New Cytotoxic Butanolides from *Lindera obtusiloba* BLUME

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Three new butanolides, 2-(1-methoxy-11-dodecenyl)-penta-2,4-dien-4-olide (1), (2*Z*,3*S*,4*S*)-2-(11-dodecenylidene)-3-hydroxy-4-methylbutanolide (2) and (2*E*,3*R*,4*R*)-2-(11-dodecenylidene)-3-hydroxy-4-methoxy-4-methylbutanolide (3), were isolated from the stems of *Lindera obtusiloba* BLUME. Their chemical structures were assigned by spectroscopic evidence. They exhibited cytotoxicity against cultured human tumor cell lines with their ED_{50} values ranging from 3.19 to 14.63 μg/ml.

Key words *Lindera obtusiloba* BLUME; Lauraceae; cytotoxicity; 2-(1-methoxyl-11-dodecenyl)-penta-2,4-dien-4-olide; (2*Z*,3*S*,4*S*)-2-(11-dodecenylidene)-3-hydroxy-4-methylbutanolide; (2*E*,3*R*,4*R*)-2-(11-dodecenylidene)-3-hydroxy-4-methoxy-4-methylbutanolide

Lindera obtusiloba BLUME (Lauraceae), a ubiquitous tree distributed mainly in Southeast Asia, has been used in traditional Chinese medicine for the treatment of bruise and extravasation.[1] Several phytosterols[2] and obtusilactones[3,4] have been identified from this plant. In the course of searching for bioactive compounds from Korean natural sources, a methanolic extract of *Lindera obtusiloba* was investigated. We previously reported five moderate cytotoxic lignans from the methylene chloride soluble fraction of this plant.[5] In continuation of our search for cytotoxic compounds, three new butanolides were isolated from the hexane soluble fraction of this plant. The present paper describes the isolation, structural characterization and cytotoxic activity of these butanolide compounds.

Compound **1** was obtained as colorless oil and its molecular formula was determined to be $C_{18}H_{28}O_3$ by HR-EI-MS (*m/z* 292.203812, M^+). The presence of one carbonyl carbon (δ 169.76) and four olefinic signals (δ 154.49, 137.76, 137.67, 97.82) in ^{13}C-NMR, UV λ_{max} of 264 nm, and the lactonic absorption band at 1775 cm^{-1} in IR spectrum suggested that compound **1** contained a penta-2,4-dien-4-olide moiety.[6] ^{1}H-NMR spectrum showed the presence of an exomethylene group at δ 4.88 (1H, d, J=2.6 Hz) and 5.20 (1H, d, J=2.6 Hz), and an olefinic proton at δ 7.22 (1H, t, J=0.5 Hz), respectively. Also, it showed the signals corresponding to a methoxy functionality at δ 3.34 (3H, s), a terminal double bond at δ 5.81 (1H, ddt, J=17.0, 10.5, 6.5 Hz), 4.93 (1H, dm, J=10.5 Hz) and 4.99 (1H, dtd, J=17.0, 2.3, 1.7 Hz), and long chain aliphatic hydrocarbons at δ 1.26 (10H, br s), 1.35 (4H, m), 1.64—1.74 (2H, m), and 2.03 (2H, m). Available chemical structures of the butanolides derived from the *Lindera* species[3,7] and our ^{1}H-NMR spectral data revealed that **1** also has a long aliphatic 11-dodecenyl chain. Based on the analysis of the ^{1}H–^{1}H correlated spectroscopy (COSY) and heteronuclear multiple bond correlation (HMBC) spectral data (Fig. 2), the structure of **1** was determined as 2-(1-methoxyl-11-dodecenyl)-penta-2,4-dien-4-olide. It is optically active ($[\alpha]_D$ +36.15°), but the configuration at C-6 remains uncertain.

Compound **2** was obtained as colorless oil, $[\alpha]_D$ −48.70° (c=0.046, $CHCl_3$). Its molecular formula was determined as $C_{17}H_{28}O_3$ by HR-EI-MS (*m/z* 280.203734, M^+). IR and NMR spectral data and the UV absorption band at 220 nm ($\log\varepsilon$=3.98) suggested that **2** was a 2-alkylidene-3-hydroxyl-4-methylbutanolide derivative.[8—10] The ^{1}H-NMR spectrum was very similar to that of litsenolide A_1,[11] except for the $[\alpha]_D$ value, chemical shifts and coupling constants of H-3 and H-4 [δ 4.66 (1H, dd, J=5.1, 1.1 Hz, H-3), 4.56 (1H, qd, J=6.5, 5.1 Hz, H-4)] (Table 1). These data suggested that **2** was a C-4 epimer of litsenolide A_1 [as (2*Z*,3*S*,4*R*)-2-(11-dodecenylidene)-3-hydroxy-4-methylbutanolide]. The geometry of alkylidene side chain was *cis* to the carbonyl group based on the upfield shift of H-6 (δ 6.57) and the downfield shift of H-7 (δ 2.76) protons, compared with the chemical shifts of H-6 (δ 7.16) and H-7 (δ 2.40) in *E*-form.[4,7] This was also confirmed in nuclear overhauser enhancement spectroscopy (NOESY) spectrum which showed the cross peaks between H-3/H-6 (Fig. 3). Based on the analysis of $[\alpha]_D$ value and ^{1}H-NMR data, the configuration at C-3 was deter-

