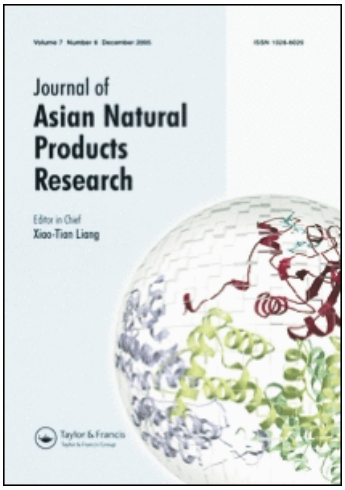

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ORIGINAL ARTICLE

Four sesquiterpenoids from *Chloranthus multistachys*

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Four new sesquiterpenoids, chlomultins A–D (**1**–**4**), were isolated from the whole plant of *Chloranthus multistachys*. Their structures were established on the basis of spectroscopic analysis.

Keywords: Chloranthaceae; *Chloranthus multistachys*; chlomultins A–D; sesquiterpenoids

1. Introduction

Chloranthus multistachys Pei (Chloranthaceae) is a perennial herb distributed in wet areas of eastern Asia [1]. Its roots have been applied as a folklore medicine to treat bone fracture in China [2]. Our previous investigations on this plant have led to the isolation of three sesquiterpenoid dimers [3,4] and two diterpenoids [5]. In continuation, four new sesquiterpenoids, chlomultins A–D (**1**–**4**), along with six known compounds, curcolonol (**5**) [6], zedoarofuran (**6**) [7], chlorantenes C (**7**) and D (**8**) [8], 1β,8β-dihydroxyeudesman-3,7(11)-dien-8α,12-olide (**9**) [9], and furanocadina-1(10),6,8-triene-4-ol (**10**) [10] were further isolated from the whole plant (Figure 1). We present herein the isolation and structural elucidation of these new compounds.

2. Results and discussion

Chlomultin A (**1**), a colorless amorphous powder, had a molecular formula $C_{15}H_{16}O_3$ as determined by the HR-EI-MS ion at *m/z* 244.1102 $[M]^+$ with eight degrees of unsaturation. Its IR absorption at 1647 cm^{-1} was assignable for the presence of ketone groups conjugated with multiple double bonds. The ^1H NMR spectrum (Table 1) exhibited three methyls (δ 1.15, 2.26, and 2.30) and an olefinic proton (δ 7.44, d, $J = 1.2$ Hz). The ^{13}C NMR spectrum with DEPT experiments revealed the presence of 15 carbon resonances comprising three methyls, two sp^3 methylenes, two sp^3 methines, two persubstituted double bonds, one trisubstituted double bond, and two carbonyls. The above-mentioned functionalities accounted for five degrees of unsaturation, and the remaining three degrees of unsaturation required **1** being tricyclic.

The combination of 2D NMR spectral data facilitated the construction of the scaffold of **1**. In the HMBC spectrum (Figure 2), the multiple correlations of Me-14/C-3, C-4 (δ_C 163.4), and C-5 (δ_C 133.5); H_2-2/C-1 and C-3; and H-1/C-4 and C-5 indicated the presence of an unsaturated five-membered ring A bearing a methyl at C-4; the HMBC correlations of Me-13/C-7 (δ_C 130.4); C-11 (δ_C 124.1)

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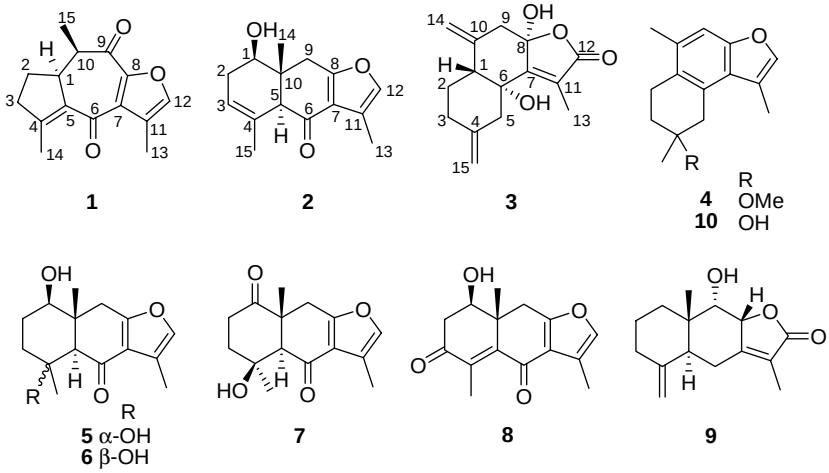

