Three Sesquiterpenoid Dimers from *Chloranthus japonicus*. Absolute Configuration of Chloraholide A and Related Compounds

Abstract: A novel sesquiterpenoid dimer, named multistalide C (**1**), together with two known congeners, shizukaols C (**2**) and D (**3**), was isolated from the whole plant of *Chloranthus japonicus* Sieb. The structures of compounds **1**-**3** were elucidated by extensive HR-ESI-MS, 1D and 2D NMR spectroscopic analysis. Compounds **1**-**3** exhibited significant toxic effects on brine shrimp larvae (*Artemia salina*). The absolute configuration of **1** was established by CD/TDDFT calculations. The related compound chloraholide A was also reinvestigated. The previous assignment of the absolute configuration of chloraholide A and several related sesquiterpenoid dimers, based on an incorrect application of the exciton chirality method, is criticized.

Xin-Wei Shi,*[a] Qiang-Qiang Lu,[a] Gennaro Pescitelli,*[b] Trpimir Ivšić,[b,c] Jun-Hui Zhou,[a] and Jin-Ming Gao*[d]

Keywords: Natural products; Structure elucidation; Electronic Circular Dichroism; TDDFT calculations; Exciton Chirality Method; Brine shrimp larvae toxicity

Introduction

The genus *Chloranthus* (Chloranthaceae) is mainly distributed over the east of Asia, and it comprises 13 species and five varieties in China.[1,2] *Chloranthus japonicus* Sieb ('yin-xian-cao' in Chinese) has been long used in traditional Chinese medicine for the treatment of bone fractures, trauma, rheumatism, and cough.[3-5] The previous phytochemical investigations on this plant have resulted in the isolation of a number of sesquiterpenoid dimers.[6-9] Some of them were reported to show multi-biological activities, such as antifungal, potent and selective tumor growth-inhibitory, inhibition of cell adhesion molecule expression and tyrosinase.[10-12]

As part of our ongoing search for new bioactive natural products from the traditional Chinese herbal medicines in the Qinba Mountains,[13-17] chemical studies of the whole plant of *C. japonicus* were carried out, leading to the discovery of a new sesquiterpenoid dimer, named multistalide C (**1**), together with two known compounds, shizukaols C and D (**2** and **3**) (Chart 1). Herein, we describe the isolation, structural identification, including the absolute configuration, and brine shrimp larvae toxicity of the three compounds. Since compound **1** is structurally related to chloraholide A (**4**), this latter compound was also reconsidered. In fact, the absolute configuration of chloraholide A[18] and several related sesquiterpenoid dimers has been assigned using the exciton chirality method,[19-21] but without the necessary prerequisites for the application of this well-known approach. The limitations of the exciton chirality method for this family of chloraholide analogues are discussed in details.

Materials and Methods

General experimental procedures: NMR Spectra were recorded with a Bruker AV-500 instrument, using TMS as internal standard. Mass spectra (HR-ESI-MS and ESI-MS) were recorded with a ThermoFisher Q-Exactive spectrometer. IR spectra were recorded with a Shimadzu FTIR-8900 instrument. UV spectra were recorded with a Shimadzu UV-2401A spectrophotometer. ECD spectra were recorded with a Jasco J-810 spectropolarimeter. Optical rotations were measured with a Rudolph Autopol-Automatic polarimeter, Silica gel (SiO_2: 100-200, 200-300 mesh) was acquired from Qingdao Marine, China. MCI gel (75-150 μm) was acquired from Mitsubishi, Japan. HPLC separations were run with RP-18 (LiChroprep, 25-40, 40-63 μm; YMC, Japan) and Sephadex LH-20 columns (Pharmacia, Sweden).

Plant material: The whole plant of *C. japonicus* was collected in Taibai mountain of Qinling area, Shaanxi province, P.R.China in 2013, and was identified by Dr. Zhou, Y. F. of Xi'an Botanical Garden, Institute of Botany of Shaanxi. A voucher sample (NO.03054) was deposited at the Plants Herbarium of Institute of Botany of Shaanxi.

[a] *Prof. X.-W. Shi, Dr. Q.-Q.Lu, Dr. J.-H. Zhou*
Xi'an Botanical Garden
Institute of Botany of Shaanxi Province
Xi'an 710061,Shaanxi, People's Republic of China
E-mail: sxw@ms.xab.ac.cn

[b] *Prof. G. Pescitelli, Dr. T. Ivšić*
Dipartimento di Chimica e Chimica Industriale
Università di Pisa
via Moruzzi 13, I-56124 Pisa, Italy
E-mail: gennaro.pescitelli@unipi.it

[c] *Dr. T. Ivšić*
Division of Organic Chemistry and Biochemistry
Ruđer Bošković Institute
10000 Zagreb, Croatia

[b] *Prof. J.-M. Gao*
Shaanxi Key Laboratory of Natural Products & Chemical Biology, College of Science
Northwest A&F University
Yangling 712100, Shaanxi, People's Republic of China
E-mail: jinminggao@nwsuaf.edu.cn

Received: ((will be filled in by the editorial staff))
Revised: ((will be filled in by the editorial staff))
Published online: ((will be filled in by the editorial staff))

