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CYTOTOXIC BUTANOLIDES FROM *LITSEA AKOENSIS*

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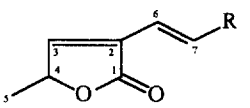
Key Word Index—*Litsea akoensis*; Lauraceae; stem bark; akolactones A and B; litseakolides A and B; litsenolide; butanolide; γ-lactone; cytotoxicity.

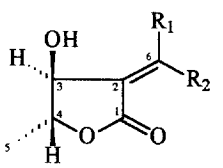
Abstract—Four new butanolides, akolactone A, akolactone B, litseakolide A and litseakolide B, along with four known butanolides, litsenolide B_2, litsenolide C_1, litsenolide C_2 and hamabiwalactone A were isolated from the stem bark of *Litsea akoensis*. Their structures were elucidated from spectral evidence. These butanolides showed cytotoxic activity against P-388, KB16, A 549 and HT-29 cancer cell lines. © 1998 Elsevier Science Ltd. All rights reserved

INTRODUCTION

Litsea akoensis is an evergreen medium-sized tree, endemic in Taiwan [1]. So far, only an alkaloid, laurolitsine, was reported from the wood of this species [2]. In a series of studies on the anticancer constituents of Formosan plants, we have screened ca 350 species for *in vitro* cytotoxicity and *L. akoensis* was one of the active species. Its stem bark showed significant cytotoxic activity against P-388, KB16, A549 and HT-29 cancer cell lines. Examination of the chloroform-soluble part of the stem bark has led to the isolation of new butanolides (**1**–**4**) and four known butanolides (**5**–**8**). The known compounds, litsenolide B_2 (**5**) [3], litsenolide C_1 (**6**) [3], litsenolide C_2 (**7**) [3] and hamabiwalactone A (**8**) [4] were identified by comparisons of their IR, UV, ^{1}H NMR or ^{13}C NMR with the corresponding literature data. This paper reports the isolation and structural elucidation of the new butanolides and the cytotoxicity of the isolates.



1 R=—$(CH_2)_{11}$—CH_3
2 R=—$(CH_2)_8$—CH=CH—CH=CH_2
8 R=—$(CH_2)_8$—C≡CH
9 R=—$(CH_2)_8$—CH=CH_2



3 R_1=—$(CH_2)_9$—CH=CH—CH=CH_2, R_2=H
4 R_1=—$(CH_2)_9$—CH=CH—CHO, R_2=H
5 R_1=—$(CH_2)_9$—C≡CH, R_2=H
6 R_1=H, R_2=—$(CH_2)_{12}CH_3$
7 R_1=—$(CH_2)_{12}CH_3$, R_2=H
10 R_1=—$(CH_2)_8$—CH=CH—CH_2CH_3, R_2=H

RESULTS AND DISCUSSION

Akolactone A (**1**) was isolated as a colourless oil. The molecular formula was determined to be $C_{19}H_{32}O_2$ by EI ($[M]^+$, *m/z* 292) and HR mass spectra. It exhibited the absorption band of an α,β-unsaturated-γ-lactone (1750 and 1660 cm^{-1}) in the IR spectrum [3]. The UV absorption at 210 nm was similar to that of hamabiwalactone B (**9**) [4] and also supported the presence of an α,β-unsaturated-γ-lactone skeleton. In the ^{1}H NMR spectrum (Table 1), the signals of an olefinic proton at δ 7.03 (*d*, *J* = 2.0 Hz), a methine proton at δ 5.02 (*qd*, *J* = 6.8, 2.0 Hz), a methyl group at δ 1.42 (*d*, *J* = 6.8 Hz), two other olefinic protons at δ 6.09 (*d*, *J* = 16.0 Hz), δ 6.79 (*dt*, *J* = 16.0, 7.2 Hz), which were identical with those of **9** [4], were attributed to H-3, H-4, H-5, H-6 and H-7, respectively.

