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Antibacterial activity of halogenated sesquiterpenes from Malaysian *Laurencia spp.*

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ABSTRACT

During our studies on Malaysian *Laurencia* species, brominated metabolites, tiomanene, acetylmajapolene B, and acetylmajapolene A were isolated from an unrecorded species collected at Pulau Tioman, Pahang along with known majapolene B and majapolene A. Acetylmajapolene A was a mixture of diastereomers as in the case of majapolene A. Tiomanene may be a plausible precursor for acetylmajapolenes B and A. In addition, three known halogenated sesquiterpenes and two known halogenated C_{15} acetogenins were found from other two unrecorded species collected at Pulau Karah, Terengganu and Pulau Nyireh, Terengganu, respectively. Some of these halogenated metabolites showed moderate antibacterial activity against some marine bacteria.

1. Introduction

In our continuing studies on chemical compositions of the red algal genus *Laurencia* from Malaysian waters (Masuda et al., 1997b, 1999, 2002; Suzuki et al., 2001; Vairappan et al., 2001; Vairappan and Phang, 2005), we examined three unrecorded species collected at different locations; sample-1 from Pulau Tioman, Pahang, sample-2 from Pulau Karah, Terengganu, and sample-3 from Pulau Nyireh, Terengganu. Sample-1 contained brominated sesquiterpenes **1–5** with a 4-bromo-3,3-dimethylcyclohexyl group. The structures of new compounds, tiomanene (**1**), acetylmajapolene B (**2**), and acetylmajapolene A (**3**), were elucidated by spectral and chemical methods. Compounds **4** and **5** were identified as majapolene B and majapolene A, respectively, which have previously been isolated from *Laurencia majuscula* (Harvey) Lucas collected off Apo Island, near the southern tip of Negros Island, Central Visayas, Philippines (Erickson et al., 1995). Acetylmajapolene A (**3**) was found to be a mixture of diastereomeric isomers as in the case of majapolene A (**5**). The sample-2 contained three known halogenated sesquiterpenes, which were identified as prepacifenol (**6**) (Sims et al., 1973), prepacifenol epoxide (**7**) (Faulkner et al., 1974), and pacifenol (**8**) (Sims et al., 1971, 1973). Furthermore, the sample-3 produced two known halogenated C_{15} acetogenins, which were identified as chlorofucin (**9**) (Howard et al., 1980) and a bromoallene (**10**) (Suzuki et al., 1989). In this paper we report the isolation and structural elucidation of these new and known halogenated metabolites as well as their antibacterial activities against marine bacteria.

2. Results and discussion

A combination of column and preparative thin-layer chromatography of the methanol extract of sample-1 collected from Pulau Tioman, Pahang gave three new brominated compounds, **1**, **2**, and **3**, in 4.4% (based on the crude extract), 5.5%, and 3.8% yield, respectively, along with known related compounds **4** (10.2%) and **5** (2.2%).

Compounds **4** and **5** were identified as majapolene B and majapolene A, respectively, by independent structure elucidation using 1D (^{1}H and ^{13}C NMR) and 2D (^{1}H–^{1}H COSY, HSQC, HMBC, and NOESY) NMR techniques together with mass spectral analyses. Majapolene B (**4**) and majapolene A (**5**), the latter of which was an inseparable mixture of diastereomeric isomers (**5a** and **5b**) (Fig. 1), have previously been isolated from *L. majuscula* collected off Apo Island, Philippines (Erickson et al., 1995).

