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Terpenoids from *Chloranthus serratus* and Their Anti-inflammatory Activities

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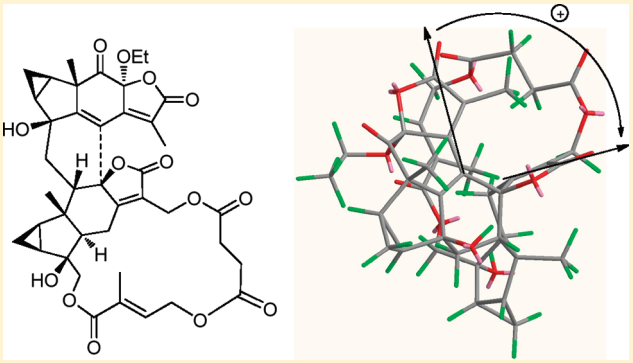
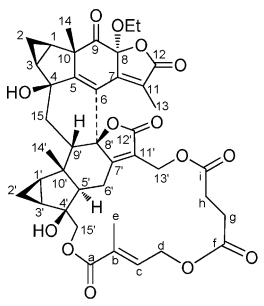
Supporting Information

ABSTRACT: Seven new terpenoids, including two sesquiterpene dimers (**1**, **2**), two norditerpenoids (**3**, **4**), and three sesquiterpenes (**5**–**7**), along with six known sesquiterpene dimers and four known sesquiterpenes were isolated from the whole plant of *Chloranthus serratus*. Their structures and relative configurations were elucidated on the basis of spectroscopic data analysis. The absolute configuration of **1** was determined by the CD exciton chirality method. These isolates were evaluated for their inhibitory effects on lipopolysaccharide-induced nitric oxide production in RAW264.7 cells. Compound **2** and two known compounds, shizukaols B and D, showed significant anti-inflammatory activities, with IC_{50} values of 0.22, 0.15, and 7.22 μM, respectively.



The genus *Chloranthus* (family Chloranthaceae) has 15 species, and nine of them are endemic to mainland China.[1] Plants in this genus have spurred considerable interest for their content of sesquiterpenes featuring novel cyclopropane or cyclobutane moieties and complex sesquiterpene dimers including macrocyclic structures, some of which exhibit antifungal, anti-HIV, and cytotoxic activities or inhibitory effects on the delayed rectifier (IK) K^+ current and on the expression of cell adhesion molecules and tyrosinase.[2−11] *Chloranthus serratus* (Thnub.) Roem. et Schult. (Chloranthaceae) is a perennial herbaceous plant that usually grows in wetlands. The whole plant has been used as a Chinese folk medicine for the treatment of bruises, bone fractures, rheumatoid arthritis, etc.[12,13] In the current study, two new sesquiterpene dimers (**1**, **2**), two new norditerpenoids (**3**, **4**), three new sesquiterpenes (**5**–**7**), six known sesquiterpene dimers, and four known sesquiterpenes were isolated from an extract of the whole plant of *C. serratus*. Their structures and relative configurations were elucidated by spectroscopic methods, mainly 1D and 2D NMR. The absolute configuration of **1** was determined by the CD exciton chirality method. The isolates were all evaluated for their inhibitory effects on lipopolysaccharide-induced nitric oxide production in RAW264.7 cells. Herein, we describe the isolation, structural elucidation, and anti-inflammatory evaluation of all these isolates.



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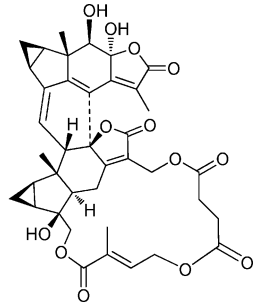

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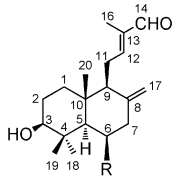

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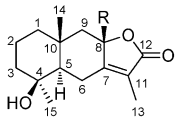

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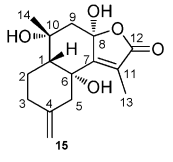

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RESULTS AND DISCUSSION

Compound **1** was assigned the molecular formula $C_{41}H_{44}O_{14}$, as deduced from its positive HRESIMS (found $[M + Na]^+$ m/z 783.2615, calcd 783.2629) and NMR data. Its ^1H NMR spectrum showed two high-field methylene signals at δ_H 0.63 (ddd, 9.2, 8.5, 5.2) and 0.95 (ddd, 8.6, 8.5, 6.9) characteristic of two cyclopropane moieties, four singlet (δ_H 0.96, 1.16, 1.76, 1.85) and one triplet (δ_H 1.08) methyl proton, and one trisubstituted olefinic proton signal at δ_H 6.64 (t, 5.2). The ^{13}C NMR (Table 1) exhibited 41 signals, which were categorized by a DEPT experiment as five methyl, ten methylene, seven methine, and nineteen quaternary carbon resonances. The HMBC correlations