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Table 1. ^{1}H NMR data for butanolides **1**–**8** (400 MHz, $CDCl_3$)

H	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
3	7.03 (1H, *d*, J = 2.0 Hz)	7.03 (1H, *d*, J = 1.6 Hz)	4.55 (1H, *br s*)	4.55 (1H, *br s*)	4.55 (1H, *br d*, J = 6.6 Hz)	4.34	4.54 (1H, *br d*, J = 6.4 Hz)	7.03 (1H, *d*, J = 1.6 Hz)
4	5.02 (1H, *qd*, J = 6.8, 2.0 Hz)	5.02 (1H, *br q*, J = 6.8 Hz)	4.50 (1H, *qd*, J = 6.9, 2.0 Hz)	4.49 (1H, *qd*, J = 6.8, 2.0 Hz)	4.50 (1H, *qd*, J = 6.6, 2.0 Hz)	(2H, *m*, H-3 and H-4)	4.50 (1H, *qd*, J = 6.4, 2.0 Hz)	5.02 (1H, *qd*, J = 6.8, 1.6 Hz)
5	1.42 (3H, *d*, J = 6.8 Hz)	1.42 (3H, *d*, J = 6.8 Hz)	1.35 (3H, *d*, J = 6.9 Hz)	1.35 (3H, *d*, J = 6.8 Hz)	1.35 (3H, *d*, J = 6.6 Hz)	1.39 (3H, *d*, J = 6.4 Hz)	1.35 (3H, *d*, J = 6.4 Hz)	1.42 (3H, *d*, J = 6.8 Hz)
6	6.09 (1H, *d*, J = 16.0 Hz)	6.09 (1H, *dd*, J = 15.0, 1.6 Hz)	7.00 (1H, *td*, J = 7.6, 1.2 Hz)	7.01 (1H, *td*, J = 8.0, 2.0 Hz)	7.00 (1H, *td*, J = 8.0, 2.0 Hz)	6.54 (1H, *td*, J = 7.8, 1.2 Hz)	7.00 (1H, *td*, J = 7.6, 1.6 Hz)	6.08 (1H, *d*, J = 16.0 Hz)
7	6.79 (1H, *dt*, J = 16.0, 7.2 Hz)	6.79 (1H, *dt*, J = 15.0, 7.2 Hz)	2.40 (2H, *m*)	2.40 (2H, *m*)	2.40 (2H, *m*)	2.76 (2H, *m*)	2.40 (2H, *m*)	6.78 (1H, *dt*, J = 16.0, 7.2 Hz)
8	2.16 (2H, *q*, J = 7.2 Hz)	2.15 (2H, *q*, J = 7.0 Hz)	1.51 (2H, *m*)	1.53 (2H, *m*)	1.51 (2H, *m*)	1.47 (2H, *m*)	1.54 (2H, *m*)	2.16 (2H, *m*)
9–14	1.42 (2H, *m*, H-9)	1.37 (2H, *m*, H-9) 1.28 (10H, *br s*, H-10–14)	1.28 (12H, *br s*, H-9–14)	1.30 (12H, *br s*, H-9–14)	1.30 (10H, *br s*, H-9–13) 1.51 (2H, *m*, H-14)			1.30 (10H, *br s*, H-9–13) 1.51 (2H, *m*, H-14)

15		2.06 (2H, *q*, J = 7.0 Hz)	2.07 (2H, *q*, J = 7.0 Hz)	2.33 (2H, *m*)	2.19 (2H, *td*, J = 6.8, 2.4 Hz)		2.18 (2H, *td*, J = 7.2, 2.8 Hz)
16	1.26 (18H, *br s*, H-10–18)	5.69 (1H, *dt*, J = 15.2, 7.2 Hz)	5.70 (1H, *dt*, J = 15.2, 7.0 Hz)	6.86 (1H, *dt*, J = 15.0, 6.8 Hz)	—	1.26 (20H, *br s*, H-9–18)	1.25 (20H, *br s*, H-9–18)	—
17		6.04 (1H, *dd*, J = 15.2, 10.2 Hz)	6.05 (1H, *dd*, J = 15.2, 10.4 Hz)	6.12 (1H, *dd*, J = 15.0, 7.8 Hz)	1.94 (1H, *t*, J = 2.4 Hz)			1.93 (1H, *t*, J = 2.6 Hz)
18		6.30 (1H, *dt*, J = 17.2, 10.2 Hz)	6.31 (1H, *dt*, J = 17.2, 10.4 Hz)	9.50 (1H, *d*, J = 7.8 Hz)	—			—
19	0.88 (3H, *t*, J = 6.8 Hz)	4.94 (1H, *dd*, J = 10.2, 1.6 Hz) 5.08 (1H, *dd*, J = 17.2, 1.6 Hz)	4.95 (1H, *dd*, J = 10.4, 1.6 Hz) 5.08 (1H, *dd*, J = 17.2, 1.6 Hz)	—	—	0.88 (3H, *t*, J = 6.8 Hz)	0.87 (3H, *t*, J = 6.8 Hz)	—
OH	—	—	*2.07 (1H)	1.98 (1H, *br s*)	1.88 (1H, *d*, J = 6.6 Hz)	2.08 (1H, *d*, J = 6.0 Hz)	1.94 (1H, *d*, J = 6.4 Hz)	—