One of the new metabolites, compound **2** was analyzed for $C_{17}H_{23}BrO_2$ by HREIMS (*m*/*z* 338.0845 (Δmmu −3.7) [M]). The ^{1}H

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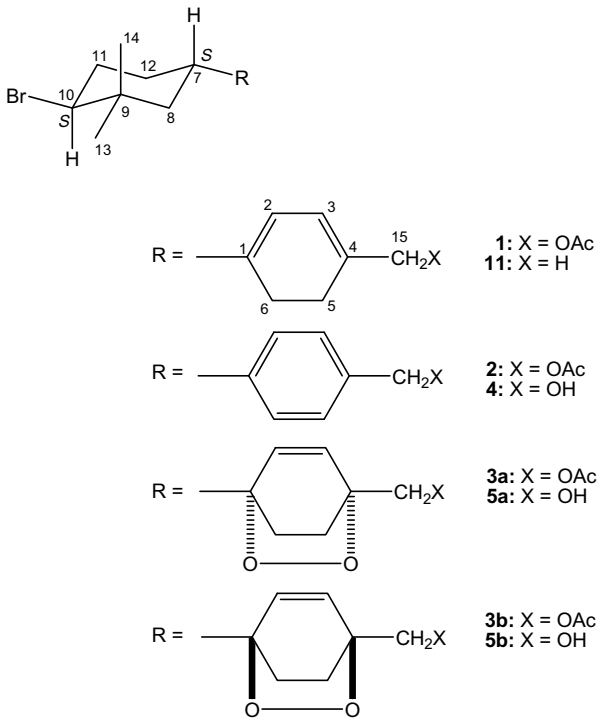

Fig. 1. Halogenated metabolites from a *Laurencia* sp. from Pulau Tioman.

NMR spectrum (Table 1) of **2** showed a close resemblance to that of majapolene B (**4**), but distinct differences were observed; e.g., in the spectrum of **2**, a signal due to an acetoxymethyl group appeared at δ_H 5.07 (2H, *s*) instead of a hydroxymethyl group at δ_H 4.66 (2H, *s*) in the spectrum of **4**. Thus, it was strongly suggested that compound **2** is an acetyl derivative of majapolene B (**4**). This was confirmed by the following chemical correlation. Treatment of **4** with acetic anhydride and pyridine yielded an acetylated product, which was identical with **2** in all respects.

Compound **3** was found to have a molecular formula of $C_{17}H_{25}BrO_4$ by HRESIMS (*m*/*z* 395.0834 (Δmmu +0.1) $[M+Na]^+$). The EIMS of **3**, however, showed no molecular ion peak and instead a prominent peak at *m*/*z* 342 and 340 $[M-O_2]^+$ and a base peak at *m*/*z* 109 $[C_8H_{13}]^+$. A facile loss of oxygen molecule (O_2) revealed that **3** contains a peroxide group as in the case of majapolene A (**5**). The ^1H and ^{13}C NMR spectra of **3** were very similar to those of majapolene A (**5**), strongly indicating that **3** is also a mixture of diastereomers. Detailed comparison of the spectral data of **3** and **5** suggested that **3** is an acetyl derivative of **5**. Treatment of **5** with acetic anhydride and pyridine yielded an acetylated product, which was identical with **3**.

The third new metabolite, compound **1**, designated as tiomanene, had a molecular formula of $C_{17}H_{25}BrO_2$ established by HRFDMS (*m*/*z* 340.1049 (Δmmu +1.1) [M]). Its IR spectrum showed the presence of an acetoxyl group at ν_{max} 1730 and 1230 cm^{-1}. The UV spectrum of **1** revealed a absorption maximum at ν_{max} 262 nm (ε 15,000), strongly indicating the presence of a 1,4-disubstituted cyclohexa-1,3-diene moiety in the molecule. The ^1H NMR spectrum (Table 2) of **1** showed signals due to two quaternary methyl groups at δ_H 1.07 and 1.08 (3H, *s*, each), an acetoxyl group at δ_H 2.08, two methylene groups at δ_H 2.10 (2H, m) and 2.14 (2H, m), which are probably allylic, a bromomethine group at δ_H 3.94 (1H, *dd*, *J* = 12.7, 4.4 Hz), an acetoxymethyl group at δ_H 4.54 (2H, *s*), and two olefinic protons at δ_H 5.62 and 5.88 (1H, *d*, *J* = 5.4 Hz, each).

