Secondary Metabolites from the Leaves of *Litsea lii* var. *nunkao-tahangensis*

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Investigation of the leaves extract of *Litsea lii* var. *nunkao-tahangensis* led to the isolation of five new butanolides, litsealiicolide A (**1**), isolitsealiicolide A (**2**), litsealiicolide B (**3**), isolitsealiicolide B (**4**), and isolitsealiicolide C (**5**), along with 17 known compounds. Their structures were determined through in-depth spectroscopic and mass-spectrometric analyses. Among the isolates, compounds **1** and **2** were cytotoxic against MCF-7, NCI-H460, and SF-268 cell lines *in vitro*. Compound **5** and isolinderanolide B (**6**) showed marginal cytotoxic activity against these three cell lines *in vitro*.

Introduction. – *Litsea lii* CHANG var. *nunkao-tahangensis* (LIAO) LIAO (Lauraceae) is an endemic variety, and distributed in the central Taiwan and Pintung County [1]. Previously, we reported new metabolites along with cytotoxicities from the *L. akoensis* HAY. [2–4] and *L. acutivena* HAY. (Lauraceae) [5][6]. In a series of studies on the cytotoxic constituents of Formosan plants, over 1,300 species were screened for *in vitro* cytotoxicity and *L. lii* var. *nunkao-tahangensis* was one of the active species. Only the leaves of this variety showed significant cytotoxic activity against MCF-7, NCI-H460, and SF-268 cancer cell lines *in vitro*. The chemical constituents of this plant have never been conducted. Investigation of its active $CHCl_3$-soluble fraction of the leaves led to the isolation of five new butanolides, litsealiicolide A (**1**), isolitsealiicolide A (**2**), litsealiicolide B (**3**), isolitsealiicolide B (**4**), and isolitsealiicolide C (**5**), together with 17 known compounds, one butanolide (**6**), one quinone, two tocopheroids, one tetralone, two sesquiterpenoids, one triterpenoid, two lignans, one flavanoid, one ionone, one steroid, one polyisoprenoid, and three aliphatics. In this article, we report on the isolation, structural elucidation of these five new compounds and the cytotoxic activities of all isolates.

Results and Discussion. – 1. *Structural Elucidation*. The $CHCl_3$-soluble fraction of the methanolic extract was fractionated by a combination of SiO_2 (normal and reverse phase), *RP-18* columns, and prep. TLC to yield 22 compounds. Their structures were elucidated by 1D- and 2D-NMR spectra and comparison with literature data.

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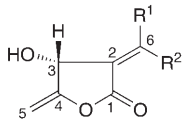

	R^1	R^2
1	H	$HC{\equiv}C(CH_2)_9$
2	$HC{\equiv}C(CH_2)_9$	H
3	H	$H_2C{=}C(CH_2)_9$
4	$H_2C{=}C(CH_2)_9$	H
5	$Me(CH_2)_{10}$	H
6	$Me(CH_2)_{12}$	H

Compound **1** was isolated as a colorless oil. From the HR-ESI-MS data, the molecular formula was determined to be $C_{17}H_{24}O_3$ (*m/z* 299.1624 ($[M+Na]^+$; calc. 299.1623)). The IR spectrum showed absorption bands for a OH group at 3432 cm^{-1} and an α,β-unsaturated γ-lactone at 1780 and 1679 cm^{-1}. On the basis of the above evidence, together with NOESY and HMBC (*Figure*) data, the structure of **1** was deduced to be (3*Z*,4*R*)-3-(dodec-11-yn-1-ylidene)dihydro-4-hydroxy-5-methylidenefuran-2(3*H*)-one, named litsealiicolide A.

The ^{1}H-NMR spectrum (*Table 1*) of **1** was similar to that of mahubynolide [7], indicating the same β-hydroxy-γ-methylene-α,β'-unsaturated γ-lactone structure and the same (*Z*)-geometry of the trisubstituted C=C bond at δ(H) 6.68 (*td*, *J* = 7.9, 2.0, H−C(6)[3])) in **1**. Compound **1** exhibited a characteristic terminal acetylene group (IR: 3307, 2123 cm^{-1}; ^{1}H-NMR: δ(H) 1.94 (*t*, *J* = 2.6, H−C(17)); ^{13}C-NMR: δ(C) 68.9 (C(17)), 84.4 (C(16))) in the side chain. The ^{1}H- and ^{13}C-NMR spectra of **1** closely resemble those of mahubynolide [7], except that the former had four CH_2 units less than the latter in the side chain. The configuration at C(3) was determined to be (3*R*) based on the correlation between the $[\alpha]_D$ value +28.9 (*c* = 0.02, $CHCl_3$) and the known (*R*) configuration at C(3) for the dextroratory 2-alkylidene-3-hydroxy-4-methylidenebutanolide derivatives [5][7][8].

