Sesquiterpene Lactones from the Root Tubers of *Lindera aggregata*

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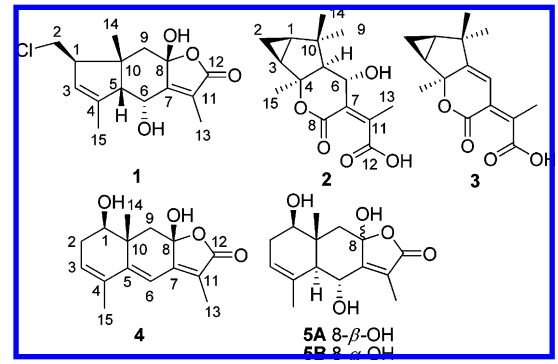
Phytochemical investigation of the root tubers of *Lindera aggregata* resulted in the isolation of five new sesquiterpene lactones, linderagalactones A−E (**1**−**5**), along with eight known sesquiterpenoids, 3-eudesmene-1β,11-diol, hydroxylindestenolide, strychnistenolide, hydroxyisogermafurenolide, atractylenolide III, linderane, neolinderalactone, and linderalactone. The structures and relative configurations of **1**−**5** were determined by spectroscopic methods, especially HRESIMS and 2D NMR techniques. The absolute configurations of **1**−**4** were defined by comparison of quantum chemical TDDFT calculated and experimental ECD spectra. Linderagalactone A (**1**) is a halogenated sesquiterpene lactone possessing a unique rearranged carbon skeleton. Linderagalactone E (**5**), linderane, hydroxylindestenolide, and linderalactone showed hepatoprotective activity against H_2O_2-induced oxidative damages on HepG2 cells with EC_{50} values of 67.5, 167.0, 42.4, and 98.0 μM, respectively.

The root tubers of *Lindera aggregata* (Sims.) Kosterm. [*L. strychnifolia* (Sieb. et Zucc.) F. Vill.] (Lauraceae), Radix Linderae, is an important traditional herbal medicine in China (Wu Yao) and Japan (Uyaku) for the treatment of renal, cystic, and rheumatic diseases.[1] Pharmacological studies on this plant have shown various bioactivities, such as superoxide anion radical scavenging, protection against post-ischemic myocardial dysfunction, antioxidation, and slowing down the progression of diabetic nephropathy in db/db mice.[2] Previous phytochemical work revealed the existence of six major types of bioactive constituents, including sesquiterpenoids, essential oils,[3] alkaloids,[4] flavonoids,[5] lignans, and condensed tannins.[6] The sesquiterpene lactones have been considered to be characteristic and are used as index components for the quality standard of Radix Linderae in China.[7] *L. aggregata* has been a rich source of as many as 37 new sesquiterpenoids.[8] In addition to the basic eudesmane, 5,10-*seco*-eudesmane (germacrane) and 2,3-*seco*-eudesmane (elemane) carbon skeletons[8,9] and three sesquiterpenes with noticeable novelties, including a bisesquiterpene, bilindestenolide,[10] an 8,9-*seco* sesquiterpene, strychnilactone,[11] and an acyclic sesquiterpene, secoaggregatalactone-A,[12] have been identified.

In our projects of quality control of genuine Traditional Chinese Medicine in Zhejiang Province, the sesquiterpenoids in Radix Linderae have been investigated phytochemically. Repeated chromatography of the 95% EtOH extract resulted in the isolation of five new sesquiterpene lactones, including a unique 2-halogenated 2,3-*seco* 1,3-linked sesquiterpene lactone, linderagalactone A (**1**), two rare 8,9-*seco* 9-deoxy 8,4-δ-lactones, linderagalactones B (**2**) and C (**3**), and two new eudesmane sesquiterpene lactones, linderagalactones D (**4**) and E (**5**), along with eight known sesquiterpenoids, 3-eudesmene-1β,11-diol, hydroxylindestenolide, strychnistenolide, 8-hydroxyisogermafurenolide, atractylenolide III, linderane, neolinderalactone, and linderalactone. In this paper, we describe their isolation, structure elucidation, and hepatoprotective activity against H_2O_2-induced oxidative damage on HepG2 cells.