Detailed analysis of the ^1H and ^{13}C NMR spectra (Table 2) as well as HSQC and ^1H–^1H COSY spectra revealed the presence of partial structural units **1a**–**1e** in the molecule (Fig. 2). Confirma-

Table 1
^{13}C NMR (100 MHz, DEPT), ^1H NMR (400 MHz), and HMBC data[a] for acetylmajapolene B (**2**)

C	^{13}C[b] (δ)	^1H (δ)	Multiplicity, (*J* in Hz)	Long range correlations
1	146.6	–	–	H-3, H-5, H-6
2	127.7	7.18	*d* (8.3)	H-3, H-6,
3	129.3	7.28	*d* (8.3)	H-5, H_2-15
4	134.6	–	–	H-2, H_2-15
5	129.3	7.28	*d* (8.3)	H-3, H_2-15
6	127.7	7.18	*d* (8.3)	H-2
7	39.5	2.84	*dddd* (12.6, 12.6, 3.4, 3.4)	H-2, H-5, H-6
8	48.6	1.84	*ddd* (12.6, 3.0, 3.0)	H_3-13, H_3-14
		1.52	*dd* (12.6, 12.6)	
9	37.8	–	–	H-10, H_2-8 H_3-13, H_3-14
10	66.3	4.04	*dd* (12.6, 4.4)	H_2-11, H_2-12, H_3-13, H_3-14
11	35.2	2.26	*m*	H-7, H-10
		2.16	*dddd* (12.9, 12.9, 12.6, 3.9)	
12	36.1	1.88	*m*	
		1.53	*m*	
13	32.4	1.09	*s*	H-10, H_3-14
14	21.2	1.16	*s*	H-10, H_3-13
15	66.8	5.07	*s*	H-3, H-5
Ac	21.7	2.09	*s*	
Ac	171.6	–	–	H_2-15

[a] Measured in chloroform – d_1.
[b] Assigned by HSQC spectrum.

Table 2
^{13}C NMR (100 MHz, DEPT), ^1H NMR (400 MHz), and HMBC data[a] for tiomanene (**1**)

C	^{13}C[b](δ)	^1H (δ)	Multiplicity, (*J* in Hz)	Long Range Correlations
1	131.4			H-3, H_2-5, H_2-6
2	117.6	5.62	*d* (5.4)	H-3, H_2-6,
3	123.7	5.88	*d* (5.4)	H_2-5, H_2-15
4	127.7			H-2, H_2-15
5	25.3	2.10	*m*	H-3, H_2-15
6	26.3	2.14	*m*	H-2
7	40.2	2.25	*dddd* (13.6, 13.6, 3.4, 3.4)	H-2, H_2-6
8	45.7	1.72	*m*	H_3-13, H_3-14
		1.26	*dd* (13.6, 13.6)	
9	37.5			H-10, H_3-13, H_3-14
10	66.6	3.94	*dd* (12.7, 4.4)	H_2-8, H_2-12, H_3-13, H_3-14
11	34.9	2.19	*m*	H-10
		2.16	*m*	
12	33.4	1.75	*m*	
		1.29	*m*	
13	32.4	1.07	*s*	H_3-14
14	21.2	1.08	*s*	H_3-13
15	68.1	4.54	*s*	
Ac	21.7	2.08	*s*	
Ac	171.7	–	–	H_2-15

[a] Measured in chloroform– d_1.
[b] Assigned by HSQC spectrum.