Extraction and Isolation: The air-dried roots and aerial parts of *C. japonicus* (20 kg) were extracted three times with MeOH (150 L) after 2-hour reflux, and the filtrate was evaporated under reduced pressure. The residue (1500 g) was diluted with H_2O (5 L) and successively extracted with petroleum ether (PE), EtOAc, and *n*-BuOH (5 L) five times. After evaporation, the PE (250 g), EtOAc (393 g), and *n*-BuOH (390 g) extracts were obtained. The EtOAc extract was chromatographed over a silica gel column (PE-EtOAc, 10:0→0:10) to yield seven fractions, A-G. Fraction B (36 g) was subjected to MCI gel column chromatography (MeOH-H_2O, 1:9→3:7→5:5→7:3→9:1) to yield five fractions B_1-B_5. The fraction B_2 (6.5 g) eluted with 60% MeOH was separated repeatedly by Sephadex LH-20 (MeOH), RP-18 column (MeOH-H_2O, 3:7→4:6→5:5→7:3→9:1), followed by purification with a silica gel column (PE-EtOAc-$CHCl_3$, 6:1:1→5:1:1→4:1:1→3:1:1) to afford compounds **1** (3 mg), **2** (20 mg) and **3** (55 mg).

Multistalide C (**1**): colorless oil; $[\alpha]_D^{23}$ = –165.5 (*c* = 1.5 in MeOH); ^{1}H NMR and ^{13}C NMR, see Table 1; IR (KBr): v = 3361, 3288, 2922, 2839, 1652, 1386, 1028 cm^{-1}; UV-vis (MeOH): λ_{max} (log ε) = 335 (0.63), 237 nm (3.9); ECD (MeOH) λ_{max} (Δε) = 342 (–5.55), 266 (+20.1), 224 nm (–13.3); ESI-MS (*m/z*): 617.2 $[M+Na]^+$; HR-MS (ESI, *m/z*): $[M+Na]^+$ calcd for $C_{33}H_{38}O_{10}Na$, 617.2465; found 617.2355. All spectra are shown in the Supporting Information.
Shizukaol C (**2**) and *Shizukaol D* (**3**): see Supporting Information.

Brine Shrimp Larvae Lethality Assay: Brine shrimp eggs (Ocean Star International, Inc., USA) were hatched in a large beaker containing artificial seawater (2.5%, pH 8.0-8.5) and were cultured at 28°C for 48 h. Compounds **1**-**3** were dissolved in dimethyl sulfoxide (DMSO) at the concentration of 2 mg/mL, and then transferred in portions of 10, 8, 6, 4, 2, 1, and 0.5 µL to a 96-well plate, and diluted with artificial seawater (15-20 nuclei larvae) to final volumes of 200 µL, using DMSO as a blank control and pdophyllotoxin as a positive control. Each test was conducted in triplicate. After 24 h incubation, the number of dead shrimp in each well was counted under a microscope. The mortality rate was calculated using the formula: M=[(A–B–N)/(G–N)]×100 where M= percent of the dead larvae after 24 h; A= number of the dead larvae after24 h; B= average number of the dead larvae in the blank control after 24 h; N= number of the dead larvae before starting the test; G= number of selected larvae for test. In addition, the LC_{50} values were calculated.

Computational Section: MMFF and preliminary DFT calculations were run with Spartan'14 (Wavefunction, Inc., Irvine CA, 2014), with standard parameters and convergence criteria. DFT and TDDFT calculations were run with Gaussian'09,[22] with default grids and convergence criteria. Conformational searches were run with the Monte Carlo algorithm implemented in Spartan'14 using Merck molecular force field (MMFF). All structures thus obtained were optimized with DFT method using B3LYP functional and 6-31G(d) basis set in vacuo. TDDFT calculations were run using CAM-B3LYP and ωB97X-D functionals and TZVP basis set, including 40 excited states (down to 159 nm with CAM-B3LYP). The two functionals led to coincident results (see Supporting Information). ECD spectra were generated using the program SpecDis[23] by applying a Gaussian band shape with 0.4-0.5 eV exponential half-width, from dipole-length rotational strengths. Transition density plots were built using a locally modified version of the Gaussian 09 development program.

Results and Discussion

STRUCTURAL IDENTIFICATION OF COMPOUNDS

Shizukaols C (**2**) and D (**3**) (Chart 1) were identified by comparison of their spectroscopic data (1H and 13C NMR, HR-ESI-MS; see Supporting Information) with reported values.[24] These two metabolites are reported from this plant for the first time.

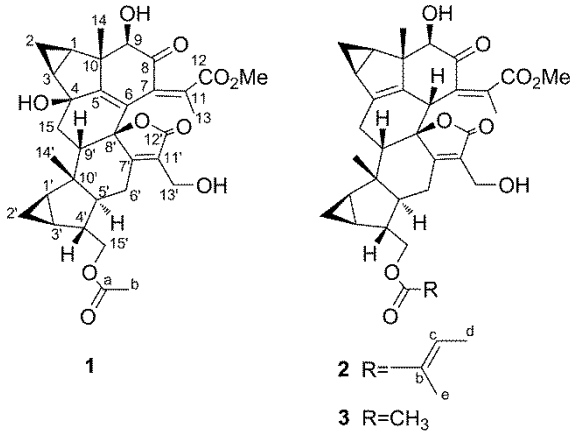


CHART 1 Compounds **1-3**.

TABLE 1 ^{1}H NMR (500 Hz) and ^{13}C NMR (125 MHz) data of compound **1** in $CHCl_3$-d_1:CH_3OH-d_4 (3:1).

