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Chloramultilide A, a highly complex sesquiterpenoid dimmer from *Chloranthus multistachys*

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Abstract—A highly complex sesquiterpenoid dimmer, chloramultilide A (**1**), was isolated from *Chloranthus multistachys*. Its structure and relative stereochemistry were mainly established by using 1D NMR and 2D NMR spectra.

The recent progress in the finding of highly complex secondary metabolites has highlighted the research field of natural product chemistry.[1] The extraordinary complexity of these secondary metabolites are the challenging subjects of natural products chemistry, organic synthesis, and biosynthesis. The previous chemical investigations on the genus of *Chloranthus* have reported a number of sesquiterpenoids and sesquiterpenoid oligomers.[2] *Chloranthus multistachys* Péi, a perennial herb mainly distributed over the east of Asia,[3] has not been previously investigated chemically. Its roots were applied to treat bone fracture in China as folklore medicine.[4] As part of our continuation on search for novel structures from Chinese folk medicine, a highly complex and novel sesquiterpenoid dimmer, namely chloramultilide A (**1**), was isolated from the whole plant of *C. multistachys*. Its structure and relative stereochemistry were determined by spectral method, especially 2D NMR spectra.

The plant material of *C. multistachys* was collected from Songyang county of Zhejiang province. 1.2 kg air-dried herb powder was percolated with 95% ethanol and the crude extract was partitioned between EtOAc and water. The EtOAc soluble fraction was then defatted over a column of MCI gel eluted with 80% aqueous methanol to collect 24 g of the defatted fraction, which was subsequently subjected to a silica gel column (petroleum/ethyl acetate = 2:1) to give a major fraction containing the sesquiterpenoid dimmers (0.69 g). The fraction of sesquiterpenoid dimmers was further purified over a silica gel column (chloroform/methanol = 50:1) to yield 50 mg of chloramultilide A (**1**) (0.0033%).

Chloramultilide A (**1**)[5] was obtained as an amorphous powder. The HR EIMS ion at m/z 748.2713 $[M]^+$ enabled us to establish its molecular formula as $C_{40}H_{44}O_{14}$ (calcd 748.2731) with 19 degrees of unsaturation. The positive mode of ESI MS at m/z 787 $[M+K]^+$ and 771 $[M+Na]^+$ further secured the determination of its molecular ion. The ^{13}C NMR with DEPT experiments displayed 40 carbon signals comprising six carbonyls (δ 199.3, 172.8, 171.8, 171.5, 169.5, and 166.8), four double bonds, five methyls, nine methylenes, seven methines, and five quaternary carbons. Among the six carbonyls, one carbonyl at δ 199.3 was assigned to a ketone group, and the others were described as ester carbonyls on the basis of their chemical shifts. The strong IR absorption bands at 3467 and 1740 cm^{-1} indicated the existences of hydroxyls and carbonyls, respectively. In the ^1H NMR spectrum, three broad singlets at δ 3.55, 2.24, and 1.86 (each 1H), which did not show any correlations in the HMQC spectrum, were indicative of the exchangeable protons, such as hydroxyls. Based on the above analyses, 10 out of 19 degrees of unsaturation were consumed by six carbonyls and four double bonds, and the remaining nine degrees of unsaturation were attributed to the presence of a nonacyclic system.

The analysis of ^1H–^1H COSY clearly outlined six protons bearing spin coupling units as drawn with bold

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Keywords: Chloramultilide A; Sesquiterpenoid dimmer; *Chloranthus multistachys*; Structural elucidation.

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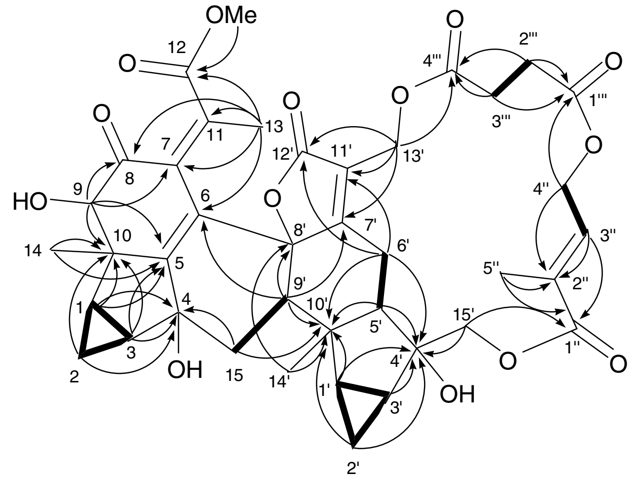


Figure 1. ^1H–^1H COSY (▬) and selected HMBC (H→C) correlations of chloramultilide A (**1**).

