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New sesquiterpene lactones from water extract of the root of *Lindera strychnifolia* with cytotoxicity against the human small cell lung cancer cell, SBC-3

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Abstract—In the present study, new sesquiterpene lactones (**1**) and (**2**) were isolated from the EtOAc soluble fraction of the water extract of Linderae Radix through bioassay-guided fractionation and isolation methods. The structure of these compounds was elucidated by spectroscopic analysis of their 2D NMR spectra, including COSY, HMBC, and HMQC techniques. Two isolates showed significant cytotoxicity against the human small cell lung cancer cell SBC-3, and lesser cytotoxicity against mouse fibroblast cell 3T3-L1.

The root of *Lindera strychnifolia* F. Vill (Lauraceae), Linderae Radix has strong fragrance and is used in Chinese folk medicine as acesodyne and antispasmodic. In present-day Japan, *L. strychnifolia* grows naturally in Shingu (Wakayama, Japan), and the water extract of the root of this plant has been used as acesodyne and antispasmodic in herbal medicine. In previous studies on the constituents of this plant, many sesquiterpenes[1–6] and alkaloids[7,8] have already been isolated from the root.

In our previous pharmacological study, we demonstrated that intake of the water extract of Linderae Radix significantly induced apoptosis in lung cancer cells and prolonged survival of tumor-bearing mice.[9] Sesquiterpenoids with cytotoxic activity against cancer cells have been isolated from some plants,[10,11] but so far have not been isolated from this plant.

Intrinsic or acquired drug resistance is a major limiting factor in the effectiveness of chemotherapy. In particular, the emergence of drug resistance during induction chemotherapy is a major obstacle to improve the treatment outcome of patients with small cell lung cancer.[12] As part of our continuing study on the cytotoxicity of the water extract of Linderae Radix against lung cancer cells and drug discovery from natural medicinal resources, the water extract of Linderae Radix was fractionated to EtOAc soluble, BuOH soluble, and water layer residue fractions. Through the 3′-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) cell proliferation assay[13]-guided fractionation and isolation methods, we isolated new sesquiterpene lactones with cytotoxic activity against cultured human small cell lung cancer cell line SBC-3 from the EtOAc soluble fraction.

Linderae Radix was collected in the spring of 2002 at Shingu City (Wakayama, Japan) and Linderae Radix (1 kg) was extracted with hot water (20 l). The lyophilized extract (76.7 g) was partitioned between EtOAc and water to give EtOAc soluble and water soluble fractions. The water soluble fraction was then partitioned

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with *n*-BuOH to give *n*-BuOH and water soluble fractions. Each fraction was evaporated to dryness and their cytotoxicity against SBC-3 (human, small cell lung cancer) cells was assayed by MTT assay.[13] The water soluble fraction did not affect the SBC-3 cells viability. The water extract of Linderae Radix and the *n*-BuOH soluble fraction were also inactive (IC_{50} values: 807.8 and 290.0 μg/ml, respectively), while the EtOAc soluble fraction was potent (IC_{50} values: 83.7 μg/ml). This result indicates that cytotoxicity of the water extract of Linderae Radix was concentrated in the EtOAc soluble fraction. Based on these results of the initial biological assay, assay-guided separation of the EtOAc soluble fraction (6.48 g) was carried out to give **1** and **2**. The EtOAc soluble fraction (6.48 g) was partitioned between $CHCl_3$ (100 ml) and water (200 ml). The $CHCl_3$ soluble fraction (3.32 g) was subjected to repeated column chromatography ($CHCl_3$/MeOH = 10:1 → 0:100, hexane/acetone = 5:1 → 4:1 ~ $CHCl_3$/EtOAc = 4:1 ~ $CHCl_3$/MeOH = 10:1, and *n*-hexane/EtOAc = 4:1 ~ *n*-hexane/2-propanol = 6:1 ~ $CHCl_3$/EtOAc = 4:1 ~ $CHCl_3$/MeOH = 10:1), and reversed phase HPLC (Develosil RPAqueous C_{30}, Ø 20 × 300 mm, MeOH/H_2O = 2:1 and MeOH/H_2O = 55:45) to purify the active fractions and gave **1** (2.4 mg) and **2** (1.8 mg) as amorphous powder.

