JOURNAL OF NATURAL PRODUCTS

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Lindenane Disesquiterpenoids with Anti-HIV-1 Activity from *Chloranthus japonicus*

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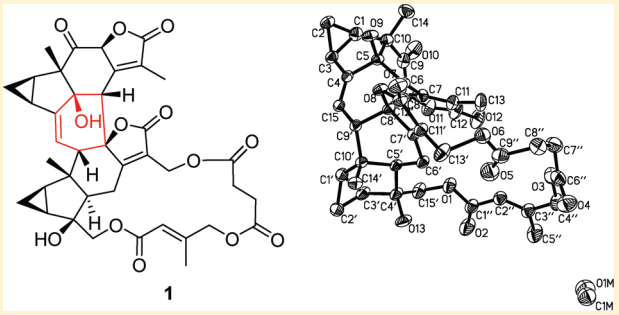
Supporting Information

ABSTRACT: Five new lindenane disesquiterpenoids, chlorajaponilides A–E (**1**–**5**), together with 11 known analogues were isolated from whole plants of *Chloranthus japonicus*. The structure and absolute configuration of **1** was confirmed by X-ray crystallography. Compounds **1** and **2** represent the first examples of lindenane disesquiterpenoids with a C-5 hydroxy group and a C-4–C-15 double bond. Compounds **8**, **9**, **11**, and **12** showed anti-HIV-1 replication activities in both wild-type HIV-1 and two NNRTIs-resistant strains. Shizukaol B (**8**) exhibited the best activity against HIV_{wt}, $HIV_{RT\text{-}K103N}$, and $HIV_{RT\text{-}K103N}$ with EC_{50} values of 0.22, 0.47, and 0.50 μM, respectively. Compounds **8**, **9**, **11**, and **12** had significant cytotoxicities against C8166 cells with CC_{50} values of 0.020, 0.089, 0.047, and 0.022, respectively, and exhibited inhibitory activities against HIV-1 with EC_{50} values of 0.0014, 0.016, 0.0043, and 0.0033 μM, respectively.

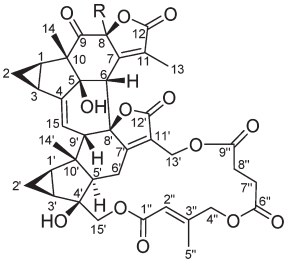


Chloranthus japonicus Sieb. (Chloranthaceae, "yin-xian-cao" in Chinese) is a perennial herb mainly distributed in the eastern region of Asia, such as mainland China, Korea, and Japan. It has long been applied for the treatment of traumatic injuries, rheumatic arthralgia, fractures, pulmonary tuberculosis, and neurasthenia in traditional Chinese medicine.[1] Previous chemical investigations resulted in the isolation of a variety of sesquiterpenoids including eudesmane-,[2,3,7−9] lindenane-,[2−6,9] germacrane-,[3−5] and acorane-,[3] as well as lindenane-type sesquiterpenoid dimers and trimers.[7,10−15] The eudesmane-type sesquiterpenoid CJ-01 showed significant inhibitory activity against chitin synthase 2 of *Saccharomyces cerevisiae*,[8] while the disesquiterpenoids shizukaol B (**8**), shizukaol F (**11**), and cycloshizukaol A (**13**) exhibited inhibition of the expression of cell adhesion molecules.[16] As part of our ongoing efforts to discover more new sesquiterpenoids and disesquiterpenoids from *Chloranthus* species,[17,18] the ethyl acetate-soluble extract of this plant was investigated, resulting in the isolation of five new lindenane disesquiterpenoids, named chlorajaponilides A–E (**1**–**5**), together with 11 known analogues, yinxiancaol (**6**),[7] shizukaols A–D (**7**–**10**),[10,11] shizukaol F (**11**),[12] shizukaol H (**12**),[12] cycloshizukaol A (**13**),[13] chloraholide B (**14**),[19] chloramultilide C (**15**),[20] and spicachlorantin B (**16**).[21] Compounds **1** and **2** represent the first examples of lindenane disesquiterpenoids with a C-5 hydroxy group and a C-4–-C-15 double bond. Compound **5** is a rare lindenane disesquiterpenoid containing a hydroperoxy group at C-4. Herein, we report the isolation and structure determination of the new compounds and their anti-HIV-1 activities.

RESULTS AND DISCUSSION

Chlorajaponilide A (**1**) was obtained as colorless prism-like crystals ($CHCl_3$–MeOH). The molecular formula $C_{39}H_{40}O_{13}$ was established by HR-ESIM displaying a quasi-molecular ion peak at m/z 739.2368 $[M + Na]^+$ (calcd 739.2366) and ^{13}C NMR data (Table 2), requiring 20 degrees of unsaturation. The IR absorptions revealed the presence of hydroxy (3477 cm^{-1}), carbonyl (1763 cm^{-1}), and olefinic (1664 cm^{-1}) functionalities. In accord with the molecular formula, 39 carbon resonances consisting of six carbonyl, four double-bond, four methyl, eight methylene (three oxygenated), eight methine (one oxygenated), and five quaternary carbons (three oxygenated) were resolved in the ^{13}C NMR and categorized by DEPT experiments (Table 2).

