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Chlojapolactone A, An Unprecedented 1,3-Dioxolane Linked-Lindenane Sesquiterpenoid Dimer from *Chloranthus japonicus*†

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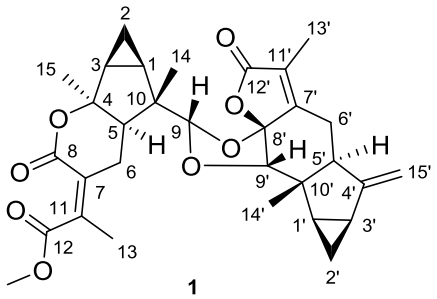
Chlojapolactone A (1), a novel lindenane sesquiterpenoid dimer with an unprecedented 1,3-dioxolane linkage, was isolated from *Chloranthus japonicus*. Its structure and absolute configuration was elucidated by combined spectral, computational, and chemical approaches. Compound 1 exhibited potential inhibitory effects on nitric oxide production in RAW 264.7 cells.

Lindenane sesquiterpenoid dimers are a class of highly complex natural products mainly occurring in plants of the genus *Chloranthus* (Chloranthaceae).[1,2] Biosynthetically they are proposed to be adducted from two lindenanes *via* Diels-Alder cycloaddition, with the fusing sites of C-6/C-8′ and C-15/C-9′ to form an additional six-membered carbon ring linkage.[1,2] So far, more than 50 lindenane dimers have been isolated.[1,2] Some of them exhibited significant biological activities, such as anticancer,[3] anti-HIV,[4] and inhibition of tyrosinase[5] and the delayed rectifier (I_K) K^+ current.[6,7] In recent years, their fascinating structures and important biological activities have attracted great interests of both natural product and synthetic chemists.[8-11]

C. japonicus Sieb., a perennial herbaceous plant growing in southern China, has been applied in the Traditional Chinese Medicine (TCM) for the treatment of rheumatic arthralgia, bone fracture, pulmonary tuberculosis, and neurasthenia.[12] Previous investigations on this plant have proved that it was a rich source of structurally diverse lindenane sesquiterpenoid dimers.[3,4] In our efforts toward novel nitric oxide (NO) inhibitors from medicinal plants,[13] a fraction of the ethanolic extract of *C. japonicus* showed an inhibitory activity of 61.21% at a concentration of 10 μg/ml against the lipopolysaccharide (LPS) induced NO production in RAW 264.7 macrophages. Subsequent chemical investigation led to the isolation of chlojapolactone A (**1**), a lindenane dimer with a unique 1,3-dioxolane linkage and comprising a rare dimaleate featured 8,9-*seco* lindenane monomer



1

Fig. 1 The structure of compound **1.**

(Fig. 1). Compound **1** represents a novel dimerization pattern of the lindenane dimer class, which is proposed to be biosynthetically formed by an aldol condensation of a rare 8,9-*seco* lindenane and a lindenane sesquiterpenoids. Bioassay verified that compound **1** had inhibition against NO production in LPS-stimulated RAW 264.7 macrophages with IC_{50} value of 14.87 μM, being comparable to the positive control quercetin (IC_{50} = 15.90 μM). Herein, the isolation, structure elucidation, biogenetic origin, and NO inhibitory activity of **1** are described.

The air-dried powder of the whole plant of *C. japonicus* (1.0 kg) was extracted with 95% EtOH at room temperature to give a crude extract, which was suspended in H_2O and successively partitioned with petroleum ether, EtOAc, and *n*-BuOH. Various column chromatographic separations of the EtOAc extract afforded compound **1** (15 mg).

Chlojapolactone A (**1**) was obtained as colorless oil. The HRESIMS displayed a *pseudo*-molecular ion at *m/z* 535.2331 $[M - H]^-$ (calcd for 535.2337), which was consistent with a molecular formula of $C_{31}H_{36}O_8$, corresponding to 14 degrees of unsaturation. The IR spectrum exhibited absorption bands for carbonyl (1751 and 1715 cm^{-1}) functionalities. The ^1H NMR spectrum showed signals for a terminal double bond [δ_H 5.03 (1H, d, *J* = 2.0 Hz) and 4.73 (1H, brs)], five methyl singlets [δ_H 1.96, 1.85, 1.62, 1.14, and 0.51 (each, 3H, s)], an *O*-methyl [δ_H 3.75 (3H, s)], two oxygenated methines [δ_H 5.15 (1H, s) and 4.30 (1H, s)], and four highly upfield-shifted protons [δ_H 0.85, 0.72, 0.69, and 0.58 (each, 1H, m)] characterized for two cyclopropane methylenes.[6,7] The ^{13}C NMR spectrum, in combinati

