Sesquiterpenes from *Chloranthus japonicus*

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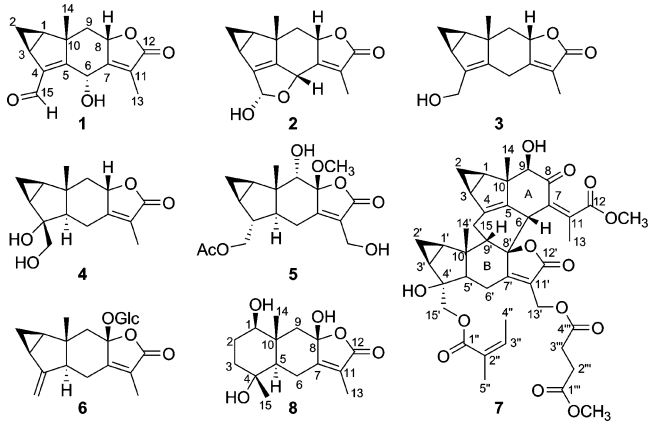
Eight new sesquiterpene lactones (chlorajapolides A−E, **1**−**5**, chlorajaposide, **6**, chlorajaponol, **7**, and chloraeudolide, **8**) were isolated from an ethyl acetate-soluble partition of the ethanol extract of the whole plants of *Chloranthus japonicus*. The structures and relative configurations of **1**−**8** were established on the basis of spectroscopic data analysis. All isolates obtained were evaluated for their cytotoxic activity against a small panel of cancer cell lines.

The genus *Chloranthus* has been placed taxonomically in the family Chloranthaceae and comprises approximately 12 species in mainland China.[1] Some species in this genus have a long history of folk use for their purported antifungal, anti-inflammatory, and antitumor activities. The reported constituents of the genus *Chloranthus* include simple coumarins, amides, and sesquiterpene lactones with an unusual 3,5,6-ring system.[2−5] Sesquiterpene lactones having a lindenane skeleton, such as the shizukanolides and chloranthalactones, are characteristic chemotaxonomically of *Chloranthus* species.[6−8] Some interesting lindenane dimers have also been isolated from this genus.[9−15]

The perennial herbaceous plant *Chloranthus japonicus* Sieb. grows in shady places and is distributed widely in many regions of the People's Republic of China.[1] This species has folk uses for the treatment of traumatic injuries, rheumatic arthralgia, fractures, pulmonary tuberculosis, and neurasthenia.[1] In the present work, investigation on its ethyl acetate-soluble extract led to the isolation of eight new sesquiterpenoids featuring an α,β-unsaturated-γ-lactone ring, including five lindenane sesquiterpenes, chlorajapolides A−E (**1**−**5**), a lindenane glucoside, chlorajaposide (**6**), a lindenane sesquiterpene dimer, chlorajaponol (**7**), and a eudesmane sesquiterpene, chloraeudolide (**8**). This paper describes the isolation and structure elucidation of these isolates and the evaluation of their cytotoxic activity against several cancer cell lines.



Results and Discussion

Chlorajapolide A (**1**) was obtained as a white, amorphous powder with a molecular formula assigned as $C_{15}H_{16}O_4$ on the basis of HRESIMS (*m*/*z* calcd 283.0946 $[M + Na]^+$; found $C_{15}H_{16}O_4Na$, 283.0941). The ^1H NMR spectrum of **1** showed four upfield protons at δ_H 0.40 (1H, dt, $J = 3.2, 4.4$ Hz), 0.93 (1H, dt, $J = 4.4, 8.0$ Hz), 1.63 (1H, ddd, $J = 8.0, 6.4, 4.4$ Hz), and 2.18 (1H, ddd, $J = 8.0, 6.4, 3.2$ Hz), characteristic of the cyclopropane ring of a lindenane sesquiterpene skeleton.[6−15] Signals at δ_H 1.88 (3H, d, $J = 1.6$ Hz) and 1.56 (3H, s) were attributed to Me-13 and Me-14. A third methyl group found commonly in lindenane sesquiterpenes was absent, and, instead, a proton signal of an aldehyde group (CHO) at δ_H 10.04 (1H, s) was present, suggesting a CHO group affixed to C-15. All 15 carbons were resolved in the ^{13}C NMR spectrum and were categorized by DEPT experiments as two methyls, two methylenes, five methines (two oxygenated and one aldehyde carbon), and six quaternary carbons (four olefinic and one ester carbonyl). The ^1H and ^{13}C NMR data of **1** (Tables 1 and 2) were similar to those of lindenane sesquiterpenes with a 4,7(11)-dien-12,8-olide structural moiety,[7,10,15] an observation confirmed from the HMBC spectrum. Thus, in the HMBC experiment of **1** (Figure 1), long-range correlations were observed between Me-13 and C-7 and C-12, between Me-14 and C-1, C-5, C-9, and C-10, between H-15 and C-3, C-4, and C-5, and between H-6 and C-4, C-5, C-7, C-8, and C-10. The relative configuration of **1** was established by a NOESY experiment (Figure 1), in which correlations of Me-14/H-2β, Me-14/H-6, and Me-14/H-8 revealed that H-6, H-8, and the cyclopropane ring are cofacial, and these were assigned arbitrarily with a β-orientation. Consequently, H-1 and H-3 were assigned as α-oriented. On the basis of the above data, **1** was proposed as (1α,3α,6β,8β)-6-hydroxy-15-al-1*H*-lindan-4,7(11)-dien-12,8α-olide.