Received: January 28, 2011
Published: June 08, 2011



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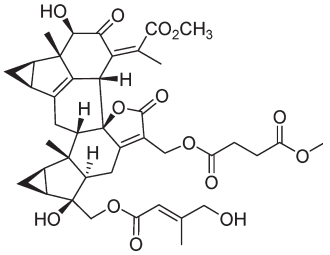
2 R = OH



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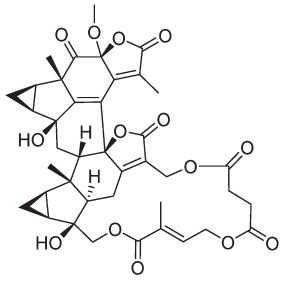

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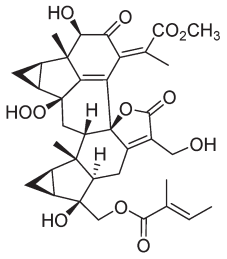

5

The ^{1}HNMR spectrum (Table 1) showed four methyl protons at δ_H 0.83 (s), 1.17 (s), 1.69 (s), and 2.07 (s) and two trisubstituted olefinic protons at δ_H 5.77 (d) and 6.15 (d). The ^{1}H−^{1}H COSY spectrum showed two sets of proton spin systems of a 1,2-disubstituted cyclopropane ring (δ_H 0.63, 1.34, 1.60, and 2.08; δ_H 0.68, 1.27, 1.60, and 1.80). The above data together with the two characteristic high-field methylene resonances at δ_H 0.63 (H-2β) and 0.68 (H-2′β) for two cyclopropane rings revealed that **1** should be a lindenane disesquiterpenoid similar to chloraholide B (**14**).[19] A major difference between the two molecules was the appearance of an additional trisubstituted double bond in compound **1**. The ^{1}H−^{1}H COSY correlation from H-15 (δ_H 6.15) to H-9′ (δ_H 2.49) and the HMBC correlations of H-15 (δ_H 6.15) with C-3 (δ_C 21.3), C-4 (δ_C 144.9), C-5 (δ_C 73.5), C-6 (δ_C 51.1), C-8′ (δ_C 90.0), C-9′ (δ_C 52.8), and C-10′ (δ_C 44.9) confirmed the trisubstituted double bond between C-4 (δ_C 144.9) and C-15 (δ_C 117.7) and the location of a hydroxy group at C-5 instead of C-4. A second major difference was the chemical shift of C-8. In comparison with the corresponding resonance (δ_C 94.0) in chloraholide B (**14**),[19] C-8 in compound **1** shifted upfield to δ_C 81.9, which was indicative of an oxymethine, not a hemiacetal carbon. C-8 was connected to C-12 via an oxygen atom to form a five-membered α,β-unsaturated lactone ring fused at C-7 and C-8 by the key HMBC correlations of H-8 (δ_H 5.45) with C-6 (δ_C 51.1) and C-12 (δ_C 171.7) and of the allylic methyl proton resonance (δ_H 1.69) with C-6 (δ_C 51.1), C-7 (δ_C 149.0), C-8 (δ_C 81.9), C-11 (δ_C 127.4), and C-12 (δ_C 171.7). The ^{1}H−^{1}H COSY and key HMBC correlations shown in Figure 1 confirmed the planar structure of **1**.

Table 1. ^{1}H NMR Data of Chlorajaponilides A−E (1−5) (δ in ppm, J in Hz)