Compound **2**, a colorless oil, with $[\alpha]_D^{24} = +18.9$, gave the $[M+Na]^+$ ion peak at *m/z* 299 in ESI-MS. The HR-ESI-MS data determined the molecular formula to be $C_{17}H_{24}O_3$ (*m/z* 299.1622 ($[M+Na]^+$; calc. 299.1623)). The IR spectrum showed absorption bands for a OH group at 3433 cm^{-1} and an α,β-unsaturated γ-lactone at 1770 and 1673 cm^{-1}. Based on the ^{1}H- and ^{13}C-NMR (*Table 1*), COSY, NOESY, HSQC and HMBC (*Figure*) experiments, the structure of **2** was elucidated as (3*E*,4*R*)-3-(dodec-11-yn-1-ylidene)dihydro-4-hydroxy-5-methylidenefuran-2(3*H*)-one, named isolitsealiicolide A.

From the spectral data, compound **2** was similar to **1**, and had the same β-hydroxy-γ-methylene-α,β'-unsaturated γ-lactone structure. A difference involves H−C(6)[3]) at δ(H) 7.07 (*td*, *J* = 8.0, 2.4) in **2** *vs.* δ(H) 6.68 in **1**, suggesting an (*E*) configuration for $\Delta^{2(6)}$. The dextrorotatory optical activity with the $[\alpha]_D^{24}$ value +18.9 (*c* = 0.12, $CHCl_3$) indicated that the configuration of C(3) was again (3*R*) [7][8].

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[3]) Arbitrary atom numbering, see *Figure*. For systematic names, see *Exper. Part.*