Compound **2**, chlorajapolide B, was obtained as a white, amorphous powder and gave a quasimolecular ion $[M + Na]^+$ at *m*/*z* 283.0952 (calcd for 283.0946) in the positive-ion HRESIMS, consistent with the molecular formula, $C_{15}H_{16}O_4$. The NMR data (Tables 1 and 2) revealed the presence of two methyls, two methylenes, five methines (three oxygenated), and six quaternary carbons (four olefinic and one ester carbonyl). Comparison of the ^{13}C NMR data of **2** with **1** revealed a close resemblance between these compounds, except that the C-15 signal was assigned to a hydroxylated carbon (δ_C 101.1) in **2** and, as a consequence, the C-5 signal was shifted upfield by about 19.0 ppm. The planar structure of **2** was verified by HMBC correlations (Figure S1, Supporting Information), in which key long-range correlations were observed between H-15 and C-3, C-4, and C-5 and between H-6 and C-4, C-5, C-8, and C-15. The relative configuration of **2** was established on the basis of a NOESY experiment (Figure S1, Supporting Information), in which correlations of Me-14/H-2β, Me-14/H-6, Me-14/H-8, H-6/H-8, and H-6/H-15 revealed that H-6, H-8, and H-15 and the cyclopropane ring are cofacial. On the basis of

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Table 1. ^{1}H NMR Spectroscopic Data of Compounds **1**−**6** and **8** (400 MHz, in CD_3OD)