* Overlapped with H-15

Table 2. ^{13}C NMR data for butanolides **1**–**3** and **5**–**8** (100 MHz, $CDCl_3$)

C	1	2	3	5	6	7	8
1	171.9	171.9	169.4	169.6	168.0	169.4	171.9
2	129.4	129.4	129.2	129.2	128.8	129.3	129.4
3	146.7	146.7	72.2	72.1	75.6	72.2	146.7
4	76.9	77.0	82.4	82.5	81.2	82.4	76.9
5	19.1	19.1	19.6	19.6	19.1	19.6	19.1
6	118.2	118.2	148.6	148.6	149.3	148.6	118.3
7	138.8	138.8	29.6	29.6	27.8	29.7	138.7
8	33.4	33.4	28.4	28.3	28.8	28.4	33.3
9	28.7	28.7					28.4
10	29.66	29.4	29.39	29.28	29.66	29.7	29.2
11	29.63	C_{10}–C_{14} 29.18	C_9–C_{14} 29.31	29.23	29.63	29.64	29.1
12	29.62	29.15	29.30	C_9–C_{14} 28.9	29.61	C_9–C_{16} 29.62	C_{10}–C_{14} 28.9
13	C_{10}–C_{16} 29.5		29.1	28.6	C_9–C_{16} 29.5	29.4	28.7
14	29.4			28.4	29.4	29.3	28.6
15	29.3	32.5	32.5	18.3	29.3		18.3
16	29.2	135.5	135.5	84.7	29.2		84.7
17	31.9	130.8	130.8	68.0	31.9	31.9	68.0
18	22.6	137.3	137.3	—	22.6	22.6	—
19	14.0	114.5	114.5	—	14.0	14.0	—

Thus, a moiety with a *trans*-olefinic group connected to the C-2 position of a 4-methyl-but-2-enolide in **1** was suggested. A dodecyl group connected to the above *trans*-olefinic group was supported by ^{13}C NMR (Table 2). According to the above observation, the structure of akolactone A was represented by the formula **1**, which was further confirmed by COSY, HETCOR and DEPT experiments.

Akolactone B (**2**) was also isolated as a colourless oil. The molecular formula was determined to be $C_{19}H_{28}O_2$ by EI ($[M]^+$, *m/z* 288) and HR mass spectrometry. It also exhibited the absorption band of an α,β-unsaturated-γ-lactone (1750 and 1650 cm^{-1}) in the IR spectrum. The ^1H NMR and ^{13}C NMR spectra (Tables 1 and 2) of **2** were very similar to those of **1**, except for the presence of the signals of an (*E*)-1, (*E*)-3-butadienyl group [δ 4.94 (1H, *dd*, J = 10.2, 1.6 Hz, H-19a), δ 5.08 (1H, *dd*, J = 17.2, 1.6 Hz, H-19b), δ 6.30 (1H, *dt*, J = 17.2, 10.2 Hz, H-18), δ 6.04 (1H, *dd*, J = 15.2, 10.2 Hz, H-17), δ 5.69 (1H, *dt*, J = 15.2, 7.2 Hz, H-16)] in **2** instead of a *n*-butyl group in **1**. Therefore, the structure of akolactone B was shown to be **2**, which was also further confirmed by COSY, HETCOR and DEPT experiments.