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† Electronic Supplementary Information (ESI) available: NMR spectra of **1–5**, detail information for ECD calculation, isolation, purification, semisynthesis, and bioassay protocols. See DOI: 10.1039/x0xx00000x

with DEPT experiments, resolved 31 carbon resonances attributable to three carbonyls (δ_C 171.0, 170.1, 167.1), three double bonds (one exocyclic and two tetrasubstituted), six methyls (one *O*-methyl), four sp^3 methylenes, eight sp^3 methines (two oxygenated), and four sp^3 quaternary carbons (two oxygenated). The aforementioned information implied that compound **1** consisted of two lindenane sesquiterpenoid units.[1,6]

Detailed 2D NMR studies (HSQC, ^{1}H–^{1}H COSY, and HMBC experiments) afforded the gross structures of these two units (**a** and **b)** as depicted in Fig. 2. Unit **b** (in blue) was readily determined to be the typical lindenane-type sesquiterpenoid, chloranthalactone E,[14,15] by direct comparison of their 1D NMR data. This was further supported by ^{1}H–^{1}H COSY and HMBC correlations (Fig. 2). As for unit **a** (in red), two structural fragments [a cyclopropane ring (C-1–C-2–C-3) and C-5–C-6] were first established by the ^{1}H–^{1}H COSY correlations (Fig. 2). The connectivities of these fragments, quaternary carbons, and other functional groups were mainly achieved by analysis of the HMBC spectrum (Fig. 2). The HMBC correlations from H_3-14 to C-1, C-5, C-9, and C-10 allowed the connection of C-1, C-5, C-9, and C-14 to the quaternary carbon C-10, while the HMBC correlations from H_3-15 to C-3, C-4, and C-5 linked C-3, C-5, and C-15 to the quaternary carbon C-4. Thus, ring A of unit **a** (Fig. 2) was established. The linkages of C-6–C-7–C-8 were revealed by HMBC correlations of H_2-6/C-7 and C-8, while the correlations from H_3-13 to C-7, C-11, and C-12 and from H_2-6 to C-7 and C-11 further attached C-7, C-12, and C-13 to C-11. The upfield-shifted carbonyl of C-8 (δ_C 167.1) and still "loose end" oxygenated quaternary carbon of C-4 (δ_C 92.5) required the existence of a lactone bridge between C-4 and C-8, which generated ring B of unit **a** (Fig. 2). Finally, the methoxy group was located at C-12 by the correlation from the *O*-methyl to C-12. Unit **a** represented a rare 8,9-*seco* lindenane skeleton.[1]

As above-mentioned structure elucidation already accounted for 13 out of the 14 degrees of unsaturation, the remaining one thus required the presence of an additional ring to link units **a** and **b**. In HMBC spectrum, a strong correlation from H-9 (δ_H 5.15) to the ketal carbon at C-8′ was observed, suggesting the presence of an oxygen bridge between C-9 and C-8′. Based on the acetal nature of C-9 (δ_C 109.7) and still "loose end" oxygenated methine C-9′ (δ_C 85.2), the existence of the other oxygen bridge between C-9 and C-9′ was proposed to form the additional 1,3-dioxolane ring. However, in regular HMBC experiment [delay time = 62.5 ms, $J_{(C,H)}$ = 8 Hz], even recorded in different deuterated solvents, the expected correlations between CH-9 and CH-9′ were not observed (ESI S15† and S20†). Thus, a modified HMBC experiment (delay time = 250 ms, $J_{(C,H)}$ = 2 Hz) was employed,[16] which generated the correlations of H-9/C-9′ and H-9′/C-9 (ESI S9†). This was also collaborated by a long rang ^{1}H–^{1}H COSY correlation[17] between H-9 and H-9′ (ESI S6†). Thus the gross structure of **1** was elucidated as depicted, with a 1,3-dioxolane ring linking the two lindenane units.

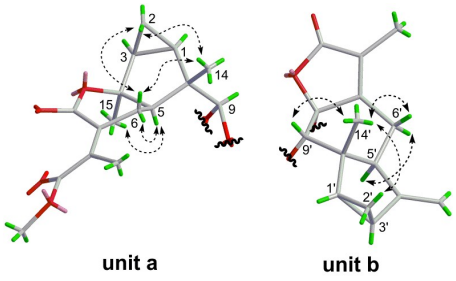


Fig. 3 Key ROESY correlations (↞⇢) of unit **a** and **b**.