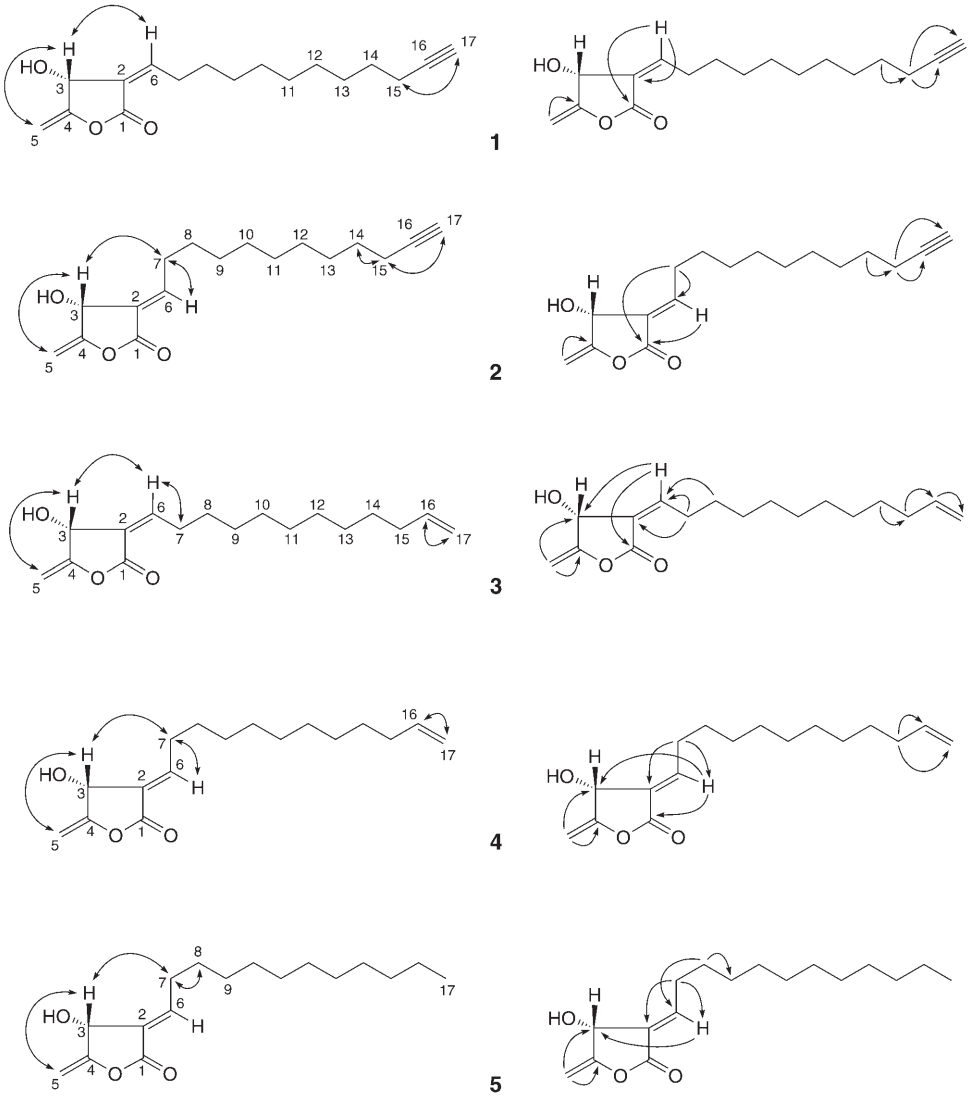

Figure. *Key NOESY* (↔) *and HMBC* (→) *correlations of compounds* **1**–**5**

Compound **3** was obtained as a colorless oil, giving the $[M+\mathrm{Na}]^+$ ion peak at *m*/*z* 301 in the ESI-MS. The HR-ESI-MS data determined the molecular formula to be $C_{17}H_{26}O_3$ (*m*/*z* 301.1778 ($[M+\mathrm{Na}]^+$; calc. 301.1780)). The IR spectrum showed absorption bands for a OH group at 3432 cm^{-1} and an α,β'-unsaturated γ-lactone at 1746 and 1639 cm^{-1}. The ^{1}H- and ^{13}C-NMR (*Table 2*), COSY, NOESY, HSQC, and HMBC (*Figure*) data established the structure of **3** as (3*Z*,4*R*)-3-(dodec-11-en-1-ylidene)dihydro-4-hydroxy-5-methylidenefuran-2(3*H*)-one, named litsealiicolide B.

Table 1. *^{1}H- and ^{13}C-NMR Data* ($CDCl_3$, 400 and 100 MHz, resp.) *of Compounds* **1** *and* **2**[3]). δ in ppm, J in Hz.

	1		**2**	
	δ(C)	δ(H)	δ(C)	δ(H)
C(1)	165.2	–	166.7	–
C(2)	126.8	–	127.3	–
H–C(3)	68.1	5.12 (br. *s*)	66.4	5.25 (br. *d*, *J* = 7.0)
HO–C(3)		2.16 (br. *s*)		2.27 (*d*, *J* = 7.0)
C(4)	157.5	–	157.6	–
CH_2(5)	90.3	4.68 (*dd*, *J* = 2.8, 1.6), 4.89 (*dd*, *J* = 2.8, 1.6)	91.4	4.72 (*dd*, *J* = 2.8, 1.6), 4.95 (*dd*, *J* = 2.8, 1.6)
H–C(6)	151.3	6.68 (*td*, *J* = 7.9, 2.0)	150.2	7.07 (*td*, *J* = 8.0, 2.4)
CH_2(7)	29.3	2.70–2.85 (*m*)	29.7	2.37–2.55 (*m*)
CH_2(8)	29.2	1.47–1.53 (*m*)	28.2	1.47–1.56 (*m*)
CH_2(9)	28.3–29.3	1.29 (br. *s*)	28.6–29.3	1.25 (br. *s*)
CH_2(10)	28.3–29.3	1.29 (br. *s*)	28.6–29.3	1.25 (br. *s*)
CH_2(11)	28.3–29.3	1.29 (br. *s*)	28.6–29.3	1.25 (br. *s*)
CH_2(12)	28.3–29.3	1.29 (br. *s*)	28.6–29.3	1.25 (br. *s*)
CH_2(13)	28.3–29.3	1.29 (br. *s*)	28.6–29.3	1.25 (br. *s*)
CH_2(14)	29.3	1.47–1.53 (*m*)	28.4	1.47–1.56 (*m*)
CH_2(15)	18.3	2.18 (*td*, *J* = 7.2, 2.6)	18.3	2.17 (*td*, *J* = 6.8, 2.6)
C(16)	84.4	–	84.8	–
H–C(17)	68.9	1.94 (*t*, *J* = 2.6)	68.1	1.94 (*t*, *J* = 2.6)

Table 2. *^{1}H- and ^{13}C-NMR Data* ($CDCl_3$, 400 and 100 MHz, resp.) *of Compounds* **3** *and* **4**[3]). δ in ppm, J in Hz.