Results and Discussion

Linderagalactone A (**1**) was isolated as an amorphous powder. Its molecular formula, $C_{15}H_{19}O_4Cl$, was determined by a negative HRESIMS pseudo-molecular ion peak at *m*/*z* 297.0902 [M − H]$^-$ (calcd 297.0899). The ^{1}H NMR spectrum of **1** exhibited three methyl group signals at δ_H 1.40, 1.87, and 1.98 (each 3H, s), which is typical for a sesquiterpene lactone containing C-3−C-4 and C-7−C-11 double bonds.[8,9] In the ^{13}C NMR spectrum, three signals at δ_C 9.1, 17.8, and 20.4 verified the existence of the above methyl groups. A hemiacetal carbon signal at δ_C 104.5 assigned to C-8 and a lactone carbonyl resonating at δ_C 172.3 showed the existence of a O═C-12−O−C-8 lactone. Four olefinic carbon signals at δ_C 159.6, 144.4, 128.8, and 122.1 represented the C-3−C-4 and C-7−C-11 double bonds. Considering the existence of a chlorine atom, two mutually coupled hydrogen signals at δ_H 3.72 (dd, J = 10.7, 5.1 Hz) and 3.51 (dd, J = 10.7, 8.1 Hz), and an overlapped carbon signal at δ_C 46.2, a −CH_2Cl moiety could be concluded.[13] Subsequent HSQC, ^{1}H−^{1}H COSY, and HMBC experiments clarified the planar structure of **1** as shown in Figure 1. All the protons were first assigned to their bonding carbons by interpretation of the HSQC data. Two spin systems, ClCH_2(2)−CH(1)−CH(3) and CH(5)−CH(6), were then fixed by analysis of its ^{1}H−^{1}H COSY spectrum (Figure 1). In the HMBC spectrum, correlations of ClCH_2 to C-1, C-3, and C-10, H-3 to C-5 and C-10, H_3-14 to C-1 and C-5, and H_2-9 to C-1 and C-5 proved the presence of the five-membered ring and the ClCH_2−CH-1 linkage (Figure 1). Two −OH protons at δ_H 6.20 (s) and 4.85 (d, J = 4.3 Hz) were assigned to 8-OH and 6-OH according to their HMBC correlations with C-8, C-9, and C-7 and C-6, C-5, and C-7, respectively. Correlations of H_3-13 to C-11, C-7, and C-12, H_3-14 to C-5 and C-1, and H_3-15 to C-4, C-3, and C-5 located the three methyl groups. Thus, the planar structure of compound **1** was determined as a halogenated sesquiterpene lactone possessing a unique rearranged carbon skeleton (Figure 1). The relative configuration of **1**, as shown in Figure 2, was elucidated by analysis of its NOESY spectrum. The key NOE correlations between H_3-14 and H_2-2, H-5, H-6, and H-9β showed



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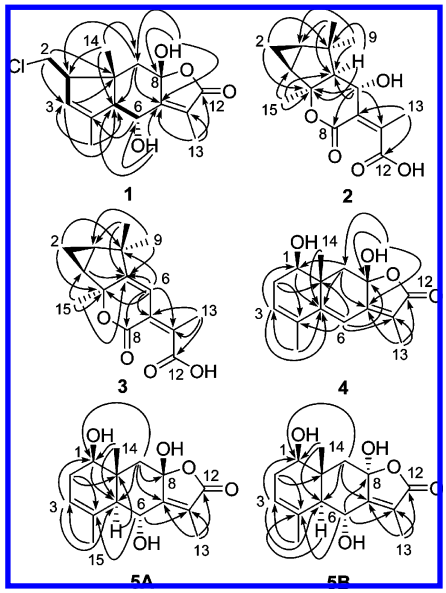

Figure 1. Key HMBC correlations (H → C) of compounds **1**−**5**.

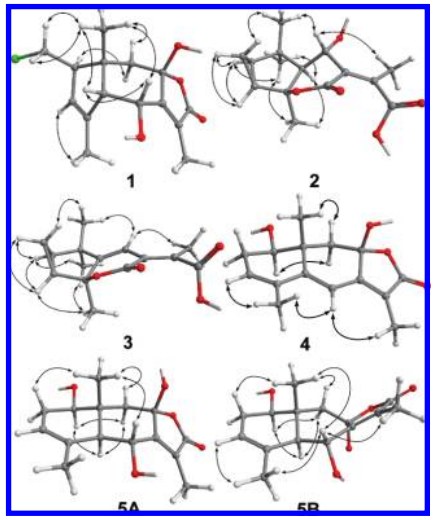

Figure 2. Lowest energy 3D conformations and key NOESY correlations (H ↔ H) of compounds **1**−**5**.

that they are cofacial and arbitrarily defined as β-oriented. Correlations between H-9α and H-1 then revealed their α-orientations.

