Secondary Metabolites from the Stem Bark of *Litsea akoensis* and Their Cytotoxic Activity

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Six new butanolides, litseadioxanins A and B (**1** and **2**, resp.) bearing a 1,2-dioxane moiety, litseatrinolides A and B (**3** and **4**, resp.), and litseakolides D_1 and D_2 (**5** and **6**, resp.), were isolated from the stem bark of *Litsea akoensis*, together with six known compounds. The structures of the new compounds were characterized by in-depth NMR-spectroscopic and mass-spectrometric analyses. Butanolides **1**–**4**, and a mixture of **6** and litsenolide E_2, and litsenolide B_1 were tested against human tumor cells, including MCF-7 (human breast adenocarcinoma), NCI-H460 (non-small-cell lung cancer), and SF-268 (glioblastoma) cell lines. Among the tested compounds, litsenolide B_1 exhibited marginal cytotoxic activity against MCF-7, NCI-H460, and SF-268 cell lines *in vitro*.

Introduction. – *Litsea akoensis* HAYATA (Lauraceae) is a medium-sized evergreen tree, endemic to Taiwan, and distributed throughout broad leaved forests at low to medium altitudes [1]. Over 1000 samples of the MeOH extracts of Formosan plants have been screened on cytotoxicity in our laboratory [2][3], and *L. akoensis* has been found to be one of the active species. Our previous study reported five new butanolides, akolactones A and B, and litseakolides A–C, and six known compounds together with their cytotoxicities from the stem bark of this species [4][5]. Careful examination of the minor constituents and the cytotoxic principles of the stem bark of this plant has led to the isolation and characterization of six additional new butanolides, litseadioxanins A and B (**1** and **2**, resp.) with a 1,2-dioxane moiety, litseatrinolides A and B (**3** and **4**, resp.), and litseakolides D_1 and D_2 (**5** and **6**, resp.), together with six known compounds, *i.e.*, litsenolides E_1, E_2, and B_1, a mixture of (13^2S)- and (13^2R)-methyl-13^2-hydroxypheophorbide b, and methyl asterrate. The structures of these compounds were determined through spectral analyses. The structural elucidation of **1**–**6**, and the cytotoxicity of the isolates, are described herein.

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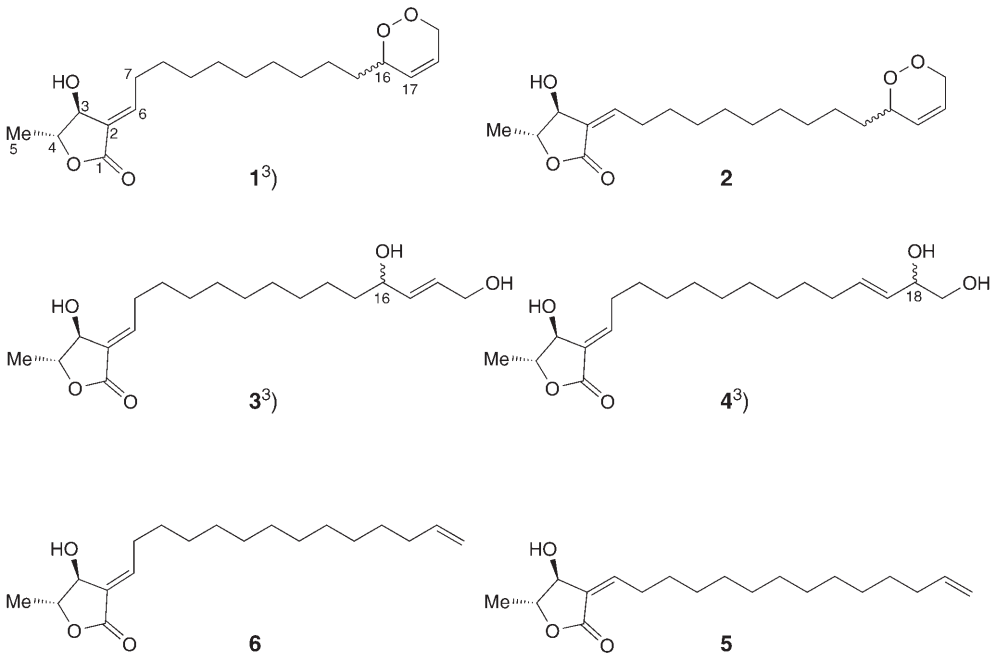

