

Two halosesquiterpenes from *Laurencia composita*†Xiao-Dong Li,^{ac} Feng-Ping Miao,^a Xiao-Rui Liang,^b Bin-Gui Wang^c and Nai-Yun Ji^{*a}Cite this: *RSC Advances*, 2013, 3, 1953Received 24th October 2012,
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Two new chamigrane sesquiterpenes, yicterpenes A (**1**) and B (**2**) each with an unprecedented halogenation at C-9 and a hemiketal unit at C-10, were isolated from the marine red alga *Laurencia composita*. The structures and absolute configurations of them were identified by NMR, ECD, and mass spectrometric methods as well as quantum chemical calculations. Compound **1** with a chlorination at C-9 was more active against some tested marine-derived organisms than **2** with a bromination at C-9.

Introduction

More than 5000 halogenated natural products have been discovered so far, and about 10% of them are derived from the marine red alga of the genus *Laurencia*, which feature remarkable molecular diversity and various bioactivities. However, most of the halogenated molecules have been assigned only relative configurations, although their specific optical rotation ($[\alpha]_D$) values are given. Moreover, it is difficult to confirm the positions of chlorination and bromination in polyhalogenated structures by only spectrometric methods.^{1–8} In our ongoing program to discover halogenated compounds from *Laurencia* species, two new 5,10-epoxy chamigrane sesquiterpenes, yicterpenes A (**1**) and B (**2**) each with an unprecedented halogenation at C-9 and a hemiketal functionality at C-10, were isolated from *L. composita*. The main subjects of this paper are the structure elucidation and bioactivity analysis of yicterpenes A (**1**) and B (**2**) aided by quantum chemical calculations of their electronic circular dichroism (ECD) spectra and specific optical rotation data as well as molecular parameters.

Results and discussion

Yicterpene A (**1**) was obtained as colorless crystals. A molecular formula of $C_{15}H_{21}BrCl_2O_3$ was established by HREIMS (m/z 398.0013 $[M]^+$, calcd for $C_{15}H_{21}BrCl_2O_3$, 398.0051), requiring four degrees of unsaturation. A broad IR absorption at 3460 cm^{-1} suggested the presence of hydroxyl groups. The 1H NMR spectrum (Table 1) along with HSQC data displayed four

methyl singlets, two double doublets ascribed to two halo(oxy)methines, and two broad singlets attributed to two exchangeable protons. The ^{13}C and DEPT NMR spectra (Table 1) demonstrated the presence of four methyls, two methylenes, three methines, and six non-protonated carbons. The above NMR data closely resembled those of pacifenol except for the presence of a signal for one additional non-protonated carbon (C-9) and the lack of signals for one of the olefinic methines.^{5,6} Thus, a chlorine atom was assigned to C-9 based on ^{13}C NMR data.⁷ A remaining hydroxyl group indicated by the molecular formula was placed at C-10 to form a hemiketal unit based on its downfield ^{13}C NMR value.⁹ The 1H - 1H COSY and HMBC correlations (Fig. 1) further verified the structure of **1**.

The relative configuration of **1** was determined by coupling constants and a NOESY experiment. H-1a and H-4a were opposite to H-2 and H-5 based on the large coupling constants (Table 1) between them. The NOE correlations of H-1a with CH_3 -13 and CH_3 -15 suggested a *syn* orientation of H-1a, CH_3 -13, and CH_3 -15, while the vicinity of H-1b, CH_3 -12, and CH_3 -14 was indicated by NOE correlations between them. The energy-minimized conformer (Fig. 2) was generated by the Dreiding force field in MarvinSketch¹⁰ and further optimized using density function theory (DFT) at the B3LYP/6-31+G(d,p) level in chloroform *via* Gaussian 09 software, which matched well with the above NOE data. Thus, the relative configuration of **1** was established.

Yicterpene B (**2**) obtained as colorless crystals was assigned a molecular formula of $C_{15}H_{21}Br_2ClO_3$ based on HREIMS (m/z 441.9537 $[M]^+$, calcd for $C_{15}H_{21}Br_2ClO_3$, 441.9546), implying four degrees of unsaturation. Its 1H , ^{13}C , HSQC, HMBC, and 1H - 1H COSY data (Table 1 and Fig. 1) exhibited close similarity with those for **1**, which revealed that **2** should be an analogue of **1**. An upfield-shifted signal (δ_C 126.7) of C-9 suggested a bromine atom was bonded to C-9 in **2** replacing the corresponding chlorine atom in **1**,¹¹ which was supported by the molecular formula. The relative configuration of **2** was also deduced to be the same as that of **1** by their identical coupling constants and NOE correlations.

