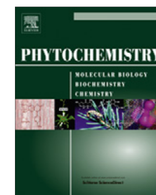
Phytochemistry 88 (2013) 112–118

Contents lists available at SciVerse ScienceDirect

Phytochemistry

journal homepage: www.elsevier.com/locate/phytochem



Sesquiterpenoids from *Chloranthus multistachys*

Hai-Yang Liu [a,*], Xin-Hui Ran [a], Ning-Bo Gong [b], Wei Ni [a], Xu-Jie Qin [a], Yan-Yan Hou [b], Yang Lü [b], Chang-Xiang Chen [a]

[a] State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, People's Republic of China
[b] Institute of Materia Medica, Chinese Academy of Medical Sciences, Beijing 100050, People's Republic of China

ARTICLE INFO

Article history:
Received 10 April 2012
Received in revised form 28 September 2012
Available online 8 January 2013

Keywords:
Chloranthus multistachys
Chloranthaceae
Sesquiterpenoids
Chloramultiol G
Multistalactones A–F

ABSTRACT

An 8,9-*seco*-lindenane disesquiterpenoid, chloramultiol G, four eudesmane sesquiterpenoids, *ent*-(3*R*)-3-hydroxyatractylenolide III and multistalactones A–C, and four guaiane sesquiterpenoids, (1*R*,4*S*,5*R*,8*S*,10*S*)-zedoalactone A and multistalactones D–F, along with 14 known compounds, were isolated from whole plant tissues of *Chloranthus multistachys*. Their structures were established by extensive NMR experiments in conjunction with mass spectrometry. Except for chloramultiol G, the absolute stereochemistries of the other eight were confirmed by single-crystal X-ray crystallography and CD spectra. Nine compounds were tested for cytotoxicity against five human tumor cell lines and for antifungal activity against four microorganisms *in vitro*, but all were inactive.

© 2012 Elsevier Ltd. All rights reserved.

1. Introduction

The genus *Chloranthus*, which includes 13 species in mainland China, has been taxonomically placed in the Chloranthaceae family (Zhou, 1993). *Chloranthus multistachys* Péi is a perennial herb that is primarily distributed over Eastern Asia and its root tissue has been used to treat bone fractures, lumbocrural pain, and pruritus in Chinese folk medicine (Jiangsu New Medical College, 1977). Recently, 16 dimeric lindenanes (Yang and Yue, 2006; Ran et al., 2010; Zhang et al., 2010a), 10 sesquiterpenoids (Zhang et al., 2010b), and nine *ent*-kaurane diterpenoids (Yang and Yue, 2008) were isolated from this species. Our interest in the identification of sesquiterpenoids from the *Chloranthus* genus (Teng et al., 2009; Ran et al., 2010; Fang et al., 2011) prompted us to further explore the chemical constituents of this title plant. Nine new sesquiterpenoids were isolated: the first 8,9-*seco*-lindenane disesquiterpenoid (**1**), four eudesmane sesquiterpenoids (**2**–**5**) and four guaiane sesquiterpenoids (**6**–**9**). In addition, 14 known compounds were identified by direct comparison with the NMR and MS spectroscopic data reported in the literature, lasianthuslactone A (**10**) (Li et al., 2006), chloraeudolide (**11**) (Wang et al., 2011), 1β,10α,4α,5β-diepoxy-6β-hydroxyglechoman-8α,12-olide (**12**) (Ei-Gamal, 2001), β-ecdysterone (**13**) (Huang et al., 2003), ajugasterone C-20,22-acetonide (**14**) (Tan et al., 2003), 24-epi-pterosterone-2,3,20,22-diacetonide (**15**) (Suksamrarn et al., 1999), 20-hydroxyecdysterone-2,3:20,22-diacetonide (**16**) (Tomas et al., 1992), rosmarinic acid (**17**) (Zeng et al., 2007), methyl rosmarinate (**18**) (Li et al., 2008a,b,c), citrusin C (**19**) (Yin et al., 2007), phenethyl-8-*O*-β-D-glucopyranoside (**20**) (Li et al., 2008a,b,c), 3,4-*O*- isopropylidene shikimic acid (**21**) (Wang et al., 2002), (**22**) (Zhu et al., 2008), and 2′-hydroxy-4,4′,6′-trimethoxychalcone (**23**) (Li et al., 2007). Herein, we report the isolation, structural determination, and bioactivities of these new sesquiterpenoids.

2. Results and discussion

Compound **1** was isolated as a yellow powder. The ESIMS spectrum showed a pseudomolecular ion peak at m/z 767 $[M+Na]^+$, and the molecular formula was established as $C_{40}H_{40}O_{14}$ by HRESIMS (767.2322 $[M+Na]^+$, calcd for 767.2315) with 21 degrees of unsaturation. The IR absorptions indicated presence of hydroxyl (3449 cm^{-1}), carbonyl (1773, 1738 cm^{-1}), and alkenyl (1637 cm^{-1}) groups. The ^1H NMR spectrum (Table 1) showed the presence of two vinylic methyl groups (δ_H 1.88 and 2.05), two tertiary methyl groups (δ_H 0.90 and 1.21), and a methoxy group (δ_H 3.66), together with two olefinic proton signals at δ_H 6.13 (d, J = 5.8 Hz) and 6.61 (t, J = 5.3 Hz) and three hydroxymethyl resonances [δ_H 4.13, 4.66 (each 1H, J = 11.5 Hz), 4.46, 4.90 (each 1H, J = 11.9 Hz), and 4.59 (1H, dd, J = 14.8, 6.5 Hz), 5.01 (ddd, J = 14.8, 6.5, 1.0 Hz)]. In accord with the molecular formula, the ^{13}C NMR spectrum (Table 1) with DEPT experiments indicated 40 carbon resonances that can be attributed to seven ester carbonyls, four methyls, one methoxy, five double bonds, eight sp^3 methylenes

* Corresponding author. Tel.: +86 871 6522 3246; fax: +86 871 6522 3245.
E-mail address: haiyangliu@mail.kib.ac.cn (H.-Y. Liu).

0031-9422/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.phytochem.2012.12.002

Table 1
^1H and ^{13}C NMR spectroscopic data for compound **1** in $CDCl_3$ (δ in ppm).