Compound **1** showed a molecular ion peak at 244 $[M]^+$ in the EI-MS spectrum and the HR-EI-MS spectrum indicated the molecular formula as $C_{15}H_{16}O_3$ (244.1102, 0.3 mmu error). The IR spectroscopic data of **1** showed the absorption of the ester carbonyl group at 1750 cm^{-1} and the ketone at 1670 cm^{-1}. The ^{13}C NMR spectrum of **1** showed 15 signals, including two methyl, three methylene, four methine, and five quaternary carbons (Table 1). Among these, one methylene (δ_C 120.1), two methine (δ_C 127.8 and δ_C 161.2), and three quaternary carbons (δ_C 122.1, δ_C 145.8, and δ_C 163.1) indicated the three olefins in the molecule. The carbonyl signal at δ_C 190.0 implied the α,β-unsaturated ketone. From the HMQC, HMBC correlations (Table 1), and H–H COSY, the planar structure was deduced as shown in Figure 1. The relative stereochemistry was determined by the NOESY spectrum (Fig. 2). The cross peaks observed between H-8 and Me-10 revealed that these are 1,3-diaxial. Thus, the structure of **1** was elucidated as a new sesquiterpene, 3-oxo-5 α*H*,8β*H*-eudesma-1,4(15),7(11)-trien-8,12-olide (Fig. 3). $[\alpha]_D^{23}$ +6.7 (*c* 0.12, MeOH).

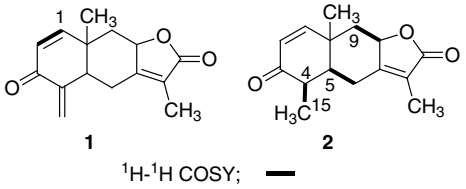

Figure 1. Planar structures of **1** and **2**.

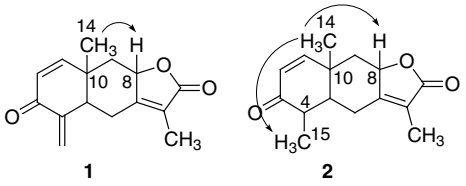

Figure 2. NOESY correlation of **1** and **2**.

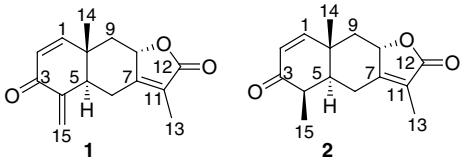

Figure 3. Structures of **1** and **2**.

Table 1. ^1H and ^{13}C NMR spectroscopic data of **1** (in CD_3OD) and **2** (in $CDCl_3$)[a]

Position	1		HMBC	2		HMBC
	δ_C	δ_H	H → C	δ_C	δ_H	H → C
1	161.2	6.91 d (10.1)	3, 10	157.4	6.79 d (10.1)	5, 9, 10
2	127.8	5.92 d (10.1)	4, 10	126.5	5.94 d (10.1)	10
3	190.0			202.9		
4	145.8			44.0	2.59–2.61 m	5, 10, 15
5	30.8	1.19 br-s	14	44.8	2.12 br-s	
6	25.7	2.98 dd (13.7, 3.3)	10	25.9	2.59–2.61 m	4, 5, 7, 10, 11, 15
		2.50–2.53 m				
7	163.1			160.3		
8	79.5			77.3	4.92 dd (6.3, 4.7)	
9	44.3	1.28–1.33 m	1, 8, 10, 14	46.4	2.38 dd (11.9, 6.3)	1, 5, 7, 8, 10
		2.45 dd (12.0, 6.4)	7, 8, 10, 14		1.30–1.32 m	8, 10, 14
10	39.9			36.9		
11	122.1			121.2		
12	178.0			174.1		
13	8.2	1.74 t (1.7)	7, 11, 12	8.4	1.85 t (1.5)	7, 11, 12
14	18.5	1.06 s	1, 9, 10	21.2	1.36 s	1, 9, 10
15	120.1	5.37 ddd (2.3, 0.8, 0.8)	3	13.3	1.24 d (7.9)	4, 5
		6.06 dd (2.3, 0.8)	3			

[a] TMS was used as internal standard; chemical shifts are shown in the scale with *J* values (Hz) in parentheses. Run at 500 or 600 MHz for ^1H and 125 or 150 MHz for ^{13}C.