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Table 1. ^{1}H (500 MHz) and ^{13}C NMR (125 MHz) Data of Compounds 1 and 2

	1[b]		2[c]	
position	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C
1	2.04, ddd (8.6, 6.9, 4.6)	26.2	1.97, ddd (8.5, 6.0, 4.0)	27.9
2α	0.95, ddd (8.6, 8.5, 6.9)	10.5	0.58, ddd (8.5, 6.3, 6.0)	10.7
2β	1.27,[a] m		1.09, m	
3	1.87, m	32.5	2.23,[a] m	23.3
4		77.9		137.4
5		162.3		155.5
6		122.6		114.7
7		150.6		153.8
8		98.7		104.5
9		201.3	3.77, d (9.0)	75.7
10		57.7		48.8
11		129.6		122.2
12		172.0		171.9
13	1.76, s	12.1	1.57, s	9.9
14	1.16, s	24.0	0.74, s	15.5
15α	1.89, m	41.2	6.27, d (4.5)	120.8
15β	2.73, dd (16.1, 6.7)			
1′	1.71, ddd (9.2, 8.5, 4.6)	27.9	1.88, ddd (9.0, 8.0, 4.0)	24.8
2′α	0.63, ddd (9.2, 8.5, 5.2)	11.0	0.54, ddd (9.0, 7.0, 5.0)	13.7
2′β	1.28,[a] m		1.03, ddd (5.5, 5.0, 3.0)	
3′	1.50, ddd (9.2, 7.5, 3.5)	30.2	1.35, ddd (9.0, 7.5, 3.5)	28.7
4′		77.6		75.4
5′	2.53, dd (12.0, 7.5)	56.5	2.21,[a] m	58.6
6′α	2.49, dd (17.8, 7.5)	25.4	2.62,[a] m	22.6
6′β	3.10, dd (17.8, 12.0)		2.72, dd (17.0, 12.0)	
7′		175.8		175.3
8′		87.8		85.5
9′	2.71, s	52.8	2.57, s	55.7
10′		48.7		47.6
11′		125.5		121.3
12′		173.6		171.5
13′α	4.57, d (12.3)	55.0	4.46,[a] m	53.8
13′β	5.27, d (12.3)		5.25, d (11.5)	
14′	0.96, s	24.6	0.86, s	24.7
15′α	3.93, d (11.5)	75.2	3.64, d (12.0)	72.5
15′β	4.54, d (11.5)		4.88, d (12.0)	
a		168.5		166.4
b		130.6		127.8
c	6.64, t (5.2)	137.9	6.48, t (6.0)	136.2
d	4.83,[a] m	62.4	4.94, dd (15.0, 6.0)	61.0
	4.65, dd (15.0, 5.2)		4.50,[a] m	
e	1.85, s	12.8	1.76, s	12.5
f		173.9		171.6
g	2.64,[a] m	29.9[a]	2.57,[a] m	28.6
	2.58,[a] m		2.56,[a] m	
h	2.68,[a] m	29.9[a]	2.60,[a] m	28.4
	2.54,[a] m		2.47,[a] m	
i		173.5		171.2
$-OCH_2-$	3.99, dq (12.1, 7.2)	64.0		
	3.71, dq (12.1, 7.2)			
$-CH_3$	1.08, t (7.2)	16.6		
8-OH			7.39, s	

[a]Overlapped with other signals. [b]Measured in MeOH-d_4. [c]Measured in DMSO-d_6.

of Me-13 with C-7, C-11, and C-12 and of H-13′ with C-11′ and C-12′ suggested the existence of two α,β-unsaturated γ-lactones in **1**. Key HMBC correlations and other NMR spectroscopic features suggested that **1** was a lindenane-type sesquiterpenoid dimer. Its NMR data resembled those of spicachlorantin A,[14] except for the downfield shifted C-8 signal ($\Delta\delta$ +4.7) and the presence of an ethoxy group [δ_H 1.08 (t, 7.2), 3.71 (dq, 12.1, 7.2), 3.99 (dq, 12.1, 7.2)], indicating ethoxylation at C-8. This was confirmed by the HMBC correlation from one of the oxymethylene protons (δ_H 3.99) to C-8 (δ_C 98.7).

The relative configuration of **1** was established by a NOESY experiment, in which correlations of Me-14/H-2β, H-2α/H-1, H-1α/H-3, H-2α/H-3, Me-14′/H-2′β, H-1′/H-2′α, H-1′/H-3′, H-2′α/H-3′, H-9′/Me-14′, H-15′α/H-5′, and H-1′/H-15′ indicated that the two cyclopropane rings and H-9′ were β-oriented and that H-5′ was α-oriented. Hence, the structure of **1** was established as shown.

