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Neoiriepentaol and nangenyne, halogenated diterpenoid and C_{15}-acetogenin from red alga *Laurencia nangii* Masuda collected in Borneo

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Abstract
The red algal genus *Laurencia* is a prolific producer of halogenated secondary metabolites. A new tricyclic dibrominated diterpenoid, neoiriepentaol (**1**) and chlorinated C_{15}-acetogenin, nangenyne (**2**), along with two known terpenoids, neoirietetraol (**3**) and dactyloxene A (**4**), were isolated from methanol crude extract of red alga *Laurencia nangii*. The structures were established based on one- and two-dimensional nuclear magnetic resonance (NMR), Fourier-transform infrared (FTIR), and high-resolution electrospray ionization mass spectrometry (HRESIMS) data. These compounds were screened against seven species of marine fungi. Compounds **1**–**3** exhibited activity against *Lagenidium thermophilum* and Haliphthoros sabahensis. Potent activity was showed by **1** with *L. thermophilum* hyphal inhibition at MIC value of 12.5 μg mL^{-1} and hyphal motility was observed at 50 μg mL^{-1} within 24 h.

Keywords Neoirieane-type · Brominated diterpenoid · C_{15}-acetogenin · *Laurencia nangii* · Red alga

Introduction

The red algae of the genus *Laurencia* (Rhodomelaceae, Ceramiales) are known to have a high degree of morphological variation within individual species and have the ability to produce structurally diverse halogenated terpenes and C_{15}-acetogenins (Suzuki and Vairappan 2005). Most species of *Laurencia* biosynthesize specific set of metabolites that is characteristic of their species (Fenical 1975; Masuda et al. 1996). These metabolites have been suggested as chemotaxonomical markers of this genus (Matsuo et al. 1995; Vairappan et al. 2014). In addition, halogenated compounds isolated from *Laurencia* have been reported to exhibit various biological activities such as anti-bacterial, anti-cancer, and anti-fouling (Al-Lihaibi et al. 2015; Kamada and Vairappan 2015, 2017; Vairappan et al. 2001). In a previous investigation of Bornean algae belonging to the genus *Laurencia*, we have reported diverse secondary metabolites (Kamada and Vairappan 2012, 2015; Vairappan et al. 2014), and some of them showed potent activities against the "ice-ice" disease bacteria (Vairappan et al. 2010) and anti-inflammation activities (Vairappan et al. 2013). In this research, we collected *L. nangii* at Mantanani Island, Sabah, Malaysia, and isolated a new tricyclic brominated diterpenoid, neoiriepentaol (**1**) and a new C_{15}-acetogenin, nangenyne (**2**), along with two other known compounds, neoirietetraol (**3**) and dactyloxene A (**4**) (Fig. 1). These compounds were tested for their anti-fungal potential against pathogens that were isolated from mud crab *Scylla tranquebarica* aquaculture farms that has been severely infected by these pathogens in Sabah, Malaysia (Hatai 2012; Lee et al. 2016, 2017). Herein, we report the structural elucidation of these metabolites and their anti-fungal activity.

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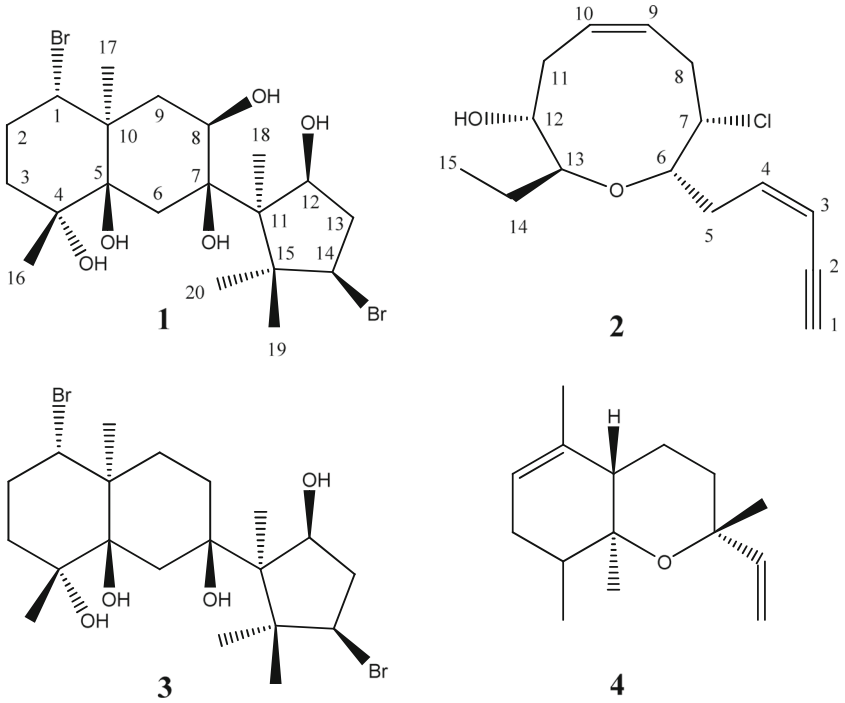

