Cytotoxic Sesquiterpene Lactones from the Root of *Saussurea lappa*

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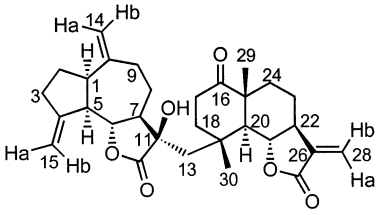
Bioassay-directed fractionation of *Saussurea lappa* led to the isolation of a novel lappadilactone (**1**) and seven sesquiterpene lactones (**2**−**8**) as cytotoxic principles against selected human cancer cell lines. Lappadilactone (**1**), dehydrocostuslactone (**2**), and costunolide (**5**) exhibited the most potent cytotoxicity with CD_{50} values in the range 1.6−3.5 μg/mL in dose- and time-dependent manners. The cytotoxicities were not specific and showed similar activities against HepG2, OVCAR-3 and HeLa cell lines. The structure−activity relationship showed that the α-methylene-γ-lactone moiety is necessary for cytotoxicity, and activity is reduced with the presence of a hydroxyl group. In addition, seven noncytotoxic compounds (**9**−**15**) were also isolated, including two novel sesquiterpenes, a guaianolide-type with a C_{17} skeleton, lappalone (**13**), and 1β,6α-dihydroxycostic acid ethyl ester (**14**). The structures of the new compounds were elucidated from spectroscopic and/or X-ray data interpretations. Some representative compounds were also tested for antibacterial activity; however, only marginal activities were observed. Therefore, compounds **1**−**8** are potential cytotoxic agents but without significant antibacterial effect.

The Chinese medicinal herb Radix Saussureae (Mu Xiang), the dried root of *Saussurea lappa* Clarke (Compositae), has been traditionally used for alleviating pain in abdominal distention and tenesmus, indigestion with anorexia, dysentery, nausea, and vomiting.[1,2] The Chinese name Mu Xiang refers to "wood fragrance", and *S. lappa* has a reputation for its fragrance and use in perfumery. The root contains up to 3% essential oil, and the principal sesquiterpene lactones isolated include dehydrocostuslactone (**2**) and costunolide (**5**).[3−5] Thousands of sesquiterpene lactones[6] have been isolated from plant sources, and many of them were shown to possess various biological and pharmacological activities including antitumor,[7−9] antimicrobial,[7,9−11] antiinflammatory,[12] and antiulcer effects.[13] More recently, compounds **2** and **5** are of particular interest since they were reported to have various immunomodulating activities.[4,14−16]



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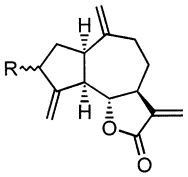
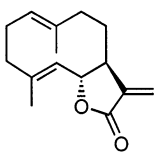


2 R = H
3 R = α-OH
4 R = β-OH



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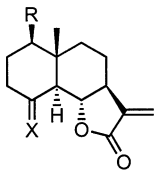
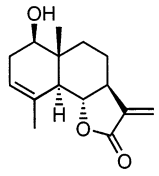
In our continuous search for plant-originated antitumor agents,[17−19] a methanolic extract of *S. lappa* was subjected to bioassay-directed fractionation using human hepatocellular carcinoma (HepG2) cell line as a monitor. Hepatoma is one of the most common cancer diseases in Taiwan.[20] The isolated compounds were further tested against human cervical epitheloid carcinoma (HeLa) and human ovarian adenocarcinoma (OVCAR-3) cell lines.



6 X = α-OH/β-CH_3 R = H
7 X = CH_2 R = OH



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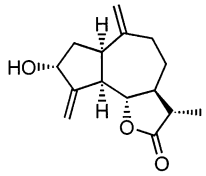

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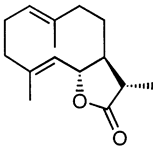

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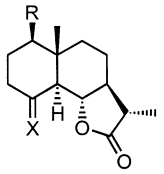

11 X = CH_2 R = OH
12 X = α-OH/β-CH_3 R = H

This paper describes the isolation and identification of eight sesquiterpene lactones (**1**−**8**) that were active against HepG2, HeLa, and OVCAR-3 cells and seven structurally related nonactive compounds (**9**−**15**). Compounds **1**, **13**, and **14** are novel, and their structures were elucidated using spectroscopic and/or X-ray data analyses. To our knowledge, compounds **6**, **11**, and **12** were isolated for the

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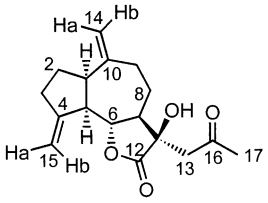
first time from *S. lappa*. According to the classification of carbocyclic skeletons, compounds **2**−**4**, **9**, and **13** are guaianolides, **5** and **10** are *trans,trans*-germacranolides, **6**−**8**, **11**, and **12** are eudesmanolides, and **14** and **15** are nonlactonic eudesmanes. Structure−activity relationships are briefly described. Some selected compounds were also tested against Gram-positive and Gram-negative bacteria including drug-resistant strains.