The absolute configuration of compound **1** was established by applying the exciton chirality method.[15] The CD spectrum of **1** exhibited positive chirality resulting from the exciton coupling between the two different chromophores of the long conjugated α,β-unsaturated γ-lactone (C-5, C-6, C-7, C-11, C-12) and the α,β-unsaturated γ-lactone (C-7′, C-11′, and C-12′). The positive chirality indicated that the transition dipole moments of the two chromophores are in a clockwise-oriented manner (Figure 1) and, hence, established the configuration of C-8′ as *R*, consistent with that of spicachlorantin A.[1] Thus, the absolute configuration of **1** was assigned as depicted.

Compound **2** was obtained as a yellowish powder, and its molecular formula was determined to be $C_{39}H_{40}O_{13}$ (HRESIMS). The ^{1}H NMR spectrum of **2** (Table 1) showed two high-field methylene signals at δ_H 0.54 (ddd, 9.0, 7.0, 5.0) and 0.58 (ddd, 8.5, 6.3, 6.0), four singlet methyl protons at δ_H 0.74, 0.86, 1.57, and 1.76, and two trisubstituted olefinic proton signals at δ_H 6.27 (d, 4.5) and 6.48 (t, 6.0). The 1D and 2D NMR spectra indicated that **2** was a lindenane sesquiterpenoid dimer with an 18-membered triester ring, structurally similar to chloramultiol F.[3] Comparison of its NMR data with those of chloramultiol F revealed a difference in that the methoxy group at C-8 in chloramultiol F was absent and, instead, an OH was present at δ_H 7.39 (1H, s) in **2**. This was supported by HMBC correlations of this OH (8-OH) with C-7, C-8, and C-9. The relative configuration of **2** was established by a ROESY experiment in which correlations of Me-14′/H-9′ and 8-OH/H-9 revealed that H-9′ and Me-14′, and 8-OH and H-9 were cofacial. Consequently, 8-OH and H-9 were assigned to be α-oriented, and H-9′ was assigned as β-oriented. The two cyclopropane rings were established as β-oriented and H-5′ was established as α-oriented from the ROESY cross-peaks of H-1/H-3, H-1/H-2α, H-2α/H-3, H-2β/Me-14, H-1′/H-3′, H-1′/H-2′α, H-2′β/Me-14′, H-2′α/H-3′ and Me-14′/H-6′β, H-5′/H-6′α. The structure of **2** was thus established as shown.

Compound **3** had the molecular formula $C_{19}H_{30}O_2$ as established by HRESIMS. Signals of four singlet methyl protons [δ_H 0.77, 0.81, 1.02, and 1.76], one oxygenated methine proton [δ_H 3.29 (dd, 8.0, 4.6)], one trisubstituted olefinic proton [δ_H 6.42 (t, 6.2)], and one formyl proton [δ_H 9.34 (s)] were observed in the ^{1}H NMR spectrum. Two protons at δ_H 4.40 (br s) and 4.86 (br s) correlated with an olefinic methylene carbon at δ_C 108.6 in the HSQC spectrum indicated an exocyclic

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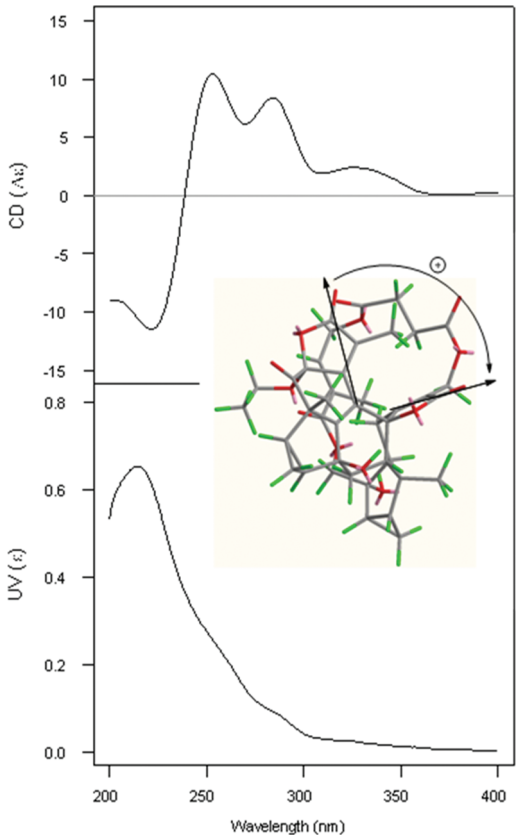

Figure 1. CD and UV spectra of compound **1** in MeOH. Stereoview of **1**: arrows denote the electric transition dipole of the chromophores.