Linderagalactone B (**2**) was obtained as an amorphous powder. The positive HRESIMS data (*m*/*z* 281.1388 $[M + H]^+$) established its molecular formula as $C_{15}H_{20}O_5$. The ^{1}H NMR spectrum of **2** exhibited a strongly shielded hydrogen signal at δ_H 0.49 (m) and three methyl singlets at δ_H 2.12, 1.66, and 1.11 (each 3H, s), showing similarities to those of strychnilactone.[11] However, an additional methyl singlet at δ_H 1.30 (3H, s) disclosed that compound **2** was probably a derivative of strychnilactone with a deoxygenated C-9 hydroxymethyl functionality. In the ^{13}C NMR spectrum, a shielded carbon signal at δ_C 6.4 and four methyl singlets at δ_C 16.5, 22.9, 33.9, and 34.6 further confirmed the above ^{1}H NMR-based deduction. This hypothesis was reinforced by the ^{13}C NMR data, in which signals due to two carbonyl groups at δ_C 169.8 and 166.0, two olefinic carbons at δ_C 140.6 and 128.0, and two oxygenated carbons at δ_C 91.9 and 67.7 suggested that compound **2** is a C-12 de-esterified, 9-deoxy derivative of strychnilactone. Further analysis of the HSQC and HMBC spectra verified the planar structure of **2**. As shown in Figure 1, in the HMBC spectra, the two methyl groups of H_3-14 and H_3-9 were corroborated by their correlations with C-10, C-1, and C-5. Correlations of H-6/C-8, H-6/C-11, H_3-13/C-12, and H_3-13/C-7 then confirmed the characteristic α,β-unsaturated dicarbonyl moiety. Finally, the relative configuration of **2** was elucidated by NOE correlations. In the NOESY spectrum, correlations of H_3-14/H-6 suggested they were cofacial and arbitrarily assigned as β-oriented. The α-oriented hydrogens were recognized by correlations of H_3-9/H-5, H_3-9/H-1, H-1/H-3, H_3-9/H_3-15, and H_3-15/H-5. Subsequently, the β-orientation of the cyclopropyl ring was determined by correlations of H-2β/H_3-14, H-2α/H-1, and H-2α/H-3. Correlations between H-6 and H_3-13 suggested a *Z* configuration for the 7,11 double bond.

The structure of linderagalactone C (**3**) was determined by a combination of HRESIMS and 2D NMR analysis, as well as by comparison of its NMR data with those of **2**. The HRESIMS data gave a molecular formula of $C_{15}H_{18}O_4$ for linderagalactone C (**3**), which suggested that **3** is a dehydrated product of **2**. This deduction was unambiguously supported by its NMR data. In the ^{1}H NMR spectrum, besides the similar hydrogen signals for four methyl groups and a three-membered ring, an olefinic hydrogen singlet at δ_H 6.16 instead of the doublet at δ_H 5.79 in **2**, along with four olefinic carbons resonating at δ_C 155.9, 138.3, 121.2, and 116.6 in the ^{13}C NMR spectrum, led to the conclusion that **3** is a 5,6-dehydrated product of **2**. The planar structure and relative configuration of **3** were confirmed by key HMBC and NOESY correlations, shown in Figures 1 and 2, respectively.

The yellow, amorphous solid, linderagalactone D (**4**), showed a pseudo-molecular ion peak at *m*/*z* 285.1107 $[M + Na]^+$ in the positive HRESIMS spectrum, consistent with a molecular formula of $C_{15}H_{18}O_4$. Seven degrees of unsaturation and the a maximum UV absorption at 303 (3.93) nm showed a highly conjugated sesquiterpene-type structure for **4**. The ^{1}H NMR spectrum exhibited three methyl singlets at δ_H 1.30, 1.81, and 1.92 (each 3H, s), typical for a eudesmane-type sesquiterpene lactone.[8,9] Two mutually coupled doublets at δ_H 1.69 (1H, d, J = 13.6 Hz) and 2.54 (1H, d, J = 13.6 Hz) might be assigned to the methylene protons at C-9. The above information, as well as the observed two olefinic proton signals at δ_H 6.50 (1H, s) and 5.83 (1H, br s), which is characteristic for two trisubstituted double-bond moieties, led to the proposal that compound **4** is likely a eudesmane sesquiterpene lactone with extended conjugation. This hypothesis was substantiated by its ^{13}C NMR spectrum and a series of 2D NMR experiments. In the ^{13}C NMR spectrum, four carbon signals at δ_C 172.7, 155.1, 118.8, and 102.1 suggested the existence of an 8-hydroxy-α,β-unsaturated-γ-lactone moiety, similar to that of **1**. The four olefin carbon signals at δ_C 152.4, 132.2, 130.0, and 113.1 then confirmed the existence of two trisubstituted double bonds. A carbon signal at δ_C 74.9 could be assigned to a hydroxy-substituted methine group. A sesquiterpene hydroxylactone with a 6,6-fused ring depicted as **4** is consistent with the above data. The overall structure of **4** was finally determined by HSQC, HMBC, and NOESY experiments. In the HMBC spectrum, correlations from CH_3-13 to C-7, C-11, and C-12 and from OH to C-8, C-7, and C-9 confirmed the 8-hydroxy-α,β-unsaturated-γ-lactone moiety. Correlations of H-6/C-4, H-6/C-11, H-6/C-8, H_3-15/C-3, H_3-15/C-4, and H_3-15/C-5 revealed the positions of the C-3−C-4 and C-5−C-6 double bonds. The remaining HMBC correlations depicted in Figure 1 confirmed the other moieties and the eudesmane skeleton of **4**. The relative configuration of **4** was determined by key NOE correlations of CH_3-14/H-9β and H-9α/H-1, as shown in Figure 2.