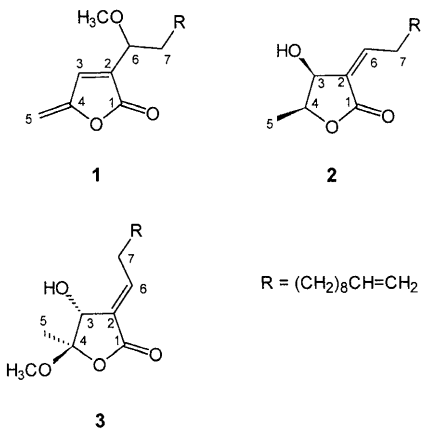

Fig. 1. Structures of Compounds **1**—**3**

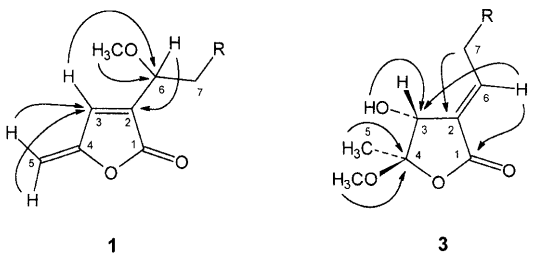

Fig. 2. HMBC Correlations of Compounds **1** and **3**

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Table 1. ^{1}H-NMR Data for Compounds **1**—**3** (500 MHz, $CDCl_3$)[a]

Position	1	2	Litsenolide A_1[11]	3
3	7.22 t (0.5)	4.66 dd (5.1, 1.1)	4.37 m	4.52 dd (7.1, 1.1)
4		4.56 qd (6.5, 5.1)	4.33 qd (6.5, 4.0)	
5	4.88 d (2.6)	1.40 d (6.5)	1.37 d (6.5)	1.61 s
	5.20 d (2.6)			
6	4.11 ddd (7.4, 4.8, 1.1)	6.57 td (8.0, 1.1)	6.53 td (7.8, 1.5)	6.97 td (8.0, 1.5)
7	1.64—1.74 m	2.76 m	2.73 dr q	2.38 m
8	1.35 m	1.47 m		1.51 br hep (7.5)
9—13	1.26 br s	1.28 br s	1.30 br s	1.28 br s
14	1.35 m	1.34 m		1.36 m
15	2.03 m	2.04 m	2.05 br q	2.04 m
16	5.81 ddt (17.0, 10.5, 6.5)	5.82 ddt (17.0, 10.2, 7.0)	4.8—6.2 m	5.81 ddt (17.0, 10.5, 6.5)
17	4.93 dm (10.5)	4.93 dm (10.2)	4.8—6.2 m	4.93 dm (10.5)
	4.99 dtd (17.0, 2.3, 1.7)	4.99 dtd (17.0, 2.2, 1.7)		4.99 dtd (17.0, 2.0, 1.5)
OCH_3	3.34 s			3.78 s
OH				2.22 d (7.1)

a) Values in parentheses are coupling constants in Hz.

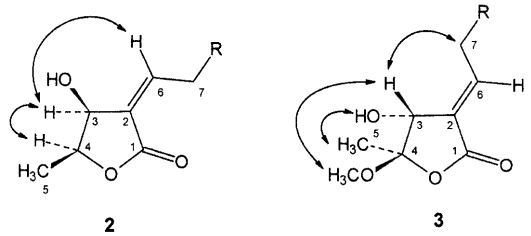

Fig. 3. NOESY Correlations of Compounds **2** and **3**

mined to be *S* as in the case of litsenolide A_1.[11] Martinez *et al.* studied the correlations between $[\alpha]_D$ value and the configuration at C-3 for 2-alkylidene-3-hydroxyl-4-mehylbutanolide derivatives; the 3*S* form has a negative $[\alpha]_D$ value, while the 3*R* form has a positive value.[10] The stereochemistry at C-4 was determined as 4*S* by the coupling constants ($J_{3,4}$=5.1 Hz) of H-3 and H-4.[10,12,13] The *cis* position of 3-OH and 4-CH_3 was also confirmed in NOESY spectrum (Fig. 3). The structure of **2** was, therefore, assigned as (2*Z*,3*S*,4*S*)-2-(11-dodecenylidene)-3-hydroxy-4-methylbutanolide.