Results and Discussion. – 1. *Structure Elucidation*. Extensive chromatographic purification of the $CHCl_3$-soluble fraction of the MeOH extract of the stem bark of *L. akoensis* afforded six new butanolides, **1**–**6**, three known butanolides, a mixture of chlorophylls, and one acetophenone derivative. The UV spectrum of each new butanolide showed maximal absorptions between 220 and 228 nm, indicating the presence of an α-alkylidene-butyrolactone moiety [4][5]. Their IR spectra displayed absorptions characteristic of an α-alkylidene-γ-lactone [4][5], the ^{1}H- and ^{13}C-NMR data indicated that compounds **1**–**4** possess an α-alkylidene-β-hydroxy-γ-methyl-γ-lactone moiety, compared with the butanolides reported in the literature [4][5]. The laevorotatory optical activity of all new butanolides **1**–**4** indicated a *rel*-(*S*)-configuration for the OH–C(3)[3]) group [6–10], the chemical shifts of H–C(3) (δ(H) *ca.* 4.5) and Me(5) (δ(H) *ca.* 1.34) of **1**–**4** were similar to those of litsenolide C_2 [4][6], and the configuration at C(4) was further confirmed to be *rel*-(*R*) by means of NOESY experiments, which showed a correlation between H–C(3) and Me(5), indicating that H–C(3) and H–C(4) are *trans* to each other. Therefore, the configurations at C(3) and C(4) of these new butanolides were deduced to be *rel*-(3*S*,4*R*) [6–10].

Compound **1** was isolated as an optically active colorless oil ($[\alpha]_D^{28} = -43.5$). The HR-ESI-MS data determined the molecular formula to be $C_{19}H_{30}O_5$ (*m*/*z* 361.1978 ($[M+Na]^+$; calc. 361.1991)). Compound **1** was similar to litsenolide C_2 [2] in its ^{1}H-NMR spectrum, and both of them had the spectral patterns of an α-alkylidene-β-hydroxy-γ-methyl-γ-lactone moiety.

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[3]) Arbitrary atom numbering.

The ^{1}H-NMR signals[3]) of a Me group at δ 1.34 (d, $J = 6.8$), an alkylidene olefinic H-atom (conjugated to a γ-lactone C=O) at δ 6.99 (td, $J = 7.6, 1.8$), and two oxygenated CH H-atoms at δ 4.55 (br. s, H–C(3)) and 4.51 (qd, $J = 6.8, 2.0$, H–C(4)) were considered as evidence for the presence of an α-alkylidene-β-hydroxy-γ-methyl-γ-lactone moiety. The (E)-geometry of the C(2)=C(6) bond was established on the basis of the ^{1}H-NMR chemical shifts of H–C(6) (δ 6.99) and CH_2(7) (δ 2.32–2.47 (m)). The downfield chemical shifts of H–C(6) can be attributed to the anisotropy effect of the C=O moiety of the lactone ring. The NOESY plot showed correlations between the H–C(3) and CH_2(7), supporting the (E)-configuration. These data are identical with those of the corresponding moiety of litsenolide C_2 [4][6]. Compared to the latter, compound **1** has an additional 3,6-dihydro-1,2-dioxin-3-yl moiety in the terminal position of the alkylidene side chain. A coupling constant $J = 10.4$ Hz between the two olefinic H-atom signals at δ 5.90 and 5.95 pointed to an unsaturated six-membered endoperoxide. In addition, based on the COSY correlations, chemical shifts (^{1}H- and ^{13}C-NMR data), and coupling constants from H–C(16) to CH_2(19) (see *Table 1*), the presence of a 3,6-dihydro-1,2-dioxin-3-yl moiety was established.

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

	1		**2**	
	δ(H)	δ(C)	δ(H)	δ(C)
C(1)	–	169.6	–	168.1
C(2)	–	129.2	–	128.8
H–C(3)	4.55 (br. s)	72.2	4.35 (br. s)	75.6
H–C(4)	4.51 (qd, $J = 6.8, 2.0$)	82.5	4.34 (qd, $J = 6.8, 2.6$)	81.2
Me(5)	1.34 (d, $J = 6.8$)	19.7	1.38 (d, $J = 6.8$)	19.1
H–C(6)	6.99 (td, $J = 7.6, 1.8$)	148.7	6.54 (td, $J = 7.6, 1.4$)	149.3
CH_2(7)	2.32–2.47 (m)	29.6	2.70–2.80 (m)	27.7
CH_2(8)	1.48–1.56 (m)	28.3	1.42–1.56 (m)	28.7
CH_2(9–13)	1.27 (br. s)	29.1–29.4	1.27 (br. s)	29.1–29.7
CH_2(14)	1.37–1.41 (m)	25.1	1.35–1.37 (m)	25.1
CH_2(15)	1.57–1.69 (m)	32.5	1.55–1.69 (m)	32.5
H–C(16)	4.56–4.62 (m)	78.6	4.56–4.62 (m)	78.6
H–C(17)	5.90 ($dddd$, $J = 10.4, 2.0, 2.0, 2.0$)	128.1	5.91 ($dddd$, $J = 10.4, 2.0, 2.0, 2.0$)	128.1
H–C(18)	5.95 ($dddd$, $J = 10.4, 2.0, 2.0, 2.0$)	123.8	5.94 ($dddd$, $J = 10.4, 2.0, 2.0, 2.0$)	123.9
CH_2(19)	4.62 ($dddd$, $J = 15.6, 2.0, 2.0, 2.0$), 4.45 ($dddd$, $J = 15.6, 2.0, 2.0, 2.0$)	69.8	4.64 ($dddd$, $J = 16.0, 2.0, 2.0, 2.0$), 4.44 ($dddd$, $J = 16.0, 2.0, 2.0, 2.0$)	69.8
3-OH	2.17 (br. s)		2.08 (br. s)	

On the basis of the above evidences, together with COSY, NOESY, and HMBC data (*Fig. 1*), the structure of **1** was deduced to be (3*E*,4*R**,5*S**)-4,5-dihydro-3-[10-(3,6-dihydro-1,2-dioxin-3-yl)decylidene]-4-hydroxy-5-methylfuran-2(3*H*)-one and named litseadioxanin A.