#	δ_H, mult (*J*, Hz)	δ_C	#	δ_H, mult (*J*, Hz)	δ_C
1	1.99 dd (12.5, 6.3)	25.9 d	1'	1.26 m	26.1 d
2α	0.95 d (4.8)	8.6 t	2'α	0.72 dd (14.3, 8.7)	16.4 t
2β	0.93 d (7.6)		2'β	0.66 d (3.7)	
3	1.85 dd (12.0, 6.4)	30.1 d	3'	1.16 m	22.6 d
4		79.4 s	4'	1.42 m	44.2 d
5		162.5 s	5'	1.58 m	53.0 d
6		125.3 s	6'α	2.59 m	25.8 t
			6'β	2.46 m	
7		141.2 s	7'		167.5 s
8		199.6 s	8'		87.6 s
9	3.81 s	77.6[a] d	9'	2.68 dd (20.3, 8.4)	54.1 d
10		50.8 s	10'		44.7 s
11		130.1 s	11'		128.4 s
12		171.2 s	12'		172.5 s
13	1.74 s	20.9 q	13'α	4.36 d (13.8)	54.5 t
14	1.09 *s*	15.8 q	13'β	4.31 d (13.8)	
15α	2.75 dd (13.4, 6.9)	41.0 t	14'	0.85 s	22.8 q
15β	1.74 m				
a		172.1 s	15'α	4.00 dd (11.1, 3.9)	66.5 t
			15'β	3.91 dd (11.1, 6.4)	
b	2.08 s	20.7 q			
OMe	3.79 s	52.7 q			

[a] Overlapped with solvent signals.

Compound **1** (Chart 1) was obtained as a colorless oil with an optical rotation of $[\alpha]^{23}_D$ -165.5 (*c*=1.5, MeOH). The molecular formula $C_{33}H_{38}O_{10}$ was determined by HR-ESI-MS showing the sodiated species at *m/z* 617.2355 $[M+Na]^+$ (calcd. for $C_{33}H_{38}O_{10}Na$, 617.2465) corresponding to 15 degrees of unsaturation. IR spectra revealed the presence of hydroxyl (3361 cm^{-1}) and carbonyl (1652 cm^{-1}) functionalities. The ^{13}C NMR and

DEPT spectra of **1** (Table 1) showed 33 signals, consisting of four carbonyls (δ_C 199.6, 172.5, 172.1 and 171.2), four methyls including an acetoxyl (δ_C 20.7), one methoxyl (δ_C 52.7), three persubstituted double bonds, six sp^3 methylenes, eight sp^3 methines, and four sp^3 quaternary carbons. The ^{1}H NMR spectrum (Table 1) showed four methyl protons at δ_H 0.85 (s), 1.09 (s), 1.74 (s) and 2.08 (s), one methoxyl at δ_H 3.79 (s), and one sp^3 methine proton at δ_H 3.81 (s). The ^{1}H and ^{13}C NMR data of **1** were very similar to those of **3,** suggesting the same skeleton. The major differences between them were that the double bond between C-4 (δ_C 142.5) and C-5 (δ_C 131.6) in **3** shifted to C-5 (δ_C 162.5) and C-6 (δ_C 125.3) in **1,** with an additional hydroxyl appearing at the quaternary carbon (C-4) (δ_C 79.4) in **1**. The HMBC correlations of H-14 and H-15 to C-5, and of H-9 to C-5, C-6 revealed the presence of the double bond at C-5 and C-6 in **1;** the location of the OH group was supported by HMBC correlations of H-2 to C-3 and C-4, and of H-15 to C-4 (Figure 1). The analysis described above and the previous studies on sesquiterpenoid dimers within this genus suggested that the compound **1** is of lindenane type.[18,24]

Analysis of 1D and 2D NMR (HSQC, HMBC, ^{1}H-^{1}H COSY and NOESY) spectral data allowed for the final structural assignment of **1**. The ^{1}H-^{1}H COSY data (Figure 1) established two sets of typical proton spin systems of two 1,2-disubstituted cyclopropane rings [H-1: δ_H 1.99 (dd, 1H, *J*=12.5, 6.3 Hz, H-1); H-2α: δ_H 0.95 (d, 1H, *J*=4.8 Hz), H-2β: δ_H 0.93 (d, 1H, *J*=7.6 Hz); H-3: δ_H 1.85 (dd, 1H, *J*=12.0, 6.4 Hz) and H-1': δ_H 1.26 (m, 1H); H-2'α: δ_H 0.72 (dd, 1H, *J*=14.3, 8.7 Hz), H-2'β: δ_H 0.66 (d, 1H, *J*=3.7 Hz); H-3': δ_H 1.16 (m, 1H)]. The connectivity of the two substructures and most of the other functional groups was accomplished by means of HMBC correlations (Figure 2). The NOESY correlation of H-3α/H_2-15 suggested that 4-OH was β-oriented.[18] Additionally, the NOESY correlations between H-9, H-15 and H-9' are characteristic of the folding of the polycyclic structure of **1**. On the basis of overall NMR evidence, the structure of **1** was determined as shown in Figure 1 and named multistalide C.