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† Electronic supplementary information (ESI) available: NMR, HREIMS, and IR spectra; Cartesian coordinates; and experimental and calculated ECD data. See DOI: 10.1039/c2ra23101h

Table 1 ^1H and ^{13}C NMR data for **1** and **2** (in CDCl_3)

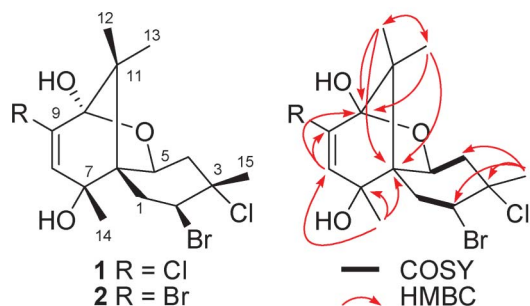
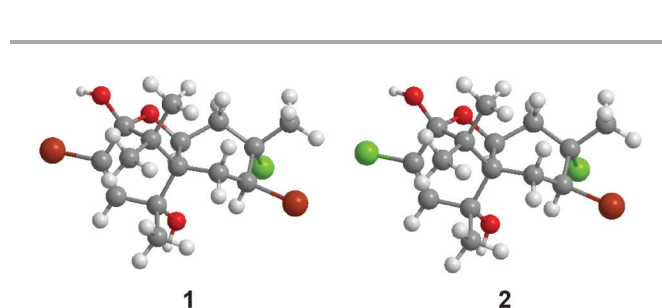
Position	1		2	
	$\delta_{\text{H}}/\text{ppm}$, mult (J/Hz)	$\delta_{\text{C}}/\text{ppm}$	$\delta_{\text{H}}/\text{ppm}$, mult (J/Hz)	$\delta_{\text{C}}/\text{ppm}$
1a	2.13, dd (14.6, 13.2)	33.5, CH_2	2.13, dd (14.6, 13.2)	33.5, CH_2
1b	2.28, dd (14.6, 3.6)		2.27, dd (14.6, 3.6)	
2	5.42, dd (13.2, 3.6)	59.5, CH	5.42, dd (13.2, 3.6)	59.4, CH
3		69.2, qC		69.2, qC
4a	2.35, dd (14.8, 12.5)	46.2, CH_2	2.35, dd (14.8, 12.5)	46.2, CH_2
4b	2.64, dd (14.8, 5.1)		2.64, dd (14.8, 5.1)	
5	4.57, dd (12.5, 5.1)	70.9, CH	4.57, dd (12.5, 5.1)	71.0, CH
6		54.4, qC		54.5, qC
7		77.6, qC		78.5, qC
8	5.74, s	131.1, CH	5.96, s	135.4, CH
9		133.4, qC		126.7, qC
10		102.6, qC		102.7, qC
11		48.8, qC		48.8, qC
12	1.02, s	23.1, CH_3	1.03, s	23.2, CH_3
13	1.22, s	20.8, CH_3	1.23, s	21.2, CH_3
14	1.54, s	25.5, CH_3	1.53, s	25.3, CH_3
15	1.79, s	33.7, CH_3	1.79, s	33.7, CH_3
OH-7	1.93, brs		2.00, brs	
OH-10	3.60, brs		3.50, brs	

In order to establish the absolute configurations of **1** and **2**, the ECD spectra of them were determined, which were in accordance with the calculated ones (Fig. 3) using the time-dependent density function theory (TD-DFT) method at the B3LYP/6-31G(d) level.^{12–14} Thus, an absolute configuration of 2*S*, 3*S*, 5*S*, 6*R*, 7*R*, and 10*R* was assigned to both **1** and **2**. It was interesting that the experimental ECD spectrum of **2** exhibited a redshift (*ca.* 15 nm) relative to that of **1**, which was deduced to arise from the lower energy differences of molecular orbitals related to electronic transitions in **2**. Moreover, the calculated ECD spectrum of **2** also exhibited a redshift (*ca.* 8 nm) relative to that of **1**, which matched a lower energy difference (6.34 eV) between LUMO and HOMO than that (6.47 eV) of **1**. Hence, ECD spectra could be indicators to distinguish chlorinated and brominated vinyl units attached to chiral centers.