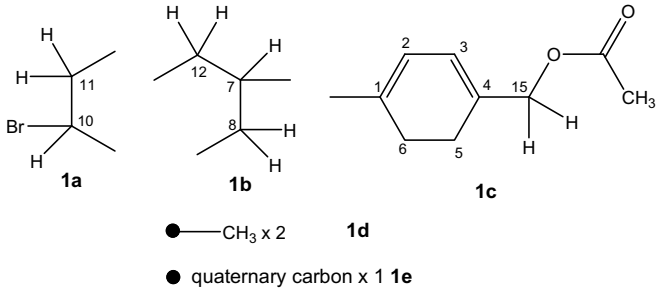

Fig. 2. Partial structural units for **1**.

tion of the partial structural units and determination of their connectivity were made by the HMBC spectrum. Mutual long-range correlations between two tertiary methyls at δ_H 1.07 (δ_C 32.4) and 1.08 (δ_C 21.2) in unit **1d**, which showed further long-range correlations to the quaternary carbon at δ_C 37.5 (unit **1e**), indicated that these methyls comprise a *gem*-dimethyl group. Moreover, this *gem*-dimethyl group showed long-range correlations to the methine carbon at δ_C 66.6 (C-10) in unit **1a** and the methylene carbon at δ_C 45.7 (C-8) in unit **1b**, thus confirming that the *gem*-dimethyl group can be inserted between C-10 and C-8. Tiomanene, which possesses five degrees of unsaturation, must consist of an additional carbocyclic ring. Therefore, C-11 in unit **1a** and C-12 in unit **1b** must be combined to form a cyclohexane ring. In addition, long-range correlation between the vinyl carbon C-2 (δ_C 117.6) in cyclohexadiene ring (unit **1c**) and the methine proton at δ_H 2.25 (H-7) confirmed the connection C-7 with C-1, thus completing a planar formula **1** for tiomanene.

The relative stereochemistry of **1** was determined by the coupling constants in the ^{1}H NMR spectrum as well as the NOESY experiment. The coupling constants of the methine proton (*dd*, J = 12.7 and 4.4 Hz) at C-10 showed that the H-10 is axial in a typical chair cyclohexane ring and hence the bromine atom adopts equatorial configuration. Moreover, the J-value (*dddd*, 13.6, 13.6, 3.4, and 3.4 Hz) of H-7 indicated that the H-7, which is adjacent to two axial and two equatorial protons, is also axial. Furthermore, a NOE correlation between H-7 and H_3-14 (δ_H 1.08) was observed in the NOESY spectrum of **1**, thus showing that tiomanene (**1**) has the same relative stereochemistry at C-7 and C-10 as compounds, **2**–**5**. It is noteworthy that tiomanene (**1**) partly changed into acetylmajapolene B (**2**) but not into acetylmajapolene A (**3**) during storage in a freezer.

To determine the absolute configurations of the isolated compounds, acetylmajapolene B (**2**) and majapolene B (**4**) were subjected to the vibrational circular dichroism (VCD). The chiroptical technique, the VCD has been developed recently as a chiral method of stereochemical analysis in which one measures the differential absorption of left versus right circularly polarized IR radiation by molecular vibrational transition (Nafie and Freedman, 2000; Freedman et al., 2003; Polavarapu and He, 2004; Taniguchi et al., 2004). In the VCD methods the absolute configuration is determined nonempirically by comparison between observed VCD spectra with the simulated VCD spectra issued from the quantum mechanical *ab initio* methods with density functional theory (DFT) calculations.

As has previously been reported (Monde et al., 2006a), the results of the VCD methods indicated that the absolute configuration is 7*S* and 10*S* in both compounds (**2** and **4**). In addition, the absolute configuration of each diastereomer of endoperoxides (**3a** and **3b**) was also established by the VCD application (Monde et al., 2006b). The complete assignment for signals in the ^{1}H and ^{13}C NMR spectra of each diastereomer are also reported in the previous paper (Monde et al., 2006b).

As mentioned above, since tiomanene (**1**) changed into acetylmajapolene B (**2**) during storage in a freezer, tiomanene has the same (7*S*,10*S*)-configuration as acetylmajapolene B.