Compound **2**, a colorless oil with $[\alpha]_D^{28} = -9.7$, gave the $[M + Na]^+$ ion peak at m/z 361 in the ESI-MS. The HR-ESI-MS data determined the molecular formula to be $C_{19}H_{30}O_5$ (m/z 361.1990 ($[M + Na]^+$; calc. 361.1991)).

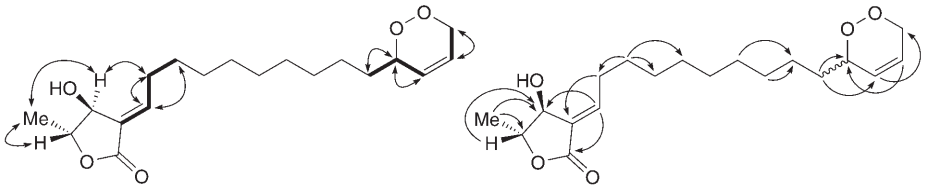

Fig. 1. *Significant COSY* (—), *NOESY* (↔), *and HMBC* (H → C) *correlations of* **1**

From the spectral evidence (^{1}H- and ^{13}C-NMR data[3]), *Table 1*), the major difference to compound **1** was the geometry of the trisubstituted C=C bond. The (*Z*)-configuration of the alkylidene side chain was evident, based on the upfield shift of the H–C(6) signal to δ(H) 6.54 (*td*, J = 7.6, 1.4 Hz) and downfield shift of the CH_2(7) signal (δ 2.70–2.80 (*m*)), compared to those of H–C(6) (δ 6.99) and CH_2(7) (δ 2.32–2.47 (*m*)) of **1**. This proposal was confirmed by a NOESY spectrum, in which H–C(3) (δ(H) 4.35) showed a correlation with H–C(6), suggesting a (*Z*)-configuration for the C(2)=C(6) bond. The laevorotatory optical activity and the chemical shifts of H–C(3) (δ(H) 4.35) and Me(5) (δ(H) 1.38) of **2** also indicated the configuration at C(3) and C(4) as *rel*-(3*S*,4*R*) [6–10].

Based on the ^{13}C-NMR, and COSY, NOESY, HSQC, and HMBC experiments (*Fig. 2*), the structure of **2** was elucidated as (3*Z*,4*R**,5*S**)-4,5-dihydro-3-[10-(3,6-dihydro-1,2-dioxin-3-yl)decylidene]-4-hydroxy-5-methylfuran-2(3*H*)-one and named litseadioxanin B.

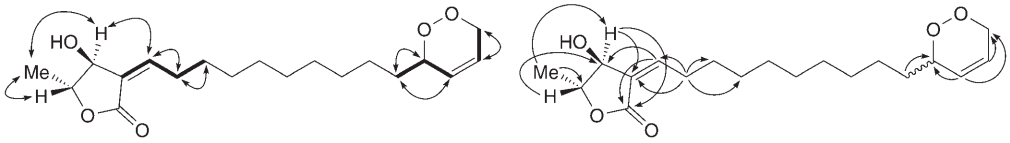

Fig. 2. *Significant COSY* (—), *NOESY* (↔), *and HMBC* (H → C) *correlations of* **2**

Compound **3** was obtained as a colorless oil with specific rotation similar to compound **1**. The molecular formula was determined to be $C_{19}H_{32}O_5$ from the HR-ESI-MS (*m*/*z* 363.2145 ([*M* + Na]$^+$; calc. 363.2147)).

The ^{1}H-, and ^{13}C-NMR spectra of **3**[3]) were similar to the one of litseadioxanin A (**1**), and both had the same signal pattern for an α-alkylidene-β-hydroxy-γ-methyl-γ-lactone moiety. The major difference was the presence of signals for a (2*E*)-1,4-dihydroxybut-2-enyl group (δ(H) 4.13 (*q*, J = 6.4 Hz, H–C(16)), 4.16 (*d*, J = 5.6 Hz, CH_2(19)), 5.74 (br. *dd*, J = 15.6, 6.4 Hz, H–C(17)), and 5.84 (*dt*, J = 15.6, 5.6 Hz, H–C(18))) attached to C(15) in **3**, instead of signals for the 3,6-dihydro-1,2-dioxin group in **1**.

The ^{1}H- and ^{13}C-NMR data (*Table 2*), and COSY, NOESY, HSQC, and HMBC data (*Fig. 3*) confirmed the structure of **3** as (3*E*,4*R**,5*S**)-4,5-dihydro-3-[(12*E*)-11,14-dihydroxytetradec-12-en-1-ylidene]-4-hydroxy-5-methylfuran-2(3*H*)-one, designated as litseatrinolide A.