Akolactone A (**1**) and akolactone B (**2**) both have laevorotatory optical activity and probably show opposite stereochemistry at C-4 with dextrorotatary **8** and **9** [4].

Litseakolide B (**3**) was isolated as a colourless oil. The molecular formula was established as $C_{19}H_{30}O_3$ by EI ($[M]^+$, *m/z* 306) and HR mass spectrometry. The IR spectrum showed the absorption bands for a hydroxyl group at 3450 cm^{-1} and α,β-unsaturated-γ-lactone at 1750 and 1680 cm^{-1}. ^1H NMR analysis showed that compound **3** has the same β-hydroxy-γ-methyl-α,β'-unsaturated-γ-lactone structure, the same *E*-form geometry of the tri-substituted double bond [δ 7.00 (1H, *td*, J = 7.6, 1.2 Hz, H-6)] and the same *trans*-relationship of the substituents at C-3 and C-4 [(δ 4.55 (1H, *br s*) and δ 4.50 (1H, *qd*, J = 6.9, 2.0 Hz)] to those of litsenolide E_2 (**10**) [4], except for the presence of an (*E*)-1, (*E*)-3-butadienyl group [δ 4.95 (1H, *dd*, J = 10.4, 1.6 Hz, H-19a), δ 5.08 (1H, *dd*, J = 17.2, 1.6 Hz, H-19b), δ 6.31 (1H, *dt*, J = 17.2, 10.4 Hz, H-18), δ 6.05 (1H, *dd*, J = 15.2, 10.4 Hz, H-17), δ 5.70 (1H, *dt*, J = 15.2, 7.0 Hz, H-16)] attached to C-15 in **3**, instead of an (*E*)-3-butenyl group in **10**. According to the above observations, the structure of litseakolide B was represented by the formula **3**, which was further confirmed by COSY and HETCOR experiments. Litseakolide B (**3**) has a laevorotatory optical activity and, hence, possesses the (3*S*, 4*R*)-configuration, like litsenolides A_1–E_2 [3, 4].

Litseakolide A (**4**) was isolated as colourless oil. The HRFAB mass spectrum gave a $[M+H]^+$ ion peak at *m/z* 309.2073 (Calcd: 309.2067) consistent with a molecular formula of $C_{18}H_{29}O_4$. The ^1H NMR spectrum of **4** revealed the presence of a β-hydroxy-γ-methyl-α,β'-unsaturated-γ-lactone with an *E*-form of geometry of the trisubstituted double bond, like those of litseakolide B (**3**). The remaining structure of **4** was clarified to be (2*E*)-dode-2-en-cyl group due to the one aldehyde proton at δ 9.50 (*d*, J = 7.8 Hz), two *trans*-olefinic protons at δ 6.12 (*dd*, J = 15.0, 7.8 Hz, H-17) and δ 6.86 (*dt*, J = 15.0, 6.8 Hz, H-16) and nine methylene groups (Table 1). Therefore, the structure of litseakolide A shown as to be **4**, which was further confirmed by COSY experiments.

Only one of the compounds isolated in this study, **6** had the trisubstituted double bond with *Z*-stereochemistry and it showed diagnostic resonances for C-7 at δ 27.8 and C-8 at δ 28.8, comparable to the *E*-

Table 3. Cytotoxicity of butanolides **1–3** and **5–8**

Compound	ED_{50} ($\mu g\ ml^{-1}$)			
	P-388	KB16	A549	HT-29
Akolactone A (**1**)	1.36	>50	2.70	>50
Akolactone B (**2**)	0.63	3.73	1.73	1.18
Litseakolide B (**3**)	0.99	2.83	2.50	1.42
Litsenolide B_2 (**5**)	1.07	>50	0.40	0.40
Litsenolide C_1 (**6**)	0.36	0.69	0.94	1.07
Litsenolide C_2 (**7**)	0.21	0.87	0.76	0.77
Hamabiwalactone A (**8**)	1.20	2.92	1.19	5.74
Mithramycin*	0.06	0.08	0.07	0.08

* Positive control

forms of compounds **3**, **5** and **7**, with C-7 at δ 29.6 or 29.7 and C-8 at δ 28.3 or 28.4 in the ^{13}C NMR spectrum through HETCOR experiments. This observation is quite different from that in the literature [4], which reported the same chemical shift for C-7 whether in the *Z*- or *E*-form in the trisubstituted double bond.