bonds (Fig. 1). The scaffold of **1** was figured out by HMBC experiment (Table 1, Fig. 1), which allowed the connection of the proton bearing spin coupling units. The HMBC correlations could link most of the bonds together, except for the bond between C-6 and C-7. Fortunately, a rare but important J^4 long-range correlation between H$_3$-13 and C-6 implied the linkage of C-6 and C-7. In the ^{13}C NMR spectrum, the downfield chemical shift of quaternary carbon C-8′ at δ 87.2 indicated the linkage of C-8′ and C-12′ via an oxygen atom to form a five-membered α,β-unsaturated lactone.[6] The HMBC correlations clearly located three carbonyls at C-8, C-12, and C-12′, three double bonds as Δ^5, $\Delta^{7(11)}$, and $\Delta^{7'(11')}$, and six oxygenated carbons at C-4, C-9, C-4′, C-8′, C-13′, and C-15′ in the sesquiterpenoid dimmer core. The only methoxyl group was placed unambiguously at C-12 based on the HMBC correlation between C-12 and OMe. The 1D and 2D NMR implied the presence of a succinyl (two carboxyls at δ 171.5 and 171.8; two methylenes at δ_C 29.2, δ_H 2.74 and 2.64; δ_C 28.8, δ_H 2.85 and 2.54) and a 4-hydroxy-2-methylbut-2-enoyl (carboxyl at δ 166.8; CH$_3$ at δ_C 12.8, δ_H 1.93; oxygenated methylene at δ_C 61.4, δ_H 5.11 and 4.63; trisubstituted double bond at δ_C 135.7 and 129.5, δ_H 6.59). The succinyl and 4-hydroxy-2-methylbut-2-enoyl were actually formed a substructural unit C-1″–C-4‴ as judged from the HMBC correlations of H$_2$-4″/C-1‴. The down-field chemical shifts of H$_2$-13′ (δ 5.26 and 4.56) and H$_2$-15′ (δ 4.39, 3.79) suggested that the substructural unit C-1″–C-4‴ was likely attached to C-13′ and C-15′ to form two ester groups. The HMBC correlations of H$_2$-13′/C-4‴, and H$_2$-15′/C-1″ linked C-13 to C-4‴ and C-15′ to C-1″ via oxygen atoms, respectively. The planar structure of **1** was thus figured out.

Table 1. NMR data for chloramultilide A (**1**) in CDCl$_3$

	δ_C	δ_H (pattern, J/Hz)	HMBC (H→C)	NOESY
1	25.3	1.99 (dd, 12.6, 6.1)	2, 3, 4, 5, 9, 10	2, 3, 9
2	8.7	0.95 (m, 2H)	1, 4, 10	1, 3, 14
3	29.9	1.84 (dd, 12.3, 6.6)	1, 2, 4, 5	1, 2, 9, 15β
4	79.1			
5	163.1			
6	124.8			
7	141.7			
8	199.3			
9	77.7	3.72 (s)	1, 5, 7, 8, 11,[a] 14	1, 3, 15α, 5′
10	50.7			
11	129.6			
12	169.5			
13	20.7	1.79 (s, 3H)	6,[a] 7, 8,[a] 11, 12	14
14	15.5	1.08 (s, 3H)	1, 2,[a] 4,[a] 5, 9, 10	1, 2, 13, OMe
15	41.4	β 2.79 (m)	4, 7′,[a] 9′, 10′	3, 15α, 9′
		α 1.71 (dd, 13.5, 10.2)	7′,[a] 9′, 10′	9, 15β, 5′
1′	26.9	1.54 (dd, 12.7, 6.3)	4′, 10′	15α, 15β, 2′α, 3′
2′	10.1	β 1.29 (m)	1′	2′α, 14′
		α 0.63 (m)	1′, 4′, 10′	1′, 2′β, 3′
3′	28.6	1.45 (m)	4′, 15′	1′, 2′α, 5′, 15′a, 15′b
4′	76.9			
5′	57.2	1.56 (m)	4′, 6′, 9′, 10′	9, 6′α, 15′b
6′	23.7	β 2.92 (dd, 18.0, 13.1)	4′, 5′, 8′, 11′	6′α, 13′a, 14′
		α 2.42 (dd, 18.0, 6.4)	5′, 10′, 11′	5′, 6′β, 3″
7′	171.1			
8′	87.2			
9′	53.2	2.54 (br d, 10.2)	15, 5′, 7′, 8′, 10′, 11′,[a] 14′	15β, 1′, 14′
10′	45.3			
11′	124.4			
12′	172.8			
13′	54.0	a 5.26 (d, 12.2)	7′, 11′, 12′, 4‴	6′β, 13′b
		b 4.56 (d, 12.2)	7′, 11′, 12′, 4‴	13′a
14′	24.0	0.93 (s, 3H)	1′, 2′,[a] 5′, 9′, 10′	2′β, 6′β, 9′