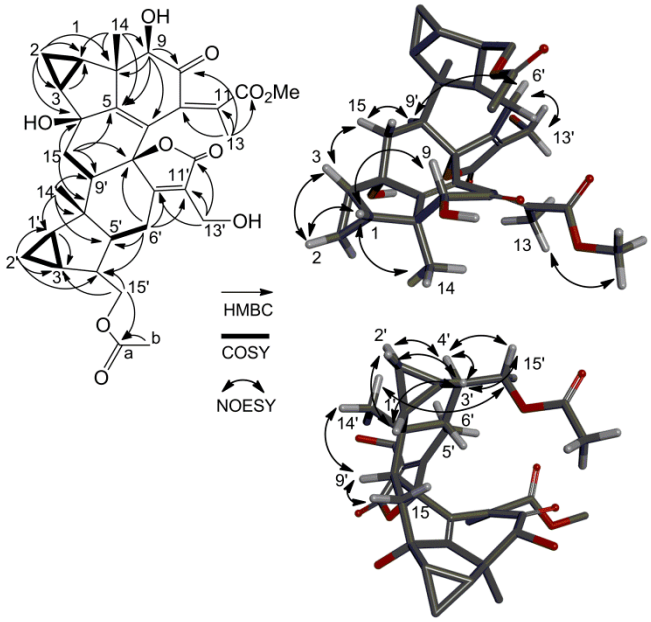


FIGURE 1 Selected HMBC, COSY and NOESY correlations of compound **1**. In the NOESY diagrams (right), the same calculated lowest-energy structure of **1** is shown in two different orientations to emphasize the correlations in different molecular portions.

Shizukaol D (**3**) and multistalide C (**1**) are biosynthetically related by double bond migration from $\Delta^{4(5)}$ to $\Delta^{5(6)}$ and oxidation at C-4.[25,26] Shizukaol D is probably biogenetically generated from shizukaol A[7] by oxidation at C-13' and water addition at C-4'/C-15' double bond. Therefore, the biogenetic pathway to shizukaol D (**3**) and multistalide C (**1**) involves the same monomeric precursors producing shizukaol A by Diels-Alder reaction,[7,24,27] which include shizukanolide B (also known as chloranthalactone A)[28] as the dienofile.

The absolute configuration (AC) of **1** was determined by means of an established protocol based on electronic circular dichroism (ECD).[29,30] The experimental ECD spectrum of (–)-**1** was recorded in methanol (Figure 2) and compared with the spectrum calculated with TDDFT method as Boltzmann average on a set of structures representing low-energy conformers. These latter were obtained by means of a molecular-mechanics conformational search followed by geometry optimizations with DFT method at B3LYP/6-31G(d) level, which afforded 8 structures with relative energies below 3 kcal/mol and Boltzmann population >0.2% at 300K. In these energy minima (the most stable one is shown in Figure 1, all others are shown in The Supporting Information), the conformation of the relatively rigid polycyclic skeleton is well preserved. The minima differ in the orientation of the flexible chains attached at C-4' and of the hydroxyl groups, and, more importantly, in the orientation of the methyl ester group attached at C-11. This ester moiety is almost perpendicular to the C5-C11 diene system – which itself is substantially twisted – and has two possible conformations with the carbonyl pointed in two opposite directions (see structures in the Supporting Information).

TDDFT calculations were run with CAM-B3LYP and ωB97X-D functionals, which currently seem to provide the most accurate prediction of ECD spectra of moderately complex molecules.[31,32] The Boltzmann-weighted average ECD spectrum calculated with CAM-B3LYP/TZVP for (1*R*,3*S*,4*S*,9*R*,10*S*,1'*R*,3'*S*,4'*R*,5'*S*,8'*R*,9'*S*,10'*S*)-**1** is shown in Figure 2 and it is in very good agreement with the experimental ECD spectrum measured for (–)-**1**, therefore the absolute configuration of multistalide C is safely assigned.

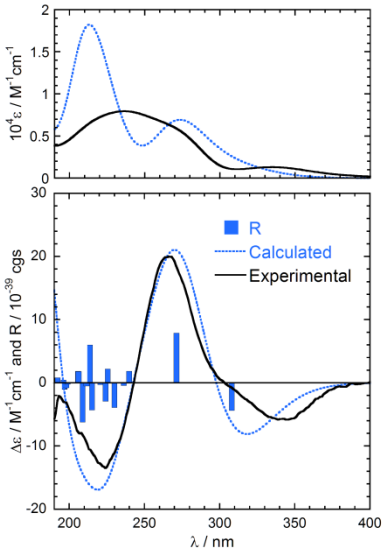


FIGURE 2 Experimental UV (top) and ECD spectra (bottom) of (–)-**1** measured in MeOH (0.34 mM, 0.05 cm cell) compared with the spectra calculated for (1*R*,3*S*,4*S*,9*R*,10*S*,1'*R*,3'*S*,4'*R*,5'*S*,8'*R*,9'*S*,10'*S*)-**1** at CAM-B3LYP/TZVP level on B3LYP/6-31G(d) geometries, as Boltzmann average at 300K for all structures with population >1%. Gaussian band-shape with 0.4 eV exponential half-width. Vertical

bars represent rotational strengths calculated for the lowest-energy structure.