Yicterpenes A (**1**) and B (**2**) also exhibited different specific optical rotations ($[\alpha]_{\text{D}} -66.2$ and -43.1° $[\text{dm g cm}^{-3}]^{-1}$), which were deduced to arise from their different halogenations. The specific rotations were then calculated at the B3LYP/aug-cc-pVDZ level,¹⁵ and **1** and **2** gave $[\alpha]_{\text{D}}$ values of -57.2 and -56.8° $[\text{dm g cm}^{-3}]^{-1}$. The calculated specific rotations

further verified the absolute configurations of **1** and **2**, although they could not make an obvious distinction between chlorination and bromination. Furthermore, the above evidence implied that quantum chemical calculations of specific rotations might be reliable to confirm the absolute configurations of the other large amounts of halogenated molecules, which were previously isolated from *Laurencia* species and assigned only relative configurations.^{1,2}

To probe into the ecological function of yicterpenes A (**1**) and B (**2**), they were assayed for inhibitory activities against marine zooplankton (*Artemia salina*) and phytoplankton (*Heterosigma akashiwo*) as well as bacteria (*Vibrio ichthyenteri*, *Proteus mirabilis*, *Enterobacter cloacae*, and *Bacillus cereus*) isolated from seawater.^{16,17} The results showed that **1** and **2** could inhibit some of these marine-derived organisms. Especially, it was interesting that **1** with a chlorination at C-9 was slightly more active against *A. salina*, *H. akashiwo*, and *B. cereus* than **2** with a bromination at the same position, which was deduced to be related to their different charge distributions, bond lengths, and angles around C-9 (Table S1 and S3 in ESI†).

**Fig. 1** Structures and key ^1H - ^1H COSY and HMBC correlations of **1** and **2**.**Fig. 2** Energy-minimized conformers of **1** and **2** (in CHCl_3).

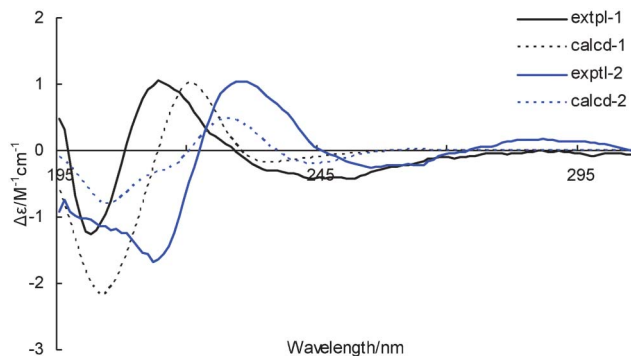


Fig. 3 Experimental and calculated ECD spectra of **1** and **2** (in MeOH).

Conclusions

Two new halosesquiterpenes, yicterpenes A (**1**) and B (**2**) each with an unprecedented halogenation at C-9 and a hemiketal unit at C-10, were isolated from the marine red alga *L. composita*. The chlorination and bromination at C-9 in **1** and **2** resulted in different spectral characteristics and bioactivities, which were further explained by quantum chemical calculations. In turn, quantum chemical methods were suggested to be reliable to evidence the structures and absolute configurations of polyhalogenated molecules.

Experimental section

General experimental procedures

NMR spectra were recorded in CDCl_3 at 500 and 125 MHz for ^1H and ^{13}C , respectively, on a Bruker Avance III 500 NMR spectrometer using TMS as internal standard. The high resolution mass spectra were determined on an Autospec Premier P776 mass spectrometer. The IR spectra were obtained on a JASCO FT/IR-4100 Fourier Transform Infrared spectrometer. The UV spectra were measured on a TU-1810 Spectrophotometer. The optical rotations were determined on a JASCO P-1020 polarimeter. Melting points were measured using an X-4 micro-melting point apparatus. Electronic circular dichroism (ECD) spectra were recorded on a Chirascan CD Spectrometer. Quantum chemical calculations were operated *via* Gaussian 09 software (IA32W-G09RevC.01). HPLC separation was carried out on an Elite HPLC system (P270 pump, UV230+ detector, Dalian Elite Analytical Instruments Co., Ltd, Dalian, China) using an Eclipse XDB-C18 ($5\mu\text{m}$, $9.4 \times 250\text{ mm}$) column. Column chromatography was performed with silica gel (200–300 mesh, Qingdao Haiyang Chemical Co., Qingdao, China) and Sephadex LH-20 (Pharmacia). TLC was carried out with precoated silica gel plates (GF-254, Qingdao Haiyang Chemical Co., Qingdao, China). All solvents were of analytical grade.