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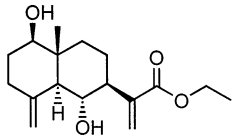

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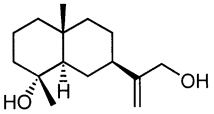

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Results and Discussion

The concentrated methanolic extract was chromatographed on silica gel, and the resulting fractions were subjected to the bioassay for cytotoxicity against the HepG2 cell line as a monitor. Repeated chromatography of the bioactive fractions resulted in the isolation of 15 compounds (**1**−**15**). The structures of new compounds lappadilactone (**1**) and lappalone (**13**) and 1β,6α-dihydroxycostic acid ethyl ester (**14**) were determined from ^{1}H and ^{13}C NMR spectra with the aid of 1D NOE, 2D NOESY, COSY, HMQC, and HMBC experiments and/or X-ray data interpretations. The known compounds were identified as dehydrocostuslactone (**2**),[14] 3-epizaluzanin C (**3**),[21] zaluzanin C (**4**),[21] costunolide (**5**),[22] arbusculin A **(6)**,[23] reynosin (**7**),[24] santamarin (**8**),[25,26] 11β,13-dihydro-3-epizaluzanin C (**9**),[21] 11β,13-dihydrocostunolide (**10**),[27] 11β,13-dihydroreynosin (**11**),[14] colartin (**12**),[23] and 4α-hydroxy-4β-methyldihydrocostol (**15**)[28,29] from ^{1}H and ^{13}C NMR data comparison with those reported in the literature. The pure compounds were then subjected to cytotoxic and antimicrobial activity evaluation.

Compound **1** was obtained as colorless needles from MeOH/EtOAc, mp 260 °C (dec). The IR spectrum exhibited absorption bands at 1761 cm^{-1} for the γ-lactone ring, 1642 and 894 cm^{-1} for exomethylene double bonds, and 1714 cm^{-1} for the carbonyl group of cyclohexanone. The EIMS of **1** gave a molecular ion peak at *m*/*z* 494 and fragment peaks at *m*/*z* 476 [M − H_2O]$^+$ and 229 [M − H_2O − $C_{15}H_{19}O_3$]$^+$. The HREIMS revealed a molecular formula of $C_{30}H_{38}O_6$. The ^{13}C and DEPT NMR spectra of **1** showed the presence of 30 carbons (Table 1), which were assigned to two methyl, 12 methylene (three olefinic at δ 118.1, 112.2, and 109.6), seven methine (two oxygenated at δ 84.1 and 80.7), and nine quaternary carbon (one carbonyl carbon at δ 214.3, one ester carbon at δ 177.2, and one α,β-unsaturated ester carbon at δ 169.9, three olefinic at δ 151.3, 149.6, and 138.2, and one oxygenated at δ 77.1). The ^{1}H NMR spectrum exhibited six olefinic proton groups at δ 6.10, 5.44, 5.19, 5.04, 4.88, and 4.79, two oxymethine protons at δ 4.21 and 4.05, two tertiary methyl signals at δ 1.22 and 1.35, a broad methine quartet at δ 2.87, and a broad methine triplet at δ 2.72 (Table 1). The connectivity of ^{1}H and ^{13}C signals was determined by HMQC spectrum,

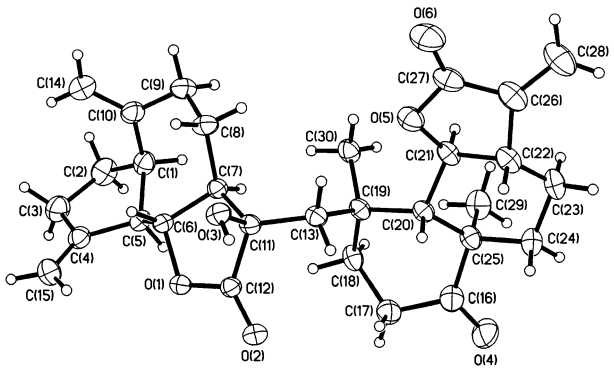

Figure 1. Perspective drawing of lappadilactone (**1**).

and the further correlation of ^{1}H and ^{13}C chemical shifts was assigned by inspection of COSY and HMBC spectra (Table 1). The above data suggested that **1** was a guaianolide-type sesquiterpene linking with a eudesmanolide-type sesquiterpene. The inter-sesquiterpenoid linkage was determined by HMBC spectrum, which showed the correlation between H-13 and C-7, C-11, C-12, C-18, C-19, C-20, and C-30, indicating that **1** consists of a carbon−carbon linkage. Therefore, compound **1** is a new dimeric sesquiterpene lactone, and the trivial name lappadilactone is given.