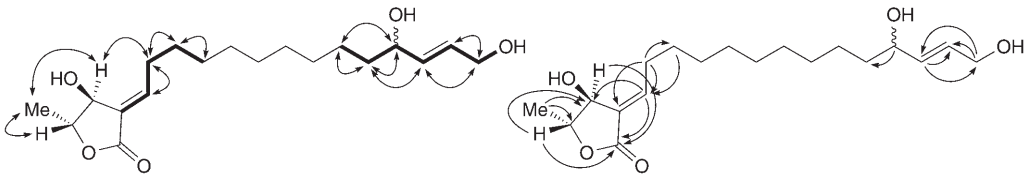

Fig. 3. *Significant COSY* (—), *NOESY* (↔), *and HMBC* (H → C) *correlations of* **3**

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

	3		**4**	
	δ(H)	δ(C)	δ(H)	δ(C)
C(1)	–	169.8	–	169.8
C(2)	–	129.3	–	129.2
H–C(3)	4.52 (br. *s*)	72.2	4.53 (br. *s*)	72.1
H–C(4)	4.49 (*qd*, *J* = 6.6, 2.0)	82.5	4.48 (*qd*, *J* = 6.8, 2.0)	82.6
Me(5)	1.35 (*d*, *J* = 6.6)	19.7	1.34 (*d*, *J* = 6.8)	19.7
H–C(6)	7.00 (*td*, *J* = 7.6, 1.8)	148.7	6.98 (*td*, *J* = 7.6, 2.0)	148.7
CH_2(7)	2.35 (*q*, *J* = 7.6), 2.44 (*q*, *J* = 7.6)	29.7	2.33 (*q*, *J* = 7.6), 2.44 (*q*, *J* = 7.6)	29.7
CH_2(8)	1.46–1.56 (*m*)	28.3	1.47–1.55 (*m*)	28.3
CH_2(9–13)	1.28 (br. *s*)	29.1–29.3	1.26 (br. *s*)	29.1–29.6
CH_2(14)	1.26–1.34 (*m*)	25.2	1.26–1.35 (*m*)	28.8
CH_2(15)	1.46–1.56 (*m*)	37.1	2.03 (*q*, *J* = 6.9)	32.5
H–C(16)	4.13 (*q*, *J* = 6.4)	72.3	5.76 (*dt*, *J* = 15.6, 6.9)	134.4
H–C(17)	5.74 (br. *dd*, *J* = 15.6, 6.4)	129.7	5.43 (*dd*, *J* = 15.6, 6.9)	128.2
H–C(18)	5.84 (*dt*, *J* = 15.6, 5.6)	134.4	4.19 (*td*, *J* = 6.9, 3.4)	73.1
CH_2(19)	4.16 (*d*, *J* = 5.6)	63.0	3.62 (*dd*, *J* = 11.2, 3.4), 3.47 (*dd*, *J* = 11.2, 6.9)	66.6
3-OH, 16- or 18-OH, 19-OH	1.70 (br. *s*)		2.20 (br. *s*)	

Compound **4** was obtained as a colorless oil, showing the $[M + Na]^+$ ion peak at m/z 363 in the ESI-MS. The HR-ESI-MS data determined the molecular formula to be $C_{19}H_{32}O_5$ (m/z 363.2145 ($[M + Na]^+$; calc. 363.2147)).

The ^{1}H- and ^{13}C-NMR spectra of **4**[3]) also displayed signals for the *trans*-relationship of the substituents at C(3) and C(4), and an (*E*)-geometry of the trisubstituted C(2)=C(6) bond, similar to those of **3**, and for the same α-alkylidene-β-hydroxy-γ-methyl-γ-lactone moiety. However, the absence of signals of a (2*E*)-1,4-dihydroxybut-2-enyl moiety indicated that **4** differs from **3** in the side chain. This partial structure of **4** was clarified to be a (1*E*)-3,4-dihydroxybut-1-en-1-yl group, due to ^{1}H-NMR signals for an oxygenated CH_2 group at δ 3.47 (*dd*, *J* = 11.2, 6.9, H_a–C(19)), 3.62 (*dd*, *J* = 11.2, 3.4, H_b–C(19)), one oxymethine at δ 4.19 (*td*, *J* = 6.9, 3.4, H–C(18)), and two *trans*-olefinic H-atoms at δ 5.43 (*dt*, *J* = 15.6, 6.9, H–C(17)) and 5.76 (*dt*, *J* = 15.6, 6.9 Hz, H–C(16)). COSY Experiments showed correlations between CH_2(19) and H–C(18), H–C(18) and H–C(17), and H–C(17) and H–C(16), supporting the existence of a 3,4-dihydroxybut-1-enyl moiety.