Compound **2** showed a molecular ion peak at 246 $[M]^+$ in the EI-MS spectrum and the HR-FT-MS spectrum showed a pseudo-molecular ion peak at 269.1167 ($C_{15}H_{18}O_3Na$, 1.9 mmu error), and the molecular formula of **2** was determined as $C_{15}H_{18}O_3$. The IR spectroscopic data of **2** show the absorption of the ester carbonyl group at 1750 cm^{-1} and the ketone at 1670 cm^{-1}. The ^{13}C NMR spectrum of **2** showed 15 signals, including three methyl, two methylene, five methine, four quaternary, and two carbonyl carbons (Table 1). In comparison with **1**, the NMR spectra of **2** indicated the signals of the CH_3–CH unit instead of exomethylene in **1**. As the signals of other positions of **2** were similar to those in **1**, the planar structure was proposed as indicated in Figure 1. Thus, the structure of **2** was elucidated as a new sesquiterpene, 3-oxo-4,5α*H*,8β*H*-eudesma-1,7(11)-dien-8,12-olide (Fig. 3). $[\alpha]_D^{23}$ +35.3 (*c* 0.09, MeOH).

Cytotoxic activity of **1** and **2** against SBC-3 (JCRB0818, human, small cell lung cancer) and mouse normal cell 3T3-L1 (JCRB9014, fibroblast) was evaluated, and the results are summarized in Table 2. Isolated **1** and **2** were found to show cytotoxicity against SBC-3 in a dose-dependent manner (Fig. 4), and less cytotoxicity against normal cell 3T3-L1. The cytotoxic activity against SBC-3 cells of **1** was much stronger than that of **2**; furthermore, the IC_{50} value of **1** against SBC-3 cells was similar to positive control, cisplatin. The exomethylene at C-4 of **1** instead of the methyl group of **2** may contribute to the cytotoxicity.

Intrinsic or acquired drug resistance is a major limiting factor in the effectiveness of chemotherapy. Cisplatin used as positive control in this study, is a cancer chemotherapeutic agent with a substantial activity against a variety of human malignancies including lung cancer. In patients with small cell lung cancer, 65–95% overall response rates and 35–50% complete response rates were reported after treatment with cisplatin plus etoposide.[12,14] However, only a small fraction of patients remain in remission beyond 2 years. The emergence of drug resistance during induction chemotherapy is a major obstacle to improve the treatment outcome of patients with small cell lung cancer, so the development of alternative treatment option (e.g., new medications) after the emergence of drug resistance is expected.

Therefore, these isolated compounds are promising candidates for additional biological evaluation to further define their potential as a cancer chemotherapeutic agent, and applying **1** in the treatment of lung cancer to overcome resistance against cancer chemotherapeutic agents is a promising treatment option.

Table 2. Cytotoxic activities of compounds **1** and **2** and cisplatin against SBC-3 cells

Compound	SBC-3 [IC_{50} (μM)]
1	7.2
2	32.2
Cisplatin	8.6

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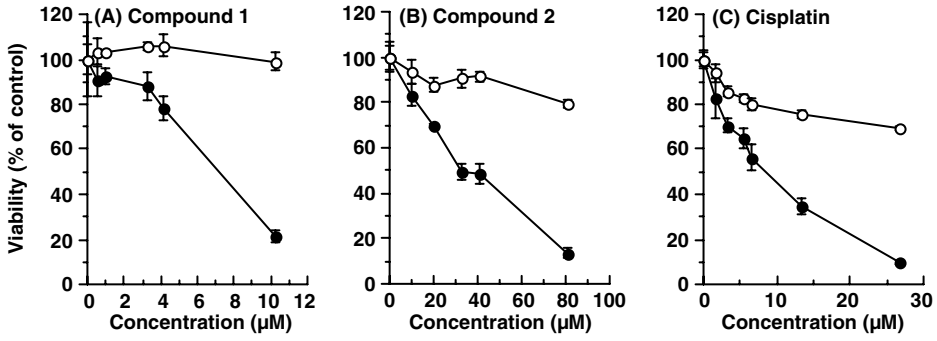

Figure 4. Cytotoxicity of isolated compounds against SBC-3 and 3T3-L1 cells. SBC-3 (●) and 3T3-L1 (○) cells at a concentration of 1×10^5 cells/ml were seeded into a 96-multi-well plate. After incubation for 24 h, the medium was removed, and then, increasing concentrations of (A) **1**, (B) **2**, and (C) cisplatin were added and incubated for 48 h. Viability was determined by the MTT method.[13] Each value represents means ± SD of four wells.

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