position	1[a,b]	2[c,d]	3[b,c]	4[b,c]	5[a,b]
1	1.34 m	1.52 m	2.06 m	2.15 m	2.00 m
2α	1.60 m	2.04 m	0.99 m	1.28 m	1.23 m
2β	0.63 m	0.78 m	0.28 m	1.02 m	0.95 m
3	2.08 m	2.24 m	1.84 m	1.78 m	1.85 m
6	3.04 s	3.69 s	3.92 d		
8	5.45 s				
9			3.97 s		3.75 s
13	1.69 s	2.00 s	1.88 s	1.81 s	1.78 s
14	1.17 s	1.55 s	0.99 s	1.15 s	1.02 s
15α	6.15 d (4.0)	6.30 d (3.0)	2.78 m	2.67 m	3.07 dd (14.3, 7.1)
15β			2.60 m	1.79 m	1.60 m
1′	1.80 m	2.13 m	1.59 m	1.59 m	1.58 m
2′α	1.27 m	1.58 m	0.71 m	1.31 m	1.18 m
2′β	0.68 m	0.74 m	1.32 m	0.65 m	0.60 dt (8.9, 5.6)
3′	1.60 m	2.00 m	1.41 m	1.47 m	1.60 m
5′	1.51 dd (13.4, 5.6)	3.36 m	1.86 m	2.28 dd (12.4, 6.8)	1.60 m
6′α	2.92 dd (20.2, 13.5)	3.05 d (5.1)	2.71 m	3.01 dd (18.1, 12.5)	2.89 dd (17.3, 13.2)
6′β	2.44 m	3.02 d (5.1)	2.50 dd (18.5, 6.0)	2.41 dd (18.1, 6.6)	2.25 dd (17.3, 6.6)
9′	2.49 d (3.1)	2.54 d (3.2)	1.84 m	2.60 dd (10.2, 6.8)	2.59 dd (10.1, 7.3)
13′a	5.14 d (12.4)	5.37 d (12.3)	4.99 d (13.5)	5.38 d (12.0)	4.42 d (13.6)
13′b	4.54 d (12.4)	4.92 d (12.3)	4.78 d (13.5)	4.50 d (12.0)	4.36 d (13.6)
14′	0.83 s	1.02 s	0.86 s	0.95 s	0.98 s
15′a	4.67 d (12.0)	5.15 d (10.9)	4.30 d (12.0)	4.73 d (11.7)	4.15 d (11.4)
15′b	3.38 d (12.0)	4.41 d (10.9)	3.68 d (12.0)	3.80 d (11.7)	3.93 d (11.4)
2″	5.77 d (1.1)	6.23 s	5.97 d (1.5)		
3″				6.62 m	6.84 m
4″a	4.66 s (2H)	4.83 d (16.4)	4.16 s (2H)	4.67 d (6.2, 2H)	1.84 s
4″b		4.61 d (16.4)			
5″	2.07 s	2.08 s	2.12 s	1.89 s	1.85 s
7″a	2.68 m (2H)	2.90 m	2.66 m (2H)	2.71 m	
7″b		2.81 m		2.51 m	
8″a	2.43 m (2H)	2.64 m	2.75 m (2H)	2.67 m	
8″b		2.00 m		2.49 m	
8-OMe				3.46 s	
12-CO*Me*			3.72 s		3.78 s
6″-CO*Me*			3.66 s		

[a] Recorded at 400 MHz. [b] Measured in $CDCl_3$. [c] Recorded at 500 MHz. [d] Measured in pyridine-d_5.

dx.doi.org/10.1021/np200087d |J. Nat. Prod. 2011, 74, 1408–1413

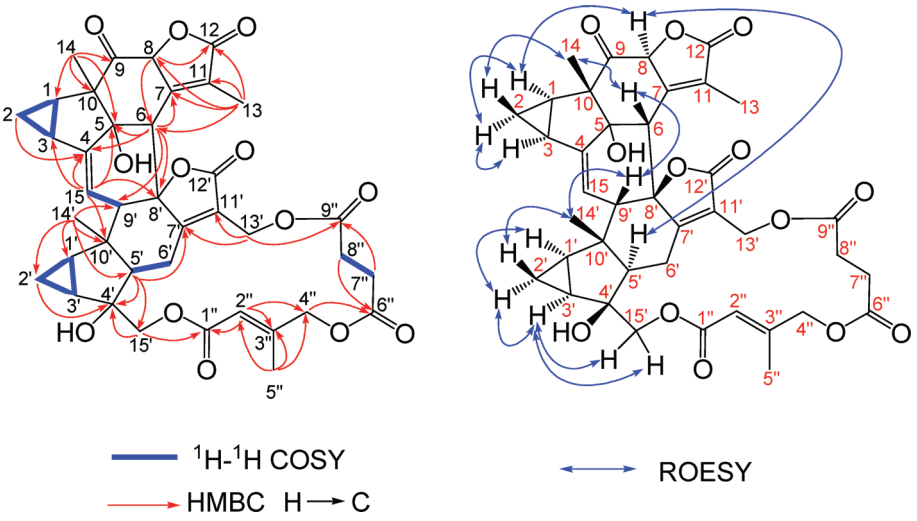

Figure 1. Selected 2D NMR correlations of chlorajaponilide A (**1**).