ABSOLUTE CONFIGURATION OF CHLORAHOLOLIDE A AND RELATED LINDENANE SESQUITERPENOID DIMERS

The established absolute configuration of multistalide C is the same for the corresponding chirality centers not only of its biosynthetic precursor chloranthalactone A,[28] but also of several related sesquiterpenoid dimers sharing the same heptacyclic core. For only a few members of this family, the AC's have been determined by X-ray crystallography[11,33] or total synthesis.[26] Most commonly, however, the AC has been assigned on the basis of ECD spectroscopy by applying the exciton chirality method (ECM),[25,34-41] following a report concerning chlorahololide A (**4**, diagram in Figure 3) where this method was applied for the first time to this class of compounds.[18] However, we argue here that the ECM not suitable for chlorahololide A and related compounds for several reasons explained below, and reconsider the ECD spectrum and the AC assignment of this compound. Chlorahololide A (**4**) has the same heptacyclic core as multistalide C (**1**) and the same main chromophores, namely the unsaturated γ-lactone and the conjugated π-electron system spanning carbon atoms C-5 to C-8, C-11 and C-12, while it differs from **1** in the attachment of the acetate group at C-6', the C-4'/C-15' methylene group, and the absence of C-8 hydroxyl group. Not surprisingly therefore, the ECD spectra of compounds **1** and **4** are very similar (Figure 2 and 3). Applying the same computational protocol described above, we found for **4** only two DFT low-energy structures populated at 300K, differing (as in the case of **1**) in the orientation of the methyl ester group attached at C-11 (see structures in the Supporting Information). The Boltzmann-weighted average ECD spectrum calculated with CAM-B3LYP/TZVP for (1*R*,3*S*,4*S*,10*S*,1'*R*,3'*S*,5'*S*,6'*R*,8'*S*,9'*S*,10'*S*)-**4** is shown in Figure 3. The agreement with the experimental spectrum confirms the previously determined absolute configuration.[18,26] This latter was originally based on the application of the non-degenerate exciton chirality method[19-21] after assigning the two strongest bands in the ECD spectrum, with maxima at 220 nm and 255 nm, to the π-π* transitions of the unsaturated γ-lactone and the twisted π-electron system, respectively. The two transition moments, drawn in Figure 4a, would define a positive chirality and hence a positive ECD exciton couplet as found experimentally.[18]

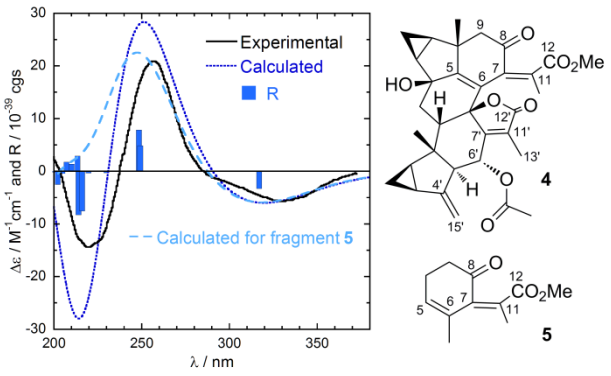


FIGURE 3 Experimental ECD spectrum of (–)-**4** in MeOH (adapted with permission from ref. 18. Copyright 2007 American Chemical Society) compared with the spectrum calculated for (1*R*,3*S*,4*S*,10*S*,1'*R*,3'*S*,5'*S*,6'*R*,8'*S*,9'*S*,10'*S*)-**4** at CAM-B3LYP/TZVP level on B3LYP/6-31G(d) geometries, as Boltzmann average at 300K for all structures with population >1%. The dashed line is the spectrum calculated for the fragment **5** in the same conformations found for **4**. Gaussian band-shape with 0.5 eV exponential half-width. Vertical bars represent rotational strengths calculated for the lowest-energy structure.

However, a correct application of the exciton chirality method, including the situation when non-equivalent chromophores are concerned (non-degenerate exciton coupling), necessitates some essential prerequisites:[19-21,42]

(a) The molecular conformation, with special attention to the inter-chromophoric arrangement, must be known with accuracy;[29]

(b) The two electric-dipole allowed transitions, exciton-coupled to each other, should be well separated in energy from other transitions, that is, the two ECD bands must be clearly assigned to the considered chromophores;

(c) The direction of the two electric transition moments must be exactly known;

(d) The two chromophores should be intrinsically achiral, which, in the case of conjugated π-systems, essentially means planar, to minimize ECD signals not due to the exciton coupling.

A careful analysis of our calculation results on chlorahololide A (**4**) and of the original paper[18] clearly reveals that none of the above criteria is really satisfied in the ECM application to this compound. In fact:

(a') The molecular conformation used to establish the chirality between the transition dipoles (Figure 4a),[18] although possibly generated by molecular modeling and apparently respectful of NOE contacts, has apparently not been ascertained by conformational analysis and geometry optimizations;

(b') As seen in Figure 3, the ECD band between 200-240 nm is due to the superposition of several different transitions, and none of them can be assigned in a prevalent way to the unsaturated γ-lactone π-π* transition;

(c') The π-electron system involving carbons C-5–C-8, C-11 and C-12 is a quite complicated chromophore, and the direction of the electric transition moment for its 255 nm band cannot be taken for granted. In the quoted paper,[18] this is drawn as shown in Figure 4a, that is with an arrow going approximately from the middle of the C-5/C-6 double bond to the middle of ester C=O bond, almost neglecting the contribution from the C-8 carbonyl group. However, a transition density plot of such a transition (Figure 4b), calculated on a molecular fragment (**5**) representing the chromophore in the same conformation found for **4**, reveals that the transition is delocalized on the whole conjugated moiety, including the C-8 carbonyl group, and the correct direction of the transition moment is rather parallel to the exocyclic C-7/C-11 double bond (Figure 4c);

(d') The same π-electron system is not planar but heavily twisted around both C-6/C-7 and C-11/C-12 single bonds. The values measured on DFT geometries are 121-124° for the C-5/C-11 dihedral angle (both conformers) and –111° and +76° for the C-7/O(=C) dihedral angle (lowest energy and second energy minimum, respectively). As a consequence, such a chromophore is definitely intrinsically chiral. In fact, both the n-π* and π-π* transitions calculated for fragment **5** are associated with strong rotational strengths which account almost entirely for the ECD of compound **4** above 250 nm (see dotted and dashed lines in Figure 3).