Fig. 1 Structural diversity of halogenated chemicals (**1**–**4**) isolated from *Laurencia nangii* Masuda

Experimental

General experimental procedures

The value of optical rotation was recorded using AUTOPOL IV automatic polarimeter (Rudolph Research Analytical, USA). The IR absorption was measured using Thermo Nicolet Avatar FTIR spectroscopy (Thermo, Japan). The ECA JEOL 600 MHz NMR spectroscopy (JEOL, Japan) was used to measure ^{1}H and ^{13}C NMR, HSQC, ^{1}H-^{1}H COSY, HMBC, and NOESY experiments in $CDCl_3$ incorporated with tetramethylsilane (TMS) as an internal standard. The liquid chromatography-electrospray ionization-ion trap-time of flight-mass spectrometry (LC-ESI-IT-TOF-MS) (Shimadzu, Japan) was used to measure *m/z* of **1** and **2**. Preparative thin layer chromatography (PTLC) glass plate coated with silica gel (Merck, Germany; Kieselgel 60 F_{254}) and normal phase silica gel gradient mode column chromatography (70–230 mesh; Merck, Germany) were used for compound isolation. Analytical TLC (Merck Kieselgel 60 F_{254}) was used to develop spots that were visualized by UV light (254 and 365 nm) and sprayed with a 5% phosphomolybdic acid-ethanol solution. All solvents are analytical grade (Fisher Scientific, USA).

Plant materials

Specimens of *Laurencia nangii* Masuda were collected from the coastal waters of Mantanani Island (6° 43′ 08.52″ N, 116° 20′ 25.99″ E), Sabah, Malaysia, in March 2015. A voucher specimen (BORH63959) was deposited in the BORNEENSIS Herbarium of Institute for Tropical Biology and Conservation (BORH), Universiti Malaysia Sabah.

Extraction and isolation

Air dried specimen (108 g) was extracted with methanol (MeOH) at room temperature (24 °C) for 3 days. The crude extract was suspended in distilled water (H_2O) (150 mL) and partitioned with ethyl acetate (EtOAc) (50 mL × 3). After removal of the organic solvent, the EtOAc fraction (1.0 g) was chromatographed on a Si gel column using hexane and EtOAc system as eluent with increasing polarity (Hex/EtOAc: 9:1, 8:2, 7:3, 5:5 and 100% EtOAc) to yield five fractions. Fraction 2 (308.7 mg) was subjected to PTLC with toluene to yield **4** (10.3 mg; 1.0%). Fraction 3 (387.7 mg) was subjected to PTLC with $CHCl_3$ to yield **2** (112.2 mg; 11.3%). Fraction 4 (95.5 mg) was subjected to PTLC with hexane/EtOAc (2:1) and the residue was further purified by preparative HPLC to yield **1** (5.1 mg; 0.5%) and **3** (2.6 mg; 0.3%). The HPLC was operated using a C18 column under following conditions: 0–40 min, gradient elution of 50–100% acetonitrile (MeCN), 40–50 min 100% MeCN, UV measurement at 210 nm, and oven temperature at 40 °C.