No.	δ_H (multi, *J* in Hz)	δ_C	HMBC(H → C)	ROESY
1	1.94 m	30.1	C-3, 4, 5, 9, 10	H-2α, H-3
2α	1.02 m	13.02	C-3, 4, 10	H-1, 3
2β	0.34 m		C-1, 3, 4, 10	Me-14
3	2.29 m	21.0	C-1, 4, 5, 10, 15	H-1, H-2α
4		140.2		
5		148.3		
6		115.1		
7		137.3		
8		163.1		
9		173.2		
10		54.2		
11		147.4		
12		164.9		
13	2.05 s	11.6	C-6, 7, 8, 11, 12	
14	1.21 s	19.6	C-1, 5, 9, 10	H-2β
15	6.13 d (5.8)	118.9	C-2, 3, 5, 1′, 8′, 9′, 10′	
1′	1.95 m	26.0	C-2′, 3′, 4′, 5′, 9′, 10′	H-2′α, H-3′
2′α	0.72 m	10.7	C-1′, 3′, 4′, 10′	H-1′, 3′
2′β	1.29 m		C-1′, 3′, 4′, 10′	Me-14′
3′	1.56 m	28.9	C-1′, 2′, 4′, 5′, 10′, 15′	H-1′, H-2′α, H_2-15′
4′		77.5		
5′	2.54 m	58.0	C-1′, 3′, 4′, 6′, 7′, 9′, 10′, 14′, 15′	H-1′, H-3′, H_2-15′
6′α	2.58 m	23.4	C-4′, 5′, 8′, 10′, 11′, 12′	
6′β	2.67 m		C-4′, 8′, 11′, 12′	Me-14′
7′		170.9		
8′		87.7		
9′	2.60 m	53.5	C-4, 5, 6, 15, 1′, 5′, 7′, 8′, 10′, 14′	Me-14′
10′		47.9		
11′		123.8		
12′		173.0		
13′a	4.90 d (11.9)	54.5	C-7′, 11′, 12′	
13′b	4.46 d (11.9)		C-7′, 11′, 12′	
14′	0.90 s	24.6	C-1′, 2′, 5′, 9′, 10′	H-2β
15′a	4.66 d (11.5)	73.1	C-3′, 4′, 5′, 10′, 1″	H-3′
15′b	4.13 d (11.5)		C-3′, 5′, 1″	H-3′
1″		167.3		
2″		129.4		
3″	6.61 t (5.3)	135.5	C-1″, 2″, 5″	
4″a	5.01 ddd (14.8, 6.5, 1.0)	61.5	C-1″, 2″, 3″, 5″, 6″	H-5″
4″b	4.59 dd (14.8, 6.5)		C-1″, 2″, 3″, 6″	H-5″
5″	1.88 s	12.9	C-1″, 2″, 3″	H_2-4″
6″		171.6		
7″	2.68 m, 2.51 m	28.6	C-6″, 8″, 9″	
8″	2.60 m, 2.56 m	28.8	C-6″, 7″, 9″	
9″		172.0		
OMe	3.66 s	52.8	C-9	

(three oxygenated carbons), six sp^3 methines, and four sp^3 quaternary carbons (two oxygenated carbons), respectively. These functionalities accounted for 12 degrees of unsaturation, and the remaining nine degrees of unsaturation required that **1** be nonacyclic. The presence of two proton spin systems from 1,2-substituted cyclopropane ring moieties, which were established by analogs of the ^1H–^1H COSY spectrum (H-1: δ_H 1.94; H_α-2: δ_H 1.02; H_β-2: δ_H 0.34; H-3: δ_H 2.29 and H-1′: δ_H 1.95; H_α-2′: δ_H 0.72; H_β-2′: δ_H 1.29; H-3′: δ_H 1.56) (Fig. 2), was distinctive for a lindenane-sesquiterpenoid dimer (Kawabata and Mizutani, 1992). The aforementioned spectroscopic features were similar to those of chloramultiol F (Ran et al., 2010), except for the absence of an oxymethine and a hemiacetal carbon and the presence of two additional ester carbonyls at δ_C 163.1 and 173.2. Thus, it is supposed that compound **1** was a 8,9-*seco* derivate of chloramutiol F, which was confirmed by the HMBC spectrum. In the HMBC spectrum (Fig. 2), the long-range correlations of the carbonyl (δ_C 173.2) with H-1 (δ_H 1.94, m), Me-14 (δ_H 1.21, s), and a methoxy group (δ_H 3.66) indicated the existence of the COOMe moiety, which was attached to C-10 of **1**. The HMBC correlations from Me-13 (δ_H 2.05) to C-6 (δ_C 115.1), C-7 (δ_C 137.3), C-8 (δ_C 163.1), C-11 (δ_C 147.4), and C-12 (δ_C 164.9) implied the existence of a 2-methylmaleic anhydride group in compound **1**, which was established to be on C-6. ROESY correlations (Fig. 2) suggested the same relative configuration as that of chloramutiol F. Based on all the information above, the structure of **1** was determined to be that shown, and the compound was named chloramultiol G (Fig. 1).

Compound **2** gave a quasi-molecular ion at *m*/*z* 287.1255 $[M+Na]^+$ (calcd 287.1259) in the positive-ion HRESIMS, which was consistent with a molecular formula of $C_{15}H_{20}O_4$, possessing 6 degrees of unsaturation. The ^1H NMR spectrum (Table 2) showed the presence of two methyl singlets at $_H$ 1.01 (s) and 1.79 (s), two exo-methylene protons at δ_H 4.77 (s) and 5.07 (s), and an oxymethine proton at δ_H 4.25 (m). The ^{13}C NMR spectrum (Table 3) showed 15 carbon signals that correspond to an eudesm-7(11)-en-12,8-olide sesquiterpenoid containing one ester carbonyl (δ_C 174.4), one terminal double bond, one tetrasubstituted double bond, an acetal (δ_C 105.5), two methyls, four methylenes, two methines (one oxygenated), and one quaternary carbon, respectively. These data strongly resembled those of (3*S*)-3-hydroxyatractylenolide III (Kitajima et al., 2003) and (3*R*)-3-hydroxyatractylenolide III (Xu et al., 2010), which suggested that these three compounds shared the same skeleton. However, compound **2** possessed a negative optical rotation value ($[\alpha]_D^{25}$ −206.4 (*c*

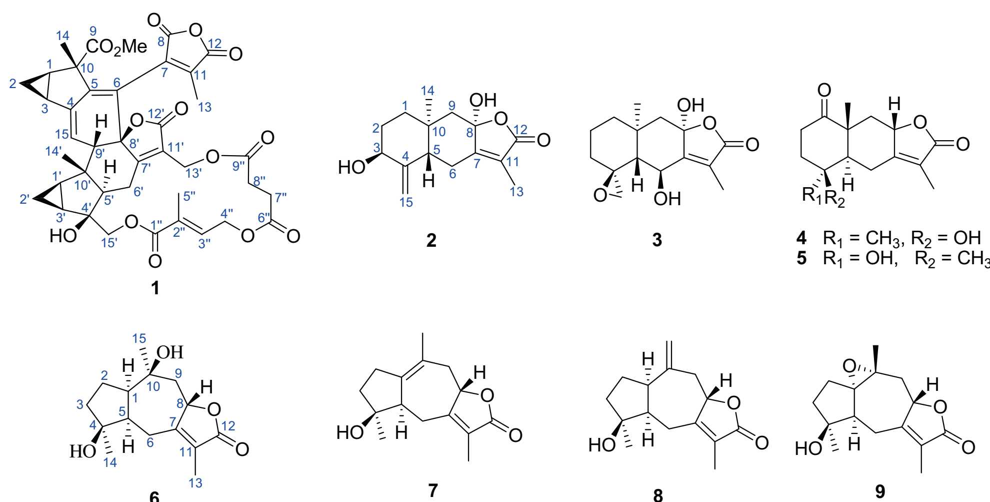


Fig. 1. Structures of compounds **1**–**9**.

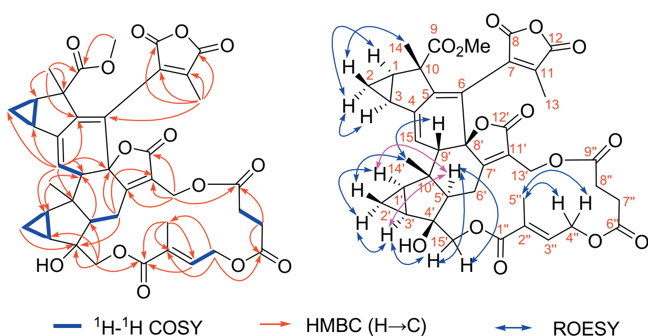


Fig. 2. Selected 2D NMR correlations of **1**.