The ^{1}H- and ^{13}C-NMR data (*Table 2*), and COSY, NOESY, HSQC, and HMBC data (*Fig. 4*) established the structure of **4** as (3*E*,4*R**,5*S**)-4,5-dihydro-3-[(11*E*)-13,14-dihydroxytetradec-11-en-1-ylidene]-4-hydroxy-5-methylfuran-2(3*H*)-one, named litseatrinolide B.

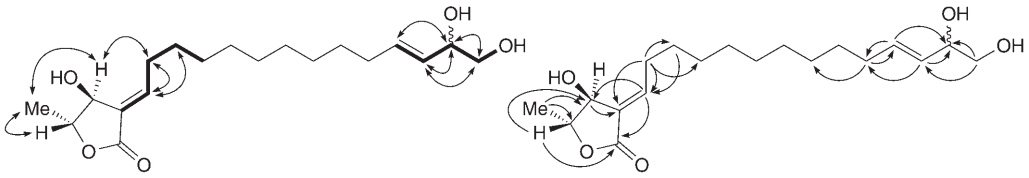

Fig. 4. *Significant COSY* (—), *NOESY* (↔), *and HMBC* (H → C) *correlations of* **4**

The relative configuration at C(16) in **1**–**3** and at C(18) in **4** are presently unknown.

Compound **5** was obtained as a colorless oily mixture with the structurally related compound litsenolide E_1 in a 1:1.1 ratio with $[\alpha]_D^{28} = -7.4$ ($c = 0.007$, $CHCl_3$). Due to the similar polarity and molecular size of these compounds, it was not possible to separate **5** from litsenolide E_1.

The major signals in the ^{1}H-NMR spectrum[3]) were identical with those of litsenolide E_1. The signals of the minor compound **5** were similar to those of litsenolide E_1, except that, at C(15) of **5**, a but-3-en-1-yl group (δ(H) 4.99 (br. *d*, $J = 16.2$, H_a–C(19)), 4.93 (br. *d*, $J = 10.2$, H_b–C(19)), 5.81 (*ddt*, $J = 16.2$, 10.2, 6.8, H–C(18)), 2.02–2.06 (*m*, H–C(17)), and 1.27 (br. *s*, H–C(16))) was present instead of the (1*E*)-but-1-en-1-yl group (δ(H) 5.42 (*dt*, $J = 15.2$, 5.6, H–C(16)), 5.38 (*dt*, $J = 15.2$, 6.0, H–C(17)), 2.02–2.06 (*m*, H–C(18)), and 0.96 (*t*, $J = 7.4$, H–C(19))) of litsenolide E_1. As observed in the COSY spectrum (*Fig. 5*), CH_2(19) was coupled to the olefinic H–C(18) H-atom, which was coupled to CH_2(17), which was also coupled to the aliphatic CH_2(16) H-atoms. This confirmed the presence of a but-3-en-1-yl group attached to C(15) of compound **5**. The absolute configuration at C(3) was determined to be (*S*) based on the correlation between the $[\alpha]_D$ value and the known configuration at C(3) of known 2-alkylidene-3-hydroxy-4-methylbutanolide derivatives [6–10], and

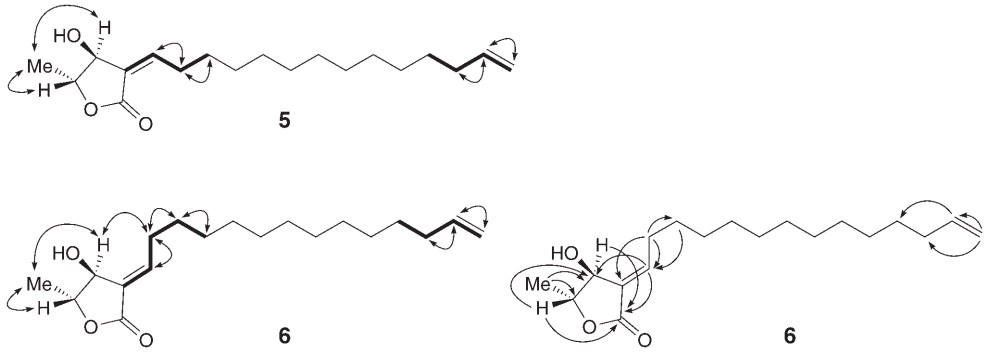

Fig. 5. *Significant COSY* (—), *and NOESY* (↔) *correlations of* **5** *and* **6**, *and HMBC* (H → C) *correlations of* **6**

the configuration at C(4) was further confirmed to be (*R*) from the NOESY plot, which showed no correlation between H–C(3) and H–C(4), indicating that they are *trans* to each other.

Further spectral data (*Table 3*) established the structure of **5** as (3*Z*,4*S*,5*R*)-4,5-dihydro-4-hydroxy-5-methyl-3-(tetradec-13-en-1-ylidene)furan-2(3*H*)-one, named litsenolide D_1.

Table 3. *^{1}H-* (400 MHz) *and ^{13}C-NMR* (100 MHz) *Data of* **5** *and* **6** *in $CDCl_3$*[3]). δ in ppm, *J* in Hz.