Erickson et al. (1995) have described in the literature that a 1,3-cyclohexadiene (**11**), which may presumably be derived from bromonium ion-induced cyclization of a bisabolene system, is a plausible immediate precursor to majapolenes A and B. Thus the present finding of tiomanene (**1**) should confirm their proposed biogenetic pathway. Acetylmajapolene B (**2**) may be derived from tiomanene by dehydrogenation, whereas each diastereomer (**3a** and **3b**) of acetylmajapolene A may arise enzymatically or chemically from tiomanene by the 1,4-addition of oxygen molecule to the conjugated diene system.

Chemical composition of the species from Pulau Tioman, Pahang (sample-1) was very similar to that of the Philippine *L. majuscula* (Erickson et al., 1995).

The sample-2 collected from Pulau Karah, Terengganu gave oxygenated chamigrane-type sesquiterpenes **6**, **7**, and **8** in 4.0%, 16.0%, and 10.0% yield, respectively. Compound **6** was identified as prepacifenol by comparison of spectral data with those of the authentic sample, which was isolated from prepacifenol race (prepacifenol-producing strains) of *Laurencia nipponica* Yamada (Masuda et al., 1997a). Prepacifenol has initially been isolated from *Laurencia pacifica* Kylin and *Laurencia filiformis* (C. Agardh) Montagne (Sims et al., 1973). Compound **7** was identified as prepacifenol epoxide, which has initially been isolated from the sea hare *Aplysia californica* (Faulkner et al., 1974). Furthermore, compound **8** was identified as pacifenol, which has first been isolated from *Laurencia tasmanica* Hooker et Harvey (Sims et al., 1971, 1973). The chemical composition of the species from Pulau Karah (sample-2) was very similar to that of *Laurencia composita* Yamada (Masuda et al., 1996) from Japanese waters.

The sample-3 collected at Pulau Nyireh, Terengganu gave two halogenated C_{15} acetogenins **9** and **10** in 6.5% and 3.8% yield, respectively. Compound **9** was identical with chlorofucin, which has previously found in *Laurencia snyderae* Dawson collected from La Jolla, California (Howard et al., 1980) and in *Laurencia pannosa* Zanardini collected from An Thoi, Phu Quoc Island, Kien Giang Province, Vietnam (Suzuki et al., 1996). (3*Z*)-Chlorofucin was also isolated from the Malaysian *L. pannosa* collected at Pulau Talang-Talang Kecil (1°53′50″N, 109°45′59″E), Kuching, Sarawak (Suzuki et al., 2001). In addition, compound **10** was identical with a known bromoallene previously found in *Laurencia intricata* Lamouroux from Bikuni, Hokkaido, Japan (Suzuki et al., 1989) and in a *Laurencia* sp. from Chinzei, Saga Prefecture, Japan (Suzuki et al., 2005) (see Figs. 3 and 4).

The antibacterial activity for compounds obtained from Pulau Tioman was tested against six marine bacteria isolated from algal surface. The results of the paper disc diffusion assay are shown in Table 3. Tiomanene (**1**) showed weak antibacterial activities against four tested bacterial strains with MIC values of 40 μg disc^{-1}, respectively. Acetylmajapolene B (**2**) was inactive against all the tested strains. Acetylmajapolene A (**3**), majapolene B (**4**), and majapolene A (**5**) showed moderate antibacterial activities against all the tested strains with MIC values between 10 and 40 μg disc^{-1}. Detailed antibacterial activity of these compounds is shown in Table 3.

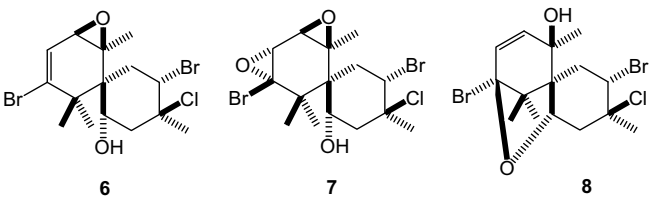

Fig. 3. Halogenated metabolites from a *Laurencia* sp. from Pulau Karah.