Neoiriepentaol (1)

Colorless oil; $[\alpha]_D^{28.0}$ − 25.0 (*c* 0.30, $CHCl_3$); IR ν_{max} (cm^{-1}): 3610, 3410, 3006, 1485, 1386, 1370, 1210, and 938; ^{1}H and ^{13}C-NMR spectral data: see Table 1; HRESIMS: *m/z* 513.0859 $[M+H]^+$ (calcd for $C_{20}H_{35}O_5Br_2$, 513.0846).

Table 1 ^{1}H-, ^{13}C-NMR, and HMBC data for neoiriepentaol (**1**)

	1		
C	^{13}C (δ)	^{1}H (δ) multiplicity, J (Hz)	HMBC correlations
1	63.9	4.68 (1H, dd, J = 4.1, 12.4 Hz)	C-17
2	30.3	2.37 (1H, dt, J = 4.1, 12.4 Hz)	C-1
		2.00 (1H, m)	
3	38.0	2.16 (1H, m)	C-1
		1.34 (1H, m)	
4	75.5		
5	78.4		
6	33.6	2.19 (1H, m)	
		2.00 (1H, m)	
7	84.7		
8	70.8	4.34 (1H, dd, J = 4.8, 11.7 Hz)	
9	42.3	2.17 (1H, m)	C-5, C-7, C-8, C-10
		1.63 (1H, m)	C-5, C-7, C-8, C-10
10	45.0		
11	48.3		
12	83.4	4.27 (1H, dd, J = 5.5, 8.3 Hz)	C-11, C-18
13	42.1	2.77 (1H, ddd, J = 8.3, 8.3, 14.4 Hz)	C-11, C-14, C-15
		2.21 (1H, m)	
14	62.0	4.13 (1H, dd, J = 8.3, 11.7 Hz)	
15	52.5		
16	27.1	1.25 (3H, s)	C-3, C-4, C-5
17	19.9	1.30 (3H, s)	C-1, C-5, C-9, C-10
18	21.8	0.99 (3H, s)	C-7, C-11, C-12, C-15
19	23.4	1.46 (3H, s)	C-11, C-14, C-15, C-20
20	24.2	1.12 (3H, s)	C-11, C-14, C-15, C-19
OH		6.30 (s)	C-6
OH		5.59 (s)	C-4, C-5, C-6
OH		4.78 (br s)	

Note: recorded at 600/150 MHz in deuterated chloroform $CDCl_3$, δ in ppm

Nangenyne (2)

Colorless oil; $[\alpha]_D^{28.0}$ − 136.8 (*c* 0.50, $CHCl_3$); IR ν_{max} (cm^{-1}): 3402, 3290, 2110, 1060, and 938; ^{1}H and ^{13}C-NMR spectral data: see Table 2; HRESIMS: *m/z* 291.1115 $[M+Na]^+$ (calcd for $C_{15}H_{21}ClO_2Na$, 291.1122) and 269.1301 $[M+H]^+$ (calcd for $C_{15}H_{22}ClO_2$, 226.1303).

Anti-fungal assay

The minimum inhibitory concentration (MIC) of hyphal inhibition on *Fusarium moniliforme* NJM 8995, *Fusarium oxysporum* NJM 0179, *Fusarium solani* NJM 8996, *Exophiala* sp. NJM 1551, *Ochroconis humicola* NJM 1503, *Lagenidium thermophilum* IPMB 1401, and *Haliphthoros sabahensis* IPMB 1402 were carried out by incorporating the compound solutions (100, 50, 25, 12.5 μg mL^{-1}) onto peptone yeast extract glucose starch (PYGS) agar in petri dish. Next, inoculation of fungal agar block onto petri dish was done using cork borer. The procedure was modified from known method (Munchan et al. 2009). The MIC can be observed as the lowest concentration with no hyphal growth of fungi after incubation at 25 °C for day 3 and day 7. The minimum fungicidal concentration (MFC) was determined by immerse the agar blocks (fungal hyphae) in various concentrations of compound solutions (100, 50, 25, 12.5 μg mL^{-1}) for 10 and 30 min; 1, 2, and 24 h. The agar blocks were washed and

