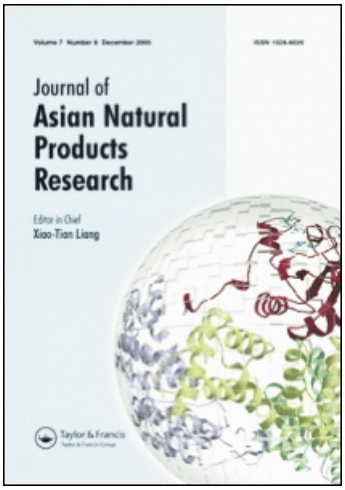

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Chemical constituents of the red alga *Laurencia tristicha*

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Six new sesquiterpenes, 10-hydroxy-epiaplysin (**1**), 10-hydroxy-aplysin (**2**), 10-hydroxy-debromoepiaplysin (**3**), aplysin-9-ene (**4**), epiaplysinol (**5**) and debromoepiaplysinol (**6**), together with 13 known compounds (**7**–**19**), have been isolated from the red alga *Laurencia tristicha*. The structures of **1**–**6** were determined by spectroscopic methods including IR, EI-MS, HREI-MS, and 1D and 2D NMR techniques. All compounds were obtained from this species for the first time and were tested for cytotoxic activities against several human cancer cell lines including lung adenocarcinoma (A549), stomach cancer (BGC-823), hepatoma (Bel 7402), colon cancer (HCT-8) and HeLa cell lines. Compound **6** showed selective cytotoxicity against HeLa cell line with IC_{50} 15.5 μM, cholest-5-en-3β,7α-diol (**14**) was toxic to all tested cell lines with IC_{50} values of 16.8, 5.1, 0.5, 0.5, and 0.3 μM, respectively, and other compounds were inactive ($IC_{50} > 10$ μg/ml).

Keywords: Red alga; *Laurencia tristicha*; Chemical constituents

1. Introduction

Red algae of the genus *Laurencia* are known to produce a great variety of metabolites consisting mainly of sesquiterpenes [1–5], C_{15}-acetogenins [6,7] and a few di- and triterpenes [8]. The majority of these secondary metabolites are characterised by their relatively high degree of halogenation [9]. As part of our program to systematically assess the chemical and biological diversity of seaweeds distributed in the China sea [10–12], the red alga *Laurencia tristicha* Tseng, Chang, E.Z. et B.M. Xia, belonging to the Rhodomelaceae family and widely distributed along the coast of the south China sea, was collected from the Naozhou Island, Zhanjiang City. Subsequent chemical investigation of this material has resulted in isolation and structural elucidation of six new natural products (**1**–**6**) along with 13 known compounds (**7**–**19**) (Scheme 1). By comparing the measured data with the corresponding literature data the known compounds were readily identified as aplysinol (**7**) [13,14], aplysin (**8**) [15,16],