	3		**4**	
	δ(C)	δ(H)	δ(C)	δ(H)
C(1)	165.3	–	166.8	–
C(2)	126.8	–	127.3	–
H–C(3)	68.8	5.11 (br. *s*)	66.4	5.25 (br. *d*, *J* = 7.4)
HO–C(3)	–	2.49 (*d*, *J* = 7.6)	–	2.39 (*d*, *J* = 7.4)
C(4)	157.5	–	157.7	–
CH_2(5)	90.3	4.73 (*dd*, *J* = 2.8, 1.6), 4.88 (*dd*, *J* = 2.8, 1.6)	91.3	4.73 (*dd*, *J* = 2.8, 1.3), 4.94 (*dd*, *J* = 2.8, 1.3)
H–C(6)	151.3	6.69 (*td*, *J* = 7.6, 2.0)	150.0	7.08 (*td*, *J* = 7.6, 2.0)
CH_2(7)	28.3	2.70–2.85 (*m*)	28.9–29.7	2.36–2.56 (*m*)
CH_2(8)	28.6	1.43–1.52 (*m*)	28.3	1.49–1.56 (*m*)
CH_2(9)	28.9–29.4	1.28 (br. *s*)	28.9–29.7	1.28 (br. *s*)
CH_2(10)	28.9–29.4	1.28 (br. *s*)	28.9–29.7	1.28 (br. *s*)
CH_2(11)	28.9–29.4	1.28 (br. *s*)	28.9–29.7	1.28 (br. *s*)
CH_2(12)	28.9–29.4	1.28 (br. *s*)	28.9–29.7	1.28 (br. *s*)
CH_2(13)	28.9–29.4	1.28 (br. *s*)	28.9–29.7	1.28 (br. *s*)
CH_2(14)	28.9–29.4	1.33–1.39 (br. *s*)	28.9–29.7	1.33–1.39 (*m*)
CH_2(15)	33.8	2.04 (br. *q*, *J* = 6.8)	33.7	2.04 (br. *q*, *J* = 6.8)
H–C(16)	114.1	5.81 (*ddt*, *J* = 17.1, 10.0, 6.8)	114.1	5.81 (*ddt*, *J* = 17.0, 10.0, 6.8)
CH_2(17)	139.2	4.93 (*ddd*, *J* = 10.0, 2.0, 1.6), 4.99 (*ddd*, *J* = 17.1, 2.0, 1.6)	139.2	4.93 (*ddt*, *J* = 10.0, 2.0, 1.0), 4.99 (*ddt*, *J* = 17.0, 2.0, 1.8)

The ^{1}H-NMR spectrum of **3** was closely similar to that of obtusilactone [9], indicating the same β-hydroxy-γ-methylidene-α,β'-unsaturated γ-lactone structure and the same (*Z*)-geometry of the trisubstituted C=C bond (δ(H) 6.69 (*td*, *J* = 7.6, 2.0, H–C(6))), except for the configuration of C(3)[3]). Compound **3** has a dextrorotatory optical activity ($[\alpha]_D^{26} = +35.7$ ($c = 0.22$, $CHCl_3$)) and shows an opposite configuration at C(3)[3]) with laevorotatory obtusilactone ($[\alpha]_D^{26} = -47.5$ ($c = 0.0016$, $CHCl_3$)) [9], hence, (3*R*)-configuration is suggested. Comparison of the ^{13}C-NMR and DEPT spectra of **3** with those of obtusilactone [9], and mahubenolide [7], allowed to determine the accuracy of the structure of **3**.

Compound **4** was obtained as a colorless oil. The molecular formula was determined to be $C_{17}H_{26}O_3$ from the HR-ESI-MS mass spectrum (*m*/*z* 301.1778 ($[M + Na]^+$; calc. 301.1780)). The IR spectrum showed absorption bands for a OH group at 3433 cm^{-1} and an α,β-unsaturated γ-lactone at 1766 and 1680 cm^{-1}. The ^{1}H- and ^{13}C-NMR (*Table 2*), COSY, NOESY, HSQC, and HMBC (*Figure*) data confirmed the structure as (3*E*,4*R*)-3-(dodec-11-en-1-ylidene)dihydro-4-hydroxy-5-methylidenefuran-2(3*H*)-one, named isolitsealiicolide B.

From the spectral evidence, compound **4** was similar to those of isoobtusilactone [9], and also had the same β-hydroxy-γ-methylene-α,β'-unsaturated γ-lactone structure. The dextrorotatory optical activity ($[\alpha]_D^{26} = +17.8$ ($c = 0.31$, $CHCl_3$)) once again indicated the configuration at C(3)[3]) as (3*R*) [7][8].