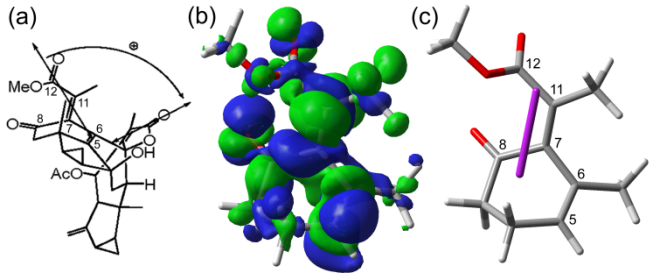


FIGURE 4 (a) Application of the ECM to chloraholide A (**4**): the arrows depict the supposed directions of the electric dipole moments (adapted with permission from ref. [18]. Copyright 2007 American Chemical Society). (b,c) Transition density plot for the first π-π^* band calculated at CAM-B3LYP/TZVP level for fragment **5** (Figure 3) in the same lowest-energy conformation found for **4**, and direction of the electric transition dipole moment.

In summary, the couplet-like feature appearing in the ECD spectrum of multistalide C (**1**) and chloraholide A (**4**) between 200-290 nm is not due to the exciton coupling between the two above discussed transitions and cannot be used for a configurational assignment based on the exciton chirality method. The same reasoning applies to all configurational assignments reported in the literature for several related sesquiterpenoid dimers, all of which were based on a similar exciton chirality analysis plagued by the same faults: in all cases, none of the requisites (a)-(d) above is satisfied, and the problems corresponding to points (a')-(d') above are encountered.[25,34-41] In some cases, moreover, ECD spectra of different lindenane-type compounds were correlated in an empirical manner even if the chromophoric systems were not the same among the various compounds.[34-38,40] In conclusion, we discourage the use of the exciton chirality method and empirical spectral correlations for this class of compounds, and recommend that full ECD calculations are performed.

BRINE SHRIMP LARVAE TOXICITY ASSAY

The brine shrimp larvae lethality assay is considered as a useful tool for preliminary assessment of toxicity with compounds and extracts.[43,44] The three compounds **1**-**3** were evaluated for brine shrimp larvae (*Artemia salina*) toxicity, and **2** and **3** showed significant growth inhibitory activities with median lethal concentration (LC_{50}) values of 19.0 and 11.7 µg/mL, respectively, compared with the positive control podophyllotoxin (6.0 µg/mL), whereas **1** displayed weak activity (LC_{50} 31.2 µg/mL).

Conclusions

A novel dimeric sesquiterpenoid, multistalide C (**1**), together with two known dimers, shizukaols C (**2**) and D (**3**), were isolated from *Chloranthus japonicus* Sieb for the first time. Compounds **2** and **3** showed remarkable growth inhibitory of brine shrimp larvae. The structure of multistalide C (**1**) was fully elucidated, including its absolute configuration. A literature survey on absolute configurational assignments of related lindenane-type sesquiterpenoid dimers revealed that in almost all cases the exciton chirality method was applied, starting from the first report on chloraholide A (**4**). This latter compound was therefore reconsidered and it was demonstrated that its ECD spectrum is not dominated by the exciton coupling mechanism. Other pitfalls in the reported applications of the exciton chirality method to this class of compounds were also noticed. Also in consideration of the relatively rigid heptacyclic skeleton, the absolute configuration of chloraholide-type compounds may be easily assigned by means of TDDFT//DFT calculations.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (Grant No. 31200257), the Overall Science and Technology Innovation program of Shaanxi Province (Grant No. 2012KTCL02-07), the West Light Foundation of The Chinese Academy of Sciences (Grant No. 2012DF05), the Science and Technology Program of Shaanxi Academy of Sciences (Grant No. 2012k-04) and the Science and Technology Research and Development Projects of Shaanxi Province (Grant No. 2013KJXX-74). T. I. gratefully acknowledges FP7-REGPOT-2012-2013-1, grant agreement number 316289 – InnoMol. G.P. thanks Lorenzo Cupellini and Daniele Padula (Univresity of Pisa) for density plots.

Supporting information

Additional supporting information may be found in the online version of this article at the publisher's website.

REFERENCES AND NOTES

1. Flora of China Editorial Committee. Flora of China (Zhongguo Zhiwu Zhi). Volume 20. Beijing: Science; **1982**. p 85-86.

2. Xu Y-J. Phytochemical and Biological Studies of *Chloranthus* Medicinal Plants. Chem Biodivers **2013**;10:1754-1773.

3. Kawabata J, Fukushi E, Mizutani J. Sesquiterpene dimers from *Chloranthus japonicus*. Phytochemistry **1995**;39:121-125.

4. Kawabata J, Fukushi Y, Tahara S, Mizutani J. Studies on the chemical constituents of Chloranthacee plants. Part III. Structures of Novel Sesquiterpene Alcohols from *Chloranthus japonicus* (Chloranthaceae). Agric Biol Chem **1984**;48:713-717.

5. Yuan T, Zhang C-R, Yang S-P, Yin S, Wu W-B, Dong L, Yue J-M. Sesquiterpenoids and Phenylpropanoids from *Chloranthus serratus*. J Nat Prod **2008**;71:2021-2025.

6. Wu B, Qu H, Cheng Y. New Sesquiterpenes from *Chloranthus japonicus*. Helv Chim Acta **2008**;91:725-733.

7. Kawabata J, Fukushi Y, Tahara S, Mizutani J. Studies on the chemical constituents of Chloranthacee. Part 7. Shizukaol a, a sesquiterpene dimer from *Chloranthus japonicus*. Phytochemistry **1990**;29:2332-2334.