	5	**6**	
	δ(H)	δ(H)	δ(C)
C(1)	–	–	169.5
C(2)	–	–	129.2
H–C(3)	4.36 (br. *s*)	4.54 (br. *s*)	77.2
H–C(4)	4.29 (*q*, *J* = 6.0)	4.50 (*qd*, *J* = 6.5, 1.8)	82.5
Me(5)	1.39 (*d*, *J* = 6.0)	1.35 (*d*, *J* = 6.5)	19.7
H–C(6)	6.54 (*td*, *J* = 7.8, 1.6)	7.00 (*td*, *J* = 8.0, 1.6)	148.7
CH_2(7)	2.62–2.84 (*m*)	2.33–2.41 (*m*)	29.7
CH_2(8)	1.46–1.56 (*m*)	1.49–1.55 (*m*)	28.9
CH_2(9–16)	1.27 (br. *s*)	1.26 (br. *s*)	29.1–29.6
CH_2(17)	2.02–2.06 (*m*)	2.02–2.08 (*m*)	33.8
H–C(18)	5.81 (*ddt*, *J* = 17.2, 10.2, 6.8)	5.81 (*ddt*, *J* = 17.0, 10.2, 6.8)	139.2
CH_2(19)	4.99 (br. *d*, *J* = 16.2),	4.99 (*ddt*, *J* = 17.0, 3.6, 2.0),	114.1
	4.93 (br. *d*, *J* = 10.2)	4.93 (*ddt*, *J* = 10.2, 2.0, 1.2)	
3-OH	1.98 (br. *s*)	1.99 (br. *s*)	

A mixture of litsenolide E_2 and compound **6** was obtained as a colorless oil in a 1.3:1 ratio (^{1}H-NMR) with $[\alpha]_D^{28} = -41.7$ ($c = 0.08$, $CHCl_3$). The HR-ESI-MS data determined the molecular formula to be $C_{19}H_{32}O_3$ (*m*/*z* 331.2250 ($[M+Na]^+$; calc. 331.2249)).

The major signals in the ^{1}H-NMR spectrum[3]) were identical with those of the known litsenolide E_2. The signals of the minor compound **6** were similar to those of **5**, the major difference being the presence of signals for a trisubstituted C=C bond with an (*E*)-geometry, which was evidenced due to the H–C(6) signal at δ(H) 7.00 (*td*, *J* = 8.0, 1.6) in **6**.

Based on further spectral evidence, the structure of **6** was elucidated as (3*E*,4*S*,5*R*)-4,5-dihydro-4-hydroxy-5-methyl-3-(tetradec-13-en-1-ylidene)furan-2(3*H*)-one, designated as litsenolide D_2, which was further confirmed by ^{13}C-NMR, and COSY, NOESY, HSQC, and HMBC experiments (*Fig. 5*).

The other known isolates, *i.e.*, litsenolide E_1 [7], litsenolide E_2 [7], litsenolide B_1 [7], a mixture of (13^2*S*)- and (13^2*R*)-methyl-13^2-hydroxypheophorbide b [11], and methyl asterrate [12], were readily identified by comparison of their physico-chemical, spectroscopic, and mass-spectrometric data with the corresponding literature values.

2. *Biological Studies.* Compounds **1**–**4**, a mixture of **6** and litsenolide E_2, and litsenolide B_1 were evaluated for their *in vitro* cytotoxicity against three human cancer cell lines, MCF-7 (breast), NCI-H460 (lung), and SF-268 (CNS), with actinomycin D as positive control [13]. Except for litsenolide B_1, none of the other compounds showed

significant *in vitro* cytotoxic activity against the three cell lines at a concentration of 50 μM. As can been seen from *Table 4*, litsenolide B_1 showed marginal cytotoxic activity with IC_{50} values of 11.77, 9.57, and 12.16 μg ml^{-1} against MCF-7, NCI-H460, and SF-268 cell lines, respectively.

Table 4. IC_{50} *Values of Litsenolide* B_1 *on the Cytotoxicity against NCI-H460, MCF-7, and SF-268 Cell Lines*

Name	IC_{50} [μg/ml][a])		
	MCF-7	NCI-H460	SF-268
Litsenolide B_1	11.77	9.57	12.16
Actinomycin D[b])	0.13	0.01	0.02

[a]) The concentration inhibiting 50% of tumor cell growth after 72 h at 37°. [b]) Positive control.

Conclusions. – In the successive study on cytotoxic constituents from the stem bark of *Litsea akoensis*, we focused on the minor secondary metabolites appearing in the stem bark of this plant. The six metabolites **1**–**6** found in this study are new, naturally occurring compounds. Interestingly, this is the first report of a structure with an α-alkylidene butyrolactone connected to an unusual 1,2-dioxane moiety, compared to other structures with long alkyl side chains isolated from this plant [4–10].

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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; ν in cm^{-1}. ^1H-, ^{13}C-, and 2D-NMR spectra: *Varian Unity-Plus-400* and *INOVA-500* spectrometers; δ in ppm rel. to Me_4Si, *J* in Hz. EI-MS: *VG-Biotech Quatro-5022* mass spectrometer; *m*/*z* (rel. %). ESI- and HR-ESI-MS: *Bruker APEX-II* mass spectrometer; in *m*/*z*.