Figure 1. Structures of compounds **1**–**10**.

and C-12 (δ_C 144.5); and H-12/C-7, C-8 (δ_C 148.4), and C-11 permitted the establishment of the furan ring C with a methyl attached to C-11. The linkage of rings A and C via the C-6 ketone group was fixed by the J^4 HMBC correlations of Me-14/C-6 and Me-13/C-6. The HMBC correlations of Me-15/C-1, C-9 and C-10, and H-10/C-1, C-5, C-8, and C-9 enabled us to construct the seven-membered ring B. The relative configuration of **1** was established by a ROESY spectrum, in which the ROESY cross-peaks of H-1/H-10 and H-2α, and Me-15/H-2β revealed that Me-15 and H-1 were *trans*-configured. Thus, the structure of **1** was established.

Chlomultin B (**2**) had a molecular formula $C_{15}H_{18}O_3$, as determined by the HR-EI-MS ion at *m/z* 246.1268 $[M]^+$ with seven degrees of unsaturation. The IR spectrum exhibited absorptions at 3427 cm^{-1} (hydroxyl) and 1657 cm^{-1} (α,β-unsaturated ketone). The ^1H and ^{13}C NMR spectral data indicated the presence of three methyls (δ_H 0.92, 2.04, and 2.18), one carbonyl (δ_C 194.3), one trisubstituted furan ring, and one trisubstituted double bond. This analysis suggested that compound **2** featured an eudesmane-type sesquiterpene. Comparison of its NMR spectral data with those of two known compounds, curcolonol (**5**) and zedoarofuran (**6**) [6,7], showed that they are structurally related congeners, except for the changes at C-3 and C-4. In the ^{13}C NMR spectrum, the chemical shifts of C-3 (δ 121.6) and C-4 (δ 131.9) indicated the presence of a Δ^3 double bond in **2**. This was confirmed by the HMBC spectrum (Figure 3). The relative configuration of **2** was verified by the ROESY spectrum, in which the ROESY correlations of H-1/H-5 and H-14/H-2β were observed.

Chlomultin C (**3**) was isolated as a colorless oil and possessed a molecular formula $C_{15}H_{18}O_4$, as established by the HR-ESI-MS ion at *m/z* 285.1102 $[M+Na]^+$ with seven degrees of unsaturation. The ^1H NMR spectral data exhibited one methyl (δ 2.07, 3H, s) and four olefinic protons (δ 4.92, 4.96, 4.99, and 5.16). The ^{13}C NMR spectral data (Table 1) exhibited 15 carbon resonances, which were further categorized by DEPT experiments as a carbonyl (δ 171.8), two exocyclic double bonds (δ 116.0, 140.5, 112.6, and 142.6), a persubstituted double bond (δ 122.7 and 156.2), a semi-ketal (δ 103.2), an oxygenated sp^3 quaternary carbon (δ 73.7), a methyl, four sp^3 methylenes, and a methine. These observations

Table 1. ^{1}H and ^{13}C NMR data of chlomultins A–D (**1**–**4**).