Linderagalactone E (**5**) was obtained as a white, amorphous powder. The molecular formula, $C_{15}H_{20}O_5$, was established by HRESIMS at *m*/*z* 303.1219, which indicated a hydrated product of linderagalactone D (**4**). Examination of UV and IR spectra showed similarity between **4** and **5**. However, the ^{1}H and ^{13}C NMR spectra of **5** displayed duplicate signals. These spectra revealed that **5** is an isomeric mixture of **5A** and **5B** in methanol-d_4, similar to the situation in strychnistenolide.[11b] Therefore, signals of each isomer

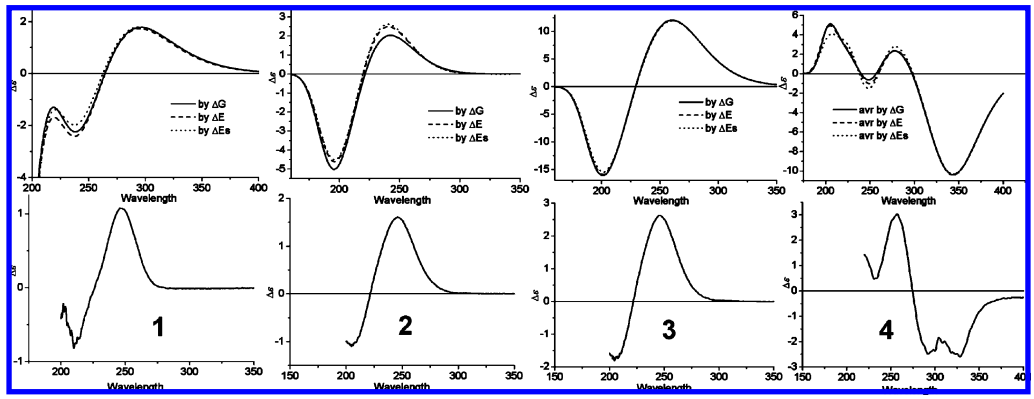

Figure 3. Experimental ECD spectra (lower) and velocity representation of B3LYP-SCRF/aug-cc-pVDZ//B3LYP-SCRF/6-31+G(d)-calculated ECD spectra (upper, conformationally averaged by relative Gibbs free energy, ΔG; internal energy, ΔE; and single point energy, ΔE_s; σ = 0.2 or 0.3 eV; more details see Supporting Information II) of linderagalactones A−D (**1**−**4**).

were assigned individually by interpretation of the HSQC, HMBC, and NOESY spectra, as well as by comparison of the 1D NMR data with those of **4** and strychnistenolide. Compared to that of **4**, the ^{1}H NMR spectrum of **5** showed two signals at δ_H 5.38 and 3.50 for H-3 and H-1, which revealed that the corresponding groups remain unchanged. Additionally, the presence of duplicated signals at δ_H 4.76 (d, J = 11.0) (**5A**) and 4.49 (d, J = 8.8) (**5B**), in combination with the MS data, indicated that **5** is a 5,6-hydration product of **4**. In the ^{13}C NMR spectrum, two lactone carbonyl carbon signals at δ_C 174.6 (C-12, **5A**) and 174.4 (C-12, **5B**) and four hydroxylated carbon signals at δ_C 77.1 (C-1, **5A**), 76.6 (C-1, **5B**), 69.7 (C-6, **5A**), and 64.8 (C-6, **5B**) confirmed the above deduction. Full assignments of the ^{1}H and ^{13}C NMR signals of **5A** and **5B**, as well as the determination of their relative configurations, were achieved by analysis of the 2D NMR correlations as depicted in Figures 1 and 2.