The relative stereochemistry of lappadilactone (**1**) was assigned by analyses of the NOESY spectrum and the correlation of protons is shown in Table 1. In the NOESY spectrum, correlations of H-5 with H-1 and H-7, and H-20 with H-13 and H-22 suggested that H-1, H-5, and H-7 had α-orientations and H-20 and H-22 had axial orientations. In addition, correlations of H-18β with Me-29 and Me-30 suggested that the cyclohexanone ring had a boat conformation. Since the chemical shift of H-7 was overlapped with that of H-13b, the stereochemistry of the hydroxy group at C-11 was indeterminable. Therefore, the stereochemistry of the hydroxy group was studied by X-ray diffraction analysis with its structural confirmation. The X-ray crystallography defined the relative configuration of **1** and gave results consistent with the data of NOESY experiments. The computer-generated perspective drawing of **1** is shown in Figure 1. The stereochemistry of the hydroxy group at C-11 was revealed in the β-orientation. Both C-18 and C-25 were disposed below the mean plane (defined by C-16, C-17, C-19, and C-20) of the cyclohexanone ring by 0.563 and 0.501 Å, respectively. In addition, the deviations of C-16 and C-19 were below the mean plane by 0.197 and 0.186 Å, respectively, whereas C-17 and C-20 were above 0.195 and 0.188 Å, respectively. This evidence indicates that the cyclohexanone ring was a twisted boat conformation.

Compound **13** was obtained as colorless plates from acetone/hexane, mp 140−141 °C. The molecular formula $C_{17}H_{22}O_4$ was determined by HREIMS. The IR spectrum of **13** indicated the existence of a γ-lactone ring (1774 cm^{-1}), a carbonyl group (1709 cm^{-1}), and the exomethylene double bonds (1640 and 892 cm^{-1}). The ^{13}C and DEPT NMR spectra displayed 17 carbon signals (Table 2), which were assigned to five quaternary (one carbonyl carbon at δ 210.0, one ester carbon at δ 176.1, two olefinic at δ 151.1 and 149.5, and one oxygenated at δ 76.3), four methine (one oxygenated at δ 84.1), seven methylene (two olefinic at δ 112.3 and 109.8), and one methyl carbon, which suggested the presence of one carbonyl group, one lactone, and two exomethylene double bonds. The structure elucidation was achieved through analyses of the HMQC and HMBC spectra. The long-range correlation between the H-13 and

Table 1. ^{1}H and ^{13}C NMR Assignments of Lappadilactone (**1**) by DEPT, NOESY, HMQC, and HMBC Experiments in $CDCl_3$[a]

position	δ_C	δ_H (J)[b]	NOESY	HMBC
1	47.7 d	2.87 bq (7.5)	H-2α, H-5, H-9α, H-14a	C-2, C-3, C-4, C-5, C-9, C-10
2	30.1 t	α 1.91 m	H-1, H-3α	C-3, C-4, C-10
		β 1.83 m	H-3*β*, H-9*β*, H-14a	C-3, C-4, C-10
3	32.2 t	α 2.43 m	H-2α	
		β 2.51 m	H-2*β*, H-14a	
4	151.4 s			
5	52.4 d	2.72 bt (9.0)	H-1, H-7, H-15b	C-6, C-15
6	84.1 d	4.21 t (9.5)	H-8*β*, H-15b	C-4, C-7, C-8
7	49.2 d	2.33 m	H-5, H-8α	
8	25.3 t	α 1.97 m	H-7	C-6, C-10
		β 1.71 m	H-6	C-6, C-10
9	35.7 t	α 2.07 m	H-1	C-1, C-7, C-8, C-10, C-14
		β 2.48 m	H-2*β*, H-14b	C-1, C-7, C-8, C-10, C-14
10	149.6 s			
11	77.1 s			
12	177.2 s			
13	46.8 t	a 2.03 d (15)	H-20	C-7, C-11, C-12, C-18, C-19, C-20, C-30
		b 2.33 d (15)	H-30	C-7, C-11, C-12, C-18, C-19, C-20, C-30
14	112.3 t	a 4.79 s	H-1, H-2*β*, H-3*β*	C-1, C-9, C-10
		b 4.86 s	H-9*β*	C-1, C-9, C-10
15	109.6 t	a 5.04 d (1.5)		C-3, C-5
		b 5.19 d (1.5)	H-5, H-6	C-3, C-5
16	214.3 s			
17	34.9 t	α 2.56 m	H-18α	C-16, C-18, C-19
		β 2.36 dt (4 & 18)	H-18*β*	C-16, C-18, C-19
18	31.6 t	α 1.97 dt (14 & 4)	H-17α	C-13, C-16, C-17, C-19, C-30
		β 1.74 dt (14 & 4)	H-17*β*, H-29, H-30	C-13, C-16, C-17, C-19, C-30
19	34.7 s			
20	53.4 d	2.12 d (11)	H-13a, H-22, H-24ax	C-13, C-16, C-19, C-21, C-22, C-25, C-29, C-30
21	80.7 d	4.05 t (11)	H-23ax, H-29, H-30	C-20, C-22, C-23, C-26
22	50.2 d	2.53 m	H-20, H-23eq, H-24ax	
23	21.1 t	ax 1.53 dq (2.5 & 13.5)	H-21, H-29	
		eq 2.07 m	H-22, H-28b	
24	35.5 t	ax 1.47 dt (2.5 & 13.5)	H-20, H-22	
		eq 2.03 m	H-29	
25	49.0 s			
26	138.2 s			
27	169.9 s			
28	118.1 t	a 6.10 d (3.5)		C-22, C-26, C-27
		b 5.44 d (3.5)	H-23eq	C-22, C-27
29	19.7 q	1.22 s	H-18*β*, H-21, H-23ax, H-24eq, H-30	C-16, C-20, C-24, C-25
30	24.4 q	1.35 s	H-13b, H-18*β*, H-21, H-29	C-13, C-18, C-19, C-20

[a] ^{1}H NMR (500 MHz), ^{13}C NMR (125 MHz), DEPT, NOESY (mix = 0.8), HMQC (J = 150 Hz), and HMBC (J = 8 Hz) experiments were measured with a Varian Unity Inova 500 instrument. [b] J = coupling constant in Hz.