position	**1**	**2**	**3**	**4**	**5**	**6**	**8**
1	1.63 (ddd, 8.0, 6.4, 4.4)	1.56 (ddd, 8.0, 6.0, 4.0)	1.52 (ddd, 7.6, 6.4, 4.4)	1.39 (m)	1.68 (dt, 4.0, 8.0)	1.41 (dt, 3.6, 7.6)	3.21 (dd, 10.4, 5.2)
2β	0.40 (dt, 3.2, 4.4)	0.40 (dt, 3.2, 4.0)	0.27 (dt, 3.2, 4.0)	0.70 (dt, 6.4, 8.4)	0.70 (dt, 5.6, 8.0)	0.70 (dt, 4.8, 3.6)	1.64 (m)
2α	0.93 (dt, 4.4, 8.0)	0.85 (dt, 4.0, 8.0)	0.80 (dt, 4.0, 7.6)	0.92 (dt, 4.0, 8.4)	0.94 (m)	0.83 (ddd, 8.8, 8.0, 4.2)	1.68 (m)
3β							1.78 (dt, 12.8, 3.6)
3α	2.18 (ddd, 8.0, 6.4, 3.2)	1.91 (ddd, 8.0, 6.4, 3.2)	1.91 (dt, 2.8, 7.6)	1.47 (m)	1.21 (m)	2.02 (m)	1.49 (dt, 5.2, 12.8)
4					1.76 (m)		
5				2.08 (dd, 14.4, 2.4)	2.23 (dt, 14.0, 2.4)	1.85 (dd, 12.8, 2.0)	1.27 (dd, 13.2, 2.8)
6β	6.13 (s)	5.75 (s)	2.73 (brd, 14.8)	2.16 (brt, 14.4)	2.05 (dd 14.0, 12.4)	2.34 (dd, 17.6, 12.8)	2.32 (dt, 1.2, 13.2)
6α			3.65 (d, 14.8)	2.80 (dd, 14.4, 2.4)	2.87 (dd, 12.4, 2.4)	2.60 (m)	2.95 (dd, 13.2, 3.2)
8	5.42 (ddq, 11.6, 6.0, 1.6)	5.35 (ddq, 11.6, 6.0, 1.6)	5.11 (m)	5.09 (m)			
9β	2.58 (dd, 11.6, 6.0)	2.50 (dd, 11.6, 6.0)	2.38 (dd, 11.2, 6.0)	2.50 (dd, 13.2, 6.8)		2.70 (d, 14.0)	2.57 (d, 13.2)
9α	1.38 (t, 11.6)	1.26 (t, 11.6)	1.26 (t, 11.2)	1.43 (m)	3.92 (s)	2.18 (d, 14.0)	1.31 (d, 13.2)
13	1.88 (d, 1.6)	1.83 (d, 1.6)	1.77 (t, 1.6)	1.78 (t, 1.6)	4.32 (d, 13.2)	1.82 (brs)	1.79 (d, 1.2)
					4.28 (d, 13.2)		
14	1.56 (s)	1.47 (s)	1.28 (s)	0.99 (s)	1.03 (s)	0.54 (s)	1.13 (s)
15	10.04 (s)	5.11 (s)	4.16 (d, 13.2)	3.56 (d, 11.6)	4.23 (dd, 11.2, 6.4)	4.97 (brs)	1.19 (s)
			4.12 (d, 13.2)	3.46 (d, 11.6)	4.13 (dd, 11.2, 5.2)	4.75 (brs)	
					3.18 (s)		
OMe					2.05 (s)		
OAc						4.22 (d, 7.6)	
1′						3.18 (t, 7.6)	
2′						3.06 (m)	
3′						3.30 (m)	
4′						3.27 (m)	
5′						3.72 (dd, 12.4, 2.4)	
6′						3.62 (dd, 12.4, 4.8)	

Table 2. ^{13}C NMR Spectroscopic Data of Compounds **1**−**6** and **8** (100 MHz, in CD_3OD)

carbon	**1**	**2**	**3**	**4**	**5**	**6**	**8**
1	29.9	30.6	28.6	27.7	25.8	29.9	80.0
2	16.0	16.9	15.8	12.0	16.4	17.2	29.0
3	20.9	23.3	24.5	30.1	23.4	24.7	41.5
4	145.6	144.3	141.4	80.4	43.7	153.5	72.3
5	163.1	144.1	138.8	69.3	56.6	55.9	56.6
6	61.2	62.1	25.1	23.9	25.2	23.4	22.3
7	163.2	164.8	164.7	165.6	162.3	158.1	163.8
8	78.1	78.7	80.2	81.5	110.0	110.2	105.5
9	48.9	50.1	48.1	48.6	77.2	51.0	52.5
10	51.8	50.1	49.0	40.1	45.4	38.7	41.3
11	124.1	121.9	119.9	121.1	130.6	128.8	121.9
12	176.0	176.6	177.0	177.0	172.6	173.7	174.6
13	8.3	8.3	8.0	8.2	54.3	8.3	8.0
14	24.0	24.1	21.7	18.7	16.2	21.5	14.3
15	188.6	101.1	58.5	65.3	67.2	106.4	22.6
OMe					50.7		
OAc					173.1		
OAc					20.8		
1′						100.2	
2′						74.6	
3′						78.2	
4′						70.9	
5′						77.9	
6′						62.4	

the above data, **2** was established as (1α,3α,6β,8β)-6,15-epoxy-15-hydroxy-1*H*-lindan-4,7(11)-dien-12,8α-olide.

Chlorajapolide C (**3**) was obtained as a white, amorphous powder and gave a quasimolecular ion $[M + Na]^+$ at *m*/*z* 269.1146 (calcd for 269.1154) in the positive-ion HRESIMS, consistent with the molecular formula, $C_{15}H_{18}O_3$. Again, the ^{1}H NMR spectrum of **3** (Table 1) showed several features of a lindenane sesquiterpene skeleton with a three-membered ring.[12,13,15] Two signals corresponding to Me-14 and Me-13 were observed at δ_H 1.28 and 1.77, respectively. In the HMBC experiment, the correlations Me-13/C-7, C-12, Me-14/C-1, C-5, C-9, C-10, H-15/C-3, C-4, C-5, and H-15/C-3, C-4, C-5 supported a 4,7(11)-dien-12,8-olide lindenane sesquiterpene structural moiety.[9] The relative configuration of **3** was established on the basis of a NOESY experiment, in which correlations of Me-14/H-2β and Me-14/H-8 revealed that H-8 and the cyclopropane ring are β-oriented. On the basis of the above data, **3** was determined as (1α,3α,8β)-15-hydroxy-1*H*-lindan-4,7(11)-dien-12,8α-olide.

