidified incubator with 5% CO₂. Human cancer cells were seeded in 96-well microtiter plates at a density of 6000/well in 100 μl culture medium. After an overnight adaptation period, 50 μg/ml (final concentration) of test compounds in serum-free medium were added to individual wells. Cells were treated with test compounds for 3 days. Cell viability was determined by the 5-(3-carboxymethoxyphenyl)-2-(4,5-dimethylthiazoyl)- 3-(4-sulfophenyl) tetrazolium salt (MTS) reduction assay. Actinomycin D 5 μM (final concentration) and DMSO 0.3 % (final concentration) were used as positive and vehicle controls. Results were expressed as a percentage of DMSO control[11].

Extraction and separation of compounds

The dried root wood (14.2 kg) was extracted with cold MeOH, and the extract was concentrated under reduced pressure. The MeOH extract (600 g) was partitioned between H₂O - CHCl₃ (1:1) to give the CHCl₃-soluble fraction (Fr. A, 280 g). The H₂O solution was partitioned between EtOAc-H₂O (1:1) to give EtOAc-soluble fraction (Fr. B, 13 g). The H₂O solution was partitioned between *n*-BuOH-H₂O (1:1) to give *n*-BuOH-soluble fraction (Fr. C, 84 g), and H₂O-soluble fraction (Fr. D, 192 g). Fraction A (100 g) was chromatographed on Si gel and eluted with *n*-hexane, and the polarity was gradually increased with EtOAc and MeOH to furnish 12 fractions (A-1 to A-12). Fr. A-4 (26.46 g) was washed with MeOH to give costunolide (**1**) (11.51 g). Part (200 mg) of the washing (13.67 g) was puritified by HPLC, eluting with *n*-hexane-EtOAc (4:1) to afford arbusculin A (**6**) (25 mg). Fraction A-7 (4.23 g) was subjected to Sephadex LH-20 and eluted with MeOH to give 6 fractions (A-7-1 to A-7-6). Fraction A -7-3 (8.2 mg)was purified by preparative TLC to produce (-)-*N*-formyldehydro- anonaine (**12**) (1.3 mg). Fraction A-8 (4.02 g) was subjected to Sephadex LH-20 and eluted with MeOH to give a mixture of 3β-hydroxylstigmast-5-en-7-one (**24**) and 3β-hydroxy-stigmasta-5,22-dien-7-one (**25**) (6.3 mg), a mixture of 6β-hydroxystigmast- 4-en-3-one (**26**) and 6β-hydroxystigmasta-4,22-dien-3-one (**27**) (7.3 mg). Fraction A-9 (4.09 g) was subjected to Si gel and eluted with CHCl₃, and then enriched with EtOAc to give santamarin (**2**) (13.3 mg), reynosin (**3**) (16.9 mg), (-)-*N*-formylanonaine (**9**) (3.3 mg), artabonatine A (**13**) (1.1 mg), and syringaldehyde (**23**) (6.3 mg). Fraction A-10 (11.69 g) was subjected to Si gel chromatography and eluted with CH₂Cl₂-acetone step gradients to give (-)-*N*-acetylanonaine (**7**) (2.7 mg). Fraction 11 (9.28 g) was subjected to Sephadex LH-20, and eluted with MeOH to give (-)-T-cadinol (**5**) (3.4 mg), (-)-*N*-acetylnornuciferine (**8**) (2.2 mg), *N-trans*-feruloyltyramine (**14**) (4.3 mg), *N-cis*-feruloyltyramine (**15**) (1.7 mg), *N-trans*-feruloylmethoxytyramine (**16**) (4.3 mg), *N-cis*-feruloylmethoxy-tyramine (**17**) (1.4 mg), (+)-5,5'-dimethoxylariciresinol (**18**) (3.3 mg), (+)-5-methoxylariciresinol (**19**) (1.9 mg), (+)-lariciresinol (**20**) (2.1 mg), (±)-syringaresinol (**21**) (113 mg), 1,2-*bis*-(4-hydroxy-3-methoxyphenyl)-1,3-ropane- diol (**22**) (3.4 mg). Fraction 12 (10.91 g) was subjected to Sephadex LH-20, and eluted with MeOH to give parthenolide (**4**) (18 mg), (-)-romucosine (**10**) (10.4 mg), and liriodenine (**11**) (13 mg).
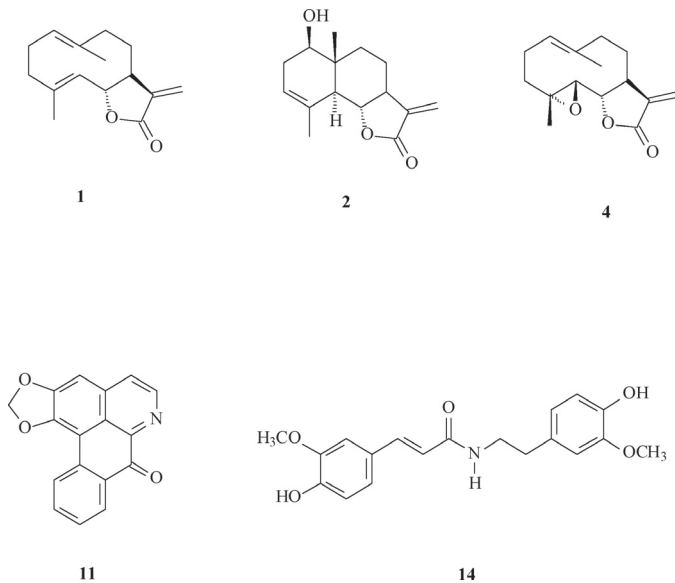
Spectroscopic data

costunolide (1): all spectroscopic data accord with ref. 6.
santamarin (2): all spectroscopic data accord with ref. 6.
parthenolide (4): all spectroscopic data accord with ref. 6.
liriodenine (11): all spectroscopic data accord with ref. 6.

