



## Multistalides A and B, two novel sesquiterpenoid dimers from *Chloranthus multistachys*

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### ABSTRACT

Two novel sesquiterpenoid dimers, multistalides A and B (**1–2**), were isolated from the whole plant of *Chloranthus multistachys*. Their structures were established on the basis of spectroscopic analysis, and the absolute configuration of **1–2** was determined by CD exciton chirality method.

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The genus *Chloranthus* (Chloranthaceae) is mainly distributed in eastern Asia,<sup>1</sup> and there are 13 species and five varieties found in China.<sup>2</sup> Most of these plants have been used as a folk medicine for the treatment of bone fracture.<sup>3</sup> Previous chemical investigations on this plant genus have resulted in the isolation of an array of sesquiterpenoids,<sup>4</sup> sesquiterpenoid oligomers,<sup>5</sup> and diterpenoids.<sup>6</sup> Recently, several studies showed that the sesquiterpenoid dimers isolated from the genus *Chloranthus* exhibited significant bioactivities.<sup>5e–i</sup>

*Chloranthus multistachys* Pei, a major species of the genus *Chloranthus*, grows favorably in the damp areas of eastern Asia.<sup>1</sup> Our preliminary study on this plant with a small amount of material has led to the isolation of one new sesquiterpenoid dimer<sup>5b</sup> and several diterpenoids.<sup>6b</sup> In a continuing phytochemical study, the whole plants of *C. multistachys* were re-examined, two new sesquiterpenoid dimers, multistalides A and B (**1–2**) were isolated. Their structures were established on the basis of spectroscopic analysis, and their absolute stereochemistry was determined by CD exciton chirality method.

The plant material of *C. multistachys* was collected from Songyang County of Zhejiang Province of China. Air-dried herb powder (1.5 kg) was extracted with 95% EtOH three times, and the crude was then partitioned between EtOAc and H<sub>2</sub>O to give an EtOAc-soluble fraction, which was further defatted over a column of MCI gel eluted with 80% methanol in water. Repeated chromatography of

the defatted fraction over silica gel, reversed phase C<sub>18</sub> silica gel, Sephadex LH-20, and semi-preparative HPLC afforded multistalides A (**1**) (16 mg, 0.0011% yield) and B (**2**) (20 mg, 0.0013% yield) (Fig. 1).

Multistalide A (**1**),<sup>7</sup> a white amorphous powder, had a molecular formula C<sub>33</sub>H<sub>38</sub>O<sub>10</sub> as determined by the HREIMS ion at 594.2470 [M]<sup>+</sup> (calcd 594.2465) with 15 degrees of unsaturation, which was supported by the positive mode of ESIMS at *m/z* 617.4 [M+Na]<sup>+</sup> and 1211.5 [2M+Na]<sup>+</sup>, and the negative mode of ESIMS at 593.5 [M–H]<sup>–</sup>. The IR spectrum exhibited absorptions for hydroxy (3437 cm<sup>–1</sup>) and carbonyl (1728 cm<sup>–1</sup>) groups. The <sup>1</sup>H NMR spectrum of **1** (Table 1) showed the presence of three tertiary methyls ( $\delta$  0.84, 0.97, 2.08, each 3H, s), one methoxy ( $\delta$  3.80, 3H, s), and one terminal double bond ( $\delta$  5.43, 6.22, each 1H, s). In accord with the molecular formula, the <sup>13</sup>C NMR spectrum (Table 1) showed 33 carbon resonances, which were further categorized by DEPT experiments into four carbonyls, four methyls, two persubstituted double bonds, one terminal double bond, six sp<sup>3</sup> methyl-

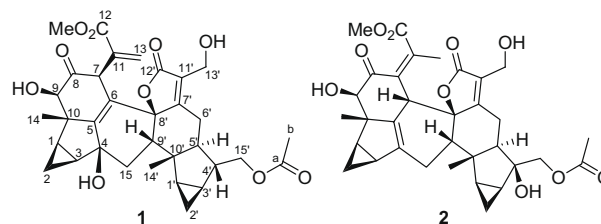


Figure 1.