olefin, which was determined to be at C-8 by the long-range HMBC correlations from the two protons to C-7 and C-9. The HMBC correlations from the formyl proton to C-13, C-14, and C-16 and from the trisubstituted olefinic proton to C-9, C-11, C-14, and C-16 suggested the presence of an isopentene group with formylation at C-14, and the HMBC cross-peak of H-9 [δ_H 1.87 (dd, 10.9, 2.5)] with C-12 further assigned it to be at C-9. The above analysis and additional 2D NMR evidence established that **3** was a labdadiene-type norditerpenoid. HMBC cross-peaks from the two *gem*-dimethyl protons at δ_H 0.81 and 1.02 (each 3H, s) to the oxymethine carbon resonance (δ_C 78.7) suggested the presence of an OH at C-3. It was determined to be β-oriented on the basis of the multiplicity of H-3 [δ_H 3.29 (dd, 8.0, 4.6)] and the observed NOESY correlations from H-18 to H-3, from H-5 to H-3, and from H-1α to H-3. Other key NOESY cross-peaks of Me-20/Me-19, Me-19/H-1β, Me-18/H-5, H-1α/H-9, and H-1α/H-3 revealed that H-3, H-5, and H-9 were α-orientated. Therefore, the structure of **3** was identified as 3β-hydroxy-15-nor-14-oxo-8(17),12-labdadien-14-al.

Compound **4** had the molecular formula of $C_{19}H_{30}O_3$. Its ^{13}C NMR spectrum also showed 19 signals, generally in agreement with those of **3** excepting the chemical shifts of C-5 ($\Delta\delta$ +1.6), C-6 ($\Delta\delta$ +45.1), C-7 ($\Delta\delta$ +9.5), and C-8 ($\Delta\delta$ −4.4) and suggesting hydroxylation at C-6, which was supported by HMBC correlations from the oxygenated methine proton at δ_H 4.43 (H-6) to C-5 and C-8. The relative configuration of 6-OH was established as β-orientated on the basis of NOESY correlations from Me-18 to H-5 and from H-5 to H-6. Configurations at other positions were elucidated in a way similar to that in **3** and were found to be the same as those of **3**. Therefore, the structure of **4** was proposed as 3β,6β-dihydroxy-15-nor-14-oxo-8(17),12-labdadien-14-al.

Compound **5** ($C_{15}H_{22}O_3$) exhibited three singlet methyl protons (δ_H 1.12, 1.13, and 1.71) and one OH proton [δ_H 4.06 (s)] in the ^1H NMR spectrum. The ^{13}C NMR spectrum of **5** showed three methyl, five methylene, two methine, and five quaternary carbon signals. HMBC correlations from Me-13 to C-7, C-11, and C-12, from Me-14 to C-1, C-9, and C-10, and from Me-15 to C-3, C-4, and C-5 indicated that it was a 7(11)-eudesmen-12,8-olide. The NMR data (in MeOH-d_4) of **5** and data of the known compound 4α-hydroxy-5α,8β(H)-eudesm-7(11)-en-8,12-olide[16] were similar except for the severely downfield shifted signal of Me-15, which might be due to the γ-gauche effect between Me-15 and Me-14 in 4α-hydroxy-5α,8β(H)-eudesm-7(11)-en-8,12-olide, suggesting a change in the relative configuration of Me-15 in **5**. This assertion was confirmed by the NOESY experiment in which correlations of Me-14/H-1β, Me-14/H-6β, Me-14/4-OH, Me-14/H-8, Me-15/H-6α, and H-5/H-6α revealed that H-8 and 4-OH were β-oriented, and thus H-5 and Me-15 were α-oriented. On the basis of the above data, **5** was elucidated as 4β-hydroxy-5α,8β(H)-eudesm-7(11)-en-8,12-olide.

Compound **6**, a white powder, had the molecular formula $C_{15}H_{22}O_4$. In the ^1H NMR spectrum of **6**, three singlet methyl protons at δ_H 1.14, 1.22, and 1.71 and two OH protons at δ_H 4.02 (s) and 7.05 (s) were evident. The NMR data of **6** were similar to those of **5**, except that H-8 was absent. Instead, an OH proton was present at δ_H 7.05 (1H, s), suggesting that an OH was attached to C-8. The relative configurations were established by the NOESY correlation of Me-14 with H-6β, H-6α with Me-15, and Me-15 with H-5α and H-9α, which inferred that 4-OH and Me-14 were cofacial and β-oriented and that Me-15 was α-oriented. Hence, compound **6** was determined to be 4β,8β-dihydroxy-5α(H)-eudesm-7(11)-en-8,12-olide.