	1[a]		**2**[a]		**3**[a]		**3**[b]	**4**[a]	
Position	δ_H, mult. (*J* in Hz)	δ_C	δ_H, mult. (*J* in Hz)	δ_C	δ_H, mult. (*J* in Hz)	δ_C	δ_H, mult. (*J* in Hz)[c]	δ_H, mult. (*J* in Hz)	δ_C
1	α 3.60, m	27.3	α 3.84, dd (10.1, 5.7)	74.4	β 2.25, m	49.5	β 2.27, d (11.0)		127.8
2α	2.21, m	45.0	2.28, m	31.6	1.79, m	24.9	2.06, dddd (25.1, 11.0, 10.4, 3.5)	a 2.64, m	24.3
2β	1.66, m		2.10, m		1.90, m		1.74, m	b 2.77, m	
3α	2.56, m	40.0	5.43, m	121.6	2.50, m	33.5	2.46, d (10.4)	a 1.83, m	31.7
3β	2.62, m				2.14, m		2.17, m	b 2.05, m	
4		163.4		131.9		142.6			72.3
5α		133.5	3.29, br s	58.3	2.89, dd (13.0, 1.7)	44.4	3.07, dd (13.0, 1.5)	a 3.12, d (16.8)	38.6
5β					2.71, d (13.0)		2.75, d (13.0)	b 3.34, d (16.8)	
6		185.5		194.3		73.7			128.2
7		130.4[c]		121.0		156.2			124.7
8		148.4		163.9		103.2			154.2
9α		192.1	2.79, d (17.2)	36.2	3.07, d (13.1)	48.0	3.28, d (12.7)	7.10, s	110.2
9β			3.02, d (17.2)		2.40, d (13.1)		2.56, d (12.7)		
10	2.80, ddd (14.9, 7.3, 1.9)	49.6		43.5		140.5			133.0
11		124.1[c]		119.0		122.7			115.9
12	7.44, d (1.2)	144.5	7.08, d (1.0)	139.3		171.8		7.23, d (1.2)	140.7
13	2.26, d (1.2)	9.9	2.18, d (1.0)	8.9	2.07, s	10.1	1.96	2.37, d (1.2)	11.3
14a	2.30, d (1.3)	17.8	0.92, s	11.3	5.22, s	116.0	5.16, d (1.0)	2.31, s	20.4
14b					5.01, s		4.99, d (1.0)		
15a	1.15, d (7.4)	12.2	2.04, dd (2.5, 1.4)	22.6	4.95, d (1.6)	112.6	4.96, d (1.8)	1.32, s	22.9
15b					4.87, d (1.6)		4.92, d (1.8)		
OMe				74.4				3.30, s	49.1

Notes: [a] Measured in $CDCl_3$.
[b] Measured in C_5D_5N.
[c] May be exchangeable in the same vertical column.

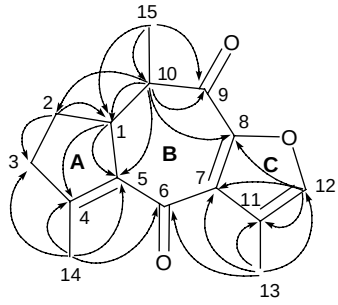

Figure 2. Selected HMBC correlations (H → C) of chlomultin A (**1**).

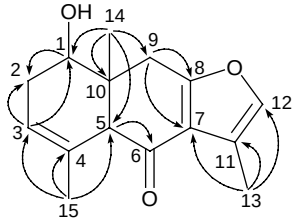

Figure 3. Selected HMBC correlations (H → C) of chlomultin B (**2**).

indicated that compound **3** is a cadinane-type sesquiterpene [11].

The planar structure of **3** was established by the HMBC spectrum (Figure 4(a)).

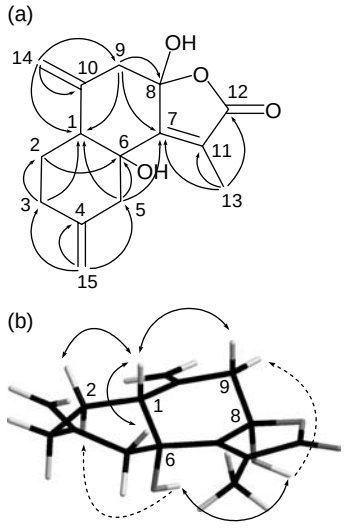

Figure 4. (a) Selected HMBC correlations (H → C) of chlomultin C (**3**). (b) Key ROESY correlations (H ↔ H) and pyridine-induced solvent shifts (⇢) of chlomultin C (**3**).