0.12, MeOH)), but (3*S*)-3-hydroxyatractylenolide III possessed a positive optical rotation value ($[\alpha]_D^{22}$ +95 (*c* 1.1, MeOH)). To assess the structure and to determine its absolute configuration, compound **2** was crystallized from acetone to afford a crystal of the stylolitic space group $P2_12_12_1$, which was analyzed by X-ray crystallography (Fig. 3). The final refinement of the Cu Kα data resulted in a Flack parameter of 0.0 (4) (Flack and Bernardinelli, 2000), which allowed unambiguous assignment of the absolute configuration of **2** to be (3*S*,5*S*,8*R*,10*S*). Furthermore, the absolute configuration of **2** was confirmed using the empirical rule for the α,β-unsaturated γ-lactone in CD spectrum. The negative Cotton effects, observed at 213 nm and 240 nm, substantiated the absolute configuration of C-8 to be *R* (Snatzke, 1968; Bodensieck et al., 2010). Therefore, compound **2** was an enantiomer of (3*R*)-3-hydroxyatractylenolide III and was named *ent*-(3*R*)-3-hydroxyatractylenolide III.

Compound **3**, obtained as a yellow powder, had a molecular formula of $C_{15}H_{20}O_5$ as determined by HRESIMS with a *m*/*z* 303.1207 $[M+Na]^+$ (calc 303.1208). The ^{1}H and ^{13}C NMR spectra were similar to those of **2**, except for the presence of an 1,1-substituted oxacyclopropane ring [δ_H 2.79 and 3.10 (each 1H, *J* = 11.8 Hz); δ_C 51.5 (t) and 62.3 (s)] and the disappearance of a $\Delta^{4,15}$ double bond. The HMBC correlations of δ_H 2.79 and 3.10 (H_2-15) with C-3 (δ_C 35.2), C-4 (δ_C 62.3), and C-5 (δ_C 53.6) implied that the epoxide ring was located between C-4 and C-15. The ^{1}H–^{1}H COSY correlation of the oxymethine proton at δ_H 4.56 with the methine proton at δ_H 1.79 (H-5) and the HMBC correlations from the oxymethine proton at δ_H 4.56 to C-4 (δ_C 62.3), C-5 (δ_C 53.6), C-7 (δ_C 158.7), C-8 (δ_C 103.2), C-10 (δ_C 37.3), and C-11 (δ_C 123.7) suggested that the oxymethine is shifted from C-3 in compound **2** to C-6 in compound **3**. The ROESY correlations of Me-14/H-6, Me-14/H-9α, Me-14/H_2-15, and H-9β/H-5 established the H-6, H_2-15, and Me-17 of α-orientation while H-5 of β-orientation. The similar patterns of Cotton effects in the CD spectra of compounds **2** and **3** indicated that the absolute configuration at C-8 in **3** was identical to that of **2**. Thus, compound **3** was determined to be (4*R*,5*R*,6*S*,8*R*,10*S*)-6,8-dihydroxy-4,15-epoxy-eudes-7(11)-en-12,8-olide, named multistalactone A.

Compound **4** was obtained as colorless square crystals (acetone). The HRESIMS of **4** exhibited a quasi-molecular ion at *m*/*z* 265.1447 $[M+H]^+$ (calcd 265.1439) from a molecular formula of $C_{15}H_{20}O_4$. Comparison of the NMR spectroscopic data of **4** with those of 1β,4β-dihydroxyeudesm-7(11)-en-12,8α-olide (Yang et al., 2007) highlighted one difference between the two compounds to be the presence of a carbonyl group (δ_C 212.9) in **4** instead of an oxymethine. The carbonyl group located at C-1 was indicated by the HMBC correlations of Me-14 (δ_H 1.46) with C-1 (δ_C 212.9), C-5 (δ_C 52.2), C-9 (δ_C 41.3), and C-10 (δ_C 48.2). The oxygenated quaternary carbon was assigned to C-4 from the HMBC correlations of Me-15 ($_H$ 1.36) to C-3 (δ_C 41.0), C-4 (δ_C 70.6), and C-5 (δ_C 52.2). A single crystal of **4** was obtained, and X-ray diffraction analysis (Fig. 3) was conducted using an anomalous dispersion with copper radiation that resulted in a Flack parameter of 0.1 (2) (Flack and Bernardinelli, 2000). This analysis indicated the absolute stereochemistry of **4** to be 4*S*,5*R*,8*S*,10*R* as shown in Fig. 1. The structure of **4** was thus elucidated as (4*S*,5*R*,8*S*,10*R*)-4-hydroxy-1-oxoeudesm-7(11)-en-12,8-olide, named multistalactone B. Its CD spectrum displayed the positive Cotton effects at 237 nm and 291 nm.

Compound **5** had the same molecular formula ($C_{15}H_{20}O_4$) as that of **4**, and their NMR (Tables 2 and 3), UV, CD, and IR data displayed high similarity, suggestive of isomers. Comparison of the ^{1}H and ^{13}C NMR spectroscopic data of **5** with those of **4** hinted that the main structural difference was the configuration at C-4 as judged from the obvious changes of chemical shifts of the carbons around C-4. The key ROESY cross-peak from H-6β to Me-15 suggested that HO-4 is in the α-orientation. Therefore, compound **5** was demonstrated to be (4*R*,5*R*,8*S*,10*R*)-4-hydroxy-1-oxoeudesm-7(11)-en-12,8-olide, named multistalactone C.

Compound **6** was obtained as a white powder with a molecular formula of $C_{15}H_{22}O_4$, as shown by HRESIMS at *m*/*z* 289.1420 $[M+Na]^+$ (calcd 289.1415). The ^{1}H NMR spectrum of **6** displayed

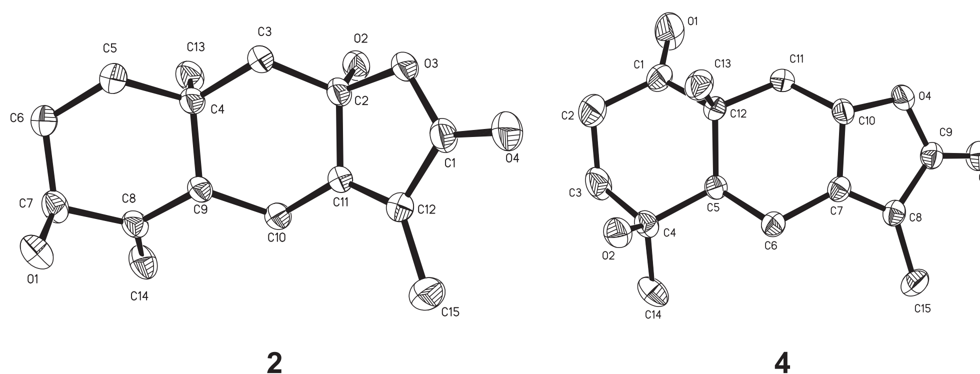


Fig. 3. X-ray crystal structures of compounds **2** and **4**.

Table 2
^{1}H NMR spectroscopic data for compounds **2**–**9** (δ in ppm, J in Hz).