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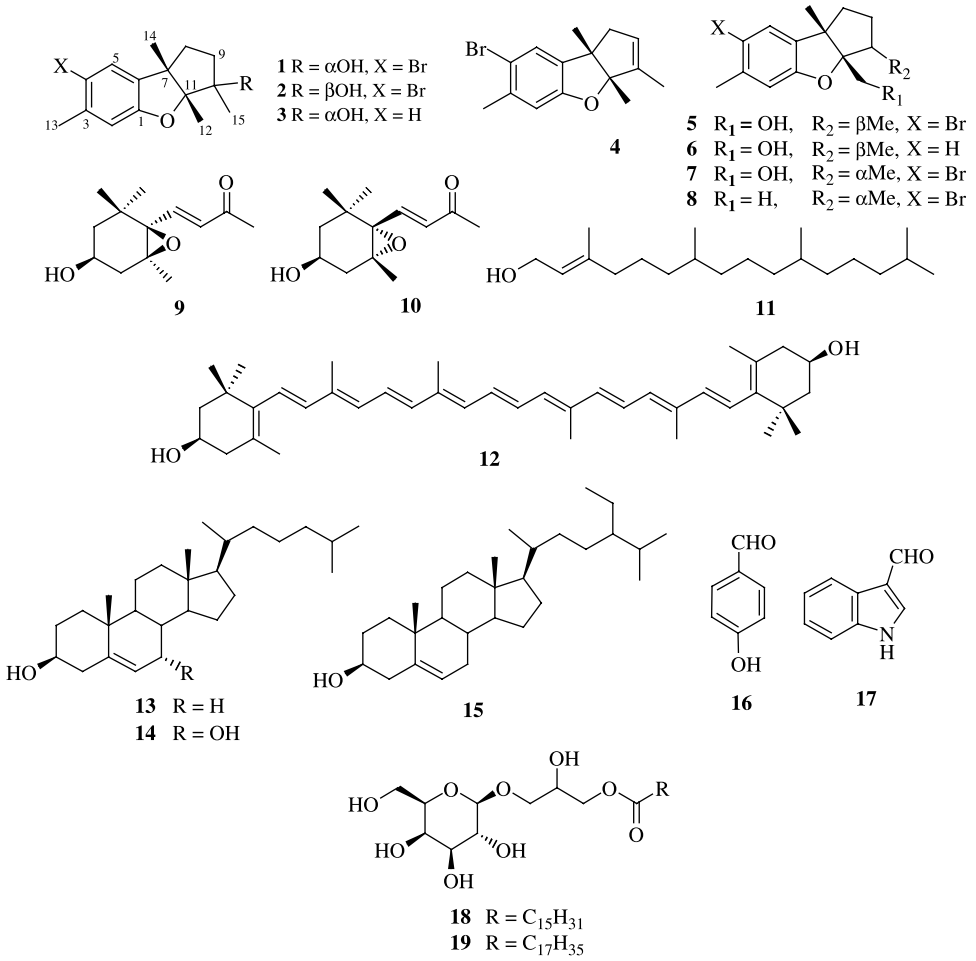

Scheme 1. Structures of **1**–**19**.

3β-hydroxy-5β,6β-epoxy-β-ionone (**9**) [17–20], 3β-hydroxy-5α,6α-epoxy-β-ionone (**10**) [19,20], phytol (**11**) [21,22], zeaxanthin (**12**) [23,24], cholesterol (**13**) [25], cholesta-5-en-3β,7α-diol (**14**) [25], β-sitosterol (**15**) [26], 4-hydroxy benzaldehyde (**16**) [27], indolyl-3-carbaldehyde (**17**) [28], 1-*O*-hexadecanoyl-3-*O*-β-D-galactopyranosylglycerol (**18**) [29], and 1-*O*-Octadecanoyl-3-*O*-β-D-galactopyra-nosylglycerol (**19**) [29]. All compounds were tested for their cytotoxicities against several human cancer cell lines including lung adenocarcinoma (A549), stomach cancer (BGC-823), hepatoma (Bel 7402), colon cancer (HCT-8) and HeLa cell lines. Compound **6** showed selective cytotoxicity against HeLa cell line with IC_{50} 15.5 μM, cholest-5-en-3β,7α-diol (**14**) was toxic to all tested cell lines with IC_{50} values of 16.8, 5.1, 0.5, 0.5, and 0.3 μM, respectively, and other compounds were inactive ($IC_{50} > 10$ μg/ml). In the previous communication we briefly reported the structural elucidation of compounds **1**–**3** [30] and 14 sesquiterpenes [31] from the title red alga; this paper describes in detail the isolation, structural elucidation, and spectroscopic data of compounds **1**–**6** from the same material.

Table 1. ^{1}H NMR data for compounds **1**–**7**[†].