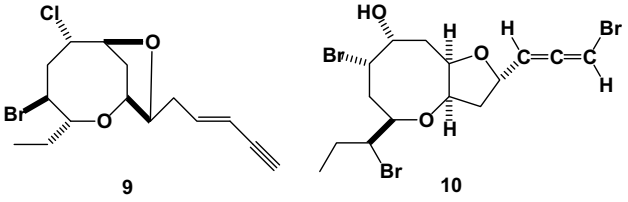

Fig. 4. Halogenated metabolites from a *Laurencia* sp. from Pulau Nyireh.

Table 3
Antibacterial activities (MIC) of halogenated sesquiterpenes from *Laurencia* spp.

	Test compounds (μg/disc)				
	1	2	3	4	5
Chromobacterium violaceum	40	–	20	20	10
Proteus mirabilis	40	–	20	20	30
Proteus vulgaris	40	–	40	20	40
Erwinia sp	–	–	20	10	30
Vibrio parahaemolyticus	40	–	30	20	30
Vibrio alginolyticus	–	–	30	20	30

Note: (–) No inhibition.

3. Experimental

3.1. General experimental procedures

IR spectra were measured on a JASCO A-102 spectrophotometer. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded in $CDCl_3$ with TMS as the internal standard by using a JEOL JNM-EX-400 spectrometer. LREIMS and HREIMS were obtained on a JEOL JMS-FABmate spectrometer. LRFDMS and HRFDMS were obtained on a JEOL JMS-SX102A spectrometer. LRESIMS and HRESIMS were obtained on a JEOL JMS-700TZ spectrometer. Optical rotations were recorded on a JASCO DIP-140 polarimeter. Si gel (Merck, Kieselgel 60, 70–230 mesh) was used for column chromatography. Si gel plates (Merck, Kieselgel 60254S) were used for preparative TLC. The known compounds were identified by comparison of spectroscopic data with those of authentic samples or those reported in the literature.

3.2. Collection

Three *Laurencia* species were gathered from different locations in Malaysian waters: sample-1, Teluk Juara, Pulau Tioman (2°46′52″N, 104°12′35″E), Pahang, on 3rd June, 1999 (SAP 094621—094625); sample-2, Pulau Karah (5°35′43″N, 103°03′50″E), Terengganu, on 23rd May, 1999 (SAP 094614—094616); sample-3, Pulau Nyireh (4°50′46″N, 103°39′55″E), Terengganu, on 28th June, 1999 (SAP 094617—094620). The voucher specimens are deposited in the Herbarium of Graduate School of Science, Hokkaido University (SAP).

3.3. Extraction and isolation of the sample-1

The partially dried alga (40 g) was extracted with MeOH. The MeOH solution was concentrated in vacuo and partitioned between Et_2O and H_2O. The Et_2O solution was washed with water, dried over anhydrous Na_2SO_4, and evaporated to leave a dark green oil (120 mg). The extract was then fractionated by Si gel column chromatography with a step gradient (hexane and EtOAc). Fraction eluted with hexane–EtOAc (9:1) was subjected to preparative TLC with hexane–EtOAc (3:1) to give **1** (5.3 mg) and **2** (6.6 mg). Fractions eluted in hexane–EtOAc (8:2), hexane–EtOAc (7:3) and hexane–EtOAc (6:4) were further separated via preparative TLC with hexane–EtOAc (3:1) to give **3** (4.6 mg), **4** (12.2 mg), and **5** (2.6 mg). Compounds **4** ($[\alpha]_D^{25}$ −16.7° ($CHCl_3$; *c* 0.20)) and **5** ($[\alpha]_D^{25}$ −23.0° ($CHCl_3$; *c* 0.20)) were identified as majapolene B and majapolene A, respectively, by comparison of spectral data with those reported in the literature for majapolenes B and A (Erickson et al., 1995).