No.	**2**[a,b]	**3**[a,d]	**4**[c,d]	**5**[a,e,]	**6**[a,b]	**7**[a,d]	**8**[c,d]	**9**[a,d]
1α	1.67 m	1.51 m			2.21 m		3.06 m	
1β	1.32 m	1.15 m						
2α	1.73 m	1.66 m (2H)	2.19 m	2.43 m	1.76 m (2H)	2.35 m	1.73 m	1.91 m
2β	1.82 m		3.14 m	2.69 m		2.51 m	2.00 m	1.66 m
3α	4.25 d (2.4)	1.86 m	1.84 m	1.88 m	1.67 m (2H)	1.73 m (2H)		1.81 m
3β		1.26 m	2.05 m	2.10 m			1.81 m (2H)	2.12 m
4								
5	2.39 m	1.79 d (10.8)	1.49 m	1.79 m	1.89 m		2.01 m	1.50 d (13.4)
6α	2.40 m	4.56 d (10.8)	2.63 d (13.6)	2.36 t (14.0)	2.69 m	2.84 dd (12.9, 1.3)	2.61 m	2.69 d (14.0)
6β	2.62 m		2.87 dd (14.2, 3.4)	3.10 dd (14.0, 3.3)	2.63 t (12.7)	1.74 m	1.84 m	1.84 m
8			4.84 dd (11.3, 6.3)	4.77 dd (11.0, 6.0)	5.10 m	4.49 d (11.4)	4.61 dd (9.4, 7.4)	4.64 d (12.9)
9α	2.22 d (13.4)	1.43 d (13.8)	2.55 m	2.62 dd (12.8, 6.1)	1.39 dd (13.3, 6.2)	2.42 d (13.2)	1.94 dd (9.1, 3.5)	1.36 t (13.3)
9β	1.49 d (13.4)	2.14 d (13.8)	1.22 t (12.3)	1.23 t (12.3)	2.52 dd (13.2, 10.9)	2.56 d (13.3, 3.6)	3.00 dd (12.1, 6.1)	2.57 dd (12.9, 2.3)
13	1.79 s	1.92 s	1.81 s	1.82 s	1.77 s	1.82 s	1.76 s	1.70 s
14	1.01 s	1.08 s	1.46 s	1.29 s	1.25 s	1.39 s	1.28 s	1.32 s
15a	5.07 s	3.10 d (11.8)	1.36 s	1.45 s	1.22 s	1.75 s	5.01 s	1.32 s
15b	4.77 s	2.79 d (11.8)					5.07 s	

[a] Recorded on 500 MHz.
[b] Measured in CD_3OD.
[c] Recorded on 400 MHz.
[d] Measured in $CDCl_3$.
[e] Measured in $CDCl_3$–CD_3OD.

Table 3
^{13}C NMR spectroscopic data for compounds **2**–**9** (δ in ppm).

No.	**2**[a,b]	**3**[c,d]	**4**[c,d]	**5**[c,e]	**6**[a,b]	**7**[a,d]	**8**[c,d]	**9**[c,d]
1	36.5	40.5	212.9	212.3	51.4	143.4	47.0	74.0
2	30.1	20.2	33.7	34.8	26.3	28.7	26.7	36.4
3	73.4	35.2	41.0	40.0	40.6	38.7	39.3	27.5
4	151.5	62.3	70.6	70.1	81.9	81.3	81.4	81.5
5	47.1	53.6	52.2	53.2	51.3	51.7	52.2	53.8
6	25.1	68.3	22.6	22.4	25.1	29.8	24.6	26.8
7	163.1	158.7	161.4	161.6	167.9	163.2	163.7	161.2
8	105.5	103.2	78.4	78.1	82.7	79.2	82.7	79.0
9	52.0	50.9	41.3	42.4	47.5	39.5	41.0	41.5
10	37.7	37.3	48.2	46.9	72.2	123.9	143.1	56.8
11	122.5	123.7	120.8	120.5	121.5	121.7	122.0	122.6
12	174.4	172.3	174.5	175.0	177.4	174.1	174.8	173.8
13	8.1	8.9	8.2	8.0	7.8	8.3	8.0	8.2
14	16.4	18.8	19.0	18.0	23.6	24.2	24.4	23.7
15	110.0	51.5	29.3	22.8	31.1	21.9	115.8	20.4

[a] Recorded on 125 MHz.
[b] Measured in CD_3OD.
[c] Recorded on 100 MHz.
[d] Measured in $CDCl_3$.
[e] Measured in $CDCl_3$–CD_3OD.

signals for three methyl groups at δ_H 1.22 (s), 1.25 (s), and 1.77 (s). The ^{13}C NMR spectrum showed 15 carbon resonances which were classified by DEPT experiments as three methyls, four methylenes, three methines (one oxygenated at δ_C 82.7), two oxygenated quaternary carbons (δ_C 72.2 and 81.9), two olefinic carbons (δ_C 121.5 and 167.9), and an ester carbonyl carbon (δ_C 177.4). These functionalities accounted for two of the five degrees of unsaturation, and the three remaining degrees of unsaturation required compound **6** to be tricyclic. The aforementioned spectroscopic features revealed that compound **6** was a guaiane sesquiterpenoid similar to zedoalactone A (Takano et al., 1995) and *ent*-zedoalactone A (Blay et al., 2000). Compound **6** possessed an $[\alpha]_D^{16}$ in methanol of +22.9, whereas the reported value for zedoalactone A was −34.4. Though both compound **6** and *ent*-zedoalactone A exhibited a positive optical rotation value, the configurations of OH-4 and OH-10 were opposite according to the ROESY experiment. In the ROESY spectrum, the correlations of H-1/H-5, H-1/Me-15, H-8/H-9β, H-1/H-9α, and H-5/Me-14 indicated that OH-4 and OH-10 were β-orientation. The CD curve of **6** exhibited a positive Cotton effect (240 nm, MeOH) that was attributed to the n–π* transition of an α,β-unsaturated γ-lactone, whereas a negative Cotton effect (218 nm, MeOH) was attributed to the π–π* transition of an α,β-unsaturated γ-lactone (Uchida and Kuriyama, 1974). Thus, the absolute configuration at C-8 was determined to be *S*. In conclusion, structure **6** was elucidated as (1*R*,4*S*,5*R*,8*S*,10*S*)-4,10-dihydroxy-guaia-7(11)-en-12,8-olide, named (1*R*,4*S*,5*R*,8*S*,10*S*)-zedoalactone A.

Compound **7** was determined to be $C_{15}H_{20}O_3$ with six degrees of unsaturation by HRESIMS at *m/z* 249.1497 $[M+H]^+$ (calcd 249.1490). Comparison of the ^1H and ^{13}C NMR spectra of **7** with those of **6** indicated that **7** differed from **6** by the presence of an additional tetrasubstituted double bond (δ_C 123.9 and 143.4) instead of the oxygenated quaternary carbon (δ_C 72.2) and methine (δ_C 51.4) functionalities of **6**. The HMBC correlations of Me-15 with C-1, C-9, and C-10 and H-5 with C-1, C-2, and C-10 suggested that the additional double bond was placed between C-1 and C-10. On the basis of the above information, the structure **7** was deduced. ROESY correlations suggested the same relative configuration as **6**. The absolute configuration of C-8 was expected to be the same as that of **6** from the positive Cotton effect at 255 nm and the negative Cotton effect at 218 nm in the CD spectrum. Consequently, the structure of **7** was determined to be (4*S*,5*R*,8*S*)-4-hydroxyguaia-1(10),7(11)-dien-12,8-olide, named multistalactone D.

