Cytotoxic Lignans of *Justicia ciliata*

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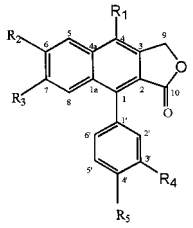
Two new naturally occurring 1-aryl-2,3-naphthalide lignans, cilinaphthalide A (**1**) and cilinaphthalide B (**2**), and nine known compounds were isolated from the whole plant of *Justicia ciliata*. Their structures were established by spectral analysis, and their cytotoxic activity was evaluated against several different cell lines. The known compound, justicidin A, showed potent cytotoxic effects against T-24, CaSki, SiHa, HT-3, PLC/PRF/5, and 212 cells in vitro.

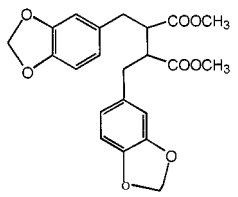
The whole plant of *Justicia ciliata* (Yamamoto) Hsieh & Huang (Acanthaceae) is used as an herbal remedy for treatment of fever and pain.[1] In a continued search for novel cytotoxic constituents from plants, two new naturally occurring 1-aryl-2,3-naphthalide lignans—cilinaphthalide A (**1**) and cilinaphthalide B (**2**)—and nine known compounds—heliobuphthalmin (**3**), chinensinaphthol methyl ether (**4**), justicidin A (**5**), neojusticin B (**6**), taiwanin E methyl ether (**7**), chinensinaphthol (**8**), diphyllin, 4′-demethyl chinensinaphthol methyl ether, and the lignan glycoside, tuberculatin—were isolated from this plant. Compounds **3**, **4**, **6**, **7**, and justicidin B were isolated previously from *J. ciliata*.[1] In the present paper the structure elucidation of the two new natural compounds, **1** and **2**, and the cytotoxicity of **1**−**8** against several cell lines, are reported.

The HREIMS of **1** indicated a molecular ion peak at *m*/*z* 396.1177, which corresponded to a molecular formula of $C_{22}H_{20}O_7$. Its IR spectrum showed absorption bands for a hydroxy group (3275 cm^{-1}), an aromatic γ-lactone (1710 cm^{-1}), and an aromatic ring (1595 cm^{-1}), and the UV spectrum exhibited absorption maxima similar to those of justicidin A.[2] The ^{1}H NMR spectrum of **1** showed proton signals ascribable to a lactone methylene group, four methoxy groups, five aromatic protons, and a phenolic proton (exchangeable with D_2O). The γ-lactone methylene proton signals appeared at δ 5.54, indicating that **1** was a 1-aryl-2,3-naphthalide lignan.[2] In the 2D NOESY spectrum of **1**, there were correlations between the proton signal of H-9 and the methoxy proton signal at δ 4.13, between the proton signal of H-5 and the methoxy proton signal at δ 4.07, between the proton signal of H-2′ and the methoxy proton signal at δ 3.88, and between the proton signal of H-8 and the methoxy proton signal at δ 3.79. These data and a negative Gibb's test for **1** clearly indicated that the phenolic group was located at C-4′, and the four methoxy signals at 3.79, 3.88, 4.07, and 4.13 were assigned to C-7, C-3′, C-6, and C-4, respectively. Consequently, **1** was characterized as 1-(4′-hydroxy-3′-methoxy)-phenyl-4,6,7-trimethoxy-2,3-naphthalide (**1**).

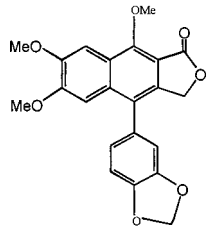
The ^{13}C NMR assignments (Table 1) of **1** were made by performing ^{1}H-decoupled and DEPT experiments, and by comparing it with the corresponding data of **5** (Table 1). The ^{13}C NMR spectrum also supported this structure assignment.



1 R_1= R_2= R_3= R_4= -OMe, R_5= -OH
2 R_1= R_2= R_3= R_4= R_5= -OMe
4 R_1= R_4= R_5= -OMe, R_2-R_3= -OCH_2O-
5 R_1= R_2= R_3= -OMe, R_4-R_5= -OCH_2O-
7 R_1= -OMe, R_2-R_3= R_4-R_5= -OCH_2O-
8 R_1= -OH, R_2-R_3= -OCH_2O-, R_4= R_5= -OMe