Based on the HR-EI-MS and ^{13}C-NMR data (*Table 3*), compound **5** has the molecular formula $C_{17}H_{28}O_3$. The IR spectrum showed absorption bands for a OH group at 3435 cm^{-1} and an α,β-unsaturated γ-lactone at 1770 and 1669 cm^{-1}. Further spectral data (*Table 3*, *Figure*) and comparison with those of lincomolide D [10] allowed to assign the structure of **5** as (3*E*,4*R*)-3-dodecylidenedihydro-4-hydroxy-5-methylidenefuran-2(3*H*)-one, and was designated as isolitsealiicolide C.

Table 3. *^{1}H- and ^{13}C-NMR Data* ($CDCl_3$, 400 and 100 MHz, resp.) *of Compound* **5**[3]). δ in ppm, *J* in Hz.

	δ(C)	δ(H)
C(1)	166.7	–
C(2)	127.3	–
H–C(3)	66.3	5.25 (br. *s*)
HO–C(3)	–	2.28 (br. *s*)
C(4)	157.6	–
CH_2(5)	91.4	4.72 (*dd*, *J* = 2.8, 1.6), 4.95 (*dd*, *J* = 2.8, 1.6)
H–C(6)	150.2	7.08 (*td*, *J* = 8.0, 2.4)
CH_2(7)	29.7	2.37–2.55 (*m*)
CH_2(8)	28.3	1.48–1.56 (*m*)
CH_2(9)	28.3–29.7	1.25 (br. *s*)
CH_2(10)	28.3–29.7	1.25 (br. *s*)
CH_2(11)	28.3–29.7	1.25 (br. *s*)
CH_2(12)	28.3–29.7	1.25 (br. *s*)
CH_2(13)	28.3–29.7	1.25 (br. *s*)
CH_2(14)	28.3–29.7	1.25 (br. *s*)
CH_2(15)	31.9	1.25 (br. *s*)
CH_2(16)	22.7	1.25 (br. *s*)
Me(17)	14.1	0.85 (*t*, *J* = 6.8)

The ^{1}H-NMR spectrum of **5** was similar to that of lincomolide D [10], indicating that **5** also has the same β-hydroxy-γ-methylidene-α,β'-unsaturated γ-lactone moiety and the trisubstituted C=C bond with the (*E*)-form geometry with the H–C(6)[3]) signal at δ(H) 7.08 (*td*, $J = 8.0, 2.4$). An undecyl group connected to the above *E*-form olefinic group was supported by ^{13}C-NMR (*Table 3*). The major difference was the positive $[\alpha]_D^{26}$ value +32.3 ($c = 0.06$, $CHCl_3$), so the configuration at C(3) was also determined to be (3*R*) [7][8].

The other known isolates, *i.e.*, isolinderanolide B (**6**) [11], α-tocopheryl quinone [12], α-tocopherol [4], α-tocospirone [13], 4-hydroxy-4,7-dimethyl-1-tetralone [14], spathulenol [15], caryophyllene oxide [16], squalene [17], piperitol [18], (–)-kusunokinin [19], 4′-hydroxy-5,7,3′-trimethoxyflavan-3-ol [20], (3*R*,6*R*,7*E*)-3-hydroxymegstigma-4,7-dien-9-one [21], β-sitosterol [22], ficaprenol-11 [23], docosanol [24], dodec-11-enoic acid [25], and dodec-11-enal [25] were readily identified by comparison with literature data.

2. *Biological Studies.* All isolated compounds were tested for cytotoxicity *in vitro* against MCF-7 (human breast adenocarcinoma), NCI-H460 (non-small-cell lung cancer), and SF-268 (glioblastoma cells) with actinomycin D as a positive control (*Table 4*). Except for **1**, **2**, **5**, and **6**, the compounds did not show significant *in vitro* cytotoxic activity against three cell lines at a concentration of 50 μM. As can been seen in *Table 4*, compounds **1** and **2** showed marginal cytotoxicity (IC_{50} values of 4.64 and 4.26 μg/ml) against the MCF-7 cell line. Compounds **1** and **2** show cytotoxicity with IC_{50} values of 3.53 and 2.74 μg/ml against the NCI-H460 cell line. Compounds **1** and **2** also exhibit cytotoxicity (IC_{50} values of 3.38 and 3.11 μg/ml) against the SF-268 cell line.