The relative configuration of **1** was assigned by the ROESY correlations shown in Figure 1. The ROESY correlations of H-1/H-3, H-1/H-2α, H-2α/H-3, H-1′/H-3′, H-1′/H-2′α, H-2′α/H-3′, H-1′/H-5′, and H-3′/H-5′ indicated they were cofacial and were arbitrarily assigned as α-oriented. As a consequence, the ROESY cross-peaks of H-2β/Me-14, H-6/Me-14, H-2′β/Me-14′, H-9′/Me-14′, and H-6/H-9′ revealed that they were β-oriented. H-8 was fixed as α-oriented, based on the ROESY correlations of H-8 with H-5′. The ROESY correlations of H_2-15′/H-3′ and H_2-15′/H-5′ suggested that OH-4′ was β-oriented. Recrystallization of **1** from $CHCl_3$−MeOH (1:5) afforded single crystals suitable for X-ray analysis. Consequently, we applied single-crystal X-ray diffraction with Cu Kα radiation to determine the final structure and absolute configuration as shown in Figure 2. On the basis of all the information above, the structure of **1** was elucidated as depicted.

Chlorajaponilide B (**2**) was obtained as a colorless, amorphous powder. Its molecular formula was determined as $C_{39}H_{40}O_{14}$ by positive-mode HR-ESIMS at 755.2309 $[M + Na]^+$ and ^{13}C NMR data (Table 2), which exceeds that of compound **1** by an oxygen atom. Comparison of the ^1H and ^{13}C NMR spectra indicated that **2** differed from **1** by the presence of a hemiacetal (δ_C 101.3) instead of an oxymethine (δ_C 81.9) moiety at C-8 in the latter. The HMBC correlations of the C-13 methyl protons with C-6, C-7, C-8, C-11, and C-12 and of H-6 with C-8 indicated the location of the hemiacetal group at C-8 (Figure 1). ROESY correlations suggested the same relative configuration as **1**. Consquently, the structure of **2** was established as shown.

Chlorajaponilide C (**3**), obtained as a yellow, amorphous powder, showed a molecular formula of $C_{41}H_{48}O_{14}$ by the positive-ion HR-ESIMS (m/z 787.2941 $[M + Na]^+$) and ^{13}C NMR data (Table 2). The NMR data of **3** (Tables 1 and 2) were similar to those of shizukaol O.[22] The only difference was the presence of one carbomethoxy resonance at δ_H 3.66 in the ^1H NMR spectrum and at δ_C 173.1 and 52.0 in the ^{13}C NMR spectrum. The correlation of the methoxy protons (δ_H 3.66) with the carboxylic carbon (δ_C 173.1) in the HMBC spectrum established the esterification position at C-6″.

Chlorajaponilide D (**4**) was obtained as a white, amorphous powder. The HR-ESIMS displayed a quasi-molecular ion peak at m/z 769.2459 $[M + Na]^+$ (calcd 769.2472), consistent with the molecular formula $C_{40}H_{42}O_{14}$. The ^1H and ^{13}C NMR data (Tables 1 and 2) revealed that this compound closely resembled spicachlorantin A[19] except for the presence of an OCH_3 group. The OCH_3 was inferred to be located at C-8 from the shift of C-8 ($\Delta\delta_C$ + 2.2) in **4** relative to spicachlorantin A, which was confirmed by the HMBC correlation of the methoxy protons at δ_H 3.46 with the dioxygenated quaternary carbon at δ_C 96.2 (C-8). Accordingly, the structure of **4** was determined as shown.

Chlorajaponilide E (**5**) had a molecular formula of $C_{36}H_{42}O_{12}$ based on HR-ESIMS (m/z 689.2577 $[M + Na]^+$) and ^{13}C NMR data (Table 2). The NMR data (Tables 1 and 2) demonstrated that **5** was similar to spicachlorantin E,[23] and the significant difference was the absence of an acetyl group in **5**. In the HMBC spectrum, the correlations of H_2-15′ (δ_H 3.93 and 4.15) with C-1″ (δ_C 168.1) unambiguously placed the angelic acid residue at C-15′. The ROESY experiment indicated that **5** had the same relative configuration as spicachlorantin E. Thus, chlorajaponilide E (**5**) was identified as 13′-de-*O*-acetylspicachlorantin E.

The disesquiterpenoids (**1**−**16**) except compound **5**, which was obtained in a limited amount, were tested for bioactivity against HIV-1 replication. The assay was performed by using a pseudotyping system with EFV (efavirenz) as a positive control. Compounds **8**, **9**, **11**, and **12** showed inhibitory effects on HIV-1 replication with an EC_{50} range 0.11−4.05 μM for wild-type HIV-1 and two non-nucleoside reverse transcriptase inhibitor resistant HIV-1 strains ($HIV_{RT\text{-}K103N}$ and $HIV_{RT\text{-}Y181C}$), as shown in Table 3. Among these compounds, shizukaol B (**8**) displayed the best activity against HIV_{wt}, $HIV_{RT\text{-}K103N}$, and $HIV_{RT\text{-}K103N}$ with EC_{50} values of 0.22, 0.47, and 0.50 μM, respectively. Since some lindenane disesquiterpenoids were reported having cytotoxicity[24,25] and the therapeutic index (TI) is an important measurement for the anti-HIV activity, compounds **8**, **9**, **11**, and **12** were tested for cytotoxicities against C8166 cells (CC_{50}), and anti-HIV-1 activities were evaluated by the inhibition assay for the cytopathic effects of HIV-1 (EC_{50}), using AZT as a positive control. The results are summarized in Table 4. Although the four compounds showed inhibitory activities against HIV-1 with EC_{50} values of 0.0014, 0.016, 0.0043, and 0.0033 μM, respectively, they exhibited significant cytotoxicity to C8166 cells and a low therapeutic index.