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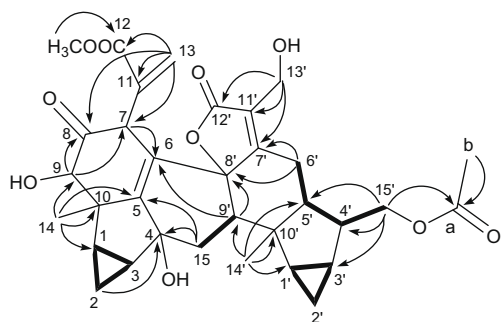
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**Table 1**  
NMR data for multistalides A and B (1–2) in CDCl<sub>3</sub>

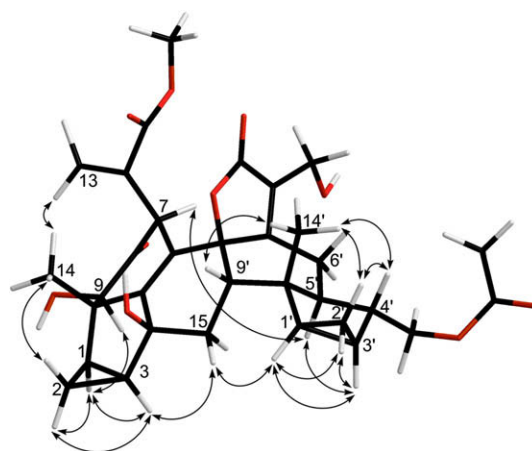
	1		2	
	$\delta_{\text{H}}$ , mult. (J in Hz)	$\delta_{\text{C}}$	$\delta_{\text{H}}$ , mult. (J in Hz)	$\delta_{\text{C}}$
1	2.04 (m)	26.3	2.08 (m)	25.9
2 $\alpha$	0.95 (m)	10.0	0.99 (m)	16.0
2 $\beta$	1.23 (m)		0.32 (m)	
3	1.86 (m)		1.83 (m)	
4		30.7		24.8
5		78.0		142.5
6		160.4		132.0
7	4.13 (s)	124.8	3.92 (s)	40.9
8		49.0		130.9
9	4.25 (s)	207.9	3.94 (s)	200.1
10		80.3		80.0
11		49.1		51.1
12		136.3		147.6
13a	5.43 (s)	167.6		171.0
13b	6.22 (s)	126.7	1.94 (s, 3H)	20.5
14	0.97 (s, 3H)	20.5	1.01 (s, 3H)	15.4
15 $\alpha$	1.86 (m)	40.4	2.81 (dd, 16.4, 1.3)	25.2
15 $\beta$	2.74 (dd, 13.1, 6.9)		2.58 (m)	
1'	1.49 (m)	25.4	1.62 (m)	25.5
2' $\alpha$	0.76 (m)	16.3	0.72 (m)	11.7
2' $\beta$	0.68 (m)		1.27 (m)	
3'	1.27 (m)	22.5	1.47 (m)	28.2
4'	1.61 (m)	45.2		77.3
5'	2.00 (m)	52.8	1.86 (m)	60.0
6' $\alpha$	2.78 (dd, 18.1, 6.4)	26.3	2.32 (dd, 18.3, 6.1)	22.3
6' $\beta$	2.63 (dd, 18.1, 12.9)		2.70 (dd, 18.3, 13.5)	
7'		164.4		168.2
8'		87.6		93.4
9'	2.63 (dd, 10.7, 6.9)	49.3	1.92 (m)	55.0
10'		44.2		44.8
11'		129.1		127.2
12'		171.0		172.4
13'a	4.22 (d, 13.8)	55.3	4.42 (d, 13.5)	54.9
13'b	4.18 (d, 13.8)		4.34 (d, 13.5)	
14'	0.84 (s, 3H)	22.7	0.88 (s, 3H)	26.2
15'a	4.25 (d, 10.9)	65.6	4.08 (d, 11.6)	71.5
15'b	4.20 (d, 10.9)		3.82 (d, 11.6)	
a		171.5		171.3
b	2.08 (s, 3H)	20.8	2.13 (s, 3H)	20.8
OMe	3.80 (s, 3H)	52.8	3.80 (s, 3H)	52.8