3



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The HREIMS of **2** indicated a molecular ion peak at *m*/*z* 410.1361, which corresponded to a molecular formula of $C_{23}H_{22}O_7$. Its IR spectrum showed absorption bands for an aromatic γ-lactone (1750 cm^{-1}) and an aromatic ring (1600 cm^{-1}), and the UV spectrum exhibited absorption maxima similar to those of **1**. The ^{1}H NMR spectrum was similar to that of **1**. It showed proton signals ascribable to a lactone methylene group, five methoxy groups, and five aromatic protons. The signal due to the γ-lactone methylene protons appeared at δ 5.55, indicating that **2** was also a 1-aryl-2,3-naphthalide lignan.[2] Based on the above

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Table 1. ^{13}C NMR (δ) for **1**, **2**, and **5** (100 MHz in $CDCl_3$)[a]

carbon	1	2	5[b]
1	135.0	134.7	134.4
1a	126.1	126.0	126.0
2	119.1	119.1	119.3
3	124.8	124.7	124.5
4	145.5	147.7	147.5
4a	130.7	130.6	130.6
5	100.5	100.5	100.6
6	151.6	151.6	151.6
7	150.3	150.3	150.3
8	106.4	106.3	106.2
9	66.5	66.5	66.6
10	169.6	169.5	169.5
1′	126.7	127.3	128.5
2′	114.2	113.6	110.8
3′	147.7	148.7	147.4
4′	146.2	148.5	147.4
5′	113.3	110.8	108.1
6′	123.3	122.7	123.6
7′			101.2
4-OMe	59.7	59.7	59.6
6-OMe	56.1	56.1	56.1
7-OMe	55.8	55.8	55.8
3′-OMe	56.1	55.8	
4′-OMe		55.9	

[a] The number of protons directly attached to each carbon was verified by DEPT experiments. [b] Signals obtained by $^1H-^1H$ COSY, HMQC, HMBC, and NOESY techniques and comparison with the corresponding data of reported data.[1]

results, **2** was characterized as 1-(3′,4′-dimethoxy)-phenyl-4,6,7-trimethoxy-2,3-naphthalide.

The ^{13}C NMR assignments (Table 1) of **2** were made by performing ^1H-decoupled and DEPT experiments and by comparing it with the corresponding data of **5**. The ^{13}C NMR spectrum also supported this structure assignment.

Cytotoxicity of **1**−**8** isolated from this plant was studied against a number of cancer cell types. The results are listed in Table 2. Compound **5** showed nearly the same cytotoxic activity against T-24, CaSki, SiHa, HT-3, and PLC/PRF/5 as did actinomycin D used as positive control. Compounds **1** and **7** exhibited significant in vitro cytotoxicity against T-24, CaSki and HT-3, and HT-3, respectively. Compounds **2**, **3**, **4**, **6**, and **8**, did not show significant cytotoxicity against the cancer cell types in Table 2.

Experimental Section

General Experimental Procedures. Melting points are reported uncorrected. UV spectra were obtained on a JASCO model 7800 UV−vis spectrophotometer, and IR spectra were recorded on a Hitachi model 260−30 spectrophotometer. ^1H (400 MHz) and ^{13}C (100 MHz) NMR spectra were recorded on a Varian Unity-400 spectrometer, and MS were obtained on a JMS−HX 100 mass spectrometer.

Plant Material. Whole plants of *J. ciliata* were collected in Pong Hwu Hsein, Taiwan, in July 1996, and a voucher specimen is on deposit at the Department of Medicinal Chemistry, School of Pharmacy, Kaohsiung Medical College.

Extraction and Isolation. The air-dried whole plant of *J. ciliata* (1.6 kg) was chipped and extracted with MeOH at room temperature. The MeOH extract (150 g) was chromatographed on Si gel to yield seven fractions. Fractions 2−6 were rechromatographed on Si gel, respectively. Fraction 2, eluted with cyclohexane−CH_2Cl_2 (3:7), yielded **3** (95 mg), and fraction 3, eluted with CH_2Cl_2, yielded **4** (127 mg) in the early fractions and **5** (65 mg) in the later fractions. Fraction 4, eluted with CH_2Cl_2, yielded **6** (35 mg) in the early fractions and **7** (42 mg) in the later fractions. Fraction 5, eluted with CH_2Cl_2−Me_2CO (7:3), yielded **1** (21 mg), **2** (34 mg), and **8** (35 mg) in the early fractions, and diphyllin (17 mg) and 4′-demethyl chinensinaphthol methyl ether (15 mg) in the later fractions. Fraction 6, eluted with CH_2Cl_2−MeOH (9.7:0.3), yielded tuberculatin (22 mg). The known compounds were identified by NMR and MS methods and by comparison with reported data.[1,3−6]