Table 2. ^{1}H and ^{13}C NMR Assignments of Lappalone (**13**) by DEPT, NOESY, HMQC, and HMBC Experiments in $CDCl_3$[a]

position	δ_C	δ_H (J)[b]	NOESY	HMBC
1	47.5 d	2.83 bq (7.5)	H-2α, H-5, H-9α, H-14a	C-4, C-5, C-6, C-10, C-14
2	30.0 t	α 1.91 m	H-1	C-3, C-4, C-10
		β 1.81 m	H-9*β*, H-14a	C-3, C-4, C-10
3	32.1 t	α 2.42 m	H-15a	C-4
		β 2.52 m	H-14a, H-15a	C-4
4	151.1 s			
5	52.0 d	2.73 bt (9.0)	H-1, H-7, H-15b	C-4, C-6
6	84.1 d	4.26 t (9.5)	H-8, H-15b	C-4
7	51.2 d	1.97 m	H-5, H-8	C-6
8	25.2 t	1.74 m	H-6, H-7, H-9α, H-9*β*, H-13a	C-6, C-7, C-9, C10, C-11
9	35.8 t	α 1.99 m	H-1, H-8	C-1, C-7, C-8, C-10, C-14
		β 2.46 m	H-2*β*, H-8, H-14b	C-1, C-7, C-8, C-10, C-14
10	149.5 s			
11	76.3 s			
12	176.1 s			
13	44.5 t	a 2.61 d (17)	H-8, H-17	C-7, C-11, C-12, C-16
		b 2.78 d (17)	H-17	C-11, C-12, C-16
14	112.3 t	a 4.78 s	H-1, H-2*β*, H-3*β*	C-1, C-9, C-10
		b 4.86 s	H-9*β*	C-1, C-9, C-10
15	109.8 t	a 5.03 d (2.0)	H-3α, H-3*β*	C-3, C-5
		b 5.18 d (2.0)	H-5, H-6	C-3, C-5
16	210.0 s			
17	32.0 q	2.29 s	H-13a, H-13b	C-13, C-16

[a] ^{1}H NMR (500 MHz), ^{13}C NMR (125 MHz), DEPT, NOESY (mix = 0.8), HMQC (J = 150 Hz), and HMBC (J = 8 Hz) experiments were measured with a Varian Unity Inova 500 instrument. [b] J = coupling constant in Hz.

C-7, C-11, C-12, and C-16 observed in the HMBC spectrum (Table 2) suggested that an acetyl moiety was attached to the guaiane skeleton at C-13 by a carbon−carbon linkage. Compound **13** was given the trivial name lappalone.

The relative stereochemistry of lappalone(**13**) was studied from the NOESY spectrum, and the correlation of protons is shown in Table 2. In the NOESY spectrum, correlations of H-5 with H-1 and H-7 suggested that H-1,

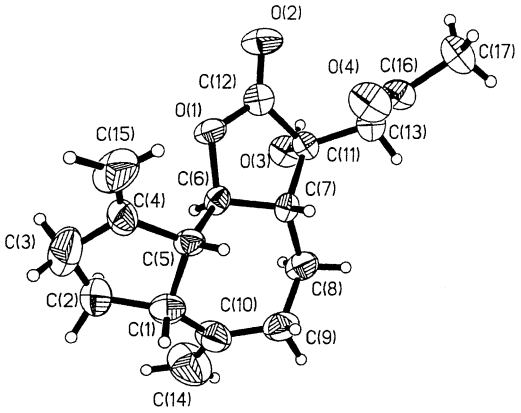

Figure 2. Perspective drawing of lappalone (**13**).

Table 3. Cytotoxicity of Compounds **1**−**15**

	CD_{50} (μg/mL)		
compound	HepG2	HeLa	OVCAR-3
1	2.4	1.8	2.5
2	3.5	3.5	2.5
3	15.0	13.5	7.5
4	34.0	22.0	15.0
5	1.6	2.0	2.0
6	10.0	7.5	7.5
7	11.0	7.5	7.5
8	7.5	10.0	10.0
9	75.0	65.0	65.0
10	75.0	85.0	75.0
11	>100.0	>100.0	95.0
12	>100.0	100.0	100.0
13	>100.0	>100.0	>100.0
14	50.0	75.0	75.0
15	>100.0	>100.0	>100.0
cisplatin	2.8	5.2	3.0

H-5, and H-7 retained α-orientations. The X-ray diffraction analysis of **13** revealed the stereochemistry of the hydroxy group at C-11 as the β-orientation with its structural confirmation (Figure 2). The relative configuration of **13** obtained by X-ray crystallography was consistent with the NOESY experimental results. Intramolecular hydrogen bonding between the ketone oxygen atom and its β-hydroxy group was not observed in the X-ray data. The ketone oxygen atom O(4) showed distances of 4.28, 2.85, and 2.64 Å to the hydroxy oxygen atoms O(3), C(11), and H(7), respectively, as shown in the Supporting Information. This revealed that the ketone oxygen atom was disposed in the opposite direction to the C(11)−O(3) bond.