Chlorajapolide D (**4**) was obtained as a white, amorphous powder. The molecular formula of **4** was determined as $C_{15}H_{20}O_4$ by the positive-ion HRESIMS of the quasimolecular ion $[M + Na]^+$ at *m*/*z* 287.1252 (calcd for 287.1259), indicating six degrees of unsaturation. Comparison of the ^{13}C NMR data of **4** (Table 2) with the aglycon part of yinxiancaoside A[11] revealed a strong resemblance, with the exception of the chemical shift of C-15. The C-15 resonance at δ_C 65.3 of **4** indicated that C-15 is substituted by a hydroxy group. The relative configuration of **4** was established by a NOESY experiment. The NOESY correlations from Me-14 to H-2β, H$_2$-15, H-8, H-6β, and H-9β indicated that they are cofacial, and these were arbitrarily assigned as β-oriented. Other correlations

Figure 1. Key HMBC and NOESY correlations observed for chlorajapolide A (**1**).

Table 3. ^{1}H and ^{13}C NMR Spectroscopic Data of Compound **7** (400 and 100 MHz, in CD_3OD)

position	δ_H (mult, *J* in Hz)	δ_C	position	δ_H (mult, *J* in Hz)	δ_C
1	1.99 (m)	26.7	6′β	2.45 (dd, 18.8, 6.0)	24.2
2β	0.32 (dt, 3.2, 4.4)	16.1	6′α	2.80 (m)	
2α	0.97 (m)		7′		174.5
3	1.93 (m)	25.3	8′		93.4
4		143.1	9′	1.81 (dd, 6.0, 2.0)	57.3
5		133.8	10′		46.1
6	3.96 (brd, 3.6)	41.9	11′		124.5
7		134.7	12′		173.5
8		202.1	13′	4.79 (d, 12.8)	56.2
9	3.88 (brs)	80.9		4.83 (d, 12.8)	
10		52.4	14′	0.89 (s)	26.8
11		146.6	15′	4.20 (d, 11.6)	72.7
12		172.2		3.78 (d, 11.6)	
13	1.81 (s)	20.1	1″		170.1
14	1.04 (s)	15.8	2″		129.5
15β	2.53 (ddd, 16.4, 6.0, 4.0)	26.0	3″	6.94 (m)	139.5
15α	2.84 (dd, 16.4, 2.0)		4″	1.85 (d, 7.2)	14.6
1′	1.67 (m)	26.4	5″	1.88 (d, 1.2)	12.4
2′β	0.73 (dt, 2.2, 8.8)	12.5	1‴		173.3
2′α	1.29 (m)		2‴	2.64 (m)	29.6
3′	1.45 (m)	29.1	3‴	2.67 (m)	29.7
4′		77.8	4‴		174.5
5′	1.79 (m)	62.4	MeO-12	3.68 (s)	52.9
			MeO-1‴	3.66 (s)	52.3

between H-5 and H-3, between H-6α and H-9α, and between H-3 and H-1 and H-2α revealed that these protons should adopt an α-orientation. Therefore, **4** was elucidated as (1α,3α,5α,8β)-4α,15-dihydroxy-1*H*-lindan-7(11)-en-12,8α-olide.