The ^1H NMR spectrum of **7** ($C_{15}H_{20}O_5$) displayed two singlet methyl signals at δ_H 1.09 and 1.90, characteristic exocyclic methylene protons at δ_H 4.65 (s) and 4.75 (s), and three OH signals at δ_H 4.72 (s), 5.06 (s), and 7.19 (s). The ^{13}C NMR spectrum of **7** showed signals of two methyl, five methylene, one methine, and seven quaternary carbons. In its HMBC spectrum, cross-peaks from H-5 to C-1, C-3, C-4, and C-6, from H-9 to C-1, C-7, C-8, and C-10, and from Me-14 to C-9 and C-10 indicated that **7** possessed a cadinane sesquiterpene skeleton incorporating a 7(11)-en-12,8-olide structural moiety. HMBC correlations from the two protons of the exocyclic olefin to C-3, C-4, and C-5 revealed that this group was attached to C-4. The three OH groups were assigned to C-6, C-8, and C-10 by the HMBC cross-peaks from 6-OH to C-5 and C-6, from 8-OH to C-8 and C-9, and from 10-OH to C-10 and C-14. The relative configuration of **7** was elucidated by NOESY experiment. NOESY correlations of H-1β/H-2β, H-2β/Me-14, H-2α/H-3α, H-9α/8-OH, 8-OH/6-OH, H-3α/10-OH, and H-9α/10-OH indicated that 6-OH, 8-OH, and 10-OH were α-oriented and that Me-14 was β-oriented. Accordingly, the structure of **7** was established as shown.

Six known sesquiterpene dimers were identified as spicachlorantin A,[14] spicachlorantin C,[17] shizukaols B and D,[18] chloramultilide A,[2] and henriol A,[6] and four known sesquiterpenes were identified as atractylenolid III,[19] lasianthuslactone A,[20] 4α-hydroxy-5α,8β(H)-eudesm-7(11)-en-8,12-olide, and 4α,8β-dihydroxy-5α(H)-eudesm-7(11)-en-8,12-olide[16] by comparison of their spectroscopic data with those reported.

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Germacrane-type sesquiterpenes are widely distributed in many plants and were considered to be key precursors for the biosynthesis of eudesmane-type and cadinane-type sesquiterpenes. Cycloaddition between C-5 and C-10 of germacrane-type sesquiterpenes affords eudesmane-type sesquiterpenoids, e.g., **5** and **6**; that between C-1 and C-6 affords cadinane-type sesquiterpenes, e.g., **7**.

All isolates were evaluated for their inhibitory effects on the release of NO from macrophages using lipopolysaccharide (LPS)-induced RAW264.7 cells as a model system. Among them, compound **2**, shizukaol B, and shizukaol D showed marked activities, with IC_{50} values of 0.22, 0.15, and 7.22 μM, respectively, as compared with that of the reference compound hexadecadrol at 0.47 μM. The other compounds, all with IC_{50} values over 10 μM, were regarded as inactive.

EXPERIMENTAL SECTION

General Experimental Procedures. Melting points were measured using an X-4 digital display micromelting apparatus and are uncorrected. Optical rotations were measured with a Jasco P-1020 polarimeter. CD spectra were obtained on a Jasco 810 spectropolarimeter. UV spectra were obtained on a Shimadzu UV-2450 spectropolarimeter. IR spectra were recorded on a Perkin-Elmer FT-IR spectrometer using KBr discs. NMR spectra were obtained on Bruker ACF-500 and JEOL ECA-500 NMR instruments, with TMS as internal standard. High-resolution mass spectra were recorded on a Shimadzu LCMS-IT-TOF, Mariner ESI-TOF, and Agilent UPLC-Q-TOF (6520B), respectively. Silica gel (Merck Kieselgel 60), Sephadex LH-20 (Pharmacia), MCI gel (75−150 μm, Mitsubishi), and RP-C_{18} (40−63 μm, Fuji) were used for column chromatography (CC). Preparative HPLC was carried out using a Shimadzu SCL-10A Series instrument with a Capcell-pak RP-C_{18} column (5 μm, 10 × 250 mm) and a SPD 6A variable-wavelength detector.

Plant Material. Whole plants of *C. serratus* were collected in May 2010 from Tiantang village, Anhui Province, People's Republic of China, and identified by Prof. Gan Yao of the Institute of Botany, Jiangsu Province, and the Chinese Academy of Sciences. A voucher specimen (CS-2010005) has been deposited in the Department of Natural Medicinal Chemistry, China Pharmaceutical University.