No.	***1***	***2***	***3***	***4***	***5***	***6***	***7***
2	6.64 s	6.61 s	6.48 s	6.67 s	6.71 s	6.48 s	6.66 s
4			6.65 d (7.5)			6.63 d (7.5)	
5	7.25 s	7.24 s	6.96 d (7.5)	7.35 s	7.31 s	6.96 d (7.5)	7.16 s
8	1.63 m	(α) 1.68 dd (12.0, 7.0)	1.61 m	(α) 2.49 d (16.0)	(α) 1.94 dd (11.0, 6.0)	(α) 1.84 dd (11.0, 6.0)	(α) 1.84 dd (12.5, 6.5)
	1.72 m	(β) 2.07 ddd (12.0, 12.0, 7.0)	1.69 m	(β) 2.68 d (16.0)	(β) 1.77 ddd (11.0, 5.5, 5.5)	(β) 1.66 ddd (11.0, 5.5, 5.5)	(β) 1.66 m
9	1.56 m	(α) 1.50 ddd (12.0, 12.0, 7.0)	1.56 m	5.47 br s	1.14 m	1.06 m	1.16 m
	1.67 m	(β) 1.61 dd (12.0, 7.0)	1.63 m		1.75 ddd (12.0, 6.0, 5.0)	1.69 ddd (12.0, 6.0, 5.0)	1.67 m
10					2.18 m	2.13 m	1.63 m
12	1.21 s	1.25 s	1.19 s	1.31 s	(a) 3.86 d (12.0) (b) 3.77 d (12.0)	(a) 3.78 d (12.0) (b) 3.72 d (12.0)	(a) 3.85 (d 12.0) (b) 3.71 (d.12.0)
13	2.27 s	2.26 s	2.23 s	2.29 s	2.35 s	2.25 s	2.32 s
14	1.37 s	1.38 s	1.34 s	1.42 s	1.59 s	1.50 s	1.48 s
15	1.16 s	1.33 s	1.16 s	1.70 brs	1.12 d (7.0)	1.06 d (7.0)	1.10 d (7.0)
O*H*	3.40 brs	3.65 brs	3.30 brs				

[†] Measured at 500 MHz in acetone-d_6. The assignments were based on ^{1}H—^{1}H COSY and HMQC experiments. Proton coupling constants (*J*) in Hz are given in parentheses.

Table 2. ^{13}C NMR data for compounds **1**–**7**†.

No.	***1***	***2***	***3***	***4***	***5***	***6***	***7***
1	158.2 s	158.5 s	158.6 s	158.1 s	160.0 s	160.5 s	158.4s
2	112.1 d	111.9 d	110.3 d	112.6 d	111.4 d	109.6 d	110.7d
3	137.6 s	137.5 s	138.5 s	137.7 s	138.0 s	138.1 s	137.1s
4	114.9 s	114.6 s	122.1d	114.6 s	114.4 s	121.6 d	114.7 s
5	127.2 d	127.3 d	123.3 d	127.6 d	127.0 d	123.1 d	126.4 d
6	138.3 s	138.1 s	135.0 s	138.9 s	137.3 s	134.7 s	136.3 s
7	52.8 s	54.7 s	52.5 s	53.6 s	55.4 s	55.0 s	54.6 s
8	40.4 t	41.6 t	40.4 t	45.9 t	43.1 t	43.0 t	42.5 t
9	38.0 t	37.7 t	38.2 t	126.5 d	32.2 t	32.2 t	31.7 t
10	81.0 s	82.3 s	81.2 s	142.1 s	41.7 t	41.8 d	42.4 d
11	100.9 s	102.5 s	99.9 s	102.7 s	101.2 s	99.9 s	100.3 s
12	15.9 q	15.2q	16.0 q	12.0 s	62.9 t	63.0 t	63.9 t
13	23.1 q	23.1 q	21.4 q	23.0 q	23.1 q	21.6 q	23.1q
14	23.5 q	23.5 q	23.7 q	23.0 q	23.1 q	23.3 q	22.9 q
15	23.0 q	22.3q	22.9 q	18.2 q	13.9 q	13.9 q	13.8 q

† Measured at 125 MHz in acetone-d_6. The assignments were based on DEPT, ^{1}H—^{1}H COSY, HMQC and HMBC experiments.