enes, nine sp<sup>3</sup> methines, and four sp<sup>3</sup> quaternary carbons. The above-mentioned functionalities accounted for seven degrees of unsaturation, and the remaining eight degrees of unsaturation required **1** being octacyclic. Two sets of spin systems being assignable to the presence of 1,2-disubstituted cyclopropane rings were evident from the <sup>1</sup>H NMR spectrum (H-1:  $\delta_{\text{H}}$  2.04; H-2 $\alpha$ :  $\delta_{\text{H}}$  0.95; H-2 $\beta$ :  $\delta_{\text{H}}$  1.23; H-3:  $\delta_{\text{H}}$  1.86 and H-1':  $\delta_{\text{H}}$  1.49; H-2' $\alpha$ :  $\delta_{\text{H}}$  0.76; H-2' $\beta$ :  $\delta_{\text{H}}$  0.68; H-3':  $\delta_{\text{H}}$  1.27). The aforementioned analysis and the fact of the existence of sesquiterpenoid dimers within this plant genus<sup>5</sup> suggested that **1** is a lindenane-type sesquiterpenoid dimer.

The extensive analysis of 1D and 2D NMR (including <sup>1</sup>H–<sup>1</sup>H COSY, HSQC, HMBC) spectral data allowed the assignment of com-



**Figure 2.** <sup>1</sup>H–<sup>1</sup>H COSY (—) and selected HMBCs (H→C) of multistalide A (**1**).

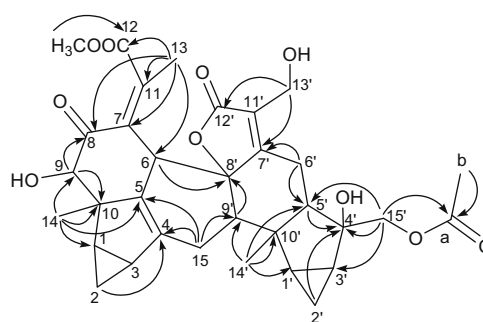


**Figure 3.** Key ROESY correlations (H↔H) of multistalide A (**1**).

pound **1**. After the assignment of all protons to their directly bonded carbon atoms via a HSQC experiment, four proton-bearing spin coupling units of the molecule as drawn with bold bonds (Fig. 2) were established by <sup>1</sup>H–<sup>1</sup>H COSY spectrum. These four structural subunits and most of other functional groups were then connected by HMBC spectrum (Fig. 2), in which the HMBCs from H<sub>2</sub>–13 to C-12, C-11, C-8, and C-7 suggested the presence of a  $\Delta^{11(13)}$  terminal double bond. The methoxyl group was placed at C-12 by the HMBC between C-12 and OMe. The presence of an  $\alpha$ ,  $\beta$ -unsaturated  $\gamma$ -lactone formed between C-8' and C-12' via an oxygen atom was evident from the down-field shifted C-8' at  $\delta_{\text{C}}$  87.6 as in the case of chloramultilide A.<sup>5b</sup> The only acetyl group was located at C-15' by the HMBC between H<sub>2</sub>–15' and the carbonyl of acetyl. The remaining three oxygenated sp<sup>3</sup> carbons were assigned to C-4 at  $\delta_{\text{C}}$  78.0, C-9 at  $\delta_{\text{C}}$  80.3, and C-13' at  $\delta_{\text{C}}$  55.3 bearing hydroxyl groups, respectively, by the multiple HMBCs.

The relative configuration of **1** was established by the performance of a ROESY experiment (Fig. 3), in which the ROESY correlations of H-1/H-3, H-1/H-2 $\alpha$ , H-3/H-2 $\alpha$ , H-1/H-9, H-1'/H-3', H-1'/H-2' $\alpha$ , H-3'/H-2' $\alpha$ , and H-3'/H-5' indicated that H-1, H-3, H-9, H-1', H-3', and H-5' were co-facial and randomly assigned in an  $\alpha$ -configuration. In consequence, the ROESY correlations of Me-14/H-2 $\beta$ , H-9'/Me-14', H-4'/Me-14', Me-14'/H-2' $\beta$ , H-2' $\beta$ /H-4', H-13a/Me-14, and H-7/H-5' revealed that Me-14, Me-14', H-4', H-9', and the C-7–C-11 bond were  $\beta$ -oriented. A  $\beta$ -direction for the C-8'–O bond was tentatively assigned on the biogenetic reasoning that compound **1** was formed via an enzymatic Diels–Alder cycloaddition of two molecular lindenane-type sesquiterpenoids.<sup>5e,8</sup>