Two exocyclic double bonds were assigned as $\Delta^{4(15)}$ and $\Delta^{10(14)}$ from the HMBC correlations of H_2-15/C-3, C-4, and C-5; and H_2-14/C-1, C-9, and C-10, respectively. The HMBC correlations of H_2-5/C-6 and H_2-2/C-6, as well as the downfield-shifted quaternary C-6 (δ 73.7) indicated the presence of HO-6. The multiple HMBC correlations of H_2-5/C-7, H_2-9/C-7, H_3-13/C-7, H_3-13/C-11, and H_3-13/C-12 indicated the presence of a $\Delta^{7(11)}$ double bond, and the linkages of an ester carbonyl (C-12, δ_C 171.8) and a methyl (CH_3-13, δ_C 10.1, δ_H 1.96) to C-11. Although there was no direct HMBC correlation available to furnish the linkage between C-8 and C-12, the remaining one degree of unsaturation and a semi-ketal assignable to C-8 (δ 103.2) on the basis of its chemical shift and the HMBC correlations of H_2-9/C-8 definitely indicated the connectivity between C-8 and C-12 via an oxygen atom to form a γ-lactone ring.

The ROESY spectrum of **3** (Figure 4(b)) showed the cross-peaks between HO-6 and HO-8, indicating that they were co-facial and arbitrarily assigned as α-oriented. The ROESY correlations of H-1/H-2β, H-9β, and H-5β indicated that H-1 was β-oriented. This was confirmed by the pyridine-induced solvent shifts [12], in which the significant pyridine-induced solvent shifts were observed [$\Delta\delta$ was defined as $\delta(CDCl_3)-\delta$(pyridine-d_5)] for H-2α ($\Delta\delta = -0.27$), H-9α ($\Delta\delta = -0.21$), and H-5α ($\Delta\delta = -0.18$). Therefore, the structure of **3** was elucidated as depicted.

Chlomultin D (**4**) was obtained as a colorless powder. The molecular formula was determined as $C_{16}H_{20}O_2$, on the basis of the HR-EI-MS ion at *m/z* 244.1466 $[M]^+$. Three methyls (δ 1.32, 2.31, 2.37), a methoxyl (δ 3.30, s, 3H), two olefinic protons (δ 7.10 and 7.23), and three methylene protons were observed in the ^{1}H NMR spectrum. The ^{1}H and ^{13}C NMR spectral data of **4** were similar to those of furanocadina-1(10),6,8-triene-4-ol [10], suggesting that they are structural analogs

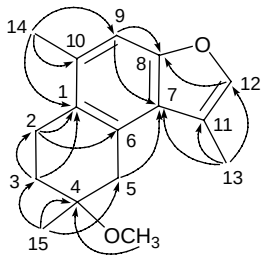

Figure 5. Selected HMBC correlations (H → C) of chlomultin D (**4**).

with the only difference being the presence of a methoxyl at C-4 instead of the hydroxyl of furanocadina-1(10),6,8-triene-4-ol. This assignment was verified by the HMBC correlation between OMe and C-4 (Figure 5).

Six known compounds were identified as curcolonol (**5**) [6], zedoarofuran (**6**) [7], chlorantenes C and D (**7** and **8**) [8], 1β,8β-dihydroxyeudesman-3,7(11)-dien-8α,12-olide (**9**) [9], and furanocadina-1(10),6,8-triene-4-ol (**10**) [10] on the basis of their NMR spectral data.

3. Experimental

3.1 General experimental procedures

IR spectra were recorded on a Perkin-Elmer 577 spectrometer with a KBr disk. UV spectra were measured on a Shimadzu UV-2550 UV-visible spectrophotometer. Optical rotations were made on a Perkin-Elmer 341 polarimeter at room temperature. NMR spectra were measured on a Bruker AM-400 spectrometer with TMS as an internal standard. EI-MS (70 eV) and ESI-MS were carried out on a Finnigan MAT 95 mass spectrometer, a Finnigan LCQ^{DECA}, and a Q-TOF Ultima (for HR-ESI-MS) instrument, respectively. All solvents used were of analytical grade (Shanghai Chemical Plant, Shanghai, China). Silica gel (200–300 mesh, Qingdao Haiyang Chemical Co. Ltd, Qingdao, China), reverse-phase C_{18} silica gel (150–200 mesh, Merck, Darmstadt, Germany), Sephadex LH-20 gel (Amersham Biosciences, Little Chanfolt, UK), and MCI gel (CHP20P, 75–150 μM, Mitsubishi Chemical Industries Ltd, Tokyo, Japan) were used for column chromatography, and pre-coated silica gel GF_{254} plates (Qingdao Haiyang Chemical Co. Ltd) were used for TLC.