2. Results and discussion

The air-dried alga material (10.5 kg) was powdered and extracted with 95% EtOH at room temperature. The EtOH extract was suspended in water and then partitioned with EtOAc. The EtOAc phase was concentrated, and then chromatographed over silica gel column using petroleum ether containing increasing proportions of ethyl acetate as eluent to yield 11 fractions on the basis of TLC analysis. The subsequent fractions were further purified by combination techniques of recrystallisation and column chromatography over Sephadex LH-20 and silica gel, as well as preparative HPLC to give compounds **1**–**6**.

Compound **1** was obtained as a colourless oil, $[\alpha]_D^{20}$ −8.1 (*c* 0.06, CH_3OH). The IR spectrum of **1** showed the presence of the hydroxy group (3564 cm^{-1}) and aromatic ring (1614, 1581 and 1485 cm^{-1}). Its EI mass spectrum exhibited characteristic monobrominated molecular ion peaks at *m/z* 312/310 (1:1) $[M]^+$. The molecular formula $C_{15}H_{19}BrO_2$ was established from the HREI-MS at *m/z* 310.0575 $[M]^+$. The ^{1}H NMR spectrum of **1** (table 1) displayed two singlets assignable to a 1,3,4,6-tetra-substituted benzene moiety at δ 7.25 (s, H-5) and 6.64 (s, H-2) and four methyl singlets at δ 1.16 (s, 3H, H_3-15), 1.21 (s, 3H, H_3-12), 1.37 (s, 3H, H_3-14) and 2.27 (s, 3H, H_3-13), in addition to multiplets attributed to two methylenes between δ 1.55 and 1.75 ppm. The ^{13}C NMR and DEPT spectra of **1** showed three quaternary carbon signals at δ 52.8 (C-7), 81.0 (C-10) and 100.9 (C-11) besides signals corresponding to the 1,3,4,6-tetra-substituted benzene moiety, four methyls and two methylenes (table 2). These spectroscopic data suggested that **1** was a hydroxylated derivative of aplysin, which was isolated firstly from the sea hare *Aplysia kurodai* [32] feeding on seaweeds of the genus *Laurencia* containing a variety of the related compounds

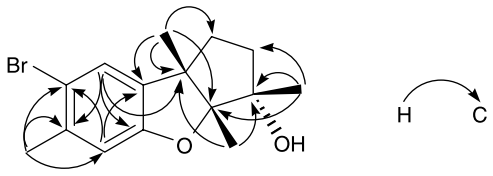

Figure 1. The key HMBC correlations of **1**.

[14]. The appearance of four methyl singlets in the ^{1}H NMR spectrum and the oxygenated quaternary carbon signal at δ_C 81.0 in the ^{13}C NMR spectrum revealed that the hydroxy group must be at C-10 of the basic structure of aplysin. In order to confirm above elucidation, gHSQC and gHMBC experiments of **1** were carried out. The proton signals in ^{1}H NMR spectrum and protonated carbon signals in ^{13}C NMR spectrum were unambiguously assigned by the gHMQC experiment (tables 1 and 2). In the gHMBC spectrum of **1** long-range correlations (figure 1) from H-2 to C-1, C-4, C-6 and C-13, from H-5 to C-1, C-3 and C-4, and from H_3-13 to C-2, C-3 and C-4 revealed the presence of the tetra-substituted benzene moiety, while correlations from H_3-15 to C-9, C-10 and C-11, from H_3-12 to C-7, C-10 and C-11, as well as H_3-14 to C-6, C-7, C-8 and C-11 demonstrated the presence of the cyclopentane moiety. In addition, HMBC correlations from H-5 to C-7 and from H_3-14 to C-6 verified the connection between C-6 and C-7. The linkage between C-1 and C-11 through an oxygen atom was concluded from the molecular composition and its six degrees of unsaturation as well as the chemical shift values of C-1 and C-11. The relative stereochemistry of **1** was determined by the NOE difference experiment. Irradiation of H_3-14 enhanced H_3-12 and H_3-15, indicating that the three methyls were in the same side of the ring system. Finally, on the basis of the biogenetic point of view the absolute configuration at C-7 was proposed to be identical to that of (−)-aplysin which has been determined by the total synthesis [33–35]. Therefore, the structure of **1** was determined as (3*S*,3α*R*,8β*S*)-(−)-2,3,3α,8β-tetrahydro-7-bromo-3-hydroxy-3,3α,6,8β-tetramethyl-1*H*-cyclopenta[*b*]benzofuran, and named 10-hydroxyepiaplysin.