Chlorajapolide E (**5**) was obtained as a white powder. The molecular formula was determined by the positive-ion HRESIMS as $C_{18}H_{24}O_7$ from the $[M + Na]^+$ signal at *m*/*z* 375.1401 (calcd for 375.1420). The ^{1}H NMR spectrum of **5** also showed several features of a lindenane sesquiterpene with a three-membered ring.[12,13,15] The signals at δ_H 1.03 (3H, s), 3.18 (3H, s), and 2.05 (3H, s) were attributed to the Me-14, methoxy, and acetyl protons, respectively. The appearance of a pair of double doublet signals at δ_H 4.23 (1H, dd, $J = 11.2$, 6.4 Hz) and 4.13 (1H, dd, $J = 11.2$, 5.2 Hz) and a pair of doublet signals at δ_H 4.32 (1H, d, $J = 13.2$ Hz) and 4.28 (1H, d, $J = 13.2$ Hz) suggested that C-13 and C-15 both occur as oxygenated methylene groups. The ^{13}C NMR (DEPT) spectrum (Table 2) showed 18 carbon resonances assignable to 15 carbon signals of a lindenane sesquiterpene skeleton, a methoxy group, and an acetyl group. The HMBC data were used to elucidate the connectivity of the different structural fragments, as well as to confirm the chemical shift assignments. In the HMBC experiment of **5** (Figure S2, Supporting Information), long-range correlations were observed between H-13 and C-7 and C-12, Me-14 and C-1, C-5, C-9, and C-10, H-15 and C-3, C-4, C-5, CH_3O-8, and C-8, and H-15 and the acetyl carbonyl. The relative configuration of **5** was established on the basis of a NOESY experiment (Figure S2, Supporting Information), in which correlations of Me-14/H-2β, Me-14/H-9, Me-14/H-4, Me-14/CH_3O-8, and CH_3O-8/H-9 showed that H-4, H-9, CH_3O-8, and the cyclopropane ring are on the same side of the molecule, and these were assigned arbitrarily with a β-orientation. Furthermore, obvious correlations were observed between H-5/H-3, H-5/H-1, and H-5/H_2-15. Consequently, H-1, H-3, and H-5 could be designated as α-oriented. On the basis of the above data, **5** was assigned as (1α,3α,4β,9β)-8β-methoxy-9α-hydroxy-15-acetyl-1*H*-lindan-4,7(11)-dien-12,8α-olide.

Chlorajaposide (**6**), a white powder, showed a molecular formula of $C_{21}H_{28}O_8$ by positive-ion HRESIMS from the $[M + Na]^+$ signal at *m*/*z* 431.1679 (calcd for 431.1682). The ^{1}H NMR spectrum of **6** exhibited several features characteristic of a lindenane sesquiterpene skeleton with a three-membered ring.[12,13,15] Two singlets corresponding to Me-14 and Me-13 were observed at δ_H 0.54 (3H, s) and 1.82 (3H, brs), respectively. The observation of two terminal olefinic protons at δ_H 4.97 and 4.75 suggested that **6** is based on a 4(15)-en-lindane system. Besides these signals, the ^{1}H and ^{13}C NMR spectra (Tables 1 and 2) also showed signals of a glucopyranosyl moiety, indicating that **6** is a lindenane sesquiterpene glucoside. Acid hydrolysis of **6** yielded D-glucose, detected by direct co-TLC comparison with an authentic sample. A large coupling constant ($J = 7.6$ Hz) for the anomeric proton (δ 4.22) of the glucose unit in the ^{1}H NMR spectrum suggested a β-configuration. The position of the glucose residue at C-8 was established by the HMBC correlation between the anomeric proton of the glucosyl moiety and C-8 of the aglycon. Its relative configuration was established using the NOESY spectrum in a similar way to **5**. Hence, **6** was determined as (1α,3α)-8β-glucopyranosyl-1*H*-lindan-4(15),7(11)-dien-12,8α-olide.