Multistalide B (**2**)<sup>9</sup> was obtained as a white amorphous powder and possessed a molecular formula C<sub>33</sub>H<sub>38</sub>O<sub>10</sub> as established by HRESIMS ion at 617.2379 [M+Na]<sup>+</sup> (calcd for C<sub>33</sub>H<sub>38</sub>O<sub>10</sub>Na



**Figure 4.** Selected HMBCs (H→C) of multistalide B (**2**).

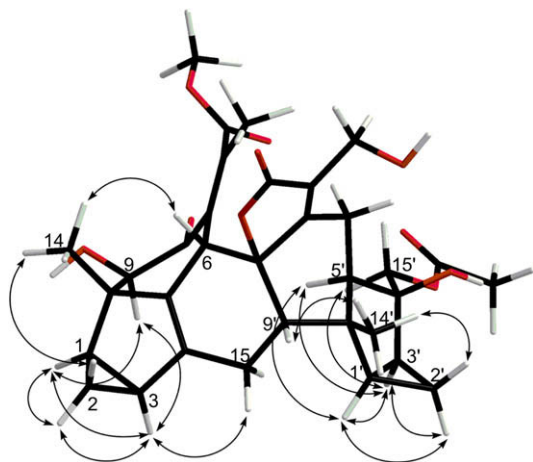


Figure 5. Key ROESY correlations (H↔H) of multistalide B (2).

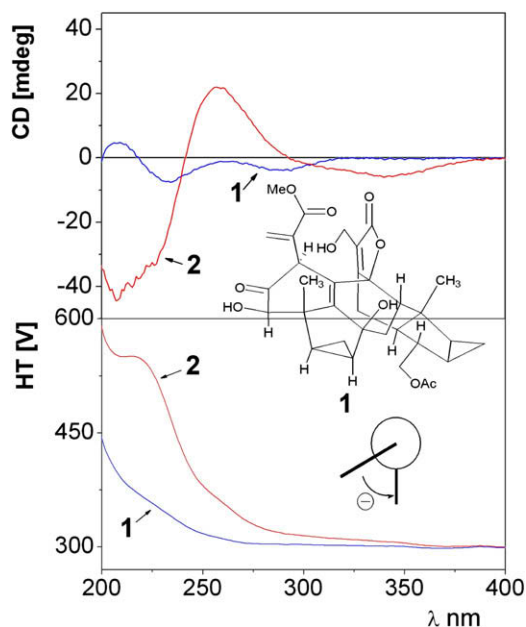


Figure 6. CD and UV spectra of multistalides A and B (1–2) measured in MeOH, and the stereoview of **1**, arrows denote the electric transition dipole of the chromophores.

617.2363). The IR spectrum exhibited absorptions of hydroxyl ( $3438\text{ cm}^{-1}$ ) and carbonyl ( $1736\text{ cm}^{-1}$ ) groups. The  $^1\text{H}$  and  $^{13}\text{C}$  NMR data of **2** (Table 1) showed many similarities to those of shizukaol D,<sup>5c</sup> except for the down-field-shifted proton (H-3':  $\Delta\delta_{\text{H}}$  0.37; H-2' $\beta$ :  $\Delta\delta_{\text{H}}$  0.44; Me-14':  $\Delta\delta_{\text{H}}$  0.22) and carbon (C-4':  $\Delta\delta_{\text{C}}$  34.3) resonances scattered around C-4' as compared with those of shizukaol D,<sup>5c</sup> suggesting that a hydroxyl group was attached to the C-4' of compound **2**. This assignment was further confirmed by 2D NMR spectra, especially HMBC spectrum (Fig. 4), in which the quaternary C-4' resonated at  $\delta_{\text{C}}$  77.3 showed HMBs with H-5', H-2' $\alpha$ , and H<sub>2</sub>-15'. The relative configuration of **2** was fixed by a ROESY experiment (Fig. 5), in which the 4'-OH was assigned as a  $\beta$ -configuration by comparing the chemical shift of C-4' ( $\delta_{\text{C}}$  77.3) of **2** with that of chloramultilide A<sup>5b</sup> bearing a  $\beta$ -OH at C-4' ( $\delta_{\text{C}}$  76.9). The presence of a 4 $\beta$ -OH in **2** was confirmed by the ROESY correlations of H<sub>2</sub>-15'/H-3' and H<sub>2</sub>-15'/H-5'.