Plant Material. The stem bark of *Litsea akoensis* was collected at Wutai, Pingtung County, Taiwan, in August 1996. A voucher specimen (no. Chen 2280) was deposited in the Herbarium of the School of Pharmacy, Kaohsiung Medical University, Kaohsiung, Taiwan, R.O.C.

Extraction and Isolation. The dried stem bark of *L. akoensis* (3.6 kg) was extracted with MeOH at r.t., and the extract was concentrated *in vacuo*. The MeOH extract (610 g) was partitioned between H_2O/$CHCl_3$ 1:1 to afford a $CHCl_3$-soluble fraction (*Fr. A*, 95 g) and H_2O-soluble fraction. The upper layer was then extracted with BuOH to obtain a BuOH-soluble fraction (*Fr. B*, 270 g) and a H_2O-soluble fraction (*Fr. C*, 230 g), resp. *Fr. A* was subjected to CC (2 kg of SiO_2, 70–230 mesh; $CHCl_3$/MeOH gradient) to yield 23 fractions: *Fr. A1*–*Fr. A23*. *Fr. A15* (8.2 g, $CHCl_3$/MeOH 95:1) was subjected to CC (355 g of SiO_2, 70–230 mesh; hexane/AcOEt gradient) to afford 23 fractions: *Fr. A15.1*–*Fr. A15.23*. *Fr. A15.9* (1.8 g) was resubmitted to CC (SiO_2; hexane/AcOEt 10:1 → 1:1) to give 9 fractions: *Fr. A15.9.1*–*Fr. A15.9.9*. *Fr. A15.9.3* (1.2 g) was submitted to CC (*RP-18* silica gel, acetone/H_2O 5:1) to afford 14 fractions: *Fr. A15.9.3.1*–*Fr. A15.9.3.14*. *Fr. A15.9.3.13* (40 mg) was submitted to CC (*RP-18* silica gel, MeOH/H_2O 5:1) to obtain 5 fractions: *Fr. A15.9.3.13.1*–*Fr. A15.9.3.13.5*. *Fr. A15.9.3.13.1* (11.6 mg, MeOH/H_2O 5:1) was purified by prep. TLC (CH_2Cl_2/AcOEt 60:1) to give a mixture of litsenolide E_1 and **5** (1.0 mg; R_f 0.44), and a mixture of litsenolide E_2 and **6** (2.1 mg; R_f 0.42). *Fr. A15.9.9* (667 mg,

hexane/AcOEt 5:1) was submitted to CC (*RP-18* silica gel, acetone/H_2O 3:1) to afford 9 fractions: *Fr. A15.9.9.1 – A15.9.9.9*. *Fr. A15.9.9.1* (43.8 mg, acetone/H_2O 3:1) was submitted to CC (*RP-18* silica gel, MeOH/H_2O 2:1) to furnish 9 fractions: *Fr. A15.9.9.1.1 – Fr. A15.9.9.1.9*. Compound **3** (7.2 mg, R_f 0.67) was purified from *Fr. A15.9.9.1.6*. *Fr. A15.9.9.1.8* (14.4 mg, acetone/H_2O 2:1) was further purified by *RP-18* prep. TLC (acetone/H_2O 2:1) to give **4** (9.2 mg; R_f 0.22) and methyl asterrate (1.2 mg; R_f 0.43). *Fr. A15.12* (189 mg, hexane/AcOEt 9:1) was submitted to CC (*RP-18* silica gel, acetone/H_2O 3:1) to afford 12 fractions: *Fr. A15.12.1 – Fr. A15.12.12*. *Fr. A15.12.9* (28.8 mg, acetone/H_2O 3:1) was purified by prep. TLC (CH_2Cl_2/acetone 70:1) to yield litsenolide B_1 (9.1 mg, R_f 0.42). *Fr. A15.14* (133 mg) was submitted to CC (3 g of SiO_2, 70–230 mesh; CH_2Cl_2/AcOEt 15:1 to 1:1) to afford eight fractions: *Fr. A15.14.1 – Fr. A15.14.8*. *Fr. A15.14.6* (40.9 mg) was submitted to CC (1.2 g of SiO_2, 70–230 mesh; hexane/AcOEt 2:1 → 1.5:1) to give 14 fractions: *Fr. A15.14.6.1 – Fr. A15.14.6.14*. *Fr. A15.14.6.5* (11.9 mg) was further purified by prep. TLC (SiO_2; CH_2Cl_2/acetone 70:1) to give **1** (4.8 mg; R_f 0.47) and **2** (6.0 mg, R_f 0.65). *Fr. A15.20* (42.6 mg, hexane/AcOEt 1:1) was submitted to CC (1.3 g SiO_2, 70–230 mesh; $CHCl_3$/acetone 20:1) to provide 11 fractions: *Fr. A15.20.1 – Fr. A15.20.11*. *Fr. A15.20.3* (17.3 mg) was further purified by prep. TLC (SiO_2, $CHCl_3$/acetone 20:1) to give a mixture of (13^2S)- and (13^2R)-methyl-13^2-hydroxypheophorbide (1.3 mg; R_f 0.63).