Table 2 ^{1}H-, ^{13}C-NMR, and HMBC data for nangenyne (**2**)

	2		
C	^{13}C (δ)	^{1}H (δ) multiplicity, J (Hz)	HMBC correlations
1	83.1	3.16 (1H, d, J = 1.4 Hz)	C-3
2	80.7		
3	111.9	5.56 (1H, dd, J = 1.4, 11.0 Hz)	C-1
4	140.9	6.01 (1H, ddd, J = 7.6, 8.3, 11.0 Hz)	C-2, C-5
5	34.9	2.90 (1H, ddd, J = 4.8, 8.3, 13.1 Hz)	C-3, C-4, C-6, C-7
		2.72 (1H, ddd, J = 7.6, 8.3, 13.1 Hz)	C-3, C-4, C-6, C-7
6	83.3	3.67 (1H, ddd, J = 2.8, 4.8, 8.3 Hz)	C-13
7	62.8	4.04 (1H, ddd, J = 2.8, 6.2, 11.0 Hz)	
8	34.5	3.06 (1H, q, J = 11.0 Hz)	C-6, C-7, C-9, C-10
		2.51 (1H, dt, J = 6.2 11.0 Hz)	C-6, C-7, C-9, C-10
9	128.2	5.59 (1H, dt, J = 6.2, 11.0 Hz)	C-8
10	130.2	5.84 (1H, dt, J = 6.2, 11.0 Hz)	
11	32.5	2.94 (1H, m)	
		2.17 (1H, td, J = 6.2, 13.1 Hz)	
12	74.3	3.95 (1H, m)	C-11
13	86.4	3.19 (1H, td, J = 4.1, 7.6 Hz)	
14	27.0	1.74 (1H, m)	C-12, C-13, C-15
		1.74 (1H, m)	C-12, C-13, C-15
15	9.4	0.97 (1H, t, J = 6.9 Hz)	C-13, C-14

Note: recorded at 600/150 MHz in deuterated chloroform $CDCl_3$, δ in ppm

incubated at 25 °C for 3rd and 7th days (Panchai et al. 2016). The control was carried out by immerse agar blocks in sterilized seawater solution without compounds.

Results

A total 1.86 g of dark green oily extract was obtained from the MeOH solution of *L. nangii* after liquid-liquid partition as described in the experimental section. Upon separation via column chromatography, PTLC, and HPLC, two new halogenated metabolites (**1**–**2**) and two known metabolites (**3**–**4**) were isolated and their structures were determined based on spectroscopic data obtained from NMR, FTIR, and HRESIMS measurements. Compound **1** was isolated as an optically active colorless oil, with $[\alpha]_D^{28}$ − 25.0 (*c* 0.30, chloroform $CHCl_3$). The molecular formula of $C_{20}H_{34}O_5Br_2$ (corresponding to three degrees of unsaturation) was deduced from the HRESIMS data (*m/z* 513.0859 $[M+H]^+$ calcd for $C_{20}H_{35}O_5Br_2$, 513.0846). The broad infrared (IR) absorption at 3410 cm^{-1} indicated the presence of a hydroxyl group. The ^{1}H-NMR data showed five tertiary methyls at δ_H 1.46, 1.30, 1.25, 1.12, and 0.99. The combination of the ^{13}C-NMR, DEPT, and HRESIMS data indicated that the two methine carbons at δ_C 63.9 and 62.0 were halo-methine in nature. Furthermore, three hydroxyl-bearing quaternary carbons (δ_C 84.7, 78.4, and 75.5) and methine carbons (δ_C 83.4 and 70.8) were observed. Based on these findings, the three degrees of unsaturation could be attributed to a tricyclic system.