Plant material

The marine red alga *L. composita* was collected from Pingtan Island of China in May, 2010 and was identified by one of the

authors (N-Y. J.). A voucher specimen (MRA100501) has been deposited at the Bio-Resource Laboratory of Yantai Institute of Coastal Zone Research, Chinese Academy of Sciences.

Extraction and isolation

The dried and powdered sample (0.7 kg) was extracted with a mixture of CHCl_3 and MeOH (1 : 1, v/v). The concentrated extract was partitioned between H_2O and EtOAc. The EtOAc extract (21.0 g) was subjected to silica gel column chromatography (CC, petroleum ether (PE)/EtOAc gradient) to give 14 fractions (Frs 1–14), monitored by TLC. Fr. 13 eluted with PE/EtOAc (5 : 1) and was further purified by CC on Sephadex LH-20 ($\text{CHCl}_3/\text{MeOH}$, 1 : 1), preparative HPLC ($\text{MeOH}/\text{H}_2\text{O}$, 4 : 1), and preparative TLC ($\text{CHCl}_3/\text{EtOAc}$, 10 : 1) to give **1** (8.6 mg) and **2** (5.1 mg).

Yicterpene A (1). Colorless crystals (CDCl_3); mp 149–151 °C; $[\alpha]_{\text{D}}^{23} -66.2$ (c 0.18, MeOH); UV (MeOH, a shoulder peak) λ_{max} ($\log \epsilon$) *ca.* 240 (2.68) nm; IR (KBr) ν_{max} 3460, 3016, 2974, 2931, 1697, 1635, 1450, 1385, 1296, 1223, 1107, 1026, 756 cm^{-1} ; ^1H and ^{13}C NMR data, see Table 1; HREIMS m/z 398.0013 $[\text{M}]^+$ (calcd for $\text{C}_{15}\text{H}_{21}\text{BrCl}_2\text{O}_3$, 398.0051).

Yicterpene B (2). Colorless crystals (CDCl_3); mp 150–152 °C; $[\alpha]_{\text{D}}^{22} -43.1$ (c 0.16, MeOH); UV (MeOH, a shoulder peak) λ_{max} ($\log \epsilon$) *ca.* 250 (3.10) nm; IR (KBr) ν_{max} 3433, 3010, 2924, 2854, 1697, 1631, 1454, 1381, 1292, 1219, 1103, 1053, 756 cm^{-1} ; ^1H and ^{13}C NMR data, see Table 1; HREIMS m/z 441.9537 $[\text{M}]^+$ (calcd for $\text{C}_{15}\text{H}_{21}\text{Br}_2\text{ClO}_3$, 441.9546).

Computational details

Conformational searches for **1** and **2** were performed *via* the Dreiding force field in MarvinSketch (optimization limit = normal, diversity limit = 0.1) regardless of rotations of methyl and hydroxyl groups, the geometries of which were further optimized at the B3LYP/6-31+G(d,p) level in chloroform, methanol (for calculations of ECD spectra and specific optical rotations), and water (for calculations of Mulliken charge distributions, bond lengths, and angles, Table S1†) to give just one conformer within a 3 kcal mol^{-1} energy threshold from the global minimum without vibrational imaginary frequencies for **1** and **2**, respectively (Table S2†). The predominant conformers of **1** and **2** were subjected to the theoretical calculations of specific optical rotations ($[\alpha]_{\text{D}}$) at the B3LYP/aug-cc-pVDZ level and ECD spectra at the B3LYP/6-31G(d) level using the time-dependent DFT method, which were drawn *via* SpecDic software with $\sigma = 0.27$ and UV shift = 25 nm (magnified by 0.07 times). All the above calculations were performed with the integral equation formalism variant polarizable continuum model (IEF-PCM) as implemented in Gaussian 09.^{18–20}

Bioassays

The inhibitory activities against marine zooplankton (*A. salina*) and marine phytoplankton (*H. akashiwo*) as well as four bacteria (*V. ichthyenteri*, *P. mirabilis*, *E. cloacae*, and *B. cereus*, at 30 $\mu\text{g}/\text{disk}$) isolated from seawater were assayed as described previously,^{16,17} with positive controls of $\text{K}_2\text{Cr}_2\text{O}_7$, $\text{K}_2\text{Cr}_2\text{O}_7$, and chloramphenicol, respectively.

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