The cytotoxic evaluation of all the purified compounds showed that compounds **1**, **2**, and **5** with an α-methylene-γ-lactone moiety were the most potent, with a CD_{50} range of 1.6−3.5 μg/mL against the HepG2, HeLa, and OVCAR-3 cell lines (Table 3). The cytotoxicities of compounds **1**−**15** against the above cancer cell lines were not previously studied. The CD_{50} values are comparable to the positive control, cisplatin. The cytotoxicity was dose-dependent for compounds **1**, **2**, and **5**, and the representative growth inhibition activities against OVCAR-3 are shown in Figure 3.

The dynamics of OVCAR-3 cell inhibition were evaluated from the time-kill assays for compound **1**. The OVCAR-3 cell line was incubated with the multiples of CD_{50} of compound **1** for different periods up to 24 h, and the results of viable cell counts as percentage of control are presented in Figure 4. At the dosage of $4\times CD_{50}$, half cell death occurred rapidly within 2 h of incubation. For the dosages of $2\times CD_{50}$ and CD_{50}, half cell death was achieved at 6 and

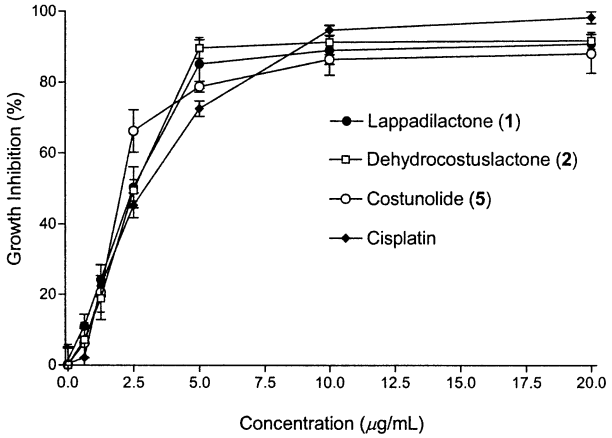

Figure 3. Cytotoxic effect against the OVCAR-3 cell line after 48 h of incubation with various concentrations of compounds **1**, **2**, **5**, and cisplatin using the MTT method. Data are expressed as the mean ± SD (n = 4).

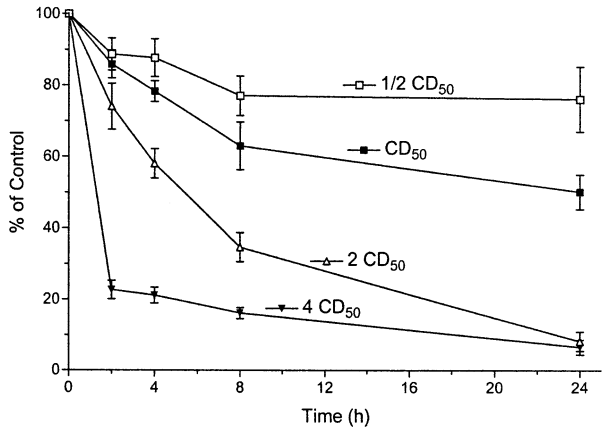

Figure 4. Time-kill assays of lappadilactone (**1**) against the OVCAR-3 cell line using the MTT method. The cells were incubated at 37 °C for 0, 2, 4, 8, and 24 h with the multiples of CD_{50} of **1**. The viable cells were expressed as percentage of control at the end of further incubation for 48 h. Data are expressed as the mean ± SD (n = 4).

24 h, respectively. At the dosage of $0.5\times CD_{50}$, the cells were barely affected even up to 24 h of incubation. Therefore, the cytotoxicity against OVCAR-3 for compound **1** was both time- and concentration-dependent.

For cytotoxic effects, lipophilicity of the compounds is considered an important factor.[30] Compounds **3**, **4**, and **6**−**8**, which were less lipophilic than compound **2**, due to the presence of the hydroxyl group, expressed less cytotoxicities with a CD_{50} range of 7.5−34.0 μg/mL (Table 3). Although the cytotoxicity was not dramatically reduced, our result is in agreement with other reports of reduction in antimycobacterial,[31] cytotoxic,[32] and fungicidal[33] activities with the presence or with the increasing number of hydroxyl groups. Interestingly, compound **3**, with the hydroxyl group at C-3 in α location, showed more potent cytotoxicity than its epimer **4**. This finding is opposite of the earlier reports of their antifungal activities; zaluzanin C (**4**) showed inhibition of mycelial growth, whereas 3-epizaluzanin C (**3**) did not show appreciable activity.[33] Therefore, depending on the nature of the biological activity, the configuration of the hydroxy group may play a different role.