Computational calculations of spectroscopic properties (OR, ECD, VCD, NMR, etc.) of organic molecules by quantum chemical methods, especially the density functional theory (DFT), have been proved to be a powerful tool for the determination of their structures and absolute configurations.[14] In the present study, the absolute configurations of linderagalactones A−D (**1**−**4**) were determined by quantum chemical calculations of their ECD spectra and optical rotations.

Briefly, conformational analyses of **1**−**4** were first carried out via Monte Carlo searching with the MMFF94 molecular mechanics force field using the SPARTAN 04 program.[15] To confirm or re-estimate stable conformations, additional scans of the potential energy surface (PES) at the AM1 level with respect to the variable dihedral angles during the conformation searching were further carried out. Subsequently, the resulting conformations were reoptimized using DFT at the B3LYP-SCRF (PCM, methanol)/6-31+G(d) level using the GAUSSIAN 03 program.[16] The B3LYP-SCRF/6-31+G(d) harmonic vibrational frequencies were further calculated to confirm their stability. The energies, oscillator strengths, and rotational strengths of the electronic excitations of all the conformers were calculated using the TDDFT methodology at the B3LYP-SCRF/aug-cc-pVDZ level, and the ECD spectra were then simulated by the overlapping Gaussian function.[17] To get the final spectra of a molecule, all the simulated spectra of the lowest energy conformations were averaged according to the Boltzmann distribution theory, in which their Gibbs free energy (G), internal energy (E), and single-point energy (E_s) were respectively adopted (Figure 3; for details of calculation, see Supporting Information II).

MMFF conformation searching and PES scan of **1** resulted in the identification of 3 × 3 × 2 = 18 lowest energy conformations (see Supporting Information II). Twelve of them whose relative Gibbs free energies within 2 kcal/mol were considered for further calculations of ECD spectra by the above protocols. In the 200−400 nm region, compared to the experimental positive first Cotton effect at 246 nm and negative second Cotton effect at 205 nm, the calculated ones showed the same pattern, but the corresponding wavelength shifted largely to 290 nm (+34 nm) and 238 nm (+33 nm), respectively (Figure 3). This phenomenon is commonly found in ECD calculations.[14] In addition, the presence of over 10 lowest energy conformations showed high flexibility for the structure of **1**, which also increased the difficulty for accurate prediction. Therefore, qualitative analysis of the result allowed the assignment of the absolute configuration of **1** as 1*R*, 5*R*, 6*R*, 8*S*, 10*R*.

Conformation searching and PES scans of dihedral angles of *O*═C(12)−C(11)═C(7) of **2** and **3** showed two dominating conformers for each. The final conformational averaged ECD spectra for **2** and **3** both showed positive first and negative second Cotton effects, which are consistent with the corresponding experimental spectra (Figure 3). The absolute configurations were therefore determined to be 1*R*, 3*S*, 4*S*, 5*S*, 6*S* for linderagalactone B (**2**) and 1*R*, 3*S*, 4*S* for linderagalactone C (**3**). Interestingly, the experimental specific optical rotations of **2** and **3** showed opposite values (−67 and +88, respectively), which were further studied computationally at the B3LYP/aug-cc-pVDZ level with the B3LYP/ aug-cc-pVDZ geometry in the gas phase. The calculated values were −123 and +91 for **2** and **3**, respectively. Although the theoretical specific rotation value of **2** was not close to the experimental one, the opposite numbers could explain the above phenomenon to some extent.

The MMFF conformational searching of **4** showed six lowest energy conformers (see Supporting Information II), as was further confirmed by PES scans of dihedral angles H−O−C(1)−C(2) and H−O−C(8)−C(9). Both the calculated and the experimental ECD spectra showed negative first Cotton effects, indicating a configuration of 1*R*, 8*S*, 10*R* for linderagalactone D (**4**) (Figure 3).

The structures of the known compounds, 3-eudesmene-1β,11-diol,[18] hydroxylindestenolide,[10] strychnistenolide,[11b] 8-hydroxyisogermafurenolide,[19] atractylenolide III,[20] linderane,[21] neolinderalactone,[22] and linderalactone,[23] were determined by comparison of their observed and reported ^{1}H NMR, ^{13}C NMR, and MS data.