Structural assignments were conducted based on ^{1}H-^{1}H COSY and HMBC spectral data. ^{1}H–^{1}H COSY experiment revealed the sequences of the correlations are shown as bold lines in Fig. 2. The two fragment units (H-1/ H_2-2/ H_2-3 and H-8/ H_2-9) together with HMBC cross peaks of H_3-16 to C-3, C-4, and C-5; H_3-17 to C-1, C-5, C-9, and C-10; H_2-9 to C-5 and C-7; 5-OH and 7-OH to C-6 have facilitated establishment of a decalin ring. Furthermore, a cyclopentyl group was found attached to the decalin ring at C-7 based on fragment unit (H-12/H_2-13/ H-14) along with HMBC correlations of H_3-18 to C-7, C-11, C-12, and C-15, and *gem*-dimethyl moiety at C-15 based on HMBC correlations of both H_3-19 and H_3-20 to C-11, C-14, and C-15 as well as to the respective carbons C-20 and C-19), as shown in Fig. 2. The structure of **1** was closely similar to neoirietetraol (**3**) which previously reported from Japanese *Laurencia yonaguniensis* Masuda except the presence of a hydroxyl group at C-8 in **1** (Takahashi et al. 2002, 2007). Three hydroxyl groups were observed in the ^{1}H NMR spectrum; nevertheless, the remaining two hydroxyl groups were not detected in ^{1}H NMR spectrum.

The relative configuration of **1** was determined through the NOESY experiment (Fig. 2) as well as the vicinal coupling constants. The methine proton at δ_H 4.68, H-1 recorded the coupling constants ($^3J_{1\text{-}2ax}$ = 12.4 Hz; $^3J_{1\text{-}2eq}$ = 4.1 Hz), implying H-1 was a typical axial proton assuming cyclohexane ring with chair conformation. Hence, the bromine atom at C-1 was equatorial. The axial configuration of the methyl group (H_3-17) at C-10 showed NOESY correlations to H_{ax}-2 (δ_H 2.37), H_{ax}-6 (δ_H 2.00), and H-8 (δ_H 4.34), which required the decalin ring to be *trans*-fused, as observed in known analogues (Takahashi et al. 2002, 2007, 2010). Therefore, the hydroxyl group at C-5 is positioned on an axial position. In addition, the 7-OH (δ_H 6.30) was found to be in a *cis* configuration with 5-OH (δ_H 5.59) due to a NOE cross peak between 5-OH and 7-OH. Relative configuration of the hydroxyl group at C-8 was assigned as β-configuration due to NOE correlations of H-8 to H_3-17 and H_3-18. This assignment is further supported when the proton-proton vicinal coupling constants ($^3J_{8ax\text{-}9ax}$ = 11.7 Hz; $^3J_{8ax\text{-}9eq}$ = 4.8 Hz) in **1** was consistent to those of neoirietetraol ($^3J_{8ax\text{-}9ax}$ = 13.2 Hz; $^3J_{8ax\text{-}9eq}$ = 4.8 Hz) (Takahashi et al. 2002). The NOEs between H-12/H_3-18, H-12/H-14, and H-14/H_3-18 revealed that H_3-18, H-12, and H-14 were located on the same plane and OH-12 and Br-14 were located on the opposite plane of the cyclopentane ring. Upon comparison with **3**, the relative configurations of **1** were determined as identical to those of neoirietetraol (**3**) except for its stereocenter at C-8 (Takahashi et al. 2002, 2007). Therefore, structure of **1** was reported to have 1*S**, 4*R**, 5*R**, 7*R**, 8*R**, 10*S**,