Table 2. ^{13}C NMR Data of Chlorajaponilides A−E (1−5) (δ in ppm)

position	1[a,b]	2[c,d]	3[b,c]	4[b,c]	5[a,b]
1	28.0, CH	30.0, CH	25.7, CH	24.7, CH	26.0, CH
2	11.1, CH_2	12.9, CH_2	15.8, CH_2	10.0, CH_2	8.2, CH_2
3	21.3, CH	23.1, CH	24.7, CH	30.5, CH	27.6, CH
4	144.9, C	143.7, C	142.2, C	77.2, C	90.4, C
5	73.5, C	74.6, C	131.7, C	161.5, C	158.6, C
6	51.1, CH	52.7, CH	40.8, CH	121.1, C	126.9, C
7	149.0, C	151.3, C	131.5, C	148.4, C	142.7, C
8	81.9, CH	101.3, C	199.8, C	96.2, C	198.8, C
9	201.9, C	205.5, C	80.1, CH	197.4, C	77.8, CH
10	61.1, C	60.6, C	51.1, C	56.5, C	50.1, C
11	127.4, C	133.1, C	147.3, C	128.5, C	129.0, C
12	171.7, C	171.8, C	171.0, C	170.3, C	170.2, C
13	10.2, CH_3	10.6, CH_3	20.4, CH_3	11.6, CH_3	21.1, CH_3
14	15.3, CH_3	17.9, CH_3	15.2, CH_3	23.3, CH_3	15.3, CH_3
15	117.7, CH	118.2, CH	25.4, CH_2	39.8, CH_2	36.6, CH_2
1′	25.4, CH	26.2, CH	25.3, CH	26.7, CH	27.2, CH
2′	10.9, CH_2	11.7, CH_2	11.6, CH_2	10.1, CH_2	10.3, CH_2
3′	26.7, CH	29.3, CH	27.8, CH	29.2, CH	29.1, CH
4′	76.5, C	77.1, C	77.2, C	77.4, C	77.5, C
5′	59.7, CH	56.8, CH	60.9, CH	55.2, CH	54.6, CH
6′	25.9, CH_2	26.2, CH_2	23.4, CH_2	23.9, CH_2	21.7, CH_2
7′	175.3, C	176.8 C	171.3, C	173.5, C	166.4, C
8′	90.0, C	91.3, C	93.3, C	85.7, C	87.5, C
9′	52.8, CH	54.1, CH	55.7, CH	51.7, CH	52.3, CH
10′	44.9, C	45.7, C	44.8, C	45.1, C	45.1, C
11′	124.2, C	124.3, C	123.4, C	123.0, C	128.4, C
12′	172.7, C	172.9, C	172.1, C	171.2, C	173.0, C
13′	54.7, CH_2	55.6, CH_2	55.7, CH_2	53.3, CH_2	54.8, CH_2
14′	25.0, CH_3	25.8, CH_3	26.5, CH_3	123.0, C	24.2, CH_3
15′	72.7, CH_2	72.9, CH_2	71.8, CH_2	73.6, CH_2	70.4, CH_2
1″	165.9, C	166.1, C	166.6, C	167.4, C	168.1, C
2″	113.4, CH	114.6, CH	112.5, CH	130.4, C	128.0, C
3″	153.5, C	152.6, C	159.5, C	135.7, CH	138.8, CH
4″	66.7, CH_2	66.8, CH_2	67.2, CH_2	61.1, CH_2	14.6, CH_3
5″	15.6, CH_3	15.7, CH_3	15.7, CH_3	12.8, CH_3	12.1, CH_3
6″	171.6, C	171.2, C	173.1, C	172.0, C	
7″	28.7, CH_2	29.3, CH_2	28.6, CH_2	28.8, CH_2	
8″	28.9, CH_2	29.6, CH_2	28.7, CH_2	28.9, CH_2	
9″	169.9, C	170.1, C	172.0, C	171.9, C	
8-OMe				54.5, CH_3	
12-CO*Me*			52.7, CH_3		52.8, CH_3
6″-CO*Me*			52.0, CH_3		

[a] Recorded at 100 MHz. [b] Measured in $CDCl_3$. [c] Recorded at 125 MHz. [d] Measured in pyridine-d_5.