Hepatoprotective effects of linderagalactones C−E (**3**−**5**), 3-eudesmene-1β,11-diol, linderane, neolinderalactone, hydroxylindestenolide, 8-hydroxyisogermafurenolide, and linderalactone were evaluated on a HepG2 cell damage model induced by H_2O_2.[24] Linderagalactone E (**5**), linderane, hydroxylindestenolide, and linderalactone showed hepatoprotective activity with EC_{50} values of 67.5, 167.0, 42.4, and 98.0 μM, respectively.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a JASCO p-1030 polarimeter. IR spectra were recorded on a Bruker

Table 1. ^{1}H NMR Data of Compounds **1**−**5** at 400 MHz (mult., *J* in Hz)

no.	**1**[a]	**2**[a]	**3**[b]	**4**[a]	**5A**[c]	**5B**[c]
1	2.30, br s	1.36, m	1.41, m	3.55, m	3.51, m	3.54, m
2	a 3.72, dd, (10.7, 5.1)	α 0.49, m	α 0.63, m	2.31, m	α 2.36, m	α 2.22, m
	b 3.51, dd, (10.7, 8.1)	β 1.31, m	β 0.22, m		β 2.03, m	β 1.94, m
3	5.45, br s	1.58, m	1.58, m	5.83, br s	5.38, br s	5.38, br s
5	3.13, d, (5.1)	2.19, d (4.3)			2.18, overlap	2.66, br s
6	5.14, br s	5.79, d (4.3)	6.16, s	6.50, s	4.76, d (11.0)	4.49, d (8.8)
9	α 1.65, d, (13.7)	1.30, s	1.30, s	α 1.69, d (13.6)	α 1.35, d, (13.7)	α 2.29, m
	β 2.10, d, (13.7)			β 2.54, d (13.6)	β 2.59, d, (13.7)	β 1.96, m
13	1.98, s	2.12, s	2.11, s	1.81, s	2.03, s	1.91, s
14	1.40, s	1.11, s	1.17, s	1.30, s	1.06, s	0.67, s
15	1.87, s	1.66, s	1.68, s	1.92, d (0.9)	1.96, s	1.94, s
OH	6.20, s			6.00, s		
OH	4.85, d, (4.3)					

[a] Acetone-d_6. [b] $CDCl_3$. [c] Methanol-d_4.

VECTOR 22 FT-IR spectrometer. UV spectra were recorded on a Hitachi U-4100 spectrometer. NMR spectra were measured on a Varian Inova-400 spectrometer with TMS as internal standard. ESIMS and HRESIMS were measured on a Waters UPLC/MS/MS ACQUITY TQD instrument and a Bruker Daltonics Bio TOF-Q mass spectrometer, respectively. Precoated silica gel GF254 plates (Qingdao Haiyang Chemical Co. Ltd., Qingdao, People's Republic of China) were used for TLC. Silica gel (200−300 mesh, Qingdao Haiyang Chemical Co. Ltd.), C_{18} reversed-phase silica gel (150−200 mesh, Merck), MCI gel (CHP20P, 75−150 μm, Mitsubishi Chemical Industries Ltd.), D-101 macroporous resin (Chemical Plant of Nankai University, Tianjin), and Sephadex LH-20 gel (Amersham Biosciences) were used for column chromatography. All solvents were of analytical grade (Shanghai Chemical Plant, Shanghai, People's Republic of China).

Plant Material. Root tubers of *L. aggregata* (5 kg) was obtained from the Factory of Traditional Chinese Medicine Sliced Tablets, Hangzhou, Zhejiang Province, People's Republic of China. A voucher specimen has been deposited in the Institute of Modern Chinese Medicine, Zhejiang University (accession number LA-2007-I).