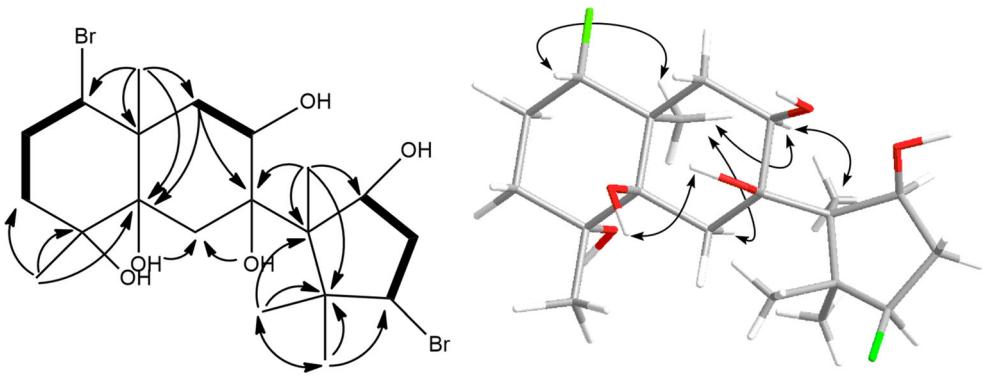

Fig. 2 ^{1}H-^{1}H COSY (–), key HMBC (→), and NOESY (↔) correlations of **1**

11*R**, 12*S**, and 14*R**. To the best of our knowledge, neoiriepentaol (**1**) is the fourth example of a halogenated diterpenoid having a neoirieane-type skeleton (Howard et al. 1982; Takahashi et al. 2002, 2007, 2010).

Compound **2** was isolated as a colorless oil with $[\alpha]_D^{28}$ − 136.8 (*c* 0.50, $CHCl_3$). The molecular formula of $C_{15}H_{21}ClO_2$ was deduced from the HRESIMS data (*m/z* 291.1115 [M+Na]$^+$ calcd for $C_{15}H_{21}ClO_2Na$, 291.1122 and 269.1301 [M+H]$^+$ calcd for $C_{15}H_{22}ClO_2$, 226.1303. The IR spectrum displayed the absorptions at 3290, 2110, and 1060 cm^{-1} indicating the presence of terminal alkyne and ether group. The structure of **2** was elucidated by comparison of the ^{1}H and ^{13}C-NMR spectral data (Table 2) with those of known metabolites, (+)-obtusenyne, and 12-*epi*-obtusenyne, as well as by 2D NMR techniques (Gopichand et al. 1981; Fujiwara et al. 1999). The main difference between their structures was the hydroxyl group at C-12 in **2** is replaced by bromine atom in obtusenyne and 12-*epi*-obtusenyne. Upon examination of their proton chemical shifts, we found a significant difference in chemical shift values of H-13 in **2** (δ_H 3.18), as compared to (+)-obtusenyne (δ_H 3.92), (3*Z*)-12-*epi*-obtusenyne (δ_H 3.92), and (3*E*)-12-*epi*-obtusenyne (δ_H 3.95) (Gopichand et al. 1981; Fujiwara et al. 1999). However, further comparison of carbon chemical shifts with 12-*epi*-obtusenyne was not possible due to absence of ^{13}C-NMR data in the literature for the published compounds. Comparison of carbon chemical shifts showed a significant different at C-12 and C-13 in **2** (δ_C 74.3, C-12; 86.4, C-13) and (+)-obtusenyne (δ_C 56.5, C-12; 76–78 obscured by $CDCl_3$ signal, C-13) (δ_C 56.8, C-12; 75.4, C-13 in C_6D_6) (Fujiwara et al. 1999). This finding concluded planar structure of **2** was similar to obtusenyne with the replacement of bromine atom at C-12 by hydroxyl group. The ^{1}H-^{1}H COSY correlations and key HMBC correlations are shown in Fig. 3. The ^{1}H–^{1}H COSY correlations indicated the presence of one spin system from H-3 to H-15. The hydroxyl group at C-12 was not observed in ^{1}H NMR spectrum.