Table 4. *Cytotoxic Effects of Test Compounds against MCF-7, NCI-H460, and SF-268 Cell Lines.* For significant activity of a pure compound, an IC_{50} value ≤ 4.0 μg/ml is required.

	Name	IC_{50} [μg/ml]		
		MCF-7	NCI-H460	SF-268
1	Litsealiicolide A	4.64 ± 0.18	3.53 ± 0.65	3.38 ± 0.41
2	Isolitsealiicolide A	4.26± 0.27	2.74 ± 0.17	3.11 ± 0.38
5	Isolitsealiicolide C	10.15± 1.10	8.45 ± 1.05	8.63 ± 0.18
6	Isolinderanolide B	> 15.4	7.77 ± 2.18	10.01 ± 0.80
	Actinomycin D[a])	0.127 ± 0.002	0.012 ± 0.002	0.020 ± 0.009

[a]) Positive control.

From the results of the cytotoxicity tests, the following conclusions can be drawn regarding these isolates: *a*) Among 22 tested compounds, only butanolides showed cytotoxicity activities. *b*) The (*Z*)-form isomer of butanolides with a β-hydroxy-γ-methylidene-α,β'-unsaturated γ-lactone group such as **1** showed similar cytotoxicity against three cell lines compared to the (*E*)-form isomer such as **2**. *c*) In comparison of butanolides **1**, **2**, **5**, and **6**, a terminal C≡C bond in **1** and **2** showed 2–3 fold cytotoxic activity than the long-chain alkyl group connected to C(6)[3]) in **5** and **6** against three cell lines.

It is interesting to note that the extracts of the leaves of *L. lii* var. *nunkaotahangensis* are composed of mainly β-hydroxy-γ-methylidene-α,β'-unsaturated γ-

lactones in contrast to *L. akoensis* [2–4], and *L. acutivena* [5][6] containing many types of butanolides. This type of butanolides was unstable in air and decomposed quickly. The (4*S*)-3-alkylidene-4-hydroxy-5-methylenebutanolide moiety is common in *Litsea* [5][10] and *Lindera* [11] species, as shown in previous studies, and is also unstable. The (4*R*)-3-alkylidene-4-hydroxy-5-methylenebutanolide moiety has been isolated from *Lindera* [10] species, but from *Litsea* species, this is the first report.

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Experimental Part

General. TLC: silica gel *60* F_{254} precoated plates (*Merck*). Column chromatography (CC): silica gel *60* (70–230 or 230–400 mesh, *Merck*). M.p.: *Yanaco* micro-melting point apparatus; uncorrected. Optical rotation: *Jasco DIP-370* polarimeter; in $CHCl_3$. UV Spectra: *Jasco UV-240* spectrophotometer; λ_{max} (log ε) in nm. IR Spectra: *Perkin-Elmer-2000* FT-IR spectrophotometer; $\tilde{\nu}$ in cm^{-1}. ^1H-, ^{13}C- and 2D-NMR spectra: *Varian-Gemini-200*, *Varian-Unity-Plus-400*, and *Varian-Mercury-400* spectrometers; δ in ppm rel. to Me_4Si, *J* in Hz. GC-MS: *Trace GC/POLARIS Q Thermo Finnigan*; in *m/z* (rel. %). EI-MS: *VG-Biotech Quatro-5022* mass spectrometer; in *m/z* (rel. %). ESI- and HR-ESI-MS: *Bruker APEX-II* mass spectrometer; in *m/z*.

Plant Material. Leaves of *L. lii* var. *nunkao-tahangensis* were collected from Tamumu Mountain, Pintung County, Taiwan, in September 2005 and identified by Prof. Dr. *Sheng-Zehn Yang* (Department of Forest Resource, Management and Technology, National Pingtung University of Science and Technology). A voucher specimen (no. Chen 6096) is deposited with the Herbarium, Faculty of Pharmacy, College of Pharmacy, Kaohsiung Medical University, Kaohsiung, Taiwan, R.O.C.