3.2 Plant material

Whole plants of *C. multistachys* Pei were collected from Songyang County of Zhejiang Province of China, and were authenticated by Dr Ding-Quan Tu of Gehu Hospital. A voucher specimen (CH-2004-1Y) has been deposited in the Shanghai Institute of Materia Medica.

3.3 Extraction and isolation

The air-dried powder of the whole plants (5 kg) of *C. multistachys* Pei was extracted with 95% EtOH (8 liters) five times at room temperature to obtain 548 g of crude extract, which was then partitioned between EtOAc and H_2O to give an EtOAc-soluble fraction (209 g). The EtOAc-soluble fraction was chromatographed over an MCI gel column (MeOH/H_2O, 50/50–90/10) to yield four fractions (A–D). Fraction A (45 g) was then subjected to silica gel column eluted with petroleum ether/EtOAc (15:1–1:1) in gradient to obtain nine fractions (A1–A9). Fraction A3 (4.3 g) was separated by a reverse-phase C_{18} silica gel column eluted with MeOH/H_2O (MeOH/H_2O, 50/50–80/20) to give two sub-fractions (A3a and A3b). Sub-fractions A3a (1.8 g) and A3b (1.4 g) were purified by a silica gel column (petroleum ether/EtOAc, 4:1) and then a Sephadex LH-20 (MeOH) column to give curcolonol (**5**: 12 mg) and chlorantene C (**7**: 10 mg), respectively. Fraction A8 (1.8 g) was subjected to a reverse-phase C_{18} silica gel column (MeOH/H_2O, 40/60–80/20) to obtain compound **3** (15 mg). Fraction A9 (3.2 g) was chromatographed over a reverse-phase C_{18} silica gel column (MeOH/H_2O, 40/60–80/20) to afford chlorantene D (**8**) (13 mg) and 1β,8β-dihydroxy

eudesman 3,7(11)-dien-8α,12-olide (**9**) (9 mg). Fraction B (12 g) was subjected to a silica gel column (petroleum ether/EtOAc, 15:1–1:1) to yield zedoarofuran (**6**) (8 mg). Fraction D (24 g) was subjected to silica gel column (petroleum ether/EtOAc, 25:1–1:1) to give eight fractions, D1–D8. Fraction D3 (0.8 g) was chromatographed over a Sephadex LH-20 column to give compound **4** (10 mg). Fraction D5 (3.6 g) was chromatographed over a reverse-phase C_{18} silica gel column (MeOH/H_2O, 50/50–70/30) to obtain compound **1** (8 mg) and furanocadina-1(10),6,8-triene-4-ol (**10**) (17 mg). Fraction D8 (3.0 g) was separated by a reverse-phase C_{18} silica gel column (MeOH/H_2O, 45/55–70/30) to yield compound **2** (8 mg).

3.3.1 *Chlomultin A (**1**)*

A white amorphous powder; $[\alpha]_D^{20} \sim 0$ ($c = 0.21$, $CHCl_3$); UV (MeOH) λ_{max} ($\log \varepsilon$): 275 (3.64), 223 (3.83) nm; IR (KBr, disk) ν_{max}: 2926, 1647, 1510, 1383 cm^{-1}; ^1H and ^{13}C NMR spectral data, see Table 1; ESI-MS *m/z*: 267.1 $[M+Na]^+$, 510.9 $[2M+Na]^+$; EI-MS *m/z*: 244 $[M]^+$(100), 229 (42), 202 (36), 187 (22); HR-EI-MS *m/z*: 244.1102 $[M]^+$ (calcd for $C_{15}H_{16}O_3$, 244.1099).