EXPERIMENTAL SECTION

General Experimental Procedures. The melting point of compound **1** was measured on an XRC-1 micromelting point apparatus and is uncorrected. Optical rotations were recorded on an Horiba SEAP-300 polarimeter. UV spectra were obtained on a Shimadzu UV-2401PC spectrophotometer. IR spectra were measured with a Bio-Rad FTS-135 spectrometer with KBr pellets. NMR spectra were recorded on Bruker AM-400, DRX-500, and Avance 600 instruments. Chemical shifts (δ) are expressed in ppm with reference to the solvent signals. ESIMS and HRESIMS were performed on an APIQSTAR TOF spectrometer. Semi-preparative HPLC was performed on an Agilent 1100 liquid chromatograph with a Zorbax SB-C_{18} (9.4 mm × 25 cm) column. Column chromatography (CC) was performed using silica gel (200−300 mesh, Qingdao Marine Chemical Inc., Qingdao, P. R. China), RP-18 gel (LiChroprep, 40−63 μm, Merck, Darmstadt, Germany), MCI gel CHP20P (75−150 μm, Mitsubishi Chemical Corporation, Tokyo, Japan), and Sephadex LH-20 (Amersham Biosciences, Sweden). Fractions were monitored by TLC, and spots were detected with a UV_{254} lamp and by heating silica gel plates sprayed with 10% H_2SO_4 in EtOH.

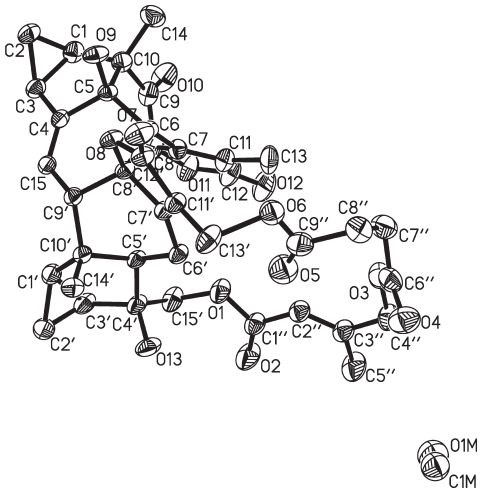

Figure 2. ORTEP drawing of chlorajaponilide A (**1**).

Plant Material. Whole plants of *C. japonicus* were collected in August 2008 from Panshi, Jilin Province, People's Republic of China, and identified by Dr. En-De Liu of Kunming Institute of Botany. A voucher sample (No. HY0003) was deposited at the State Key Laboratory of Phytochemistry and Plant Resources in West China.

Extraction and Isolation. The dried and powdered plant material (10.5 kg) was extracted three times with MeOH (40 L × 3) under reflux. The filtrate was evaporated under reduced pressure to give a residue (800 g), which was subjected to silica gel chromatography eluting with EtOAc to yield 426 g of eluate. The eluate was passed through a column containing MCI gel and eluted with gradient of MeOH−H_2O (3:7 → 5:5 → 7:3 → 1:0). The 70% MeOH fraction (109.4 g, a major fraction containing disesquiterpenoids) was chromatographed over a silica gel column ($CHCl_3$−MeOH, 100:1 → 80:1 → 60:1 → 40:1) to yield six fractions, A−F. Fraction B was subjected to silica gel CC ($CHCl_3$−MeOH, 150:1 → 120:1 → 80:1 → 60:1), then purified by semipreparative HPLC (CH_3CN−H_2O, 57:43; flow rate, 3 mL/min) to afford **3** (60 mg; t_R = 8.550 min). Fraction C (21.1 g) was separated on an RP-18 column eluted with a MeOH−H_2O gradient system (35%, 40%, 45%, 50%, and 55%) to obtain eight fractions, C_1−C_8. Shizukaol F (**11**, 200 mg) and cycloshizukaol A (**13**, 500 mg) crystallized from C_1 and C_2 in MeOH, respectively. Fraction C_3 was subjected to CC over silica gel ($CHCl_3$−MeOH, 130:1 → 120:1 → 100:1 → 60:1) to afford shizukaol A (**7**, 19 mg) and shizukaol B (**8**, 100 mg). The purification of fraction C_4 yielded spicachlorantin B (**16**, 10 mg) via CC on silica gel ($CHCl_3$−MeOH, 100:1). Chloraholide B (**14**, 18 mg) was obtained from fraction C_6 by silica gel CC ($CHCl_3$−MeOH, 120:1 → 100:1 → 80:1 → 60:1). Fraction C_7 was separated by silica gel CC ($CHCl_3$−MeOH, 100:1 → 80:1 → 60:1), then purified on Sephadex LH-20 (MeOH) to yield **1** (65 mg), **2** (14 mg), **4** (12 mg), and shizulaol D (**10**, 20 mg). Fraction D (12.7 g) was chromatographed over a silica gel column using $CHCl_3$−MeOH (150:1 → 100:1 → 80:1 → 60:1) to provide shizukaol H (**12**, 150 mg).