Compound **8** was a double bond isomer of **7**, as deduced by the same molecular formula of $C_{15}H_{20}O_3$, which was established from the HRESIMS by an *m/z* 249.1491 $[M+H]^+$ (calcd 249.1490) and the NMR spectroscopic data, which showed the presence of a terminal double bond (δ_H 5.01 and 5.07; δ_C 115.8 and 143.1) and a methine (δ_H 3.06; δ_C 47.0) and the absence of a tetra-substituted double bond and a methyl. The position of the C(10) = C(15) bond was deduced from the HMBC correlations of H_2-15 (δ_H 5.01 and 5.07) with C-1, C-9, and C-10 and H-1 with C-2, C-3, C-4, C-5, C-6, C-9, C-10 and C-15. The ROESY experiment indicated that compound **8** had the same relative configuration as compound **7**. The positive Cotton effect at 241 nm and negative Cotton effect at 222 nm suggested the absolute configuration at the 8-position as *S*. Thus, the structure of **8** was elucidated as (1*R*,4*S*,5*R*,8*S*)-4-hydroxyguaia-7(11),10(15)-dien-12,8-olide, named multistalactone E.

Compound **9** showed a quasi-molecular ion $[M+H]^+$ at *m/z* 265.1446 in the HRESIMS, which was compatible with a molecular formula of $C_{15}H_{20}O_4$ (six degrees of unsaturation). The ^1H and ^{13}C NMR spectra of **9** were similar to those of **6**, except for the absence of a methine and the presence of an oxygenated quaternary carbon (δ_C 74.0), which indicated that **9** was the 1,10-epoxidized analog of **6**. This conclusion was supported by the fact that **9** had one more degree of unsaturation and shows HMBC correlations from Me-15 to C-1, C-9, and C-10. The similar patterns of Cotton effects in the CD spectra of **6**, **7**, **8**, and **9** indicated that the absolute configuration of C-8 in compound **9** was identical to that of **6**, **7**, and **8**. So, the structure of **9** was assigned as (1*S*,4*S*,5*S*,8*S*,10*R*)-1,10-epoxy-4-hydroxyguaia-7(11)-en-12,8-olide, named multistalactone F.

Because some disesquiterpenoids and sesquiterpenoids isolated from *Chloranthus* have been reported to possess a wide range of cytotoxic activity against various cancer cell lines (Wu et al. 2007; Li et al., 2008a), the cytotoxicity of compounds **1**–**9** was tested against five tumor cell lines (HL-60, SMMC-7721, A-549, SK-BR-3, and PANC-1) with cisplatin as the positive control (Mosmann, 1983). However, none of these compounds showed any remarkable activity ($IC_{50} > 40$ μM). Furthermore, compounds **1**–**9** were also tested for their antifungal activity against four microorganisms (ATCC 25923, *Candida albicans*, *Pseudomonas aeruginosa*, and *Escherichia coli*) based on the literature (Xu et al., 2007; Yim et al., 2008; Lee et al., 2009) with vancomycin hydrochloride as positive control. Unfortunately, none of them was active.

3. Concluding remarks

The investigation of the extract of the whole plant of *C. multistachys* led to the identification of one lindenane disesquiterpenoid, six eudesmane sesquiterpenoids, four guaiane sesquiterpenoids, and one germacrane sesquiterpenoid, which included nine new structures, as well as four ecdysterone derivates and seven phenoloids. Compound **1** is the first 8,9-*seco* lindenane disesquiterpenoid that seems to be derived via the enzymatic *endo*-Diels–Alder cycloaddition of two lindenanes and an enzymatic Baeyer–Villiger oxidation. The discovery of compound **1** is a further addition to the diverse and complex class of lindenane disesquiterpenoids. The absolute stereochemistries of compounds **2**–**9** were determined by single-crystal X-ray crystallography via an anomalous dispersion with copper radiation and CD analysis. In addition, this is the first report on the isolation of ecdysterone derivates from this genus.

4. Experimental

4.1. General experimental procedures

Optical rotations were recorded on an Horiba SEAP-300 polarimeter. UV spectra were obtained on a Shimadzu UV-2401PC spectrophotometer. CD spectra were recorded with an Applied Photophysics Chirascan spectrometer. IR spectra were measured with a Bio-Rad FTS-135 spectrometer with KBr pellets. The 1D and 2D NMR spectra were measured on a Bruker AM-400 or DRX-500 spectrometer using TMS as the internal standard. FAB-MS spectra were recorded on a VG Auto Spec-300 spectrometer, and ESIMS and HRESIMS were performed on an APIQSTAR TOF spectrometer. Column chromatography (CC) was performed on silica gel (48–75 μm or 10–40 μm; Qingdao Marine Chemical Inc., 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 (40–70 μm, Amersham Pharmacia Biotech AB, Uppsala, 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.

4.2. Plant material

Whole plants of *C. multistachys* were collected in August 2007 from Xinning County, Hunan Province, People's Republic of China and identified by Dr. En-De Liu of the Kunming Institute of Botany. A voucher specimen (No. HY0001) has been deposited at the State Key Laboratory of Phytochemistry and Plant Resources in West China.

4.3. Extraction and isolation

The air-dried powder of the plant material (20 kg) was extracted three times with MeOH (90 L × 3) under conditions of reflux to give a crude extract, which was suspended in H_2O and partitioned successively with EtOAc and *n*-BuOH. The EtOAc-soluble fraction (1300 g) was subjected to an MCI gel column with MeOH–H_2O (A:B) as the gradient (A:B, 3:7 → 5:5 → 7:3 → 1:0) to give three fractions, 1–3. Fraction 1 (890 g, combined 30% and 50% MeOH fractions from MCI gel CC) was separated on a silica gel column (petroleum ether–acetone, from 10:1 to 1:1) to yield four fractions, A–D. Fraction D was applied to a silica gel column (petroleum ether–EtOAc, from 5:1 to 1:2) to afford seven fractions, D1–D7. Fraction D1 (40.0 g) was first subjected to an Rp-18 column eluted with a MeOH–H_2O (A:B) gradient system (A:B from 3:7 to 6:4) to obtain three fractions (D1a–D1c). Subsequently, fraction D1a (8.0 g) was subjected to a silica gel CC using $CHCl_3$–MeOH (from 150:1 to 10:1) as the eluent to provide fractions D1a1–D1a5. Fraction D1a1 (0.9 g) was purified on silica gel (petroleum ether–acetone, 80:1 to 10:1) and Sephadex LH-20 column (MeOH) to yield **4** (65 mg) and **7** (15 mg). Fraction D1a2 (0.8 g) was