Nonactive sesquiterpene lactones found here include lappalone (**13**) and 1β,6α-dihydroxycostic acid ethyl ester (**14**) and five known compounds, 11β,13-dihydro-3-epizaluzanin C (**9**), 11β,13-dihydrocostunolide (**10**), 11β,13-

dihydroreynosin (**11**), colartin (**12**), and 4α-hydroxy-4β-methyldihydrocostol (**15**).

Compound **10**, which is the corresponding saturated γ-lactone compound of **5**, showed approximately 40 times (CD_{50} = 75−85 μg/mL) less cytotoxicity than **5**, suggesting that the α-methylene-γ-lactone moiety is necessary for the cytotoxicity. This result is in agreement with previous reports of biological activities of sesquiterpene lactones.[7,34,35] The saturated γ-lactone compounds (**11**, **12**) containing a hydroxyl group were the least active ($CD_{50} \geq 95$ μg/mL) among this series of sesquiterpene lactones. Therefore, the α-methylene-γ-lactone moiety is an essential factor for the cytotoxicity, and the more lipophilic the compound is, the greater the cytotoxicity is.

Consistently, compound **13** has a saturated γ-lactone moiety with two hydrophilic keto and hydroxyl groups, resulting in no cytotoxicity. Compound **14**, which is the lactone ring-opened ethyl ester of **7**, showed reduction of the activity by 10-fold against HeLa (CD_{50} = 75 μg/mL) and OVCAR-3 (CD_{50} = 75 μg/mL) cell lines by comparison with **7**. A weak activity is observed against HepG2 (CD_{50} = 50 μg/mL), presumably due to an α,β-unsaturated carbonyl group. Although a similar compound with a conjugated carbonyl double bond was reported to have moderate cytotoxicities against some cancer cells,[25] the cytotoxicities observed here for **14** were not significant. Furthermore, compound **15**, which contained neither the lactone ring nor an α,β-unsaturated carbonyl group, showed no cytotoxicity.

Some sesquiterpene lactones have been shown to be antimicrobial[7,10,31,33] and could therefore be antitumor agents with antimicrobial activities. Antitumor agents with antimicrobial activity may be useful for immunocompromised cancer patients, who are prone to microbial infectious complications. Therefore some selected compounds (**2**, **5**, **7**, **8**, **12**) were subjected to evaluation of antibacterial activity against Gram-positive *Staphylococcus aureus*, methicillin-resistant *Staphylococcus aureus* (MRSA), *Enterococcus faecalis*, vancomycin-resistant enterococci (VRE), and Gram-negative *Escherichia coli*. Only costunolide (**5**) showed mild antibacterial activities with minimum inhibitory concentrations (MICs) of 100−400 μg/mL for Gram-positive bacteria, as shown in the table in the Supporting Information.

Compounds **1**−**8** expressed moderate cytotoxicities, but none of the representative tested compounds showed appreciable antibacterial activities. Therefore, compounds **1**−**8** are attractive antitumor candidates but not as antimicrobials.

Experimental Section

General Experimental Procedures. Melting points were measured using a Yanaco MP-S9 micro-melting point apparatus and uncorrected. Optical rotations were obtained on a JASCO DIP-370 digital polarimeter. IR spectra were recorded on a Nicolet Magna 560 FT-IR spectrometer. ^1H and ^{13}C NMR spectra were recorded on a Varian Unity Inova 500 spectrometer in $CDCl_3$ using the solvent as a reference standard (^1H, 7.24 ppm, and ^{13}C, 77.0 ppm). ^1H, ^{13}C, COSY, HMQC, HMBC, NOESY, and DEPT spectra were obtained using standard Varian pulse sequences. EIMS spectra were measured with a direct insertion probe on a Finnigan GCQ spectrometer at 30 eV. HREIMS data were taken on a Finnigan MAT 95S mass spectrometer. Silica gel (Kieselgel 60, Macherey-Nagel) was used for column chromatography. TLC was carried out on aluminum sheets precoated with silica gel 60 F_{254} (layer thickness 0.2 mm, Merck). The chromatograms were visualized under UV light (254 or 365 nm) or by spraying with 5% phosphomolybdic acid containing a trace of ceric sulfate in 5% H_2SO_4, followed by heating on a hot plate (120 °C).

Plant Material. The root of *S. lappa* was purchased from the Cherng-Chi Chinese herbal shop in Taipei in November 2000. A voucher specimen (NRICM 99026) is retained in the National Research Institute of Chinese Medicine, Taipei.