The relative configuration of **1** was established by ROESY experiment and by comparison of its 1D NMR data with those known lindenane monomers. In unit **a**, the correlations of H-2β/H-6β and H_3-14/H-2β and H-6β indicated that these protons were co-facial and were arbitrarily assigned as β-orientation (Fig. 3). In consequence, the ROESY cross-peaks of H-5/H-6α and H_3-15 revealed that these protons were α-oriented, which was further supported by the large coupling constant between H-5 and H-6β (J = 12.5 Hz). As for unit **b**, the ROESY correlations of H_3-14′/H-2′β, H-6′ and H-9′ indicated that they were co-facial and were arbitrarily assigned as β-oriented (Fig. 3). Consequently, the ROESY cross-peaks of H-5′/H-6′α indicated that these protons were α-oriented, which was further supported by the large coupling constant between H-5′ and H-6′β (J = 12.5 Hz). In addition, the correlation of H-9/H-9′ indicated that these protons were co-facial on the 1,3-dioxolane ring (Fig. 4). The 1D NMR data of most of the chiral centers in units **a** and **b** were consistent with those reported in their corresponding monomers.[1,2]

The configuration of C-8′ could not be directly assigned owing to its ketal nature, resulting in two possible isomers of **1** (**1a** with C-8′–O–C-12′ in β-orientation and **1b** with C-8′–O–C-12′ in α-orientation, in Fig. 4). Thus, a Chem3D molecular modeling study considering the rotatable C-9–C-10 bond was employed to rationalize the ROESY correlations crossing the two lindenane units. In the energy minimized conformation of **1a**, after optimization of the conformation around C-9–C-10 bond, the internuclear distances between H-5′ and H-5 or H_3-14 could reach the range within 3.0 Å, while these distances were greater than 4.0 Å in **1b** no matter how to rotate the two units around this bond (ESI S32†). As the strong cross-peaks of H-5′/H-5 and H-5′/H_3-14 were observed in the ROESY spectrum, **1a** was selected with favorable stereochemistry.

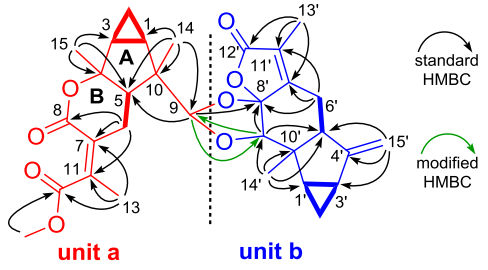


Fig. 2 ^{1}H–^{1}H COSY (bold lines) and selected HMBC (arrows) correlations of **1**.

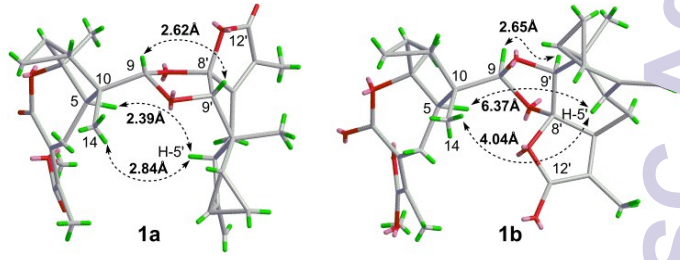


Fig. 4 Internuclear distances of H-5′/H-5 and H-5′/H_3-14 in isomers **1a** (C-8′–O–C-12′ in β-orientation) and **1b** (C-8′–O–C-12′ in α-orientation).

Table 1 ^{1}H and ^{13}C NMR data of compound **1** (in $CDCl_3$)

no.	δ_H [a]	δ_C [b]	no.	δ_H [a]	δ_C [b]
1	1.70, m	27.4	1′	1.81, m	23.6
2α	0.72, m	7.7	2′α	0.85, m	15.6
2β	0.58, m		2′β	0.69, m	
3	1.61, m	29.3	3′	1.95, m	23.5
4		92.5	4′		151.1
5	2.33, dd (12.5, 6.9)	48.1	5′	3.43, m	50.3
6α	2.45, dd (12.5, 6.9)	27.2	6′α	2.51, dd (18.0, 6.7)	22.1
6β	2.03, dd (12.5, 12.5)		6′β	2.26, dd (18.0, 12.5)	
7		128.2	7′		154.1
8		167.1	8′		109.5
9	5.15, s	109.7	9′	4.30, s	85.2
10		48.9	10′		42.6
11		137.9	11′		127.8
12		170.1	12′		171.0
13	1.96, s	16.2	13′	1.85, s	8.7
14	1.14, s	16.4	14′	0.51, s	17.1
15	1.62, s	31.1	15′a	4.73, brs	106.6
OMe	3.75, s	52.6	15′b	5.03, d (2.0)	

[a] Recorded at 400 MHz, [b] Recorded at 100 MHz, chemical shifts are in ppm, coupling constant *J* is in Hz.