Chlorajaponol (**7**), obtained as white needle crystals, showed a molecular formula of $C_{41}H_{48}O_{13}$ by positive-ion HRESIMS, from the $[M + Na]^+$ signal at *m*/*z* 771.2971 (calcd for $C_{41}H_{48}O_{13}Na^+$ 771.2993). The ^{1}H NMR spectrum of **7** (Table 3) exhibited two high-field signals (δ_H 0.32 and 0.73) characteristic of H-2β of a lindenane skeleton. Their chemical shifts and coupling patterns were quite similar to those found in H-1, H-2, and H-3 of previously isolated lindenanes.[4,5] The ^{1}H−^{1}H COSY spectrum showed two sets of proton networks of 1,2-disubstituted cyclopropane rings (δ_H 0.32, 0.97, 1.93, and 1.99; δ_H 0.73, 1.29, 1.45, and 1.67). Therefore, **7** could be assigned tentatively as a lindenane sesquiterpene dimer. Comparison of the ^{13}C NMR data of **7** (Table 3) with those of the shizukaols, lindenane dimers previously isolated from the genus *Chloranthus*,[4] revealed a strong resemblance between units A and B. The presence of angelic acid and succinic acid moieties in unit B was supported from the 2D-NMR spectroscopic data of **7**, including COSY, HSQC, and HMBC correlations. In the HMBC experiment of **7** (Figure S3, Supporting Information), long-range correlations were observed between the C-15′ methylene protons and the ester carbonyl of the angelic acid moiety and between the C-13′ methylene protons and the ester carbonyl of the succinic acid moiety. The relative configuration of **7** was determined as depicted by analysis of the NOESY spectrum (Figure S3, Supporting Information), in which NOE correlations between H-2β/H-14, H-1/H-9, H-1/H-3, H-2′β/H-14′, H-1′/H-3′, H-3′/H-5′, H-5′/H-15′, and H-3′/H-15′ were observed in units A and B. Moreover, the double bond in the angelic acid moiety was shown to be *E*, from the key NOE correlations between the olefinic proton (δ_H 6.94, m) and two olefinic methyl protons (δ_H 1.88, d, $J = 1.2$ Hz; 1.85, d, $J = 7.2$ Hz). Therefore, the structure of **7** was elucidated as depicted.

Chloraeudolide (**8**), a white powder, showed a molecular formula of $C_{15}H_{22}O_5$ by positive-ion HRESIMS from the $[M + Na]^+$ signal at *m/z* 305.1360 (calcd for 305.1365). The ^{13}C NMR spectrum displayed 15 carbon signals for a 7(11)-en-eudesma-12,8-olide comprising three methyls, four methylenes, two methines (one oxygenated), six quaternary carbons including two oxygenated, one ester carbonyl at δ_C 174.6, and two olefinic carbons (Table 2).[11] In the HMBC spectrum (Figure S4, Supporting Information), long-range correlations between H-6α and C-8, between H-13 and C-7 and C-12, between Me-14 and C-1, C-5, C-9, and C-10, and between H-15 and C-3, C-4, and C-5 were used to establish the framework of **8**. In the ^1H NMR spectrum of **8**, the large coupling constants of H-1, H-2, H-3, H-5, and H-6 ($J_{1,2ax} = 10.4$ Hz, $J_{3ax,2ax} = 12.8$ Hz, $J_{5,6ax} = 13.2$ Hz) indicated that rings A and B are in a chair conformation. The *trans*-junction between rings A and B was supported by the diagnostic upfield shifted value of the angular methyl C-14 (δ 14.3) with respect to that reported for the corresponding carbon (δ 22.7) of the synthetic *cis*-fused isomer, 1,2-dihydrotubipofuran.[16] This assertion was confirmed by the NOESY correlation of H-5/H-6α (Figure S4, Supporting Information). Since H-14 showed a NOE correlation with H-15, it was apparent that these two methyls possess a 1,3-diaxial configuration. Other correlations of H-5 with H-1 and H-9α helped to determine the hydroxy group at C-4 in the β-orientation and to distinguish the two protons of H-9. The configuration of the hydroxy at C-8 was determined as β-oriented by comparison with a known compound with the same structural residue.[15] On the basis of the above data, **8** was established as 1β,4α,8-trihydroxy-7(11)-en-eudesm-8,12-olide.

Plants in the family Chloranthaceae have been found to be rich in sesquiterpenes of the germacrane, eudesmane, and lindenane type (including sesquiterpenoid dimers and trimers).[6−15] Eudesmane and germacrane-type sesquiterpenoids are also widely distributed in monocotyledonous plants, such as Araceae and Zingiberaceae. However, the distribution of lindenane sesquiterpenes in natural sources is very limited. They have been found so far only in the plant familes Lauraceae, Chloranthaceae, and Compositae. The new isolates **1**−**7** are a series of lindenane sesquiterpene lactones, and, of them, **1** and **2** have an aldehyde and acetal group of C-15, which have not been reported for lindenane-type sesquiterpenoids previously. Furanogermacrane-type sesquiterpenes are considered to be key intermediates of other furanosesquiterpenes, which are regarded as precursors to sesquiterpene lactones.[9] Cycloaddition between two olefin bonds of some germacrane-type sesquiterpenes affords eudesmane-type sesquiterpenoids, which could be transformed to lindenane-type sesquiterpenes in plants. Enzyme-catalyzed intermolecular Diels−Alder cycloaddition of two lindenane sesquiterpenes produces lindenane sesquiterpene dimers.[4]

A 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H* tetrazolium hydrobromide (MTT) assay was used to determine the cytotoxicity of these compounds (**1**−**8**) against a panel of eight cancer cell lines. However, none of these compounds showed any marked activity ($IC_{50} < 10$ μM).