subjected to silica gel CC (petroleum ether–$CHCl_3$, 5:1) to yield **22** (35 mg). Compounds **5** (12 mg) and **12** (30 mg) were isolated from fraction D1a3 (1.1 g) by silica gel CC (petroleum ether–AcOEt, 80:1 to 10:1) and Sephadex LH-20 column (MeOH). Fraction D1a4 (1.7 g) was purified on a silica gel column (petroleum ether–acetone, 50:1 to 10:1) to give **2** (110 mg), **6** (10 mg), and **10** (15 mg); Fraction D1a5 (3.2 g) was subjected to silica gel CC (petroleum ether–AcOEt, 10:1) to yield **23** (30 mg). Fraction D2 (80.1 g) was applied to a silica gel column using $CHCl_3$–MeOH (A:B) (from A:B, 150:1 to 10:1) as the eluent to provide six fractions D2a–D2f. Compounds **1** (13 mg), **3** (25 mg), **8** (8 mg), and **9** (25 mg) were obtained from fraction D2b (5.2 g) by repeated purifications of silica gel CC (petroleum ether–AcOEt, from 50:1 to 10:1) and Sephadex LH-20 column (MeOH). Fraction D2e (16.2 g) was subjected to an Rp-18 column using a stepwise gradient-elution technique with mixtures of MeOH–H_2O (A:B) (A:B from 30:70 to 60:40) as the mobile phase, washed with MeOH, and grouped into five fractions (D2e1–D2e5). Fraction D2e1 (1.1 g) was separated using a silica gel column (petroleum ether–acetone, 50:1 to 1:1) and purified further by a Sephadex LH-20 (MeOH) column to afford **11** (15 mg). Fraction D4 (45.5 g) was subjected to an Rp-18 column (MeOH–H_2O) (A:B) (A:B from 30:70 to 60:40) to provide six fractions D4a–D4f. Fraction D4b (1.3 g) was separated on a silica gel column (petroleum ether–acetone, 10:1) and further purified on a Sephadex LH-20 column (MeOH) to provide **18** (25 mg). Fraction D7 (50.0 g) was subjected to Rp-18 CC eluted with a MeOH–H_2O (A:B) gradient system (A:B, 30:70; 40:60; 50:50; and 60:40) to obtain seven fractions, D7a–D7g. Compounds **15** (32 mg) and **16** (60 mg) were obtained from fraction D7b (85 mg) and D7c (1.5 g) by silica gel CC eluted with petroleum ether–acetone (20:1 → 0:1). Compound **21** (28 mg) was purified from D7e (0.65 g) on a Sephadex LH-20 (MeOH) column. Fraction D7f (1.1 g) was separated on a silica gel column (petroleum ether–acetone, 15:1 to 1:1) and purified with a Sephadex LH-20 (MeOH) column to afford **14** (28 mg). Then, fraction D7g (38.2 g) was applied to a silica gel column using $CHCl_3$–MeOH (from 50:1 to 10:1) as the eluent to provide fractions D7g1–D7g7. Fraction D7g3 (1.0 g) was separated on a silica gel column (petroleum ether–EtOAc, 6:1 to 0:1) and purified using a Sephadex LH-20 (MeOH) column to provide **20** (35 mg). Compound **17** (800 mg) was destained from D7g5 (1.8 g) on a Sephadex LH-20 (MeOH) column. Fraction D7g6 (3.5 g) was separated on a silica gel column ($CHCl_3$–MeOH, 20:1 to 1:1) and purified using a Sephadex LH-20 (MeOH) column to afford **13** (35 mg) and **19** (1.0 g).

*4.3.1. Chloramultiol G (**1**)*

Yellow powder: $[\alpha]_D^{20}$ −128.8 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 212 (4.50); IR (KBr) ν_{max} 3449, 2939, 1773, 1738, 1637, 1383, 1266, 1164 cm^{-1}; for ^1H and ^{13}C NMR spectroscopic data, see Table 1; positive ESIMS *m/z* 767 $[M+Na]^+$; HRESIMS *m/z* 767.2322 $[M+Na]^+$ (calcd for $C_{40}H_{40}O_{14}Na$, 767.2315).

*4.3.2. ent-(3R)-3-Hydroxyatractylenolide III (**2**)*

Colorless stylolitic crystals (Me_2CO): $[\alpha]_D^{25}$ −206.4 (*c* 0.12, MeOH); UV (MeOH) λ_{max} (log ε):219 (3.95); CD (*c* 4.60×10^{-4} M, MeOH): 213 nm ($\Delta\varepsilon$ = −12.76), 240 nm ($\Delta\varepsilon$ = −13.80); IR (KBr) ν_{max} 3431, 3241, 2925, 2855, 1740, 1697, 1655, 1422, 1384, 1219, 1011, 942, 918 cm^{-1}; for ^1H and ^{13}C NMR spectroscopic data, see Tables 2 and 3. ESIMS *m/z*: 287 $[M+Na]^+$; HRESIMS *m/z*: 287.1255 $[M+Na]^+$ (calcd for $C_{15}H_{20}O_4Na$, 287.1259).

*4.3.3. Multistalactone A (**3**)*

Yellow powder: $[\alpha]_D^{25}$ −126.5 (*c* 0.44, MeOH); UV (MeOH) λ_{max} (log ε):220 (4.06); CD (*c* 1.04×10^{-3} M, MeOH): 214 nm ($\Delta\varepsilon$ = −9.69), 242 nm ($\Delta\varepsilon$ = −10.30); IR (KBr) ν_{max} 3415, 2941, 2925, 2873, 1760, 1698, 1447, 1383, 1162, 1079 cm^{-1}; for ^1H and ^{13}C NMR ($CDCl_3$, 100 MHz) spectroscopic data, see Tables 2 and 3; ESIMS *m/z*: 303 $[M+Na]^+$; HRESIMS *m/z*: 303.1207 $[M+Na]^+$ (calcd for $C_{15}H_{20}O_5Na$, 303.1208).

*4.3.4. Multistalactone B (**4**)*

Colorless lumpish crystals (Me_2CO): $[\alpha]_D^{25}$ +154.6 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε): 220 (4.17); CD (*c* 5.98×10^{-4} M, MeOH): 237 nm ($\Delta\varepsilon$ = +6.33), 291 nm ($\Delta\varepsilon$ = +2.02); IR (KBr) ν_{max} 3464, 2925,1731, 1673, 1445, 1384, 1114, 1014 cm^{-1}; for ^1H and ^{13}C NMR ($CDCl_3$, 100 MHz) spectroscopic data, see Tables 2 and 3. FABMS *m/z*: 265 $[M+H]^+$; HRESIMS *m/z*: 265.1447 $[M+H]^+$ (calcd for $C_{15}H_{21}O_4$, 265.1439).

*4.3.5. Multistalactone C (**5**)*

White powder: $[\alpha]_D^{16}$ +91.0 (*c* 0.21, MeOH); UV (MeOH) λ_{max} (log ε): 219 (4.21); CD (*c* 7.58×10^{-4} M, MeOH): 236 nm ($\Delta\varepsilon$ = +5.26), 294 nm ($\Delta\varepsilon$ = +0.37); IR (KBr) ν_{max} 3478, 2937, 2845, 1735, 1680, 1464, 1362, 1173, 1032 cm^{-1}; for ^1H and ^{13}C NMR spectroscopic data, see Tables 2 and 3; ESIMS *m/z*: 265 $[M+H]^+$; HRESIMS *m/z*: 265.1638 $[M+H]^+$ (calcd for $C_{15}H_{21}O_4$, 265.1439).