Extraction and Isolation. The air-dried root of *S. lappa* (5 kg) was crushed and extracted with methanol (50 L) three times at 60 °C for 24 h. The methanolic extracts were combined and concentrated in vacuo to 1 L. The concentrated extract was suspended in H_2O (5 L) and then extracted with EtOAc (3 × 5 L). After concentration of the EtOAc extract, the concentrate was mixed with 500 g of silica gel (230−400 mesh). The air-dried mixture was subjected to a chromatographic column (10 cm i.d. × 100 cm) and then eluted with a stepwise gradient eluent of hexane/EtOAc (80:20, 60:40, 40:60, 20:80, 0:100) (15 L each). Fractions (1 L each) were collected, and similar fractions were combined to give four fractions (A−D). Fractions B and C showed cytotoxic activity. One tenth of the fraction B was subjected to silica gel column chromatography (4 cm i.d. × 100 cm) and eluted with hexane/EtOAc (95:5, 90:10, 85:15, 80:20, 75:25, 70:30) (4 L each) to afford three additional fractions (B1−B3). One-fourth of the fraction B2 was rechromatographed on a silica gel column (2 cm i.d. × 120 cm). Elution with hexane/EtOAc (90:10) (3 L) gave dehydrocostuslactone (**2**)[14] (950 mg) and costunolide (**5**)[22] (870 mg). Fraction C was similarly subjected to silica gel column chromatography (4 cm i.d. × 100 cm), and elution with hexane/EtOAc (90:10, 83:17, 75:25, 68:32, 60:40, 50:50) (4 L each) afforded five additional fractions (C1−C5). Fraction C2 was further fractionated by silica gel column chromatography (2 cm i.d. × 120 cm) and eluted with hexane/EtOAc (88:12) (3 L) to give arbusculin A (**6**)[23] (76 mg), 11β,13-dihydrocostunolide (**10**)[27] (1.21 g), and colartin (**12**)[23] (64 mg). Fraction C3 was separated by silica gel column chromatography (1 cm i.d. × 120 cm) and eluted with hexane/EtOAc (88:12) (2.5 L) to result in lappadilactone (**1**) (10 mg), santamarin (**8**)[25,26] (28 mg), and 11β,13-dihydroreynosin (**11**)[14] (75 mg). Fraction C4 was further fractionated by silica gel column chromatography (2 cm i.d. × 120 cm) and eluted with hexane/EtOAc (70:30) (8 L) to afford 3-epizaluzanin C (**3**)[21] (15 mg), zaluzanin C (**4**)[21] (115 mg), reynosin (**7**)[24] (108 mg), 11β,13-dihydro-3-epizaluzanin C (**9**)[21] (17 mg), lappalone (**13**) (9 mg), 1β,6α-dihydroxycostic acid ethyl ester (**14**) (75 mg), and 4α-hydroxy-4β-methyldihydrocostol (**15**)[28,29] (238 mg).

Lappadilactone (1): colorless needles; 10 mg; mp 260 °C (dec); $[\alpha]_D^{25}$ +32.2° (*c* 1.0, $CHCl_3$); IR (film) ν_{max} 3401, 2933, 2860, 1761, 1714, 1642, 1462, 1404, 1346, 1230, 1130, 968, 894 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1; HREIMS *m/z* 494.2669 (calcd for $C_{30}H_{38}O_6$ 494.2663); EIMS *m/z* 494 $[M]^+$ (3), 476 (8), 342 (17), 296 (7), 246 (10), 229 (33), 159 (100), 145 (12), 131 (20), 117 (12).

X-ray Crystal Structure Analysis of Lappadilactone (1).[36] A colorless crystal from MeOH/EtOAc of **1** with dimensions of 0.35 × 0.35 × 0.22 mm was selected for X-ray analysis. The crystallographic data were collected on a NONIUS Kappa CCD diffractometer using graphite-monochromated Mo Kα radiation. Structure analysis was made by using the SHELXTL program on a PC.[37] The compound crystallized in the space group $P2_1$, a = 7.2647(1) Å, b = 26.0697(4) Å, c = 13.7687(2) Å, monoclinic, β = 90.4462(5)°, V = 2607.55(7) Å^3, Z = 4, D_{calc} = 1.260 g/cm^3, λ = 0.71073 Å, μ(Mo Kα) = 0.086 mm^{-1}, F(000) = 1064, and T = 295 K. A total of 26 948 reflections were collected in the range 1.48° ≤ θ ≤ 27.50°, of which 10 879 unique reflections with $I > 2\sigma(I)$ were used for the analysis. The structure was solved using direct methods and refined by full-matrix least-squares on F^2 values. Non-hydrogen atoms were refined anisotropically. Hydrogen atoms were fixed at calculated positions and refined using a riding mode. The final indices were R = 0.0571, R_w = 0.1094 with goodness-of-fit = 1.054. Scattering factors were taken from *International Tables for X-ray Crystallography*.[38]

Lappalone (13): colorless solid; 9 mg; mp 140−141 °C; $[\alpha]_D^{25}$ +37.2° (*c* 0.97, $CHCl_3$); IR (film) ν_{max} 3405, 2923, 2851, 1774, 1709, 1640, 1414, 1365, 1168, 1140, 990, 892 cm^{-1}; ^1H and ^{13}C NMR, see Tables 2; HREIMS *m/z* 290.1543 (calcd for $C_{17}H_{22}O_4$ 290.1513); EIMS *m/z* 290 $[M]^+$ (9), 272 (38), 254 (76), 246 (27), 228 (29), 214 (45), 188 (100), 170 (38), 160 (85), 145 (98), 131 (83), 117 (56), 105 (70), 91 (65), 79 (51), 67 (14).