3.4. Tiomanene (**1**)

Oil: $[\alpha]_D^{24}$ −17.4° ($CHCl_3$; *c* 0.47); UV λ_{max} (EtOH) nm: 262 (ε 15,000); IR ν_{max} (neat) cm^{-1}: 1730, 1440, 1360, 1230, 1010, 960; for ^{1}H and ^{13}C NMR spectra, see, Table 2; LR-FDMS *m*/*z* (rel. int.): 343, 341 $[M+H]^+$ (30:29), 342, 340 $[M]^+$ (96:100); HR-FDMS *m*/*z*: 340.1049. Calc. for $C_{17}H_{25}{}^{79}BrO$, 340.1038 [M].

3.5. Acetylmajapolene B (**2**)

Oil: $[\alpha]_D^{25}$ −3.40° ($CHCl_3$; *c* 0.22); IR ν_{max} (neat) cm^{-1}: 1730, 1440, 1360, 1220, 1010, 960; for ^{1}H and ^{13}C NMR spectra, see, Table 1; LR-EIMS *m*/*z* (rel. int.): 340, 338 $[M]^+$ (65:65), 298, 296 (42:43) $[M-CH_2{=}C{=}O]^+$, 215 (46), 199 (92), 107 (100), 91 (28), 43 (34); HR-EIMS *m*/*z*: 338.0845. Calc. for $C_{17}H_{23}{}^{79}BrO_2$, 338.0882 [M].

3.6. Acetylmajapolene A (**3**)

Oil: $[\alpha]_D^{25}$ −14.7° ($CHCl_3$; *c* 0.18); IR ν_{max} (neat) cm^{-1}: 1750, 1460, 1395, 1370, 1240, 1050, 970; ^{1}H NMR, δ 1.05 (3H, *s*, H_3-14), 1.06 (3H, *s*, H_3-14), 1.06 (3H, *s*, H_3-13), 1.08 (3H, *s*, H_3-13), 1.18–1.30 (2H, m, Ha-8 and Ha-12), 1.41–1.55 (2H, m, Ha-5 and Ha-6), 1.72–1.88 (2H, m, Hb-8 and Hb-12), ~1.93 (1H, m, H-7), 1.98–2.16 (3H, m, Hb-5, Hb-6, and Ha-11a), 2.11 (3H, *s*, H_3-Ac), ~2.20 (1H, m, Hb-11), 3.92 (1H, *dd*, *J* = 12.6, 4.2 Hz, H-10), 3.93 (1H, *dd*, *J* = 12.6, 4.2 Hz, H-10), 4.21 (1H, *d*, *J* = 12.5 Hz, Ha-15), 4.32 (1H, *d*, *J* = 12.5 Hz, Hb-15), 6.52 (2H, *s*, H-2 and H-3), 6.53 (2H, *s*, H-2 and H-3); ^{13}C NMR, δ 171.4 (*C*OCH_3), 134.6/134.2 (C2), 133.1/133.0 (C3), 79.9 (C1), 76.5 (C4), 65.8 (C10), 65.3 (C15), 41.1/41.0 (C8), 37.9/37.8 (C7), 37.3/37.2 (C9), 34.4 (C11), 32.4/32.3 (C13), 29.0/28.9 (C12), 25.9 (C6), 25.7/25.6 (C5), 21.4 ($COCH_3$), 21.0/20.9 (C14); LR-ESIMS *m*/*z*: 397, 395 $[M+Na]^+$; LR-EIMS *m*/*z* (rel. int.): 342, 340 (3:3) $[M-O_2]^+$, 298, 296 (1:1), 282, 280 (3:3) $[M-O_2-CH_3CO_2H]^+$, 201 (2), 199 (2), 191, 189 (6:6), 109 (100), 92 (32), 67 (16), 55 (9), 43 (25); HR-ESIMS *m*/*z*: 395.0834. Calc. for $C_{17}H_{25}{}^{79}BrO_4Na$, 395.0833 $[M+Na]^+$; HR-EIMS *m*/*z*: 340.1033. Calc. for $C_{17}H_{25}{}^{79}BrO_2$, 340.1037 $[M-O_2]$ and *m*/*z*: 109.1019. Calc. for C_8H_{13}, 109.1017 $[M-C_9H_{12}BrO_4]^+$.