The absolute configuration of **1** was determined by the exciton chirality method.[18] The UV spectrum of **1** showed a strong absorption at λ_{max} 223 nm (log ε 4.20) corresponding to two moieties of α,β-unsaturated ketones. Consistent with this UV maximum, the CD spectrum of **1** showed a positive Cotton effect at 251 nm ($\Delta\varepsilon$ +0.52, n $\rightarrow \pi^*$ transition) and a negative Cotton effect at 222 nm ($\Delta\varepsilon$ –1.60, $\pi \rightarrow \pi^*$ transition) due to the transition interaction between two α,β-unsaturated ketone chromophores (Fig. 5), indicating a positive chirality for **1**. The positive chirality of **1** revealed that the transition dipole moments of two chromophores were oriented in clockwise direction, and the absolute stereochemistry of **1** was assigned as depicted.

To verify the absolute configuration assigned by the CD exciton chirality method, ECD calculations using time dependent density functional theory (TDDFT) were carried out on compound **1** (ESI S33†). The experimental ECD spectrum of **1** showed first positive and second negative Cotton effects at 251 and 222 nm respectively, which matched the calculated ECD curve for **1a** (Fig. 5), an isomer with 1*R*, 3*S*, 4*S*, 5*R*, 9*S*, 10*S*, 1′*R*, 3′*S*, 5′*S*, 8′*S*, 9′*S*, 10′*S* configuration, indicating that **1** possessed the same absolute configuration.

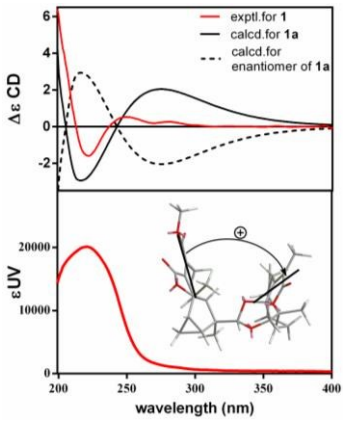
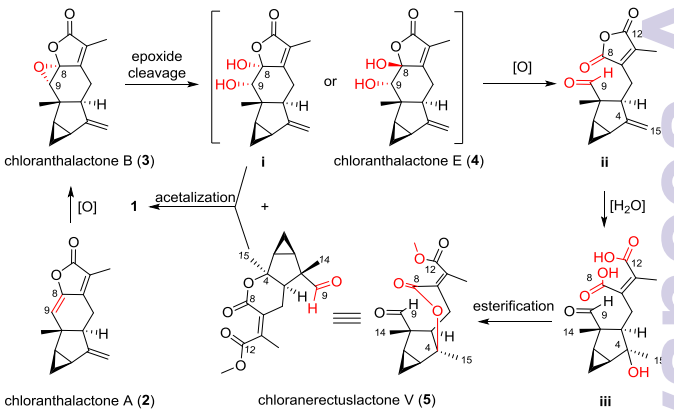


Fig. 5 CD and UV spectra of **1** (in MeOH, red lines), the stereoview of **1** in exciton chirality method model (arrows denote the electric transition dipole of the coupling chromophores), the calculated ECD spectra for **1a** (1*R*, 3*S*, 4*S*, 5*R*, 9*S*, 10*S*, 1′*R*, 3′*S*, 5′*S*, 8′*S*, 9′*S*, 10′*S*, black line), and the calculated ECD spectra for the enantiomer of **1a** (dashed line).

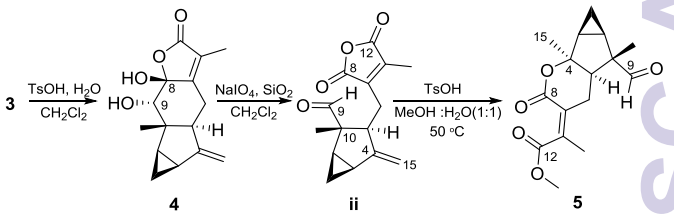
This assignment was consistent with the biogenetic origin of lindenane- type sesquiterpenoids from the genus *Chloranthus*. Th compound **1** was assigned as depicted.