Extraction and Isolation. The whole plants of *C. serratus* (1.8 kg) were extracted with 95% EtOH (4 × 6 L) for 3 h. The filtrate was evaporated under reduced pressure to give a residue (163 g), which was suspended in water and partitioned with EtOAc. Solvent was removed to afford the EtOAc fraction (101 g). The EtOAc extract was chromatographed over a silica gel column, eluted with petroleum ether−EtOAc in a gradient (1:0 to 0:1), to afford 20 fractions (1−20), monitored by TLC. Fraction 8 (2.3 g) was chromatographed on columns of silica gel, eluted with petroleum ether−EtOAc (2:1), then Sephadex LH-20 with MeOH, respectively, to afford atractylenolid III (4.3 mg). Fraction 10 (1.4 g) was chromatographed on silica gel, eluted successively with petroleum ether−EtOAc (2:1 to 1:1), to give subfractions 10a−10c. Fraction 10a was subjected to reversed-phase C_{18} silica gel CC, eluted successively with MeOH−H_2O (6:4 to 7:3), to give subfractions 10a1 and 10a2. Fraction 10a2 was separated by preparative HPLC using MeOH−H_2O (70:30, 5 mL/min) to give **3** (5.8 mg). Fraction 11 (1.5 g) was chromatographed on silica gel eluted with petroleum ether−EtOAc (2:1) to give subfractions 11a−11c. Fraction 11b was separated by preparative HPLC using MeOH−H_2O (50:50, 5 mL/min) to give lasianthuslactone A (10.9 mg). Fraction 12 (2.0 g) was chromatographed on MCI gel, eluted successively with MeOH−H_2O (1:1 to 7:3), to give subfractions 12a−12c. Fraction 12b was separated on Sephadex LH-20 with MeOH to afford **5** (7.4 mg). Fraction 13 (1.7 g) was chromatographed on MCI gel, eluted successively with MeOH−H_2O (1:1 to 7:3), to give subfractions 13a−13d. Fraction 13a was separated on Sephadex LH-20 with MeOH to afford **6** (4.8 mg). Fraction 13c was subjected to reversed-phase C_{18} silica gel, eluted with MeOH−H_2O (1:1), to give subfractions 13c1 and 13c2. Fraction 13c2 was separated by preparative HPLC using MeOH−H_2O (50:50) to give 4α-hydroxy-5α,8β(H)-eudesm-7(11)-en-8,12-olide (1.2 mg) and **4** (2.3 mg). Fraction 14 (2.1 g) was chromatographed on MCI gel, eluted successively with MeOH−H_2O (3:7 to 5:5), to give subfractions 14a−14d. Fraction 14c was separated by preparative HPLC using MeOH−H_2O (40:60) to give 4α,8β-dihydroxy-5α(H)-eudesm-7(11)-en-8,12-olide (4.7 mg) and **7** (1.5 mg). Fraction 15 (1.2 g) was subjected to MCI gel, eluted successively with MeOH−H_2O (3:7 to 5:5), to yield subfractions 15a and 15b. Fraction 15b was subjected to reversed-phase C_{18} silica gel, eluted successively with MeOH−H_2O (3:7 to 1:1), to give subfractions 15b1−15b4. Fraction 15b3 was separated by preparative HPLC using MeOH−H_2O (45:55) to give spicachlorantin C (1.7 mg). Fraction 16 (1.4 g) was subjected to MCI gel, eluted successively with MeOH−H_2O (3:7 to 5:5), to give subfractions 16a and 16b. Fraction 16a was subjected to reversed-phase C_{18} silica gel eluted successively with MeOH−H_2O (3:7 to 1:1) to give subfractions 16a1−16a4. Fraction 16a3 was separated by preparative HPLC using MeOH−H_2O (45:55) to give **1** (2.4 mg) and shizukaols B (2.3 mg) and D (1.9 mg). Fraction 17 (1.9 g) was subjected to MCI gel, eluted successively with MeOH−H_2O (3:7 to 5:5), to give subfractions 17a−17c. Fraction 17b was subjected to C_{18} silica gel, eluted successively with MeOH−H_2O (3:7 to 1:1), to give subfractions 17a1 and 17a2. Fraction 17b1 was separated by Sephadex LH-20 with MeOH to afford spicachlorantin A (9.6 mg). Fraction 17c was subjected to reversed-phase C_{18} silica gel, eluted successively with MeOH−H_2O (3:7 to 1:1), to give subfractions 17c1−17c3. Fraction 17c3 was separated by Sephadex LH-20 with MeOH to afford **2** (8.7 mg). Fraction 18 (1.5 g) was subjected to MCI gel, eluted successively with MeOH−H_2O (3:7 to 4:6), to give subfractions 18a−18c. Fraction 18a was subjected to CC on C_{18} silica gel, eluted successively with MeOH−H_2O (3:7 to 1:1), to give subfractions 18a1−18a4. Fraction 18a1 was separated by preparative HPLC using MeOH−H_2O (35:65) to give chloramultilide A (1.7 mg). Fraction 18a3 was separated using Sephadex LH-20 with MeOH to afford henriol A (5.2 mg).

Compound ***1:*** colorless powder; mp 178.5−179.5 °C; $[\alpha]^{25}_D$ +65.3 (*c* 0.15, MeOH); UV (MeOH) λ_{max} (log ε) 214 (4.40) nm; CD (MeOH, *c* 1.50 × 10^{-3}) λ_{max} ($\Delta\varepsilon$) 284 (+12.90), 253 (+16.06), 221 (−17.69) nm; IR (KBr) ν_{max} 3435, 2928, 1738, 1637, 1255, 1034 cm^{-1}; ^{13}C and ^1H NMR data, see Table 1; HRESIMS *m*/*z* 783.2615 (calcd for $C_{41}H_{44}O_{14}Na$, 783.2629).