Compound **3** was obtained as colorless oil and its molecular formula was determnined to be $C_{18}H_{30}O_4$ by HR-EI-MS (*m/z* 310.214485, M^+). IR, UV and NMR spectrum data were similar to those of compound **2**. The major difference was in the presence of a methoxy group in **3**, which was shown at δ 3.78 (3H, s) in ^{1}H-NMR spectrum. Analysis of the HMBC spectrum (Fig. 2) allowed for the position of the methoxy group. The gross structure of **3** was speculated to be 2-(11-dodecenylidene)-3-hydroxy-4-methoxy-4-methyl butanolide. The geometry of alkylidene side chain of **3** was *trans* to the carbonyl group based on the chemical shifts of H-6 (δ 6.97) and H-7 (δ 2.38) in ^{1}H-NMR spectrum.[4] The downfield shift of H-6 (δ 6.97), compared with that of H-6 (δ 6.57) in compound **2**, can be ascribed to the effect of a carbonyl group of the lactone ring. Its *trans* geometry was confirmed in NOESY spectrum which showed the cross peaks between H-3/H-7 (Fig. 3). The stereochemistry of C-3 was determined to be 3*R* based on the correlations between $[\alpha]_D$ value [+29.33 (c=0.06, $CHCl_3$)] and the configuration at C-3 for 2-alkylidene-3-hydroxyl-4-mehylbutanolide derivatives.[7,10] The stereochemistry of C-4 was determined to be 4*R* by NOESY spectrum (Fig. 3) which showed correlations between 3-OH and 4-CH_3 signals and between 4-OCH_3 and H-3 signals, indicating that 3-OH and 4-CH_3 are in the *cis* position (Fig. 3). The structure of **3** was, therefore, assigned as (2*E*,3*R*,4*R*)-2-(11-dodecenylidene)-3-hydroxy-4-methoxy-4-methylbutanolide.

Table 2. ^{13}C-NMR Data for Compounds **1**—**3** (125 MHz, $CDCl_3$)

Position	1	2	3
1	169.76	169.28	169.71
2	137.76	129.98	130.72
3	137.67	72.13	73.20
4	154.49	78.48	110.26
5	97.82	14.81	16.75
6	76.63	150.63	149.17
7	35.10	28.61	30.54
8	25.64	29.51	29.07
9—14[a]	29.63, 29.80, 30.08, 30.12, 30.16	29.62, 29.82, 29.96, 30.08, 30.14, 30.17	29.60, 29.79, 30.03 (×2), 30.12, 30.13
15	34.49	34.51	34.49
16	139.93	139.94	139.90
17	114.75	114.81	114.84
OCH_3	58.27		50.99

a) Values are interchangeable.

Table 3. Cytotoxicity of Compounds **1**—**3** against Five Cultured Human Tumor Cell Lines

Cancer Cell Lines	ED_{50} values[a]				
	A549	SK-OV-3	SK-MEL-2	XF498	HCT15
1	9.65	4.73	3.19	3.88	3.57
2	9.43	6.71	4.06	7.14	5.21
3	14.63	12.92	10.07	12.80	10.14
Doxorubicin	0.12	0.13	0.11	0.23	2.40

a) ED_{50} was defined as the concentration (μg/ml) that caused a 50% inhibition of cell growth *in vitro*.

Compounds **1** and **2** showed good cytotoxicity against five human tumor cells, A549 (non small cell lung cancer), SK-OV-3 (ovarian cancer), SK-MEL-2 (skin cancer), XF498 (CNS cancer) and HCT15 (colon cancer), whereas **3** showed moderate activity against these cell lines (Table 3).

Experimental

General NMR: in $CDCl_3$, Bruker AMX 500 and Varian UNITY INOVA 500. IR: in CCl_4, Nicolet model 205 FT-IR spectrophotometer. EI-MS: VG70-VSEQ mass spectrometer. Column chormatography: silica gel (Merck, 230—400 mesh) and Sephadex LH-20 (Pharmacia). TLC: Merck precoated Si gel F_{254} plates and RP-18 F_{254s} plates. Low pressure liguid chromatography: Merck Lichroprep Lobar®-A Si 60 (240×10 mm)

Plant Materials *Lindera obtusiloba* was collected in Suwon, Kyungi-Do, Korea in September 1997. A voucher specimen (SKK-97-001) is deposited in the College of Pharmacy at Sungkyunkwan University.