*4.3.6. (1R,4S,5R,8S,10S)-Zedoalactone A (**6**)*

White powder: $[\alpha]_D^{16}$ +22.9 (*c* 0.11, MeOH); UV (MeOH) λ_{max} (log ε): 220 (4.06); CD (*c* $8{,}12 \times 10^{-4}$ M, MeOH): 218 nm ($\Delta\varepsilon$ = −5.69), 240 nm ($\Delta\varepsilon$ = +4.30); IR (KBr) ν_{max} 3397, 2927, 2863, 1738, 1674, 1454, 1376, 1089, 1018 cm^{-1}; for ^1H and ^{13}C NMR spectroscopic data, see Tables 2 and 3; ESIMS *m/z*: 289 $[M+Na]^+$; HRESIMS *m/z*: 289.1420 $[M+Na]^+$ (calcd for $C_{15}H_{22}O_4Na$, 289.1415).

*4.3.7. Multistalactone D (**7**)*

Yellow powder: $[\alpha]_D^{25}$ −11.6 (*c* 0.27, MeOH); UV (MeOH) λ_{max} (log ε): 208 (4.22); CD (*c* 1.01×10^{-3} M, MeOH): 218 nm ($\Delta\varepsilon$ = −6.42), 255 nm ($\Delta\varepsilon$ = +3.75); IR (KBr) ν_{max} 3450, 2959, 2858, 1734, 1667, 1441, 1371, 1110, 1019 cm^{-1}; for ^1H and ^{13}C NMR spectroscopic data, see Tables 2 and 3; EIMS *m/z*: 249 $[M+H]^+$; HRESIMS *m/z*: 249.1497 $[M+H]^+$ (calcd for $C_{15}H_{21}O_3$, 249.1490).

*4.3.8. Multistalactone E (**8**)*

Yellow powder: $[\alpha]_D^{25}$ +38.7 (*c* 0.88, MeOH); UV (MeOH) λ_{max} (log ε): 219 (4.17); CD (*c* 1.01×10^{-3} M, MeOH): 222 nm ($\Delta\varepsilon$ = −1.17), 241 nm ($\Delta\varepsilon$ = +2.22); IR (KBr) ν_{max} 3432, 2933, 2870, 1738, 1675, 1450, 1387, 1105, 1029 cm^{-1}; for ^1H and ^{13}C NMR spectroscopic data, see Tables 2 and 3; ESIMS *m/z*: 271 $[M+Na]^+$; HRESIMS *m/z*: 249.1491 $[M+H]^+$ (calcd for $C_{15}H_{21}O_3$, 249.1490).

*4.3.9. Multistalactone F (**9**)*

White powder: $[\alpha]_D^{26}$ −0.4 (*c* 0.09, MeOH); UV (MeOH) λ_{max} (log ε): 219 (4.15); CD (*c* 1.33×10^{-3} M, MeOH): 203 nm ($\Delta\varepsilon$ = +1.15), 218 nm ($\Delta\varepsilon$ = −1.94), 239 nm ($\Delta\varepsilon$ = +4.56); IR (KBr) ν_{max} 3443, 2935, 2876, 1753, 1689, 1642, 1451, 1383, 1099, 1027 cm^{-1}; for ^1H and ^{13}C NMR spectroscopic data, see Tables 2 and 3; FABMS *m/z*: 265 $[M+H]^+$; HRESIMS *m/z*: 265.1446 $[M+H]^+$ (calcd for $C_{15}H_{21}O_4$, 265.1439).

*4.3.10. X-ray structure determinations and crystal structures of **2** and **4***

Diffraction intensity data were collected with a Rigaku Micro Max 002+ diffractometer employing graphite monochromated Cu Kα radiation and operating in the −κ scan mode. The crystal structure was solved by the direct method 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.

*Crystal data for compound **2**:* $C_{15}H_{20}O_4$, MW = 264.32; orthorhombic system, space group $P2_12_12_1$; crystal cell parameters a = 9.760(5) Å, b = 10.51(1) Å, c = 13.217(7) Å, V = 1355.8(16) Å^3, Z = 4, d = 1.295 g/cm^3. A crystal of dimensions 0.30 × 0.50 × 0.70 mm was used for the X-ray measurements (2_{max} = 141.62°). The total number of independent reflections measured was 2387, of which 2015 were observed ($|F|^2 \geqslant 2|F|^2$). The final indices were R_1 = 0.0543, wR_2 = 0.1223, and S = 1.033. The absolute structure could be determined properly giving a Flack parameter of 0.0(4).

*Crystal data for compound **4**:* $C_{15}H_{20}O_4$, MW = 264.32; orthorhombic system, space group $P2_12_12_1$; crystal cell parameters a = 9.257(4) Å, b = 11.034(5) Å, c = 13.416(2) Å, V = 1370.3(9) Å^3, Z = 4, d = 1.281 g/cm^3. A crystal of dimensions 0.48 × 0.68 × 0.84 mm was used for the X-ray measurements (2_{max} = 143.90°). The total number of independent reflections measured was 2585, of which 2371 were observed ($|F|^2 \geqslant 2|F|^2$).The final indices were R_1 = 0.0475, wR_2 = 0.1254 and S = 1.051. The absolute structure could be determined properly given a Flack parameter of 0.1(2).

4.4. Cytotoxicity bioassays

Cytotoxicity of compounds against HL-60 (human promyelocytic leukemia), SMMC-7721 (human hepatocellular carcinoma), A-549 (human lung adenocarcinoma), SK-BR-3 (human breast carcinomas), and PANC-1 (human pancreatic carcinoma, epithelial-like) cells was determined by the MTT assay (Mosmann, 1983).

4.5. Antifungal bioassays

Antifungal evaluations were performed according to the previously described protocol (Xie et al., 2009).

Acknowledgments

This work was 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.

Appendix A. Supplementary data

Crystallographic data for compounds **2** and **4** have been deposited at the Cambridge Crystallographic Data Centre (Deposition No. CCDC-871086 and 871087). Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.phytochem.2012.12.002.

References

Blay, G., Bargues, V., Cardon, L., García, B., Pedro, J.R., 2000. Stereoselective synthesis of 7,11-guaien-8,12-olides from santonin. Synthesis of podoandin and (+)-zedolactone A. J. Org. Chem. 65, 6703–6707.

Bodensieck, A., Fabian, W.M.F., Kunert, O., Belaj, F., Jahangir, S., Schühly, W., Bauer, R., 2010. Absolute configuration of eremophilane sesquiterpenes from *Petasites hybridus*: comparison of experimental and calculated circular dichroism spectra. Chirality 22, 308–319.

Ei-Gamal, A.A., 2001. Sesquiterpene lactones from *Smyrnium olusatrum*. Phytochemistry 57, 1197–1200.

Fang, P.L., Cao, Y.L., Yan, H., Pan, L.L., Liu, S.C., Gong, N.B., Lü, Y., Chen, C.X., Zhong, H.M., Guo, Y., Liu, H.Y., 2011. Lindenane disesquiterpenoids with anti-HIV-1 activity from *Chloranthus japonicus*. J. Nat. Prod. 74, 1408–1413.

Flack, H.D., Bernardinelli, G., 2000. Reporting and evaluating absolute-structure and absolute-configuration determinations. J. Appl. Crystallogr. 33, 1143–1148.

Huang, X.C., Guo, Y.W., Zhou, W.L., Zuo, J.P., Wang, Z.T., 2003. Ecdysteroids from the stems of *Diploclisia glaucescens*. Nat. Prod. Res. Dev. 15, 93–97.

Jiangsu New Medical College, 1977. Dictionary of Chinese Traditional Medicine. Shanghai Science and Technology Press, Shanghai, p. 654.