Compound ***2:*** yellowish powder; decomposed at 281.5 °C; $[\alpha]^{25}_D$ −22.9 (*c* 0.34, MeOH); UV (MeOH) λ_{max} (log ε) 237 (3.52) nm; IR (KBr) ν_{max} 3434, 1752, 1712, 1658, 1384, 1249, 1155, 1009 cm^{-1}; ^{13}C and ^1H NMR data, see Table 1; HRESIMS *m*/*z* 739.2373 (calcd for $C_{39}H_{40}O_{13}Na$, 739.2361).

Compound ***3:*** colorless oil; $[\alpha]^{25}_D$ −17.8 (*c* 0.27, MeOH); UV (MeOH) λ_{max} (log ε) 229 (3.78) nm; IR (KBr) ν_{max} 3448, 2942, 1595, 1385, 1091 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 1.80 (m, H-1α), 1.28 (t, *J* = 13.2 Hz, H-1β), 1.77 (m, H-2α), 1.63 (m, H-2β), 3.29 (dd, *J* = 8.0, 4.6 Hz, H-3), 1.15 (dd, *J* = 10.0, 4.0 Hz, H-5), 1.75 (m, H-6α), 1.43 (ddd, *J* = 8.6, 8.0, 5.2 Hz, H-6β), 2.03 (td, *J* = 13.2, 5.2 Hz, H-7α), 2.42 (m, H-7β), 1.87 (dd, *J* = 10.9, 2.5 Hz, H-9), 2.38 (dd, *J* = 16.6, 13.2, H-11α), 2.52 (dd, *J* = 16.6, 13.2 Hz, H-11β), 6.42 (t, *J* = 6.2 Hz, H-12), 9.34 (s, H-14), 1.76 (s, 16-CH_3), 4.40 (br s, H-17), 4.86 (br s, H-17), 1.02 (s, 18-CH_3), 0.81 (s, 19-CH_3), 0.77 (s, 20-CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 37.3 (C-1), 27.9 (C-2), 78.7 (C-3), 39.2 (C-4), 54.6 (C-5), 23.8 (C-6), 37.8 (C-7), 147.7 (C-8), 56.2 (C-9), 39.3 (C-10), 24.5 (C-11), 156.0 (C-12), 139.2 (C-13), 195.3 (C-14), 9.5 (C-16), 108.6 (C-17), 28.4 (C-18), 15.5 (C-19), 14.5 (C-20); HRESIMS *m*/*z* 289.2168 (calcd for $C_{19}H_{29}O_2$, 289.2173).

Compound ***4:*** colorless powder; mp 193.5−194.5 °C; $[\alpha]^{25}_D$ −21.1 (*c* 0.38, MeOH); UV (MeOH) λ_{max} (log ε) 226 (3.60) nm; IR (KBr) ν_{max} 3423, 2936, 1667, 1633, 1357, 1219, 1092, 1031 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 1.80 (t, *J* = 4.0 Hz, H-1α), 1.28 (td, *J* = 13.2, 4.0 Hz, H-1β), 1.68 (m, H-2α), 1.74 (m, H-2β), 3.21 (t, *J* = 3.5 Hz, H-3), 1.06 (m, H-5), 4.43 (br s, H-6α), 2.38 (m, H-7), 1.87 1.93 (dd, *J* = 10.3, 2.3 Hz, H-9), 2.48 (ddd, *J* = 8.9, 6.9, 5.0 Hz, H-11α), 2.54 (m, H-11β), 6.43 (t, *J* = 6.3 Hz, H-12), 9.36 (s, H-14), 1.75 (s, 16-CH_3), 4.78 (br s, H-17), 5.04 (br s, H-17), 1.13 (s, 18-CH_3), 1.20 (s, 19-CH_3), 1.08 (s, 20-CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 39.6 (C-1), 28.1 (C-2), 78.8 (C-3), 39.9 (C-4), 56.2 (C-5), 68.9 (C-6), 47.3 (C-7),

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143.3 (C-8), 56.8 (C-9), 40.3 (C-10), 24.3 (C-11), 155.0 (C-12), 139.3 (C-13), 195.1 (C-14), 9.4 (C-16), 111.8 (C-17), 27.9 (C-18), 16.6 (C-19), 17.1 (C-20); HRESIMS *m/z* 305.2115 (calcd for $C_{19}H_{29}O_3$, 305.2122).