Extraction and Isolation. The dried sliced tablets of root tuber of *L. aggragata* (5 kg) were ground and extracted with 95% EtOH (×3, 7 days for each extraction) at room temperature. After removal of the solvent under reduced pressure, a crude extract (500 g) was obtained, which was dissolved in 2 L of H_2O to form a suspension and extracted three times with EtOAc to give an EtOAc-soluble fraction (180 g). The EtOAc fraction was subjected to a macroporous resin D-101 column eluted with aqueous EtOH (25%, 50%, 60%, 70%, 80%, 95%) to afford six fractions (Fr-A−Fr-F). Fr-B was chromatographed on a silica gel column eluted with EtOAc/MeOH (from 25:1 to 0:1) to afford eight fractions (Fr-B1−Fr-B8). Fr-B2 was further isolated by a silica gel column eluted with petroleum ether/2-propanol/formic acid (105:20:1) to yield **2** (6 mg). Fr-B4 was chromatographed over silica gel eluted with petroleum ether/EtOAc/MeOH (15:2:1) followed by a Sephadex LH-20 gel column (40% aqueous EtOH) to yield **4** (90 mg). Fr-B6 was further purified on an ODS column eluted with 25% EtOH to afford **5** (45 mg). Fr-C was subjected to a silica gel column eluted with EtOAc/MeOH (from 120:1 to 0:1) to yield 19 fractions (Fr-C1−Fr-C19). 3-Eudesmene-1β,11-diol (15 mg) was obtained from Fr-C11 after silica gel column chromatography (petroleum ether/EtOAc, 8:1). Fr-C16 was chromatographed on an ODS column eluted with 30% aqueous EtOH to yield **1** (5 mg). Fr-C19 was further purified on a silica gel column eluted with petroleum ether/EtOAc/formic acid (15:5:1) and a Sephadex LH-20 gel column (EtOH) to yield **3** (12 mg). Fr-D was subjected to a silica gel column eluted with petroleum ether/$CHCl_3$/MeOH (from 60:2:1 to 3:2:1) to yield 16 fractions (Fr-D1−Fr-D16). Hydroxylindestenolide (750 mg) was obtained by recrystallization of Fr-D2 in acetone. Fr-D16 was further purified by preparative TLC (petroleum ether/EtOAc/formic acid, 63:20:1) to give strychnistenolide (25 mg). Fr-E was subjected to a silica gel column eluted with petroleum ether/EtOAc (from 10:1 to 1:1) and subsequently on an ODS column (45% aqueous EtOH) to yield an mixture of 8-hydroxyisogermafurenolide and atractylenolide III (45 mg), as well as linderalactone (9 mg). Fr-F was subjected to a silica gel column eluted with petroleum ether/$CHCl_3$/MeOH (from 160:2:1 to 3:2:1) to yield six fractions (Fr-F1−Fr-F6). Fr-F1 was recrystallized in acetone to afford linderane (50 mg). Neolinderalactone (95 mg) was obtained by silica gel chromatography (petroleum ether/EtOAc, 25:1) of Fr-F2.

Table 2. ^{13}C NMR Data of Compounds **1**−**5** at 100 MHz

no.	**1**[a]	**2**[a]	**3**[b]	**4**[a]	**5A**[c]	**5B**[c]
1	57.1	32.2	28.9	74.9	77.1	76.6
2	46.2	6.4	7.8	33.1	33.7	32.8
3	128.8	28.2	23.3	130.0	124.1	122.4
4	144.4	91.9	91.0	132.2	135.6	135.2
5	60.8	58.0	155.9	152.4	55.3	49.3
6	68.8	67.7	116.6	113.1	69.7	64.8
7	159.6	140.6	138.3	155.1	164.1	160.0
8	104.5	166.0	165.2	102.1	105.9	105.9
9	47.8	34.6	29.4	43.6	47.2	48.6
10	46.2	44.1	42.0	42.6	40.2	38.5
11	122.1	128.0	121.2	118.8	123.9	126.4
12	172.3	169.8	173.4	172.7	174.6	174.4
13	9.1	16.5	17.3	8.0	9.3	8.1
14	20.4	22.9	25.4	16.6	11.8	14.2
15	17.8	33.9	29.4	19.7	24.4	22.6

[a] Acetone-d_6. [b] $CDCl_3$. [c] Methanol-d_4.

Linderagalactone A (1): white, amorphous powder; $[\alpha]^{25}_D$ −63 (*c* 0.47, CH_3OH); UV (MeOH) λ_{max} (log ε) 220 (3.96) nm; CD (MeOH) 246 (Δε +1.07), 210 (Δε −0.82) nm; IR (KBr disk) ν_{max} 3417, 1597, 1385, 1350, 764 cm^{-1}; ^{1}H NMR (acetone-d_6, 400 MHz) and ^{13}C NMR (acetone-d_6, 100 MHz), see Tables 1 and 2; ESIMS (negative) *m/z* 333 $[M + Cl]^-$, 299 $[M - H + 2]^-$ Cl isotope peak, 297 $[M - H]^-$, 279 $[M - H - H_2O]^-$, 261 $[M - H - 2H_2O]^-$; HRESIMS (negative) *m/z* 297.0902 $[M - H]^-$ (calcd for $C_{15}H_{18}O_4Cl$, 297.0899).

Linderagalactone B (2): white, amorphous powder; $[\alpha]^{25}_D$ −67 (*c* 0.32, CH_3OH); UV (MeOH) λ_{max} (log ε) 219 (3.55), 233 (3.53) nm; CD (MeOH) 246 (Δε +1.61), 205 (Δε −1.10) nm; IR (KBr disk) ν_{max} 3420, 1594, 1382, 1351, 1234, 1082, 765 cm^{-1}; ^{1}H NMR (acetone-d_6, 400 MHz) and ^{13}C NMR (acetone-d_6, 100 MHz), see Table 1; ESIMS (positive) *m/z* 281 $[M + H]^+$; ESIMS (negative) *m/z* 279 $[M - H]^-$; HRESIMS (positive) *m/z* 281.1388 $[M + H]^+$ (calcd for $C_{15}H_{21}O_5$, 281.1384).