The *cis* geometry double bond at C-3/C-4 was determined from coupling constant J_{3-4} = 11.0 Hz. The (3*Z*) configuration was consistent when acetylenic proton at δ_H 3.16 (d, *J* = 1.4 Hz, H-1) and olefinic proton δ_H 5.57 (dd, *J* = 11.0, 1.4 Hz, H-3) were closely identical to those of (3*Z*)-12-*epi*-obtusenyne (δ_H 3.18, d, *J* = 2.0 Hz; 5.57, dd, *J* = 11.0, 2.0 Hz) than (3*E*)-12-*epi*-obtusenyne (δ_H 2.85, d, *J* = 2.0 Hz; 5.62, dd, *J* = 16.0, 2.0 Hz) (Gopichand et al. 1981). The relative configuration of the asymmetric centers was determined based on the NOESY experiment and proton-proton vicinal coupling constants. The strong NOE correlation between H-6 and H-7 suggested a *cis* configuration (Fig. 3). The methines H-12 and H-13 have a *trans* configuration due to the absence of NOE correlation between H-12 and H-13. The proton H-12 showed NOE correlation to H_2-14 and H_3-15, further supporting the β-configuration of these protons. The β assignment at H-12 was consistent with the reported 12-*epi*-obtusenyne (Gopichand et al. 1981). On the contrary, the relative configurations of **2** and (+)-obtusenyne were identical for all stereocenters except the C-12, this can be explained by their sign of optical rotation in **2** ($[\alpha]_D^{28}$ − 136.8) and (+)-obtusenyne ($[\alpha]_D^{19}$ + 151), hence further supported the assignment of β relative configuration at H-12 in **2** (Fujiwara et al. 1999). Therefore, the relative configurations of C-6, C-7, C-12, and C-13 in **2** were identical to those of 12-*epi*-obtusenyne. Hence, compound **2** was reported as (3*Z*,6*S**,7*S**,9*Z*,12*R**,13*S**)-7-chloro-12-hydroxy-5,13-epoxypentadeca-3,9-trien-1-yne, named as nangenyne. To the best of our knowledge, this is the first isolation of a nine-membered C_{15}-acetogenin with hydroxyl functionalities at the position C-12 isolated from *Laurencia* species.

The known compounds were identified as neoirietetraol (**3**) and dactyloxene A (**4**) by comparing their observed and reported spectroscopic data (Schmitz et al. 1978; Takahashi et al. 2002, 2007). All four isolated compounds were tested for their fungistatic and fungicidal effect on hyphae against seven marine-derived fungal strains *Fusarium moniliforme* NJM 8995, *Fusarium oxysporum* NJM 0179, *Fusarium solani* NJM 8996, *Exophiala* sp. NJM 1551, *Ochroconis humicola* NJM 1503, *Lagenidium thermophilum* IPMB

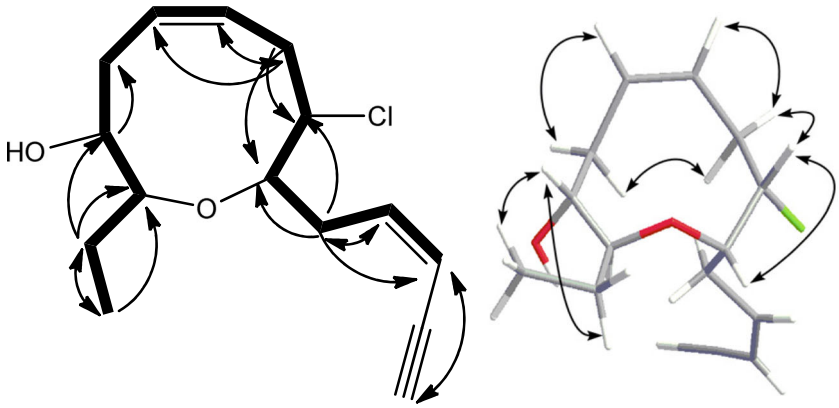

Fig. 3 ^{1}H-^{1}H COSY (–), key HMBC (→), and NOESY (↔) correlations of **2**

Table 3 Fungistatic effect of **1–4** against hyphae of seven fungal strains

Strains	MIC (μg mL^{-1})			
	1	2	3	4
F. moniliforme	100	100	100	–
F. oxysporum	100	100	100	–
F. solani	100	100	100	–
H. sabahensis	50	50	25	–
Exophiala sp.	100	100	100	–
O. humicola	100	100	100	–
L. thermophilum	12.5	25	25	–

Positive control: itraconazole MIC 2.2 μg mL^{-1}

1401, and *Haliphthoros sabahensis* IPMB 1402 as shown in Table 3. The result showed that compounds **1**–**3** were active inhibiting hyphal growth of *L. thermophilum* and the most active compound (**1**) with MIC 12.5 μg mL^{-1}. In addition, compound **1**–**3** inhibited hyphal growth on *H. sabahensis* at MIC 25 μg mL^{-1}.