Based on the co-isolated known lindenanes **2–4**,[14,15,19] a plausible biosynthesis of chlojapolactone A was proposed in Scheme 1. Chloranthalactone A (**2**), a major component isolated in the study, was considered as the precursor. **2** was modified by several steps of transformations, involving epoxidation of Δ^8, epoxy ring-opening, and oxidative cleavage of C-8–C-9 diol, to generate intermediate **i** and **ii**. Intermediate **ii** underwent hydrolysis and hydration then followed by intramolecular esterification to produce chloranerectuslactone V (**5**). Finally, the aldehyde **5** was acetalized with diol **i** to afford **1**. To support the proposed biosynthetic pathway and to further secure the structure of unit **a**, compound **5** was biomimetically semisynthesized from **3** (Scheme 2). Interestingly, just after we finished the synthetic work, chloranerectuslactone V (**5**) was reported as a novel natural produ from *C. erectus* by other group.[20] The efforts to semisynthesize **i** f further condensation with **5** was failed, as **i** was very labile, which could only be detected in the acid-catalyzed epoxy ring-openi solution of **3** and quickly isomerized to **4** during the purificatio process. The existence of **i** was demonstrated by isolation of its 8-*O*-methyl derivative in a TsOH/MeOH/H_2O reaction system (ESI S2.5†). Above chemical evidences together with the absence of **5** in th crude extract of this plant suggested that **1** was a genuine natural product.

Chlojapolactone A (**1**) was evaluated for its inhibitory effect on nitric oxide (NO) production of LPS-activated RAW 264.7 macrophages. Under the subtoxic concentration (100 μM), **1** exhibited potentia inhibition against NO production with IC_{50} value of 14.87 μM, being comparable to the positive control quercetin (IC_{50} = 15.90 μM).



Scheme 1 Proposed biosynthetic pathway for **1**.



Scheme 2 Semisynthesis of chloranerectuslactone V (**5**).

Conclusions

In summary, our bioassay guided investigation into the chemistry of the traditional Chinese medicinal plant *Chloranthus japonicus* led to the discovery of chlojapolactone A (**1**), a novel lindenane dimer featuring a rare 1,3-dioxolane linkage between a 8,9-*seco* lindenane and a lindenane sesquiterpenoid. To the best of our knowledge, dimers incorporating a 1,3-dioxolane linkage are rare in nature, known examples of which are limited to the sesquiterpene dimers volvalerelactone B from *Valeriana officinalis* var. *latifolia*[21] and cinnafragrin A and B and capsicodendrin from *Cinnamosma fragrans*.[22] Lindenane-type dimers reported previously were exclusively formed through direct carbon-carbon coupling of two monomers. Chlojapolactone A comprising a rare 8,9-*seco*-lindenane unit represented the first example of lindenane dimer coupled *via* an acetal bridge. A plausible biosynthetic route for **1** was proposed and partially mimicked by biomimetic semisynthesis. Compound **1** exhibited potential inhibitory effects on nitric oxide (NO) production induced by lipopolysaccharide (LPS) in RAW 264.7 cells, which may account for the inhibitory effect of the crude extract and explain the anti-inflammatory function of *C. japonicus* in folk medicine. Further studies of compound **1** regarding its effects on other anti-inflammatory signal pathways are under investigation.

Acknowledgments

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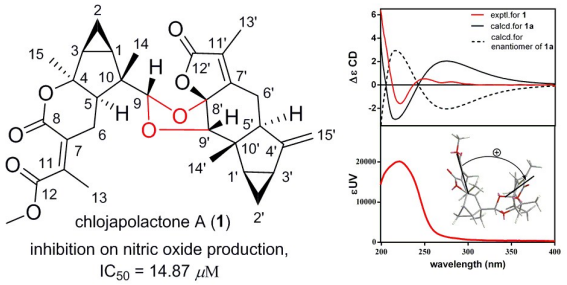
22. L. Harinantenaina and S. Takaoka, *J. Nat. Prod*., 2006, **69**, 1193.
23. Chlojapolactone A (**1**)**.** colorless oil; $[\alpha]^{20}_{D}$ −67.20 (*c* 0.1, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 223 (4.20) nm; IR (KBr) ν_{max} 1751, 1711, 1648, 1080, 960 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1 and ESI Table S1;† positive ESIMS *m/z* 559.3 $[M + Na]^+$, negative ESIMS *m/z* 535.5 $[M - H]^-$; HRESIMS *m/z* 535.2331 $[M - H]^-$ (calcd for $C_{31}H_{35}O_8$, 535.2337).

Graphical Abstract:



Chlojapolactone A (**1**), a novel lindenane sesquiterpenoid dimer with an unprecedented 1,3-dioxolane linkage, was isolated from *Chloranthus japonicus*.