3.7. Acetylation of majapolene B (**4**)

Acetylation of **4** (2 mg) was carried out with acetic anhydride (1 mL) and pyridine (1 mL) at room temperature for 12 h in the usual manner to afford the corresponding acetate in almost quantitative yield. The resulting acetate was identical with **2** in all respect.

3.8. Acetylation of majapolene A (5)

Acetylation of **5** (2 mg) was carried out with acetic anhydride (1 mL) and pyridine (1 mL) at room temperature for 12 h in the usual manner to afford the corresponding acetate in almost quantitative yield. The resulting acetate was identical with **3** in all respect.

3.9. Extraction and isolation of the sample-2

The dried alga (90 g) was extracted with MeOH. The MeOH solution was concentrated in vacuo and partitioned between Et_2O and H_2O. The Et_2O solution was washed with water, dried over anhydrous Na_2SO_4, and evaporated to leave a dark green oil (400 mg). The extract was then fractionated by Si gel column chromatography with a step gradient (hexane and EtOAc). Fractions eluted with hexane–EtOAc (9:1) and hexane–EtOAc (8:2) gave **6** (16 mg), whose spectral properties were identical with those of the authentic prepacifenol from *L. nipponica* (Masuda et al., 1997a). Fractions eluted with hexane–EtOAc (8:2) and hexane–EtOAc (7:3) gave **7** (64 mg), whose spectral properties were identical with those of the authentic prepacifenol epoxide from *L. composita* (Masuda et al., 1996). Fraction eluted with hexane–

EtOAc (7:3) gave **8** (40 mg), whose spectral properties were identical with those of the authentic pacifenol from *L. composita* (Masuda et al., 1996).

3.10. Extraction and isolation of the sample-3

The dried alga (27 g) was extracted with MeOH. The MeOH solution was concentrated in vacuo and partitioned between Et_2O and H_2O. The Et_2O solution was washed with water, dried over anhydrous Na_2SO_4, and evaporated to leave a dark green oil (137 mg). The extract was then fractionated by Si gel column chromatography with a step gradient (hexane and EtOAc). Fraction eluted with hexane–EtOAc (8:2) gave **9** (8.9 mg; $[\alpha]_D^{25}$ −18.5° ($CHCl_3$; *c* 0.80)), whose spectral properties were identical with those of the authentic chlorofucin (Suzuki et al., 1996). Fraction eluted with hexane–EtOAc (7:3) gave **10** (5.2 mg; $[\alpha]_D^{25}$ +121° ($CHCl_3$; *c* 0.50)), whose spectral properties were identical with those of the authentic bromoallene from *L. intricata* (Suzuki et al., 1989).

3.11. Antibacterial bioassay

The antibacterial bioassay for compounds obtained from Pulau Tioman was carried out using six species of marine bacteria isolated from algal surface collected in the Malaysia waters. These bacteria are *Chromobacterium violaceum*, *Proteus mirabilis*, *Proteus vulgaris*, *Erwinia* sp., *Vibrio parahaemolyticus*, and *Vibrio alginolyticus*.

One loopful of each organism was precultured in 20 mL of peptone water (3% NaCl) overnight. The turbidity of the culture was adjusted to an optical density (OD) McFarland 0.5 (Sonnerwirth and Jarett, 1980; Hindler et al., 1990). Then 0.1 ml of the precultured bacterial suspension was used to seed Nutrient Agar plates (3% NaCl). Paper discs (Whatman, 6 mm) impregnated with various amounts of the respective isolated compound were placed on the seeded agar plates and the diameters of the inhibitory zones were measured after the plates were incubated at 28°C for 24 h.

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