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

9. Kuang H-X, Xia Y-G, Yang B-Y, Wang Q-H, Lü S-W. Lignan constituents from *Chloranthus japonicus* Sieb. Arch Pharm Res **2009**;32:329-334.

10. Yim NH, Hwang EI, Yun BS, Park KD, Moon JS, Lee SH, Sung ND, Kim SU. Sesquiterpene Furan Compound CJ-01, a Novel Chitin Synthase 2 Inhibitor from *Chloranthus japonicus* Sieb. Biol Pharm Bull **2008**;31:1041-1044.

11. 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. Lindenane Disesquiterpenoids with Anti-HIV-1 Activity from Chloranthus japonicus. J Nat Prod **2011**;74:1408-1413.

12. Hu R-K, Yan H, Hao X-J, Liu H-Y, Wu J-R. Shizukaol D isolated from *Chloranthus japonicus* inhibits AMPK-dependent lipid content in hepatic cells by inducing mitochondrial dysfunction. PloS One **2013**;8:e73527.

13. Tang J-J, Zhang F-Y, Wang D-M, Tian J-M, Dong S, Gao J-M. Semisynthesis and Antifeedant Activity of New Derivatives of a Dihydro-β-Agarofuran from *Parnassia wightiana*. Int J Mol Sci **2013**;14:19484.

14. Liu H-W, Yu X-Z, Padula D, Pescitelli G, Lin Z-W, Wang F, Ding K, Lei M, Gao J-M. Lignans from *Schisandra sphenathera* Rehd. et Wils. and semisynthetic schisantherin A analogues: Absolute configuration, and their estrogenic and anti-proliferative activity. Eur J Med Chem **2013**;59:265-273.

15. Wei S-P, Gao J-M, Wu W-J, Ji Z-Q, Zhang J-W. Simultaneous Identification of Multiple Celangulins from the Root Bark of *Celastrus angulatus* Using High-performance Liquid Chromatography–Diode Array Detector–Electrospray Ionisation–Tandem Mass Spectrometry. Phytochem Anal **2012**;23:23-33.

16. Wang D-M, Zhang C-C, Zhang Q, Shafiq N, Pescitelli G, Li D-W, Gao J-M. Wightianines A–E, Dihydro-β-agarofuran Sesquiterpenes from *Parnassia wightiana*, and Their Antifungal and Insecticidal Activities. J Agric Food Chem **2014**;62:6669-6676.

17. Gao J-M, Wu W-J, Zhang J-W, Konishi Y. The dihydro-b-agarofuran sesquiterpenoids. Nat Prod Rep **2007**;24:1153-1189.

18. Yang S-P, Gao Z-B, Wang F-D, Liao S-G, Chen H-D, Zhang C-R, Hu G-Y, Yue J-M. Chloraholides A and B, Two Potent and Selective Blockers of the Potassium Channel Isolated from *Chloranthus holostegius*. Org Lett **2007**;9:903-906.

19. Berova N, Harada N, Nakanishi K. Exciton Coupling. In: Lindon JC, editor. Encyclopedia of Spectroscopy and Spectrometry. Oxford: Elsevier; **1999**. p 470-488.

20. Harada N, Nakanishi K. Circular Dichroic Spectroscopy - Exciton Coupling in Organic Stereochemistry. Mill Valley, CA: University Science Books; **1983**.

21. Harada N, Nakanishi K, Berova N. Electronic CD Exciton Chirality Method: Principles and Applications. In: Berova N, Polavarapu PL, Nakanishi K, Woody RW, editors. Comprehensive Chiroptical Spectroscopy. Hoboken (NJ): John Wiley & Sons, Inc.; **2012**. p 115-166.

22. Frisch MJ, Trucks GW, Schlegel HB, Scuseria GE, Robb MA, Cheeseman JR, Scalmani G, Barone V, Mennucci B, Petersson GA, Nakatsuji H, Caricato M, Li X, Hratchian HP, Izmaylov AF, Bloino J, Zheng G, Sonnemberg JL, Hada M, Ehara M, Toyota K, Fukuda R, Hasegawa J, Ishida M, Nakajima T, Honda Y, Kitao O, Nakai H, Vreven T, Montgomery J, J. A., Peralta JE, Ogliaro F, Bearpark M, Heyd JJ, Brothers E, Kudin KN, Staroverov VN, Kobayashi R, Normand J, Raghavachari K, Rendell A, Burant JC, Iyengar SS, Tomasi J, Cossi M, Rega N, Millam JM, Klene M, Knox JE, Cross JB, Bakken V, Adamo C, Jaramillo J, Gomperts R, Stratmann RE, Yazyev O, Austin AJ, Cammi R, Pomelli C, Ochterski JW, Martin RL, Morokuma K, Zakrzewski VG, Voth GA, Salvador P, Dannenberg JJ, Dapprich S, Daniels AD, Farkas O, Foresman JB, Ortiz JV, Cioslowski J, Fox DJ. Gaussian 09, Revision D.01. Wallingford CT: Gaussian, Inc.; 2013.

23. Bruhn T, Schaumlöffel A, Hemberger Y, Bringmann G. SpecDis: Quantifying the Comparison of Calculated and Experimental Electronic Circular Dichroism Spectra. Chirality **2013**;25:243-249.