Cilinaphthalide A (1): colorless crystal; mp 221−222 °C; Gibb's test (negative); UV (CH_3Cl) λ_{max}(log ϵ) 250 (4.05), 260 (4.06), 265 (4.05), 275 (4.04), 315 (3.98), 350 (3.75) nm; IR (KBr) ν_{max} 3275 (OH), 1710, 1595 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 3.79 (3H, s, OMe-7), 3.88 (3H, s, OMe-3′), 4.07 (3H, s, OMe-6), 4.13 (3H, s, OMe-4), 5.54 (2H, s, H-9), 5.76 (1H, s, OH-4′), 6.86 (1H, dd, J = 8.4, 2.0 Hz, H-6′), 6.87 (1H, d, J = 2.0 Hz, H-2′), 7.06 (1H, d, J = 8.4 Hz, H-5′), 7.11 (1H, s, H-8), 7.55 (1H, s, H-5); ^{13}C NMR, see Table 1; EIMS (70 eV) m/z $[M]^+$ 396 (100), 381 (10), 353 (9), 338 (2), 335 (5), 321 (46), 182 (11); HREIMS m/z 396.1177 (calcd for $C_{22}H_{20}O_7$, 396.1209).

Cilinaphthalide B (2): colorless crystal; mp 202.5−203.5 °C; UV (CH_3Cl) λ_{max} (log ϵ) 260 (3.58), 290 (3.57), 305 (3.57), 325 (3.56), 350 (3.56) nm; IR (KBr) ν_{max} 1750, 1600 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 3.77 (3H, s, OMe-7), 3.87 (3H, s, OMe-4′), 3.98 (3H, s, OMe-3′), 4.07 (3H, s, OMe-6), 4.13 (3H, s, OMe-4), 5.55 (2H, s, H-9), 6.88 (1H, d, J = 1.6 Hz, H-2′), 6.93 (1H, dd, J = 8.0, 1.6 Hz, H-6′), 7.02 (1H, d, J = 8.0 Hz, H-5′), 7.09 (1H, s, H-8), 7.55 (1H, s, H-5);^{13}C NMR, see Table 1; EIMS (70 eV) m/z $[M]^+$ 410 (100), 395 (12), 380 (6), 367 (17), 336 (43), 189 (11); HREIMS m/z 410.1361 (calcd for $C_{23}H_{22}O_7$, 410.1365).

Tumor Cell Growth Inhibition Assays. A microassay for cytotoxicity was performed using a MTT assay.[7,8] Briefly, 1−3 × 10^3 cells/100 μL were seeded in 96-well microplates (Nunck, Roskilde, Denmark) and preincubated for 6 h in order to allow cell attachment. This medium was then aspirated, and 100 μL fresh medium containing various concentrations of test drug were added to the cultures. The cells were incubated with each drug for 6 days. Cell survival was evaluated by adding 10 μL tetrazolium salt solution (1 mg MTT/mL in PBS). After 4 h incubation at 37 °C, 100 μL DMSO were added to dissolve the precipitate of reduced MTT. Microplates were then shaken for 15 min, and the absorbance was determined at 550 nm with a multiwell scanning spectrophotometer (Dynex MR 5000, Chantilly, VA).

PLC/PRF/5 cells were established from a human hepatoma and known to produce HBs Ag continuously in culture fluids.[9] Human hepatoma PLC/PRF/5, T24 cells, human cervical carcinoma, HT-3, SiHa, and CaSki cells were maintained in Dulbecco's modified Eagle medium (DMEM; Gibco BRL, Grand Island, NY),[7,8] containing 10% fetal bovine serum (FBS; Gibco BRL), 2 mM 1-glutamine, 100 units/mL penicillin, and 100 μg/mL streptomycin. The 212 cells (an inducible Ha-*ras* oncogene transformed the NIH/3T3 cell line) were maintained in minimum essential alpha medium (MEM; Gibco BRL), containing 10% calf serum (Gibco BRL) and antibiotics.[10] For the microassay, the growth medium was supplemented with 10 mM HEPES buffer pH 7.3 and incubated at 37 °C in a CO_2 incubator.

Table 2. Cytotoxicity of Lignans Isolated from *Justicia ciliata* (ED_{50} values in μg/mL)[a]

	cell line					
compound	T-24	CaSki	SiHa	HT-3	PLC/PRF/5	212
1	1.3	3.0	6.4	0.3	12.0	>8.0
5	2.0×10^{-3}	3.0×10^{-3}	7.4×10^{-3}	1.8×10^{-3}	2.2×10^{-3}	22.7×10^{-3}
7	5.0	>8.0	6.1	2.6	6.8	>8.0
actinomycin D	1.5×10^{-3}	1.9×10^{-3}	8.1×10^{-4}	5.6×10^{-4}	1.4×10^{-3}	*b*

[a] For significant activity of the pure compounds, an ED_{50}<4.0 μg/mL is required. [b] Not determined.

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