Linderagalactone C (3): white, amorphous powder; $[\alpha]^{25}_D$ +88 (*c* 0.99, MeOH); UV (MeOH) λ_{max} (log ε) 222 (3.74), 275 (3.80) nm; CD (MeOH) 245 (Δε +2.63), 204 (Δε −1.81) nm; IR (KBr disk) ν_{max} 3422, 1705, 1629, 1596, 1390, 1351, 1234, 1084, 781 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz,) and ^{13}C NMR ($CDCl_3$, 100 MHz), see Table 1; ESIMS (positive) *m/z* 285 $[M + Na]^+$, 263 $[M + H]^+$; HRESIMS (positive) *m/z* 285.1101 $[M + Na]^+$ (calcd for $C_{15}H_{18}O_4Na$, 285.1097).

Linderagalactone D (4): yellow, amorphous solid; $[\alpha]^{24}_D$ −141 (*c* 0.74, MeOH); UV (MeOH) λ_{max} (log ε) 220 (3.64), 303 (3.93) nm; CD (MeOH) 328 (Δε −2.60), 292 (Δε −2.48), 257 (Δε +3.03) nm; IR (KBr disk) ν_{max} 3421, 1592, 1384, 1350, 766 cm^{-1}; ^{1}H NMR (acetone-d_6, 400 MHz) and ^{13}C NMR (acetone-d_6, 100 MHz), see Tables 1 and 2; ESIMS (positive) *m/z* 285 $[M + Na]^+$, 263 $[M + H]^+$, 245 $[M + H - H_2O]^+$, 227 $[M + H - 2H_2O]^+$; HRESIMS (positive) *m/z* 285.1107 $[M + Na]^+$ (calcd for $C_{15}H_{18}O_4Na$, 285.1097).

Linderagalactone E (5): white, amorphous powder; $[\alpha]^{24}_D$ +25 (*c* 1.9, MeOH); UV (MeOH) λ_{max} (log ε) 225 (3.82), 312 (2.77) nm; IR (KBr disk) ν_{max} 3421, 1594, 1384, 1350, 766 cm^{-1}; ^{1}H NMR (methanol-d_4, 400 MHz) and ^{13}C NMR (methanol-d_4, 100 MHz), see Tables 1 and 2; ESIMS (positive) *m/z* 303 $[M + Na]^+$; HRESIMS (positive) *m/z* 303.1219 $[M + Na]^+$ (calcd for $C_{15}H_{20}O_5Na$, 303.1203).

Hepatoprotective Activity Assay. Human hepatoma HepG2 (ATCC) cells were cultured according to the method described by Kinjo et al.[24] Following cultivation of the HepG2 cells, a DMSO solution containing the test compounds at various concentrations (6.25, 12.5, 25, 50, 100, and 200 μM) was added to the wells and incubated at 37 °C for 1 h. The final concentration of DMSO in the solution in each well was 0.5%, and those samples containing DMSO only were used as a control. After the 1 h incubation, 1.9 mM H_2O_2 was added to each well and the cells were continuously incubated at 37 °C for 3 h. Subsequently, the solution in each cell was drawn out and the cells were rinsed with a PBS solution. Leuco Crystal Violet (150 μL, 0.2 μM in EtOH) was added to each well. Ten minutes later, the solution was removed and cells were rinsed with H_2O ($\times$3) immediately. SDS (0.5%, 180 μL) was added to each well, and the cells were shaken for 5 min at room temperature. Cell viability was determined by measuring the absorption using a 96-well microtiter plate reader at 550 nm. Results are expressed as percentage protection, i.e., the percentage increase in cell viability relative to the viability of cells treated with the H_2O_2 alone. The percentage protection is calculated as [(Sample − Control)/(Reference − Control)] × 100. Reference is the absorbance value of those wells not challenged with H_2O_2 and experimental material. Control is the absorbance value of wells challenged with H_2O_2 and not treated with the tested compunds. The viability of HepG2 cells treated with H_2O_2 (1.9 mM) for 3 h was 50% (IC_{50}), as was determined at the beginning of the assay by using the same protocol (concentrations used, 0.23, 0.46, 0.93, 1.85, and 3.7 mM). Data were statistically assessed by using a linear regression model to get the final EC_{50} values.

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Supporting Information Available: Spectroscopic data (1D and 2D NMR, ESIMS, and IR spectra) of **1**−**5** (Supporting Information I); computational calculation details and the original data for each conformer of **1**−**4** (Supporting Information II). This material is available free of charge via the Internet at http://pubs.acs.org.

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