24. Kawabata J, Mizutani J. Dimeric sesquiterpenoid esters from *Chloranthus serratus*. Phytochemistry **1992**;31:1293-1296.

25. Kim S-Y, Kashiwada Y, Kawazoe K, Murakami K, Sun H-D, Li S-L, Takaishi Y. Spicachlorantins C–F, hydroperoxy dimeric sesquiterpenes from the roots of *Chloranthus spicatus*. Tetrahedron Lett **2009**;50:6032-6035.

26. Lu Y-S, Peng X-S. A Concise Construction of the Chloraholide Heptacyclic Core. Org Lett **2011**;13:2940-2943.

27. Yang L, Yue G, Yuan C, Du B, Deng H, Liu B. Synthetic Studies toward Lindenane-Type Sesquiterpenoid Dimers. Synlett **2014**;25:2471-2474.

28. Kawabata J, Tahara S, Mizutani J, Furusaki A, Hashiba N, Matsumoto T. Shizukanolides, Two Sesquiterpenoids from *Chloranthus japonicus* (Chloranthacese). Agric Biol Chem **1979**;43:885-887.

29. Pescitelli G, Di Bari L, Berova N. Conformational aspects in the studies of organic compounds by electronic circular dichroism. Chem Soc Rev **2011**;40:4603-4625.

30. Pescitelli G, Kurtán T, Flörke U, Krohn K. Absolute Structural Elucidation of Natural Products-A Focus on Quantum-Mechanical Calculations of Solid-State CD Spectra. Chirality **2009**;21:E181-E201.

31. Gunasekaran P, Perumal S, Menéndez JC, Mancinelli M, Ranieri S, Mazzanti A. Axial Chirality of 4-Arylpyrazolo[3,4-b]pyridines. Conformational Analysis and Absolute Configuration. J Org Chem **2014**;79:11039-11050.

32. Berardozzi R, Guido CA, Capozzi MAM, Cardellicchio C, Di Bari L, Pescitelli G. Circular Dichroism and TDDFT Investigation of Chiral Fluorinated Aryl Benzyl Sulfoxides. Eur J Org Chem **2015**;2015:5554-5562.

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

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

35. Yang S-P, Gao Z-B, Wu Y, Hu G-Y, Yue J-M. Chloraholides C–F: a new class of potent and selective potassium channel blockers from *Chloranthus holostegius*. Tetrahedron **2008**;64:2027-2034.

36. He X-F, Yin S, Ji Y-C, Su Z-S, Geng M-Y, Yue J-M. Sesquiterpenes and Dimeric Sesquiterpenoids from *Sarcandra glabra*. J Nat Prod **2010**;73:45-50.

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

38. Kim S-Y, Kashiwada Y, Kawazoe K, Murakami K, Sun H-D, Li S-L, Takaishi Y. Spicachlorantins G-J, New Lindenane Sesquiterpenoid Dimers from the Roots of *Chloranthus spicatus*. Chem Pharm Bull **2011**;59:1281-1284.

39. Zhang M, Iinuma M, Wang J-S, Oyama M, Ito T, Kong L-Y. Terpenoids from *Chloranthus serratus* and Their Anti-inflammatory Activities. J Nat Prod **2012**;75:694-698.

40. Ni G, Zhang H, Liu H-C, Yang S-P, Geng M-Y, Yue J-M. Cytotoxic sesquiterpenoids from *Sarcandra glabra*. Tetrahedron **2013**;69:564-569.

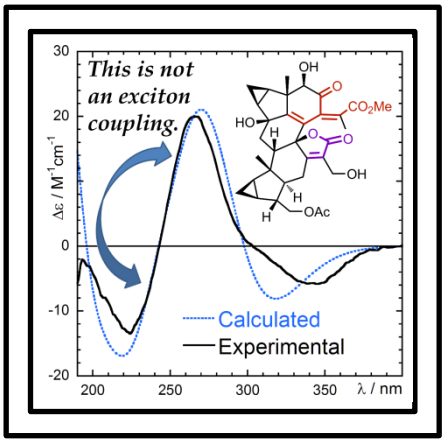
41. Wang P, Luo J, Zhang Y-M, Kong L-Y. Sesquiterpene dimers esterified with diverse small organic acids from the seeds of *Sarcandra glabra*. Tetrahedron **2015**;71:5362-5370.

42. Berova N, Di Bari L, Pescitelli G. Application of electronic circular dichroism in configurational and conformational analysis of organic compounds. Chem Soc Rev **2007**;36:914-931.

43. Li X-J, Zhang Q, Zhang A-L, Gao J-M. Metabolites from *Aspergillus fumigatus*, an Endophytic Fungus Associated with *Melia azedarach*, and Their Antifungal, Antifeedant, and Toxic Activities. J Agric Food Chem **2012**;60:3424-3431.

44. Qin J-C, Zhang Y-M, Gao J-M, Bai M-S, Yang S-X, Laatsch H, Zhang A-L. Bioactive metabolites produced by *Chaetomium globosum*, an endophytic fungus isolated from *Ginkgo biloba*. Bioorg Med Chem **2009**;19:1572-1574.

Graphical Abstract



A new sesquiterpenoid dimer, Multistalide C, was isolated from *Chloranthus japonicus* Sieb and its absolute structure determined. Although displaying a couplet-like feature in the ECD spectrum, the use of exciton chirality method for multistalide C and several related chloraholide-type sesquiterpenoid dimers reported in the literature is not suitable because of the intrinsic chirality of the composite conjugated chromophore and the complexity of the ECD spectrum.