Table 1 (*continued*)

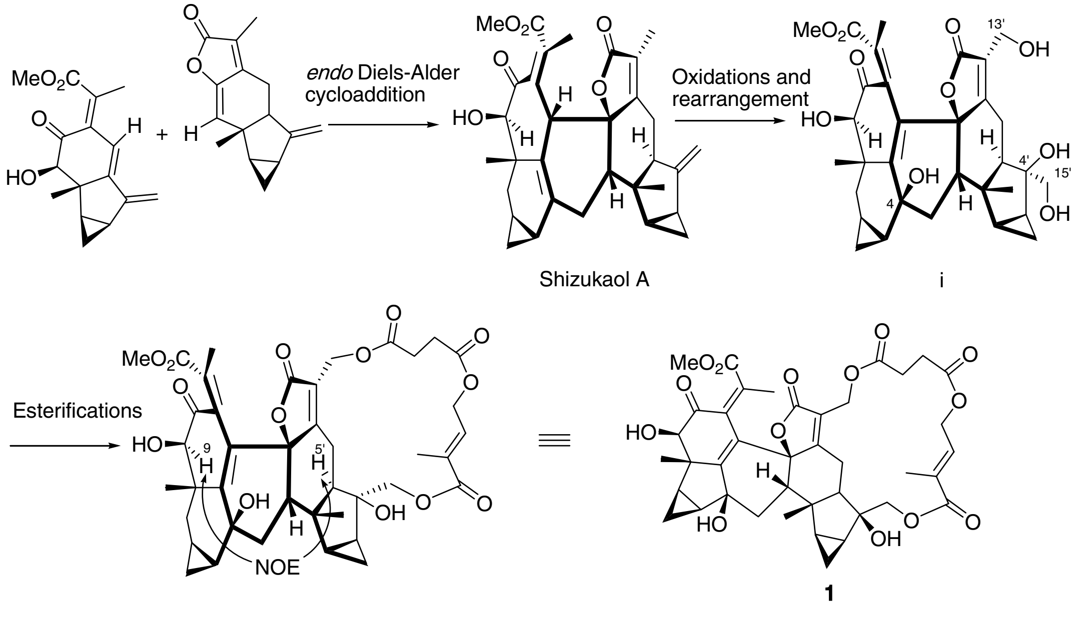
	δ_C	δ_H (pattern, *J*/Hz)	HMBC (H→C)	NOESY
15′	73.4	a 4.39 (d, 11.6)	3′, 4′, 5′, 1″	3′, 15′b
		b 3.79 (d, 11.6)	5′, 1″	3′, 5′, 15′a
1″	166.8			
2″	129.5			
3″	135.7	6.59 (t, 5.9)	1″, 2″, 5″	6′α, 4″a, 4″b, 5″
4″	61.4	a 5.11 (dd, 14.4, 4.8)	2″, 3″, 1‴	3″, 4″b, 5″
		b 4.63 (dd, 14.4, 7.2)	2″, 3″, 1‴	3″, 4″a, 5″
5″	12.8	1.93 (s, 3H)	1″, 2″, 3″	3″, 4″a, 4″b
1‴	171.5			
2‴	28.8	a 2.85 (m)	1‴, 3‴, 4‴	2‴b, 3‴a, 3‴b
		b 2.54 (m)	1‴, 3‴, 4‴	2‴a, 3‴a, 3‴b
3‴	29.2	a 2.74 (dd, 13.5, 7.2)	1‴, 2‴, 4‴	2‴a, 2‴b, 3‴b
		b 2.64 (dd, 16.8, 7.2)	1‴, 2‴, 4‴	2‴a, 2‴b, 3‴a
4‴	171.8			
OMe	52.5	3.72 (s)	11,[a] 12	14
OH		3.55		
		2.24		
		1.86		

In the vertical column of HMBC.

[a] Stand for J^4 correlations.

To support the gross structure of this sesquiterpenoid dimmer, the biogenetic origin of chloramultilide A (**1**) was proposed (Scheme 1). Two molecular lindenane sesquiterpenoids via an intermolecular Diels–Alder cycloaddition yielded the basic dimer core, shizukaol A.[7] The oxidation of C-6, C-13′, C-4′, and C-13′ were subsequently occurred, and then the rearrangement of Δ^4 to Δ^6 gave the key intermediate **i**. The intermediate **i** was finally acylated to afford chloramultilide A (**1**).