Experimental Section

General Experimental Procedures. The optical rotations were recorded on a Perkin-Elmer 341 polarimeter. IR spectra were recorded on a Shimadzu FTIR-8400S spectrometer. NMR spectra were recorded on a Bruker DPX 400 NMR instrument (400 MHz for ^1H NMR and 100 MHz for ^{13}C NMR). Chemical shifts are given as δ values with reference to tetramethylsilane (TMS) as internal standard, and coupling constants are given in Hz. The HRESIMS data were conducted on an IonSpec Ultima 7.0T FTICR mass spectrometer. Preparative HPLC (Waters, Delta 600-2487) was performed on a Hypersil-ODS II (10 μm, 20 × 300 mm, Yilite, Dalian, People's Republic of China).

Plant Material. Whole plants of *C. japonicus* were collected in August 2008 from Suiling district, Heilongjiang Province, People's Republic of China, and identified by Prof. Zhenyue Wang of Heilongjiang University of Chinese Medicine. A voucher specimen (20080079) has been deposited at Heilongjiang University of Chinese Medicine, Harbin, People's Republic of China.

Extraction and Isolation. The whole plants of *C. japonicus* (20 kg) were ground and extracted with 95% EtOH (3 × 10 L) for 2 h. The EtOH extract (850 g) was concentrated under reduced pressure, dissolved in water (10 L), and extracted successively with petroleum ether (60−90 °C), $CHCl_3$, EtOAc, and *n*-BuOH. Solvent was removed to afford petroleum ether (50 g), $CHCl_3$ (80 g), EtOAc (120 g), and *n*-BuOH (300 g) extracts. Of these, the EtOAc extract (100 g) was repeatedly column chromatographed on silica gel with a gradient of $CHCl_3$/MeOH (1:0 to 0:1), to afford five major fractions: Fr_1 (5 g), Fr_2 (15 g), Fr_3 (20 g), Fr_4 (8 g), and Fr_5 (12 g). Fr_3 (20 g) was subjected to additional silica gel chromatography, by elution with $CHCl_3$/MeOH (50:1 to 0:1), to afford subfractions A_1-A_{13}. Subfraction A_3 (1.2 g) was subjected to ODS column chromatography with MeOH/H_2O (3:7 to 1:0) and finally purified by semipreparative HPLC on a Hypersil-ODS Π column (10 μm, 20 × 300 mm, flow rate, 8 mL/min, UV detection, 225 nm), eluted with MeOH/H_2O (7:3), to afford **1** (57 mg, t_R 19 min), **2** (33 mg, t_R 18 min), **3** (65 mg, t_R 16 min), and **4** (36 mg, t_R 22 min). Fr_5 (12 g) was purified by silica gel chromatography, eluted with $CHCl_3$/MeOH (30:1 to 0:1), to afford subfractions B_1-B_{10}. Subfraction B_2 (0.5 g) was purified in a similar treatment to that of subfraction A_3, with MeOH/H_2O (65:35) as HPLC mobile phase, to afford **7** (42 mg, t_R 65 min). Subfraction B_6 (1.3 g) was chromatographed over a ODS column with a gradient of MeOH/H_2O (3:7 to 1:0) and finally purified by semipreparative HPLC (Hypersil-ODS Π column), eluted with MeOH/H_2O (2:3), to afford **5** (28 mg, t_R 37 min), **6** (49 mg, t_R 22 min), and **8** (31 mg, t_R 28 min).

Chlorajapolide A (1): white, amorphous powder; $[\alpha]^{20}_D$ +121.5 (*c* 0.1, MeOH); IR (KBr) ν_{max} 3341, 2943, 2838, 2321, 2047, 1736, 1669, 1113, 1028, 655 cm^{-1}; ^1H NMR and ^{13}C NMR, see Tables 1 and 2; HRESIMS *m/z* 283.0941 $[M + Na]^+$ (calcd for $C_{15}H_{16}O_4Na$, 283.0946).