The cytotoxic activity of seven butanolides were tested *in vitro* against P-388, KB16, A549 and HT-29 cell lines; all showed significant activity (Table 3). In the group of compounds **1**, **2** and **8**, the long-chain alkyl group connected to C-7 in **1** decreased the cytotoxicity against four tested cell-lines. In another group of compounds with a β-hydroxy-γ-methyl-α,β'-unsaturated-γ-lactone, the geometry of the tri-substituted double bond showed no obvious difference in cytotoxicity between **6** (*Z*-form) and **7** (*E*-form). Furthermore, a terminal triple bond in **5** increased cytotoxic activity against A549 and HT-29 cell lines, but decreased it against P-388 and KB16 cell lines.

EXPERIMENTAL

Mps: uncorr. ^1H (400 MHz) and ^{13}C NMR (100 MHz): $CDCl_3$, chemical shifts are given in δ with TMS as int. standard. MS: direct inlet system. Optical rotations: $CHCl_3$. UV: EtOH. IR: neat. Silica gel of 60–230 and 230–400 mesh (Merck) were used for CC and 60 F-254 for TLC.

Plant material

Stem bark of *L. akoensis* Hayata was collected from Wutai, Pingtung Hsien, Taiwan, in August 1996. A voucher specimen is deposited in the Herbarium of the School of Pharmacy, Kaohsiung Medical College, Taiwan, Republic of China.

Extraction and separation

Dried stem bark (3.6 kg) was extracted with MeOH and the extract concd under red. pres. The MeOH extract was partitioned between H_2O–$CHCl_3$ (1:1) to afford a $CHCl_3$-sol. fr. (fr. A, 95 g). The H_2O-sol. portion was then partitioned with *n*-BuOH again to obtain a *n*-BuOH-sol. fr. (fr. B, 270 g) and a H_2O-sol. fr. (fr. C, 230 g). Fraction A (95 g) was chromatographed over silica gel, eluting with $CHCl_3$, gradually increasing the polarity with MeOH, to obtain 22 frs (A1–A22). Fraction A4 (2.35 g, $CHCl_3$) was resubjected to silica gel CC using *n*-hexane and *n*-hexane–EtOAc mixts to yield 29 frs (A4-1–A4-29). Fraction A4-13 (0.098 g, *n*-hexane–EtOAc, 100:7) was purified by prep. TLC (*n*-hexane–EtOAc, 5:1) to yield **1** (3.3 mg, R_f 0.63). Fraction A9 (2.72 g, $CHCl_3$) was rechromatographed on silica gel using *n*-hexane and *n*-hexane-EtOAc mixts to yield 24 frs (A9-1–A9-24). Fraction A9-8 (0.623 g, *n*-hexane–EtOAc, 4:1) was rechromatographed on silica gel and eluted with *n*-hexane and *n*-hexane-EtOAc mixts to yield 17 frs (A9-8-1–A9-8-17). Fraction A9-8-8 (56.4 mg, *n*-hexane–EtOAc, 17:3) was purified by prep. TLC (CH_2Cl_2–EtOAc, 20:1) to yield **6** (2.6 mg, R_f 0.5) and **7** (15.2 mg, R_f 0.4). Fraction A9-11 (0.195 g, *n*-hexane–EtOAc, 4:1), was rechromatographed on silica gel and eluted with CH_2Cl_2 and CH_2Cl_2–EtOAc mixts to obtain 28 frs (A9-11-1–A9-11-28). Fraction A9-11-7 (1.6 mg, CH_2Cl_2–EtOAc, 20:1) was purified by prep. TLC (CH_2Cl_2–MeOH, 40:1) to yield **4** (0.7 mg, R_f 0.5). Fraction A10 (2.7 g, $CHCl_3$) was rechromatographed on silica gel using CH_2Cl_2 and CH_2Cl_2–EtOAc mixts to yield 25 frs (A10-1–A10-25). Fraction A10-19 (0.155 g, CH_2Cl_2–EtOAc, 20:1) was purified by prep. TLC (*n*-hexane–Me_2CO, 3:1) to yield **3** (4.0 mg, R_f 0.38). Fraction A13 (2.59 g, $CHCl_3$) was rechromatographed on silica gel using CH_2Cl_2 and CH_2Cl_2–EtOAc mixts to yield 26 frs (A13-1–A13-26). Fraction A13-2 (0.056 g, CH_2Cl_2) was purified by prep. TLC (*n*-hexane–EtOAc, 10:1) to yield **2** (6.3 mg, R_f 0.42). Fraction A13-3 (0.028 g, CH_2Cl_2) was purified by prep. TLC (*n*-hexane–EtOAc, 10:1) to yield **8** (5.2 mg, R_f 0.5). Fraction A13-7 (0.277 g, CH_2Cl_2) was purified by prep. TLC (*n*-hexane–EtOAc, 2:1) to yield **5** (15.7 mg, R_f 0.4).