Extraction and Isolation. Dried leaves (8.3 kg) of *L. lii* var. *nunkao-tahangensis* were extracted repeatedly with MeOH. The extract was concentrated under reduced pressure, and the residue (1.6 kg) was partitioned with $H_2O/CHCl_3$ 1:1. The $CHCl_3$-soluble fraction (*Fr. A*, 550 g) was separated. The H_2O-soluble fraction was further extracted with BuOH to afford a BuOH-soluble part (*Fr. B*, 200 g) and a H_2O-soluble one (*Fr. C*, 220 g). Part of *Fr. A* (100 g) was chromatographed on CC (2.5 kg, SiO_2, 230–400 mesh; hexane/AcOEt gradient) to give 25 fractions: *Fr. A1–Fr. A25*. *Fr. A1* (2.0 g) was subjected to CC (25 g SiO_2, 230–400 mesh; hexane/Me_2CO gradient) to yield 15 fractions: *Fr. A1.1–Fr. A1.15*. *Fr. A1.3* was purified further by prep. TLC (SiO_2; hexane/AcOEt 4:1) to give caryophyllene oxide [16] (6.7 mg). *Fr. A9* (9.9 g) was purified by CC (280 g SiO_2, 230–400 mesh; CH_2Cl_2/MeOH gradient) to afford 40 fractions: *Fr. A9.1–Fr. A9.40*. *Fr. A9.10* (1.2 g) was washed by MeOH to yield docosanol [24] (13.2 mg). The washing of *Fr. A9.10* was fractionated by CC to afford 60 fractions: *Fr. A9.10.1–Fr. A9.10.60*. *Fr. A9.10.6* was subjected to CC to give ficaprenol-11 [23] (8.6 mg), and dodec-11-enoic acid [25] (3.7 mg). *Fr. A9.10.15* was repeatedly by CC and by recrystallization or prep. TLC to afford **5** (4.5 mg), **6** (3.2 mg), spathulenol [15] (6.3 mg), and squalene [17] (3.8 mg). *Fr. A10* (7.6 g) was chromatographed over CC (200 g SiO_2, 230–400 mesh; hexane/Me_2CO gradient) to produce 52 fractions: *Fr. A10.1–Fr. A10.52*. *Fr. A10.15* (27.5 mg) and *Fr. A10.20* (44.2 mg) were purified by prep. TLC (SiO_2; hexane/AcOEt 15:1) to furnish α-tocopherol [4] (5.7 mg) and α-tocospirone [13] (3.3 mg). *Fr. A10.26* (1.6 g) was subjected to CC (60 g SiO_2, 230–400 mesh; hexane/Me_2CO gradient) to obtain 40 fractions: *Fr. A10.26.1–Fr. A10.26.40*. *Fr. A10.26.9* (314.3 mg), eluting with hexane/Me_2CO 20:1, was further separated by CC and prep. TLC (SiO_2; hexane/AcOEt 15:1) to give **3** (150.1 mg), **4** (120.4 mg), β-sitosterol [22] (33.7 mg), and dodec-11-enal [25] (2.1 mg). *Fr. A10.26.21* (53.7 mg) was subjected to CC (1.5 g SiO_2, 230–400 mesh; CH_2Cl_2/AcOEt gradient) and then purified by prep. TLC (SiO_2; hexane/AcOEt 3:1) to give **1** (2.4 mg), and **2** (12.1 mg). *Fr. A10.26.32* (15 mg) was further purified by prep. TLC (SiO_2; hexane/CH_2Cl_2/MeOH 3:1:0.2) to afford α-tocopheryl quinone [12] (3.4 mg). *Fr. A16* (5.8 g) was applied to a *RP-C18* CC, eluting with MeOH and H_2O (2:1) to obtain 29 fractions: *Fr. A16.1–Fr. A16.29*. *Fr. A16.3* (1.2 g) was applied to a *RP-C18* CC (30 g), eluting with MeCN and H_2O (1:1), to obtain 17 fractions: *Fr. A16.3.1–Fr. A16.3.17*. *Fr. A16.3.1*, *Fr. A16.3.5*, and *Fr. A16.3.12* were

repeatedly subjected to CC and purified by prep. TLC to afford piperitol [18] (4.8 mg), (−)-kusunokinin [19] (2.6 mg), and 4′-hydroxy-5,7,3′-trimethoxyflavan-3-ol [20] (3.1 mg). *Fr. A16.5* (526 mg) was subjected to CC and purified by prep. TLC to afford 4-hydroxy-4,7-dimethyl-1-tetralone [14] (2.1 mg) and (3*R*,6*R*,7*E*)-3-hydroxy-4,7-megstigmadien-9-one [21] (2.4 mg).