X-ray Crystal Structure Analysis of Lappalone (13).[36] A colorless crystal from acetone/hexane of **13** with dimensions of 0.34 × 0.30 × 0.10 mm was selected for X-ray analysis. The crystallographic data were collected on a Bruker-AXS SMART 1000 CCD diffractometer using graphite-monochromated Mo Kα radiation. Structure analysis was made by using the SHELXTL program on a PC.[37] The compound crystallized in the space group $P2_1$, a = 5.7198(12) Å, b = 7.8626(16) Å, c = 17.340(3) Å, monoclinic, β = 92.715(5)°, V = 778.9(3) Å^3, Z = 2, D_{calc} = 1.238 g/cm^3, λ = 0.71073 Å, μ(Mo Kα) = 0.087 mm^{-1}, $F(000)$ = 312, and T = 293 K. A total of 4484 reflections were collected in the range 2.85° ≤ θ ≤ 26.05°, of which 2911 unique reflections with $I > 2\sigma(I)$ were used for the analysis. The structure was solved using direct methods and refined by full-matrix least-squares on F^2 values. Non-hydrogen atoms were refined anisotropically. Hydrogen atoms were fixed at calculated positions and refined using a riding mode. The final indices were R = 0.0620, R_w = 0.1023 with goodness-of-fit = 1.003. Scattering factors were taken from *International Tables for X-ray Crystallography*.[38]

1*β*,6α-Dihydroxycostic acid ethyl ester (14): yellow oil; 23 mg; $[\alpha]_D^{25}$ +52° (*c* 1.86, MeOH); IR (film) ν_{max} 3419, 2934, 2869, 1759, 1709, 1649, 1621, 1456, 1378, 1320, 1265, 1171, 1156, 1008, 994, 897 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 6.23 (1H, s, H-13a), 5.65 (1H, s, H-13b), 4.96 (1H, s, H-15a), 4.70 (1H, s, H-15b), 4.17 (2H, q, J = 7 Hz, OCH_2), 3.94 (1H, t, J = 10 Hz, H-6), 3.44 (1H, dd, J = 4.5, 11.5 Hz, H-1), 2.55 (1H, q, J = 10 Hz, H-7), 2.28 (1H, m, H-3eq), 2.02 (1H, m, H-3ax), 1.93 (1H, br d, J = 13 Hz, H-9eq), 1.81 (1H, m, H-2eq), 1.81 (1H, J = 10 Hz, H-5), 1.62 (2H, m, H-8), 1.54 (1H, m, H-2ax), 1.27 (3H, t, J = 7 Hz, CH_3CH_2), 1.17 (1H, m, H-9ax), 0.74 (3H, s, H-14); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 167.6 (s, C-12), 145.0 (s, C-4), 142.8 (s, C-11), 124.7 (t, C-13), 108.4 (t, C-15), 78.7(d, C-1), 69.1 (d, C-6), 60.8 (t, OCH_2), 55.3 (d, C-5), 47.5 (d, C-7), 41.8 (s, C-10), 36.2 (t, C-9), 34.9 (t, C-3), 31.7 (t, C-2), 26.2 (t, C-8), 14.1 (q, CH_3CH_2), 11.5 (q, C-14); HREIMS *m/z* 294.1826 (calcd for $C_{17}H_{26}O_4$ 294.1831); EIMS *m/z* 294 $[M]^+$ (6), 276 (10), 258 (19), 248 (22), 230 (43), 215 (25), 202 (52), 185 (43), 174 (41), 159 (98), 145 (100), 135 (50), 119 (67), 107 (50), 93 (45), 79 (46), 67 (38), 55 (32).

Antibacterial Activity. Bacteria, culture conditions, and the antibacterial activity evaluation for compounds **2**, **5**, **7**, **8**, **12**, and positive controls were the same as previously outlined.[39]

Cytotoxicity Assay. The cell line culture conditions[19] and MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] colorimetric assay for CD_{50} were carried out according to the procedures previously described.[39]

Time-kill assays were performed for compound **1** against OVCAR-3 cell lines. The cells were seeded at a density of 5 × 10^3 cells per well (0.1 mL) in 96-well microplates and incubated for 24 h, and the multiples of CD_{50} of compound **1** were added. The cells were then incubated for 0, 2, 4, 8, and 24 h with the compounds. At the end of each incubation, the compounds were washed away and fresh medium was added. All the cells were further incubated for 48 h at 37 °C. Then the viable cells were estimated as percentage of control from optical density data at 570 nm using the MTT method.[39]

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Supporting Information Available: The results of an antibacterial activity study of **2**, **5**, **7**, **8**, and **12** and the distance between atoms of compound **13**. This information is available free of charge via the Internet at http://pubs.acs.org.

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(36) Crystallographic data for compounds **1** and **13** reported in this paper have been deposited with the Cambridge Crystallographic Data Centre (deposition numbers CCDC 204997 for **1** and 204996 for **13**). Copies of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44 1223 336033 or e-mail: deposit@ccdc.cam.ac.uk).
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