Chlorajapolide B (2): white, amorphous powder; $[\alpha]^{20}_D$ +142.3 (*c* 0.1, MeOH); IR (KBr) ν_{max} 3346, 2945, 2831, 2330, 2041, 1755, 1340, 1113, 1028, 663 cm^{-1}; ^1H NMR and ^{13}C NMR, see Tables 1 and 2; HRESIMS *m/z* 283.0952 $[M + Na]^+$ (calcd for $C_{15}H_{16}O_4Na$, 283.0946).

Chlorajapolide C (3): white, amorphous powder; $[\alpha]^{20}_D$ +175.4 (*c* 0.1, MeOH); IR (KBr) ν_{max} 3342, 2943, 2831, 2596, 2523, 1752, 1488, 1115, 1028, 642 cm^{-1}; ^1H NMR and ^{13}C NMR, see Tables 1 and 2; HRESIMS *m/z* 269.1146 $[M + Na]^+$ (calcd for $C_{15}H_{18}O_3Na$, 269.1154).

Chlorajapolide D (4): white, amorphous powder; $[\alpha]^{20}_D$ −52.0 (*c* 0.1, MeOH); IR (KBr) ν_{max} 3342, 2943, 2831, 2361, 2041, 1716, 1317, 1122. 1028, 644 cm^{-1}; ^1H NMR and ^{13}C NMR, see Tables 1 and 2; HRESIMS obsd *m/z* 287.1252 $[M + Na]^+$ (calcd for $C_{15}H_{20}O_4Na$, 287.1259).

Chlorajapolide E (5): white, amorphous powder; $[\alpha]^{20}_D$ −74.9 (*c* 0.1, MeOH); IR (KBr) ν_{max} 3342, 2945, 2831, 2527, 2044, 1751, 1340, 1113, 1028, 619 cm^{-1}; ^1H NMR and ^{13}C NMR, see Tables 1 and 2; HRESIMS *m/z* 375.1401 $[M + Na]^+$ (calcd for $C_{18}H_{24}O_7Na$, 375.1420).

Chlorajaposide (6): white, amorphous powder; $[\alpha]^{20}_D$ +88.6 (*c* 0.1, MeOH); IR (KBr) ν_{max} 3354, 2945, 2833, 2598, 2521, 1747, 1421, 1113, 1026, 638 cm^{-1}; ^1H NMR and ^{13}C NMR, see Tables 1 and 2; HRESIMS *m/z* 431.1679 $[M + Na]^+$ (calcd for $C_{21}H_{28}O_8Na$, 431.1682).

Chlorajaponol (7): white, amorphous powder; $[\alpha]^{20}_D$ −166.7 (*c* 0.1, MeOH); IR (KBr) ν_{max} 3428, 2929, 2375, 1737, 1638, 1439, 1365, 1269, 1159, 993 cm^{-1}; ^1H NMR and ^{13}C NMR, see Table 3; HRESIMS *m/z* 771.2971 $[M + Na]^+$ (calcd for $C_{41}H_{48}O_{13}Na$, 771.2993).

Chloraeudolide (8): white, amorphous powder; $[\alpha]^{20}_D$ +12.1 (*c* 0.1, MeOH); IR (KBr) ν_{max} 3342, 2943, 2831, 2594, 2523, 1752, 1420, 1117, 1028, 645 cm^{-1}; ^1H NMR and ^{13}C NMR, see Tables 1 and 2; HRESIMS *m/z* 305.1360 $[M + Na]^+$ (calcd for $C_{15}H_{22}O_5Na$, 305.1365).

Cytotoxicity Bioassays. The cytotoxic activity of each compound against eight cultured human tumor cells was examined in vitro at the Tumor Hospital of Harbin Medical University. The tumor cell lines used were Lovo (colorectal cancer cells), Hepg2 (hepatoma cells), OS-RC-2 (renal carcinoma cells), A549 (lung epithelial carcinoma cells), SKOV3 (ovarian carcinoma cells), HeLa (cervical cancer cells), MCF-7 (breast cancer cells), and SGC-7901 (gastric adenocarcinoma cells). The protocol of the cytotoxicity bioassays was provided in a previously published paper.[11] 5-Fluorouracil was employed as the positive control.

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Supporting Information Available: ^{1}H NMR, ^{13}C NMR, and HRESIMS spectra of **1**−**8**. This material is available free of charge via the Internet at http://pubs.acs.org.

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