Table 3. Inhibitory Effect of Compounds 8, 9, 11, and 12 on HIV-1 Replication[a]

compound	VSVG[b]/HIVwt EC_{50} (μM)	VSVG/$HIV_{RT\text{-}K103N}$ EC_{50} (μM)	VSVG/$HIV_{RT\text{-}Y181C}$ EC_{50} (μM)
8	0.22	0.47	0.50
9	0.98	1.36	1.00
11	0.11	3.39	4.05
12	0.83	2.35	0.86
EFV	0.0008	0.024	0.002

[a] The percentages in this table indicate the infectivity compared to the same amount solvent as 100%. [b] VSV-G: vesicular stomatitis virus G protein.

The 50% MeOH fraction obtained from the MCI gel column was subjected to an RP-18 column (MeOH−H_2O, 35% → 60%) to give five fractions G−K. Fraction I was subjected to silica gel CC using $CHCl_3$−MeOH (100:1 → 80:1 → 60:1) to afford chloramultilide C (**15**, 35 mg) and yinxiancaol (**6**, 28 mg). Shizukaol C (**9**, 18 mg) and **5** (6 mg; t_R = 5.203 min) were obtained from fraction J by silica gel CC eluted with $CHCl_3$−MeOH (100:1) and purified by semipreparative HPLC (CH_3CN−H_2O, 48:52; flow rate, 3 mL/min).

Chlorajaponilide A (1): colorless prisms (CH_3OH−$CHCl_3$, 5:1, v/v); mp 277−280 °C; $[\alpha]^{20}_D$ +59.2 (*c* 0.1, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 241 (4.22) nm; IR (KBr) ν_{max} 3477, 2931, 1763, 1664, 1441, 1370, 1325, 1265, 1216, 1138, 1049, 1023 cm^{-1}; ^1H NMR data ($CDCl_3$, 400 MHz), see Table 1; ^{13}C NMR ($CDCl_3$, 100 MHz), see Table 2; positive ESIMS *m/z* 739 $[M + Na]^+$; positive HRESIMS *m/z* 739.2368 $[M + Na]^+$ (calcd for $C_{39}H_{40}O_{13}Na$, 739.2366).

X-ray Single-Crystal Structure Determination of 1. Crystal data: $C_{39}H_{40}O_{13}\cdot CH_3OH$, MW = 716.74 (no solvent of crystallization); monoclinic system, space group $P2_1$; crystal cell parameters a = 9.840(5) Å, b = 18.655(7) Å, c = 10.168(5) Å, β = 101.518(16)°, V = 1828.9(15) $Å^3$, Z = 2, d = 1.360 g/cm^3. A crystal of dimensions 0.07 × 0.14 × 0.21 mm was used for X-ray measurements on a macroMAX002+ diffractometer with a graphite monochromator (ω−κ scans, $2\theta_{max}$ = 144.84°), using Cu Kα radiation. The total number of independent reflections measured was 6672, of which 6120 were observed ($|F|^2 \geq 2\sigma|F|^2$). The crystal structure of **1** was solved by the direct method with SHELXS-97, expanded by using difference Fourier techniques, and refined by the program and method NOMCSDP and full-matrix least-squares calculations. The hydrogen atoms were fixed at their calculated positions. The final indices were R_1 = 0.0653, wR_2 = 0.1873, S = 1.043°. The absolute structure could be determined properly giving a Flack parameter of 0.1(2). Crystallographic data for the structure of **1** have been deposited in the Cambridge Crystallographic Data Centre as supplementary publication CCDC-809528 (available free of charge at http://www.ccdc.cam.ac.uk/deposit or from the DDCD, 12 Union Rd., Cambridge CB21EZ, UK; fax (+44) 1223-336-033; or deposit@ccdc.cam.ac.uk).

Chlorajaponilide B (2): colorless, amorphous powder; $[\alpha]^{20}_D$ +3.0 (*c* 0.06, MeOH); UV (MeOH) λ_{max} (log ε) 218 (4.29) nm; IR (KBr) ν_{max} 3437, 2928, 1745, 1663, 1637, 1222, 1162, 1039 cm^{-1}; ^1H NMR data (pyridine-d_5, 500 MHz), see Table 1; ^{13}C NMR (pyridine-d_5, 125 MHz), see Table 2; positive ESIMS *m/z* 755 $[M + Na]^+$; positive HRESIMS *m/z* 755.2309 $[M + Na]^+$ (calcd for $C_{39}H_{40}O_{14}Na$, 755.2315).