The absolute stereochemistry of multistalide A (**1**) was determined on the basis of CD spectrum (Fig. 6). The Cotton effects (206 and 234 nm) centered at 221 nm corresponding to the UV maximum of  $\alpha,\beta$ -unsaturated  $\gamma$ -lactone (C-7', C-11', and C-12', Woodward's rules showed ca. 227 nm).<sup>10</sup> The first negative Cotton effect at 234 nm and the second positive Cotton effect at 206 nm arising from the exciton coupling between the  $\alpha,\beta$ -unsaturated  $\gamma$ -lactone and the  $\alpha,\beta$ -unsaturated ester group (C-11, C-12, and C-13, Woodward's rules showed ca. 203 nm)<sup>10</sup> indicated a negative chirality for **1**. The absolute configuration of **1** was thus established as depicted, which is consistent with those of chlorahololides A and B<sup>5e</sup> isolated from this genus.

The CD curve of multistalide B (**2**) was very similar to those of the known sesquiterpenoid dimers, chlorahololides A and B,<sup>5e</sup> in the range of 210–265 nm (Fig. 6). The first positive Cotton effect at 257 nm and the second negative Cotton effect at 207 nm showed a positive chirality for compound **2**. The absolute configuration of **2** was thus defined as depicted.

## Acknowledgments

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## Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2009.12.001.

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- Multistalide A (**1**): white, amorphous powder;  $[\alpha]_{\text{D}}^{20}$   $-124$  (c 0.370,  $\text{CHCl}_3$ ); UV (MeOH)  $\lambda_{\text{max}}$  (log  $\epsilon$ ) 221 (4.26) nm; CD (MeOH)  $\lambda_{\text{max}}$  ( $\Delta\epsilon$ ) 292 (–3.78), 264 (–1.14), 234 (–7.37), 206 (4.63) nm; IR (KBr, disc)  $\lambda_{\text{max}}$  3437, 2922, 1728, 1630, 1441, 1383, 1238, 1113  $\text{cm}^{-1}$ ;  $^1\text{H}$  and  $^{13}\text{C}$  NMR see Table 1; EIMS 70 eV  $m/z$  594 [M]<sup>+</sup> (51), 577 (65), 499 (100), 403 (88), 105 (86); HREIMS  $m/z$  594.2470 [M]<sup>+</sup> (calcd for  $\text{C}_{33}\text{H}_{38}\text{O}_{10}$ , 594.2465).
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- Multistalide B (**2**): white, amorphous powder;  $[\alpha]_{\text{D}}^{20}$   $-113$  (c 0.155,  $\text{CHCl}_3$ ); UV (MeOH)  $\lambda_{\text{max}}$  (log  $\epsilon$ ) 218 (4.08) nm; CD (MeOH)  $\lambda_{\text{max}}$  ( $\Delta\epsilon$ ) 257 (21.8), 207 (–43.2) nm; IR (KBr, disc)  $\lambda_{\text{max}}$  3438, 2924, 1736, 1436, 1379, 1238, 1086  $\text{cm}^{-1}$ ;  $^1\text{H}$  and  $^{13}\text{C}$  NMR see Table 1; ESI MS (positive)  $m/z$  617.4 [M+Na]<sup>+</sup>, 1211.5 [2M+Na]<sup>+</sup>; HRESIMS  $m/z$  617.2379 [M+Na]<sup>+</sup> (calcd for  $\text{C}_{33}\text{H}_{38}\text{O}_{10}\text{Na}$ , 617.2363).
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