Compound **5**: white powder; mp 162.5−164.0 °C; $[\alpha]^{25}_D$ +20.0 (*c* 0.57, MeOH); UV (MeOH) λ_{max} (log ε) 210 (3.55) nm; IR (KBr) ν_{max} 3487, 2925, 1734, 1676, 1457, 1378, 1104, 1043, 1013 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) δ 1.08 (m, H-1α), 1.42 (m, H-1β), 1.30 (m, H-2α), 1.78 (m, H-2β), 1.24 (m, H-3α), 1.59 (m, H-3β), 0.99 (m, H-5), 2.33 (t, *J* = 13.2 Hz, H-6α), 2.78 (dd, *J* = 3.5, 13.2 Hz, H-6β), 4.95 (m, H-8), 0.87 (t, *J* = 12.1 Hz, H-9α), 1.96 (dd, *J* = 12.1, 6.3 Hz, H-9β), 1.71 (s, 13-CH_3), 1.13 (s, 14-CH_3), 1.12 (s, 15-CH_3), 4.06 (s, 4-OH); ^{13}C NMR (125 MHz, DMSO-d_6) δ 41.0 (C-1), 17.9 (C-2), 41.1 (C-3), 70.3 (C-4), 52.3 (C-5), 21.8 (C-6), 165.4 (C-7), 78.4 (C-8), 50.1 (C-9), 35.2 (C-10), 118.6 (C-11), 174.5 (C-12), 8.4 (C-13), 19.3 (C-14), 30.3 (C-15); HRESIMS *m/z* 273.1467 (calcd for $C_{15}H_{22}O_3Na$, 273.1461).

Compound **6**: white powder; mp 176.0−177.0 °C; $[\alpha]^{25}_D$ +61.6 (*c* 0.64, MeOH); UV (MeOH) λ_{max} (log ε) 227 (3.32) nm; IR (KBr) ν_{max} 3373, 2943, 1375, 1688, 1408, 1321, 1188, 1136, 1112 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) δ 1.03 (m, H-1α), 1.38 (m, H-1β), 1.79 (m, H-2), 1.28 (m, H-3α), 1.57 (m, H-3β), 0.98 (m, H-5), 2.29 (m, H-6α), 2.67 (dd, *J* = 2.3, 13.2 Hz, H-6β), 1.25 (m, H-9α), 1.95 (m, H-9β), 1.71 (s, 13-CH_3), 1.22 (s, 14-CH_3), 1.14 (s, 15-CH_3), 4.02 (s, 4-OH), 7.05 (s, 8-OH); ^{13}C NMR (125 MHz, DMSO-d_6) δ 40.6 (C-1), 17.5 (C-2), 41.2 (C-3), 70.0 (C-4), 54.2 (C-5), 22.0 (C-6), 162.9 (C-7), 104.0 (C-8), 53.6 (C-9), 34.7 (C-10), 119.8 (C-11), 171.8 (C-12), 7.8 (C-13), 19.3 (C-14), 30.2 (C-15); HRESIMS *m/z* 289.1412 (calcd for $C_{15}H_{22}O_4Na$, 289.1410).

Compound **7**: white powder; mp 198.5−200.5 °C; $[\alpha]^{25}_D$ −12.4 (*c* 0.34, MeOH); UV (MeOH) λ_{max} (log ε) 218 (3.58) nm; IR (KBr) ν_{max} 3339, 2939, 1726, 1385, 1225, 1086 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) δ 1.51 (dd, *J* = 12.1, 4.8 Hz, H-1), 1.72 (m, H-2α), 2.01 (m, H-2β), 2.04 (m, H-3α), 2.33 (m, H-3β), 2.65 (m, H-5α), 2.53 (m, H-5β), 1.73 (m, H-9α), 2.41 (m, H-9β), 1.90 (s, 13-CH_3), 1.09 (s, 14-CH_3), 4.65 (s, H-15), 4.75 (s, H-15), 4.02 (s, 6-OH), 7.05 (s, 8-OH), (s, 10-OH); ^{13}C NMR (125 MHz, DMSO-d_6) δ 50.9 (C-1), 21.8 (C-2), 33.8 (C-3), 145.0 (C-4), 44.9 (C-5), 74.0 (C-6), 159.4 (C-7), 104.6 (C-8), 50.3 (C-9), 71.6 (C-10), 120.5 (C-11), 172.5 (C-12), 10.3 (C-13), 28.7 (C-14), 110.7 (C-15); HRESIMS *m/z* 303.1201 (calcd for $C_{15}H_{20}O_5Na$, 303. 1203).

Anti-inflammatory Bioassay. The protocol of the anti-inflammatory bioassays was provided in a previously published paper with hexadecadrol as the reference.[21]

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Tables S1 and S2 (^1H and ^{13}C NMR data of compounds **3**−**7**), Figures S1−S6 (selected 2D NMR correlations of compounds **1**−**5**, **7**), and ^1H NMR, ^{13}C NMR, and HRESIMS spectra of **1**−**7** are available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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