Chlorajaponilide C (3): yellow, amorphous powder; $[\alpha]^{20}_D$ −168.0 (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 220 (4.59) nm; IR (KBr) ν_{max} 3449, 2927, 1736, 1659, 1603, 1439, 1376, 1280, 1222, 1157, 1085, 990 cm^{-1}; ^1H NMR data ($CDCl_3$, 500 MHz), see Table 1; ^{13}C NMR ($CDCl_3$, 125 MHz), see Table 2; positive ESIMS *m/z* 787 $[M + Na]^+$; positive HRESIMS *m/z* 787.2949 $[M + Na]^+$ (calcd for $C_{41}H_{48}O_{14}Na$, 787.2941).

Table 4. Anti-HIV-1 Activities of Compounds 8, 9, 11, and 12

compound	cytotoxicity, CC_{50} (μM)	anti-HIV-1 activity, EC_{50} (μM)	therapy index (TI), CC_{50}/EC_{50}
8	0.020	0.0014	14.39
9	0.089	0.016	5.56
11	0.047	0.0043	10.93
12	0.022	0.0033	6.67
AZT	4.07	0.000004	1017500

Chlorajaponilide D (4): colorless, amorphous powder; $[\alpha]^{20}_D$ −1.8 (*c* 0.06, MeOH); UV (MeOH) λ_{max} (log ε) 219 (4.25) nm; IR (KBr) ν_{max} 3468, 2932, 1783, 1441, 1361, 1251, 1152, 1032, 975 cm^{-1}; ^1H NMR data ($CDCl_3$, 500 MHz), see Table 1; ^{13}C NMR ($CDCl_3$, 125 MHz), see Table 2; positive ESIMS *m/z* 769 $[M + Na]^+$; positive HRESIMS *m/z* 769.2459 $[M + Na]^+$ (calcd for $C_{40}H_{42}O_{14}Na$, 769.2472).

Chlorajaponilide E (5): yellow, amorphous powder; $[\alpha]^{20}_D$ −152.2 (*c* 0.08, MeOH); UV (MeOH) λ_{max} (log ε) 219 (4.45) nm; IR (KBr) ν_{max} 3443, 2935, 1738, 1648, 1438, 1382, 1267, 1135, 1081, 976 cm^{-1}; ^1H NMR data ($CDCl_3$, 400 MHz), see Table 1; ^{13}C NMR ($CDCl_3$, 100 MHz), see Table 2; positive ESIMS *m/z* 689 $[M + Na]^+$; positive HRESIMS *m/z* 689.2577 $[M + Na]^+$ (calcd for $C_{36}H_{42}O_{12}Na$, 689.2573).

Anti-HIV-1 Activity Assay by Pseudotyped Viruses. Vesicular stomatitis virus glycoprotein (VSV-G) plasmid was co-transfected with env-deficient HIV vector (pNL4-3.luc.R-E$^-$, pNL4-3.luc.R$^-$E$^-_{RT\text{-}K103N}$, or pNL4-3.luc.R$^-$E$^-_{RT\text{-}Y181C}$) into 293T cells by using a modified $Ca_3(PO_4)_2$ method.[26−28] Briefly, plates were washed with PBS, and fresh media was added 16 h after transfection. Supernatant that contains pseudotyped virions (VSVG/HIV-wt, VSVG/$HIV\text{-}_{RT\text{-}K103N}$, or VSVG/$HIV\text{-}_{RT\text{-}Y181C}$) was harvested and filtered through a 0.45 μm filter 48 h post-transfection. Viral solution was quantified by p24 concentrations, which were detected by ELISA (ZeptoMetrix, Cat.: 0801111) and diluted to 0.2 ng p24/mL, which can be used directly or stored at −80 °C. One day prior to infection, 293T cells were plated on 24-well plates at a density of 6 × 10^4 cells per well. Compounds were dissolved in DMSO and added into target cells 15 min ahead of infection. Forty-eight hours post-infection, infected cells were lysed in 50 μL of Cell Lysis Reagent (Promega). Luciferase activity of cell lysate was measured by a Sirius luminometer (Berthold Detection System) according to the manufacture's instructions.

Anti-HIV-1 Assay. Cytotoxicity against C8166 cells (CC_{50}) was assessed using the MTT method, and anti-HIV-1 activity was evaluated by the inhibition assay for the cytopathic effects of HIV-1 (EC_{50}).[29]

■ ASSOCIATED CONTENT

Supporting Information. 1D and 2D NMR (HSQC, COSY, HMBC, ROESY) spectra for chlorajaponilides A−E (**1**−**5**) are available free of charge via the Internet at http://pubs.acs.org.

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■ ACKNOWLEDGMENT

This work was financially supported by National Natural Science Funding of China (No. 81072543), the Key Project of

Knowledge Innovation Project of CAS (No. KSCX2-EW-Q-10), and funds (No. P2009-KF04) from the State Key Laboratory of Phytochemistry and Plant Resources in West China.

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