The relative configuration of **1** was fixed by using NOESY spectrum (Fig. 2). The NOESY correlations of H-1/H-3, H-1/H-9, H-3/H-9, H-9/H-15α, H-2β/H_3-14, H-1′/H-3′, H-1′/H-2′α, H-3′/H-2′α, H-5′/H-15α, and H_3-14′/H-2′β, indicated that the stereochemistry of H-1 (α), H-3 (α), CH_3-14 (β), H-1′ (α), H-3′ (α), H-5′ (α), and CH_3-14′ (β) was consistent with those of natural lindenane type sesquiterpenoid, and H-9 adopted α-configuration. Consideration on the biogenetic origin of chloramultilide A (**1**) (Scheme 1), two molecular lindenane type sesquiterpenoids approached each other in a cis-mannar and mostly gave a predominated *endo*-cycloaddition product in Diels–Alder cycloaddition reaction,[8] and the *endo*-cycloaddition product (**1**) was supported by a key cross-peaks between H-9 and H-5′ in the NOESY spectrum. For Diels–Alder cycloaddition product, the O-8′ of ester group and H-9′ were definitely in the same direction and was defined as β-oriented. Although there were no NOESY correlations available to fix the geometry of $\Delta^{7(11)}$ double bond, the biogenetic precursor of chloramultilide A (**1**), shizukaol,[7] would retain the *Z*-geometry for $\Delta^{7(11)}$ double bond in the



Scheme 1. Biogenetic pathway proposed for chloramultilide A (**1**).

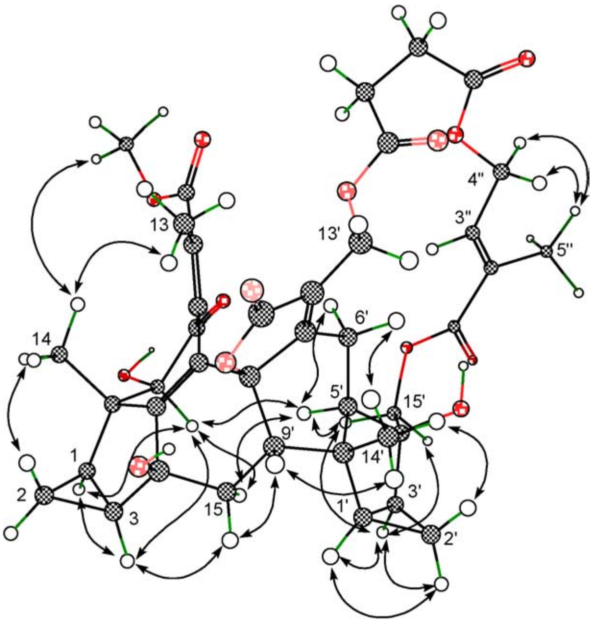


Figure 2. Key NOESY (↔) correlations of chloramultilide A (**1**).

transformation of **1**. The NOESY correlations of H_2-15′ to H-3′ implied a α-configuration for CH_2-15′. The NOESY cross-peaks of H_2-4″ to CH_3-5″ showed the 2″*E*-geometry for $\Delta^{2''}$ double bond. A computer modeled 3D structure of **1** (Fig. 2, on which the NOESY correlations were depicted) was generated by using the molecular modeling program CS Chem 3D Pro Version 6.0, using MM2 force field calculations for energy minimization. The relative stereochemistry and the favorable conformation of **1** offered by computer modeling were consistent with those of **1** assigned by NOESY spectrum.

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5. *Chloramultilide A* (**1**). Amorphous white powder; $[\alpha]_D^{20}$ −93.2 (*c* 0.6, $CHCl_3$); UV (CH_3OH) λ_{max} (log ε) 218 (4.25); IR (KBr) λ_{max} cm^{-1} 3467, 2937, 1740, 1437, 1369, 1267, 1169, 978; ^{1}H and ^{13}C NMR see Table 1; EIMS 70 eV *m*/*z* (rel int) 748 $[M]^+$ (2), 730 (7), 698 (20), 648 (7), 630 (7), 586 (4), 554 (7), 532 (9), 514 (17), 482 (17), 437 (25), 409 (29), 393 (35), 365 (28), 349 (27), 289 (25), 263 (25), 246 (37), 213 (49), 202 (30), 119 (34), 99 (75), 69 (53), 56 (100); ESI MS (positive) *m*/*z* (rel int) 787 $[M+K]^+$ (26), 771 $[M+Na]^+$ (100); HR EIMS *m*/*z* 748.2713 ($C_{40}H_{44}O_{14}$, calcd 748.2731).
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