Litseadioxanin A (=*(3*E*,4*R**,5*S**)-4,5-Dihydro-3-[10-(3,6-dihydro-1,2-dioxin-3-yl)decylidene]-4-hydroxy-5-methylfuran-2(3*H*)-one*; **1**). Colorless oil. $[\alpha]_D^{28} = -43.5$ ($c = 0.33$, $CHCl_3$). UV (MeOH): 220 (4.45). IR (Neat): 3428 (OH), 1741, 1677 (α,β-unsaturated γ-lactone). ^{1}H- and ^{13}C-NMR: see *Table 1*. ESI-MS: 361 ($[M+Na]^+$). HR-ESI-MS: 361.1978 ($[M+Na]^+$, $C_{19}H_{30}NaO_5^+$; calc. 361.1991).

Litseadioxanin B (=*(3*Z*,4*R**,5*S**)-4,5-Dihydro-3-[10-(3,6-dihydro-1,2-dioxin-3-yl)decylidene]-4-hydroxy-5-methylfuran-2(3*H*)-one*; **2**). Colorless oil. $[\alpha]_D^{28} = -9.7$ ($c = 0.08$, $CHCl_3$). UV (MeOH): 228 (4.15). IR (Neat): 3437 (OH), 1746, 1673 (α,β-unsaturated γ-lactone). ^{1}H- and ^{13}C-NMR: see *Table 1*. ESI-MS: 361 ($[M+Na]^+$). HR-ESI-MS: 361.1990 ($[M+Na]^+$, $C_{19}H_{30}NaO_5^+$; calc. 361.1991).

Litseatrinolide A (=*(3*E*,4*R**,5*S**)-4,5-Dihydro-3-[(12*E*)-11,14-dihydroxytetradec-12-en-1-ylidene]-4-hydroxy-5-methylfuran-2(3*H*)-one*; **3**). Colorless oil. $[\alpha]_D^{28} = -42.1$ ($c = 0.21$, $CHCl_3$). UV (MeOH): 222 (4.32). IR (Neat): 3399 (OH), 1738, 1675 (α,β-unsaturated γ-lactone). ^{1}H- and ^{13}C-NMR: see *Table 2*. ESI-MS: 363($[M+Na]^+$). HR-ESI-MS: 363.2145 ($[M+Na]^+$, $C_{19}H_{32}NaO_5^+$; calc. 363.2147).

Litseatrinolide B (=*(3*E*,4*R**,5*S**)-4,5-Dihydro-3-[(11*E*)-13,14-dihydroxytetradec-11-en-1-ylidene]-4-hydroxy-5-methylfuran-2(3*H*)-one*; **4**). Colorless oil. $[\alpha]_D^{28} = -40.9$ ($c = 0.33$, $CHCl_3$). UV (MeOH): 222 (4.47). IR (Neat): 3409 (OH), 1737, 1675 (α,β-unsaturated γ-lactone). ^{1}H- and ^{13}C-NMR: see *Table 2*. ESI-MS: 363 ($[M+Na]^+$). HR-ESI-MS: 363.2145 ($[M+Na]^+$, $C_{19}H_{32}NaO_5^+$; calc. 363.2147).

Litsenolide D_1 (=*(3*Z*,4*S*,5*R*)-4,5-Dihydro-4-hydroxy-5-methyl-3-(tetradec-13-en-1-ylidene)furan-2(3*H*)-one*; **5**). Colorless oil. UV (MeOH): 227 (4.28). IR (Neat): 3427 (OH), 1737, 1673 (α,β-unsaturated γ-lactone). ^{1}H-NMR: see *Table 3*.

Litsenolide D_2 (=*(3*E*,4*S*,5*R*)-4,5-Dihydro-4-hydroxy-5-methyl-3-(tetradec-13-en-1-ylidene)furan-2(3*H*)-one*; **6**). Colorless oil. UV (MeOH): 220 (4.46). IR (Neat): 3426 (OH), 1738, 1677 (α,β-unsaturated γ-lactone). ^{1}H- and ^{13}C-NMR: see *Table 3*. ESI-MS: 331 ($[M+Na]^+$). HR-ESI-MS: 331.2250 ($[M+Na]^+$, $C_{19}H_{32}NaO_3^+$; calc. 331.2249).

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 acids (*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, resp. 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 4-{5-[3-(carboxymethoxy)phenyl]-3-(4,5-dimethyl-1,3-thiazol-2-yl)-2*H*-tetrazol-3-ium-2-yl}benzenesulfonate (MTS) reduction assay [13], and the experiment was performed as recommended by the manufacturer (*Promega*, Madison, WI, USA). DMSO (0.1% final concentration) was 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. A value of $IC_{50} \leq 4$ µg ml^{-1} is considered to be indicative of significant cytotoxicity. The values represent averages of three independent experiments, each with duplicate samples.

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