Litsealiicolide A (*=(3Z,4R)-3-(Dodec-11-yn-1-ylidene)-4,5-dihydro-4-hydroxy-5-methylidene-2(3H)-furanone*; **1**). Colorless oil. $[\alpha]_D^{24} = +28.9$ ($c = 0.02$, $CHCl_3$). UV (MeOH): 224 (3.55). IR (neat): 3432 (OH), 3307, 2123 (C≡CH), 1780, 1679 (α,β-unsaturated γ-lactone). ^{1}H- and ^{13}C-NMR: *Table 1*. ESI-MS: 299 ($[M+Na]^+$). HR-ESI-MS: 299.1624 ($[M+Na]^+$, $C_{17}H_{24}NaO_3^+$; calc. 299.1623).

Isolitsealiicolide A (*=(3E,4R)-3-(Dodec-11-yn-1-ylidene)-4,5-dihydro-4-hydroxy-5-methylidenefuran-2(3H)-one*; **2**). Colorless oil. $[\alpha]_D^{24} = +18.9$ ($c = 0.12$, $CHCl_3$). UV (MeOH): 223 (3.76). IR (neat): 3433 (OH), 3300, 2115 (C≡CH), 1770, 1673 (α,β-unsaturated γ-lactone). ^{1}H- and ^{13}C-NMR: *Table 1*. ESI-MS: 299 ($[M+Na]^+$). HR-ESI-MS: 299.1622 ($[M+Na]^+$, $C_{17}H_{24}NaO_3^+$; calc. 299.1623).

Litsealiicolide B (*=(3Z,4R)-3-(Dodec-11-en-1-ylidene)-4,5-dihydro-4-hydroxy-5-methylidenefuran-2(3H)-one*; **3**). Colorless oil. $[\alpha]_D^{26} = +35.7$ ($c = 0.22$, $CHCl_3$). UV (MeOH): 227 (3.07). IR (neat): 3432 (OH), 1746, 1639 (α,β-unsaturated γ-lactone). ^{1}H- and ^{13}C-NMR: *Table 2*. ESI-MS: 301 ($[M+Na]^+$). HR-ESI-MS: 301.1778 ($[M+Na]^+$, $C_{17}H_{26}NaO_3^+$; calc. 310.1780).

Isolitsealiicolide B (*=(3E,4R)-3-(Dodec-11-en-1-ylidene)-4,5-dihydro-4-hydroxy-5-methylidenefuran-2(3H)-one*; **4**). Colorless oil. $[\alpha]_D^{26} = +17.8$ ($c = 0.31$, $CHCl_3$). UV (MeOH): 220 (3.41). IR (neat): 3433 (OH), 1766, 1680 (α,β-unsaturated γ-lactone). ^{1}H- and ^{13}C-NMR: *Table 2*. ESI-MS: 301 ($[M+Na]^+$). HR-ESI-MS: 301.1778 ($[M+Na]^+$, $C_{17}H_{26}NaO_3^+$; calc. 301.1780).

Isolitsealiicolide C (*=(3E,4R)-3-Dodecylidene-4,5-dihydro-4-hydroxy-5-methylidenefuran-2(3H)-one*; **5**). Colorless oil. $[\alpha]_D^{26} = +32.3$ ($c = 0.06$, $CHCl_3$). UV (MeOH): 223 (3.41). IR (neat): 3435 (OH), 1770, 1669 (α,β-unsaturated γ-lactone). ^{1}H- and ^{13}C-NMR: *Table 3*. ESI-MS: 303 ($[M+Na]^+$). HR-ESI-MS: 303.1937 ($[M+Na]^+$, $C_{17}H_{28}NaO_3^+$; calc. 303.1936).

Biological Assay. MCF-7 (human breast adenocarcinoma), NCI-H460 (non-small-cell lung cancer), and SF-268 (glioblastoma cells) 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 37° in a humidified incubator with an atmosphere of 5% CO_2. Human cancer cells were seeded in 96-well microtiter plates in 100 µl culture medium at cell number/well of 6500, 2500, and 7500 for MCF-7, NCI-H460, and SF-268, respectively. After an overnight adaptation period, the cells were treated with at least eight different concentrations of test compounds in a CO_2 incubator for 72 h. The number of viable cells was estimated using the 5-[(3-carboxymethoxy)phenyl]-2-(4,5-dimethylthiazoyl)-3-(4-sulfophenyl)tetrazolium salt (MTS) reduction assay [26] and the experiment was performed according to the manufacturer's recommendations (*Promega*, Madison, WI, USA). DMSO 0.1% (final concentration) were used as vehicle control. Results were expressed as a percentage of DMSO control. The results of these assays were used to obtain the dose-response curves, from which IC_{50} values were determined. The values represent averages of three independent experiments, each with duplicate samples.

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