RESULTS AND DISCUSSION

The EtOAc-soluble fraction of the methanol extract was fractionated by a combination of silica gel, Sephadex LH-20, and prep. TLC to yield 27 compounds, their structures were elucidated by 1D and 2D NMR spectra and comparison with literature data. Amomg above isolates, costunolide (**1**) was the major constituent from this study, and was also previously isolated from the root bark of the same plant by Ogura et al. (1978)[6]. From this study, we can reveal costunolide also exists in root wood as a major composition.

The cytotoxic effects of the isolates from the root wood of *M. compressa* were tested *in vitro* against MCF-7, NCI-H460 and SF-268 cancer cell lines. The cytotoxic data are shown in Table 1. The clinically used anticancer agent, mithramycin, was used as positive control. As can been shown from Table 1, compounds **2—4**, **6**, and **10** showed no cytotoxic activities, and the major constituent **1** and **11** were only shown weakly active.

From the results of the cytotoxic tests, the following conclusions can be drawn regarding these isolates: (a) Among the sesquiterpenes, only costunolide (**1**) as major constituent, showed marginal cytotoxicity against these three cancer cell lines. Comparing the cytotoxicities of costunolide (**1**), santamarin (**2**), and parthenolide (**4**), to the previous cytotoxic data of these three compounds on KB (IC$_{50}$ = 0.69, 1.1, and 0.45 μg/ml)[6] revealed that KB cell is more susceptible to these three compounds than MCF-7, NCI-H460, and SF-268 cell. (b) The aporphine, liriodenine (**11**) exhibited significant cytotoxic activity against these three cancer cell lines. This compound also can inhibit the proliferation of human hepatoma cell lines[12]. (c) Costunolide (**1**) exhibited more cytotoxic than parthenolide (**4**), and this revealed that epoxidation of the C-4,5 bond of **4** would result in reduced of cytotoxicity activity. Another known compound, *N-trans*-feruloylmethoxytyramine (**14**) with cytotoxicity against P-388 and HL-60 cancer cell lines was already reported[13].

The known isolates including six sesquiterpenes, costunolide (**1**)[6], santamarin (**2**) [6], reynosin (**3**)[6], parthenolide (**4**)[6], (-)-T-cadinol (**5**)[14] and arbusculin A (**6**)[15]; seven alkaloids, (-)-*N*-acetylanonaine (**7**)[16], (-)-*N*-acetylnornuciferine (**8**)[16], (-)-*N*-formylanonaine (**9**)[17], (-)-romucosine (**10**)[18], liriodenine (**11**)[9], (-)-*N*-formyldehydroanonaine (**12**)[19] and artabonatine A (**13**)[20]; four amides, *N-trans*-feruloyltyramine (**14**)[9], *N-cis*-feruloyltyramine (**15**)[9], *N-trans*-feruloylmethoxytyramine (**16**)[21] and *N-cis*-feruloylmethoxy-tyramine (**17**)[21]; five lignans, (+)-5,5'-dimethoxylariciresinol (**18**)[22], (+)-5-methoxylariciresinol (**19**)[23], (+)-lariciresinol (**20**)[22], (±)-syringaresinol (**21**)[9] and 1,2-*bis*-(4-hydroxy-3-methoxyphenyl)-1,3-ropanediol (**22**)[24]; one benzenoid, syringaldehyde (**23**)[25]; a mixture of 3β-hydroxylstigmast-5-en-7-one (**24**) and 3β-hydroxy-stigmasta-5,22-dien-7-one (**25**)[26], a mixture of 6β-hydroxystigmast-4-en-3-one (**26**) and 6β-hydroxystigmasta-4,22-dien-3-one (**27**)[27], were identified by comparison of physical and spectroscopic data (UV, IR, ^{1}H-NMR, [α]$_D$, and Mass spectroscopic data) to corresponding authentic samples or literature values. Among the isolates, 16 compounds (**5, 6, 10, 12, 13, 16-20, 22-27**) were first isolated from this species.

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1   **2**   **4**

11   **14**

Table. 1 Cytotoxic effects of compounds isolated from the root wood of *Michelia compressa* against MCF-7, NCI-H460 and SF268 cell lines

Compounds	IC$_{50}$ (μg/ml)		
	MCF-7	NCI-H460	SF-268
Costunolide (**1**)	5.26	6.95	5.31
Santamarin (**2**)	17.42	15.18	10.48
Reynosin (**3**)	10.93	13.96	10.76
Parthenolide (**4**)	>50	>50	>50
T-Cadinol (**6**)	>50	>50	>50
(-)-Romucosine (**10**)	>50	>50	>50
Liriodenine (**11**)	2.75	3.21	3.22

[a] Positive control : Actinomycin D
[b] For significant activity of pure compound an IC$_{50}$ ≤ 4.0 μg/ml is required.