Kawabata, J., Mizutani, J., 1992. Dimeric sesquiterpenoid esters from *Chloranthus serratus*. Phytochemistry 31, 1293–1296.

Kitajima, J., Kamoshita, A., Ishikawa, T., Takano, A., Fukuda, T., Isoda, S., Ida, Y., 2003. Glycosides of *Atractylodes japonica*. Chem. Pharm. Bull. 51, 152–157.

Lee, Y.M., Moon, J.S., Yun, B.S., Park, K.D., Choi, G.J., Kim, J.C., Lee, S.H., Kim, S.U., 2009. Antifungal activity of CHE-23C, a dimeric sesquiterpene from *Chloranthus henryi*. J. Agric. Food Chem. 57, 5750–5755.

Li, B., Zhang, D.M., Luo, Y.M., 2006. A new sesquiterpene lactone from the roots of *Lasianthus acuminatissimus*. Acta Pharm. Sin. 41, 426–430.

Li, X.M., Li, J.M., Zhang, Y., Wang, B.G., 2007. Chemical constituents of *Aglaia odorata*. Zhong Cao Yao 38, 356–357.

Li, C.J., Zhang, D.M., Luo, Y.M., Yu, S.S., Li, Y., Lu, Y., 2008a. Bis-sesquiterpenes and diterpenes from *Chloranthus henryi*. Phytochemistry 69, 2867–2874.

Li, L., Xu, L.J., He, Z.D., Yang, Q.X., Peng, Y., Xiao, P.G., 2008b. Chemical study on ethyl acetate portion of *Ehretia thyrsiflora*, Boraginaceae species of Kudingcha. Zhongguo Zhong Yao Za Zhi 33, 2121–2123.

Li, Y., Zhao, Q.C., Guo, T., Wu, L.J., 2008c. Chemical constituents of *Clerodendron cyrtophyllum* Turcz. Chin. J. Med. Chem. 18, 371–373.

Mosmann, T., 1983. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. J. Immunol. Methods 65, 55–63.

Ran, X.H., Teng, F., Chen, C.X., Wei, G., Hao, X.J., Liu, H.Y., 2010. Chloramultiols A–F, lindenane-type sesquiterpenoid dimers from *Chloranthus multistachys*. J. Nat. Prod. 73, 972–975.

Snatzke, G., 1968. Circular dichroism and optical rotatory dispersion. Principles and application to the investigation of the stereochemistry of natural products. Angew. Chem. Int. Ed. 7, 14–25.

Suksamrarn, A., Yingyongnarongkul, B., Charoensuk, S., 1999. Regioselective synthesis of 24-epi-pterosterone. Tetrahedron 55, 255–260.

Takano, I., Yasuda, I., Takeya, K., Itokawa, H., 1995. Guiane sesquiterpene lactones from *Curcuma ameruginosa*. Phytochemistry 40, 1197–1200.

Tan, C.Y., Wang, J.H., Li, X., Du, Y.G., Bai, X.F., 2003. Chemical constituents of *Cyanotis arachnoidea*. Acta Pharm. Sin. 38, 760–762.

Teng, F., Zong, H.M., Chen, C.X., Liu, H.Y., 2009. Four new eudesmane sesquiterpenoid lactones from *Chloranthus serratus*. Helv. Chim. Acta 92, 1298–1303.

Tomas, J., Camps, F., Coll, J., Mele, E., Pascual, N., 1992. Synthesis of 25-fluroponasterone A, a fluorinated analogue of 20-hydroxyecdysone. Tetrahedron 48, 9809–9818.

Uchida, I., Kuriyama, K., 1974. The π–π* circular dichroism of α,β-unsaturated γ-lactones. Tetrahedron Lett. 43, 3761–3764.

Wang, J., Peng, S.M., Wang, M.K., Chen, N.Y., Ding, L.S., 2002. Chemical constituents of *Hyperricum monogynum*. Zhongguo Zhong Yao Za Zhi 27, 120–122.

Wang, Q.H., Kuang, H.X., Yang, B.Y., Xia, Y.G., Wang, J.S., Kong, L.Y., 2011. Sesquiterpenes from *Chloranthus japonicus*. J. Nat. Prod. 74, 16–20.

Wu, B., He, S., Wu, X.D., Wu, D.K., Pan, Y.J., 2007. Cadinane and eudesmane sesquiterpenoids from *Chloranthus henryi*. Helv. Chim. Acta 90, 1586–1592.

Xie, B.B., Liu, H.Y., Ni, W., Chen, C.X., 2009. Ypsilandrosides C–G, five new spirostanol saponins from *Ypsilandra thibetica*. Steroids 74, 950–955.

Xu, Y.J., Tang, C.P., Ke, C.Q., Zhang, J.B., Weiss, H.C., Gesing, E.R., Ye, Y., 2007. Mono- and di-sesquiterpenoids from *Chloranthus spicatus*. J. Nat. Prod. 70, 1987–1990.

Xu, Y.J., Tang, C.P., Tan, M.J., Ke, C.Q., Wu, T., Ye, Y., 2010. Sesquiterpenoids and diterpenoids from *Chloranthus anhuiensis*. Chem. Biodivers. 7, 151–157.

Yang, S.P., Yue, J.M., 2006. Chloramultilide A, a highly complex sesquiterpenoid dimmer from *Chloranthus multistachys*. Tetrahedron Lett. 47, 1129–1132.

Yang, S.P., Yue, J.M., 2008. Terpenoids from *Chloranthus multistachys*. Nat. Prod. Res. 22, 1163–1168.

Yang, S.P., Zhang, C.R., Chen, H.D., Liao, S.G., Yue, J.M., 2007. Sesquiterpenoids from *Chloranthus spicatus* (Thunb.) Makino. Chin. J. Chem. 25, 1892–1895.

Yim, N.H., Hwang, E.I., Yun, B.S., Park, K.D., Moon, J.S., Lee, S.H., Sung, N.D., Kim, S.U., 2008. Sesquiterpene furan compound CJ-01, a novel chitin synthase 2 inhibitor from *Chloranthus japonicus* SIEB. Biol. Pharm. Bull. 31, 1041–1044.

Yin, Y.Q., Luo, J.G., Kong, L.Y., 2007. Chemical constituents from *Ipomoea batatas*. Zhong Cao Yao 38, 508–510.

Zeng, G.Y., Yuan, W.M., Tan, J.B., Tan, G.S., Zhou, Y.J., 2007. Chemical constituents of *Sarcandra glabra*. Central South Pharmacy 5, 459–461.

Zhang, S., Yang, S.P., Yuan, T., Lin, B.D., Wu, Y., Yue, J.M., 2010a. Multistalides A and B, two novel sesquiterpenoid dimers from *Chloranthus multistachys*. Tetrahedron Lett. 51, 764–766.

Zhang, S., Su, Z.S., Yang, S.P., Yue, J.M., 2010b. Four sesquiterpenoids from *Chloranthus multistachys*. J. Asian Nat. Prod. 12, 522–528.

Zhou, Z.K., 1993. Origin, systematics and distribution of Chloranthaceae. Acta Bot. Yunnan. 1993 (15), 321–331.

Zhu, L.P., Li, Y., Yang, J.Z., Zuo, L., Zhang, D.M., 2008. Studies on chemical constituents of *Sarcandra glabra*. Zhongguo Zhong Yao Za Zhi 33, 155–157.