3.3.2 *Chlomultin B (**2**)*

A white amorphous powder; $[\alpha]_D^{20}$ −7 ($c = 0.11$, $CHCl_3$), UV (MeOH) λ_{max} ($\log \varepsilon$): 260 (3.71) nm; IR (KBr, disk) ν_{max}: 3429, 2928, 1657, 1379, 1051 cm^{-1}; ^1H and ^{13}C NMR spectral data, see Table 1; ESI-MS *m/z*: 247.1 $[M+H]^+$; EI-MS *m/z*: 246 $[M]^+$(62), 122 (100), 107 (65), 94 (26); HR-EI-MS *m/z*: 246.1268 $[M]^+$ (calcd for $C_{15}H_{18}O_3$, 246.1256).

3.3.3 *Chlomultin C (**3**)*

A white amorphous powder; $[\alpha]_D^{20}$ + 8 ($c = 0.12$, MeOH), UV (MeOH) λ_{max} ($\log \varepsilon$): 199 (4.28) nm; IR (KBr, disk) ν_{max}: 3458, 2929, 1776, 1655, 1437, 1221, 1113, 1010 cm^{-1}; ^1H and ^{13}C NMR spectral data, see Table 1; ESI-MS *m/z*: 285.0 $[M+Na]^+$; HR-ESI-MS *m/z*: 285.1102 $[M+Na]^+$ (calcd for $C_{15}H_{18}O_4Na$, 285.1103).

3.3.4 *Chlomultin D (**4**)*

A white amorphous powder; $[\alpha]_D^{20}$ −5 ($c = 0.17$, $CHCl_3$), UV (MeOH) λ_{max} ($\log \varepsilon$): 254 (3.94), 207 (4.38) nm; IR (KBr, disk) ν_{max}: 2926, 1803, 1741, 1616, 1454, 1101, 846, 782, 594 cm^{-1}; ^1H and ^{13}C NMR spectral data, see Table 1; ESI-MS *m/z*: 267.1 $[M+Na]^+$; EI-MS *m/z*: 244 $[M]^+$(36), 212 (61), 197 (55), 172 (100); HR-EI-MS *m/z*: 244.1466 $[M]^+$ (calcd for $C_{16}H_{20}O_2$, 244.1463).

Acknowledgements

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References

[1] Z.K. Zhou, *Acta Bot. Yunnan.* **15**, 321 (1993).
[2] Editorial Committee (over 300 members) of the Administration Bureau of Traditional Chinese Medicine, *Chinese Materia Medica (Zhonghua Bencao)* (Shanghai Science and Technology, Shanghai, 1998), Vol. 3, p. 451.
[3] S.P. Yang and J.M. Yue, *Tetrahedron Lett.* **47**, 1129 (2006).
[4] S. Zhang, S.P. Yang, T. Yuan, B.D. Lin, Y. Wu, and J.M. Yue, *Tetrahedron Lett.* **51**, 764 (2010).
[5] S.P. Yang and J.M. Yue, *Nat. Prod. Res.* **22**, 1163 (2008).
[6] W.J. Syu, C.C. Shen, M.J. Don, J.C. Ou, G.H. Lee, and C.M. Sun, *J. Nat. Prod.* **61**, 1531 (1998).
[7] H. Matsuda, T. Morikawa, I. Toguchida, K. Ninomiya, and M.Y. Yoshikawa, *Chem. Pharm. Bull.* **49**, 1558 (2001).
[8] T. Yuan, C.R. Zhang, S.P. Yang, S. Yin, W.B. Wu, L. Dong, and J.M. Yue, *J. Nat. Prod.* **71**, 2021 (2008).

[9] A.A. El-Gamal, *Phytochemistry* **57**, 1197 (2001).
[10] T. Shen, W.Z. Wan, X.N. Wang, L.M. Sun, H.Q. Yuan, X.L. Wang, M. Ji, and H.X. Lou, *Helv. Chim. Acta* **91**, 881 (2008).
[11] X.A. Dominguez, S. Hafez, V.H. Sanchez, and J. Slim, *Phytochemistry* **27**, 1863 (1988).
[12] P.V. Demarco, E. Farkas, D. Doddrell, B.L. Mylari, and E. Wenkert, *J. Am. Chem. Soc.* **90**, 5480 (1968).