The fungicidal results (Table 4) showed that compound **1** was able to kill the hyphae of *L. thermophilum* at the concentration of 50 μg mL^{-1} with 24 h exposure time. While, compounds **2** and **3** showed hyphal killing effect on *L. thermophilum* with a higher concentration of 100 μg mL^{-1} within 24 h. Furthermore, compound **3** also could kill hyphae of *H. sabahensis* at 100 μg mL^{-1} in 24 h. However, compound **4** did not show any anti-fungal activity against the tested marine fungi.

Discussion

High mortality among mud crab *Scylla tranquebarica* eggs and larvae in the hatchery at Sabah, Malaysia, was due to the fungal infection caused by *H. sabahensis* and *L. thermophilum* (Hatai 2012; Lee et al. 2016, 2017). Previous attempt in solving this issue using formalin, trifluralin, and malachite green have detrimental impact on aquatic ecosystems, aquatic life and human activities (Schreier et al. 1996; Kitancharoen et al. 1997a, b; Fuangsawat et al. 2011). Two new halogenated compounds, neoiriepentaol (**1**) and nangenyne (**2**) along with two known compounds, neoirietetraol (**3**) and dactyloxene A (**4**) were isolated from *L. nangii* Masuda in Borneo. These red alga-derived secondary metabolites **1**–**3** showed hyphal inhibition against *H. sabahensis* and *L. thermophilum*. The most active compound (**1**) could kill the hyphae of *L. thermophilum* at the concentration of 50 μg mL^{-1} within 24 h. This finding has given new idea on solving the fungal infection of *H. sabahensis* and *L. thermophilum* at hatchery. Chemicals with similar chemical skeleton of **1** such as neoirietetraol (**3**) was reported to exhibit anti-bacterial activities against marine bacteria *Alcaligenes aquamarinus* and *Escherichia coli* at 100 μg disk^{-1} (Takahashi et al. 2002). It is worth to mention that the rare neoirieane skeleton was reported only in neoirieone and neoirietetraol (**3**) isolated from species of *Laurencia* in 1982 and 2002, respectively (Howard et al. 1982; Takahashi et al. 2002; Hill 2003; Blunt et al. 2004; El Gamal 2010). Hence, this is the third representative of neoirieane-type diterpenoid, neoiriepentaol (**1**) isolated from

Table 4 Fungicidal effect of **1–3** on hyphae

Strains	Compounds	Exposure time	Control	Concentration (μg mL^{-1})			
				12.5	25	50	100
H. sabahensis	**3**	10 min	+	+	+	+	+
		30 min	+	+	+	+	+
		1 h	+	+	+	+	+
		2 h	+	+	+	+	+
		24 h	+	+	+	+	
L. thermophilum	**1**	10 min	+	+	+	+	+
		30 min	+	+	+	+	+
		1 h	+	+	+	+	+
		2 h	+	+	+	+	+
		24 h	+	+	+		
	2	10 min	+	+	+	+	+
		30 min	+	+	+	+	+
		1 h	+	+	+	+	+
		2 h	+	+	+	+	+
		24 h	+	+	+	+	
	3	10 min	+	+	+	+	+
		30 min	+	+	+	+	+
		1 h	+	+	+	+	+
		2 h	+	+	+	+	+
		24 h	+	+	+	+	

Note: (+) represent growth; () represent no growth

Laurencia nangii, representing another species from genus *Laurencia*. The carbon skeleton of neoirieone was derived from dictyolane (formally known as prenyleudesmane) through two bromonium ion-induced cyclization and a series of classical rearrangement (Howard et al. 1982). In addition, the hydroxyl group at C-12 in 9-membered ring C_{15}-acetogenin **2** was relatively unique due to most reported 9-membered ring C_{15}-acetogenins have bromine atom at C-12 instead of hydroxyl moiety such as obtusenyne (Kokkotou et al. 2014), laurendecumallene A (Ji et al. 2007), itomanallene A (Jeong et al. 2010), isolaurallene (Furusaki et al. 1985), neolaurallene (Suzuki et al. 1984), 3-*Z*- and 3-*E*-12-*epi*-obtusenyne (Gopichand et al. 1981) as shown in review paper of Wanke et al. (2015). Thereby, both of these new halogenated secondary metabolites are unique in their structure and showed chemical diversity in red alga *L. nangii* in Borneo.

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