Extraction and Isolation The dried and chopped stems of *Lindera obtusiloba* (5.8 kg) were extracted with MeOH three times at room temperature. The resultant MeOH extract (200 g) followed by successive solvent partition gave hexane (20 g), CH_2Cl_2 (10 g), EtOAc (20 g) and *n*-BuOH (40 g) soluble fractions. The hexane fraction was the most active in the sulforhodamine B (SRB) assay.[14] Thus, the hexane soluble fraction (20 g) was chromatographed through a silica gel column using the gradient solvent system of hexane–EtOAc (15 : 1)→hexane–EtOAc (1 : 2) to give seven subfractions (A—G). The subfraction B (1.8 g) was chromatographed with silica gel eluting with hexane : EtOAc (4 : 1), followed by CH_2Cl_2 to give three subfractions. The second subfraction was rechromatographed over silica gel eluted with CH_2Cl_2 and further purified with silica gel Lobar®-A column (hexane : EtOAc=15 : 1) to yield **1** (5 mg). The subfraction E (1.7 g) was chromatographed with a silica gel column (CH_2Cl_2 : acetone=15 : 1—10 : 1 step gredient) to give four subfractions. The second subfraction (220 mg) was rechromatographed with silica gel (hexane : EtOAc=3 : 1) to yield two fractions. Each subfraction was further purified with Sephadex LH-20 column chromatography (CH_2Cl_2 : MeOH=1 : 1) to afford **2** (5 mg) and **3** (13 mg).

2-(1-Methoxyl-11-dodecenyl)-penta-2,4-dien-4-olide (**1**): Colorless oil. $[\alpha]_D$ +36.15° (*c*=0.052, $CHCl_3$). UV λ_{max} (EtOH) nm (log ε): 264 (4.08). IR (neat) cm^{-1}: 2927, 2855, 1775, 1652, 1291. EI-MS *m/z* (rel. int.): 292 (M^+, 0.9), 140 (21.6), 139 (100), 111 (10.8). HR-EI-MS *m/z*: 292.203812 (Calcd for $C_{18}H_{28}O_3$: 292.203845). ^{1}H-NMR: Table 1; ^{13}C-NMR : Table 2

(2*Z*,3*S*,4*S*)-2-(11-Dodecenylidene)-3-hydroxy-4-methylbutanolide (**2**): Colorless oil. $[\alpha]_D$ −48.70° (*c*=0.046, $CHCl_3$). UV λ_{max} (EtOH) nm (log ε): 221 (3.98). IR (neat) cm^{-1}: 3427, 2926, 2855, 1739, 1675, 1183. EI-MS *m/z* (rel. int.): 280 (M^+, 3.5), 262 (0.9), 198 (20.2), 164 (24.0), 155 (43.5), 142 (33.0), 137 (58.6), 123 (20.7), 109 (31.2), 81 (72.5), 70 (100). HR-EI-MS *m/z*: 280.203734 (Calcd for $C_{17}H_{28}O_3$: 280.203845). ^{1}H-NMR : Table 1 ;^{13}C-NMR : Table 2.

(2*E*,3*R*,4*R*)-2-(11-Dodecenylidene)-3-hydroxy-4-methoxy-4-methylbutanolide (**3**): Colorless oil. $[\alpha]_D$+29.33° (*c*=0.06, $CHCl_3$). UV λ_{max} (EtOH) nm (log ε): 221 (4.08). IR (neat) cm^{-1}: 3423, 2927, 2855, 1746, 1680, 1286. EI-MS *m/z* (rel. int.): 310 (M^+, 1.0), 292 (2.2), 153 (11.8), 140 (58.1), 123 (16.2), 109 (15.4), 70 (100). HR-EI-MS *m/z*: 310.214485 (Calcd for $C_{18}H_{30}O_4$: 310.214410). ^{1}H-NMR: Table 1; ^{13}C-NMR: Table 2.

Test for Cytotoxicity *in Vitro* Sulforhodamin B (SRB) bioassay was used as a cytotoxicity screening method. Activities of each compound were monitored at several concentration levels against five cultured human tumor cells; A549 (non small cell lung adenocarcinoma), SK-OV-3 (ovarian), SK-MEL-2 (skin melanoma), XF498 (CNS) and HCT15 (colon) *in vitro*.[14]

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