Akolactone A (**1**). Colourless oil. $[\alpha]_D^{28}$: $-13.2°$ (ca 0.10, $CHCl_3$). UV λ_{max} nm (log ε): 210 (3.19). IR ν_{max}

cm^{-1}: 1750, 1660 (α,β-unsaturated-γ-lactone). EIMS *m/z* (rel. int.): 292 $[M]^+$ (10), 179 (10), 152 (11), 137 (66), 123 (38), 111 (17), 107 (26), 105 (19). HRMS: $C_{19}H_{32}O_2$. Found: 292.2386. Calcd: 292.2403. ^1H NMR: Table 1. ^{13}C NMR: Table 2.

Akolactone B (**2**). Colourless oil. $[\alpha]_D^{27}$: $-10.0°$ (ca 0.10, $CHCl_3$). UV λ_{max} nm (log ε): 221 (3.21). IR ν_{max} cm^{-1}: 1750, 1650 (α,β-unsaturated-γ-lactone). EIMS *m/z* (rel. int.): 288 $[M]^+$ (1), 191 (13), 177 (9), 161 (9), 147 (19), 145 (14), 137 (23), 133 (32), 131 (16), 121 (35). HRMS: $C_{19}H_{28}O_2$. Found: 288.2090. Calcd: 288.2089. ^1H NMR: Table 1. ^{13}C NMR: Table 2.

Litseakolide B (**3**). Colourless oil. $[\alpha]_D^{27}$: $-40.0°$ (ca 0.08, $CHCl_3$). UV λ_{max} nm (log ε): 224 (3.42). IR ν_{max} cm^{-1}: 3450 (OH), 1750, 1680 (α,β-unsaturated-γ-lactone). EIMS *m/z* (rel. int.): 306 $[M]^+$ (0.02), 167 (20), 163 (23), 149 (100), 129 (16), 123 (11), 121 (10), 111 (11), 105 (26). HRMS: $C_{19}H_{30}O_3$. Found: 306.2209. Calcd: 306.2195. ^1H NMR: Table 1.^{13}C NMR: Table 2.

Litseakolide A (**4**). Colourless oil. UV λ_{max} nm (log ε): 218 (4.74). EIMS *m/z* (rel. int.): 308 $[M]^+$ (0.08), 149 (17), 137 (11), 133 (10), 125 (16), 123 (15), 121 (14), 111 (23), 109 (18). HRFAB-MS: $C_{18}H_{29}O_4$. Found: 309.2073 $[M+H]^+$. Calcd: 309.2067. ^1H NMR: Table 1.

Cytotoxicity assay. Activities of compounds **1–3** and **5–8** against P-338 (mouse lymphocytic leukaemia), KB16 (human nasopharyngeal carcinoma), A549 (human lung carcinoma) and HT-29 (human colon adenocarcinoma) cells were assayed by the MTT [3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl tetrazolium bromide] colorimetric method [6, 7].

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