Compound **2** was obtained as colourless needles (MeOH), mp 48–50°C, $[\alpha]_D^{20}$ −50.1 (*c* 0.81, CH_3OH). Its IR and EI-MS spectral features were very similar to those of **1**. The comprehensive analysis of ^{1}H—^{1}H gCOSY, gHSQC and gHMBC spectra of **2** revealed that **2** possessed a planar structure completely identical to that of **1** though the ^{1}H NMR spectrum of **2** (table 1) was distinctively different from that of **1**, especially in that the partially overlapped and unresolved H_2-8 and H_2-9 in the ^{1}H NMR spectrum of **1** were clearly separated from each other and became resolvable in the ^{1}H NMR spectrum of **2**. A comparison of the ^{1}H NMR data between **2** and **1** indicated that H-8β, H_3-12 and H_3-15 of **2** were downfield shifted by $\Delta\delta$ 0.35, 0.04 and 0.17, respectively, suggesting that **2** was a C-10 epimer of **1**. This was supported by the further comparison of ^{13}C NMR data between these two compounds (table 2) showing that C-12 and C-15 of **2** were shielded respectively by $\Delta\delta$ 0.7 ppm. Furthermore, in the NOE difference experiment of **2** H_3-14 and the hydroxy proton were enhanced by irradiation of H_3-12, demonstrating that H_3-12, H_3-14 and the hydroxy group were in the same side of the cyclopentane ring. Therefore, the structure of **2** was determined as (3*R*,3α*R*,8β*S*)-(−)-2,3,3α,8β-tetrahydro-7-bromo-3-hydroxy-3,3α,6,8β-tetramethyl-1*H*-cyclopenta[*b*]benzofuran, and designated as 10-hydroxyaplysin.

Compound **3** was obtained as a colourless oil, $[\alpha]_D^{20}$ −19.7 (*c* 0.10, CH_3OH). The IR spectrum of **3** showed absorption bands for the hydroxy group (3566 cm^{-1}) and aromatic ring (1620, 1593 and 1500 cm^{-1}). The EI mass spectrum of **3** gave a molecular ion peak at *m*/*z* 232 $[M]^+$, in combination with the NMR data (table 1), the molecular formula of **3** was determined as $C_{15}H_{20}O_2$ that was a bromine atom less than **1** and **2**. The ^{1}H NMR spectrum of **3** was very similar to that of **1** except that the signals attributed to the 1,3,4,6-tetra-substituted aromatic moiety of **1** were replaced by signals attributed to a 1,3,6-trisubstituted aromatic moiety at δ 6.96 (1H, d, J = 7.5 Hz, H-5), 6.65 (1H, d, J = 7.5 Hz, H-4) and 6.48 (1H, s, H-2). These data demonstrated that **3** was a debrominated product of **1**, which was supported by the

^{13}C NMR data of **3** (table 2) and further confirmed by the comprehensive analysis of 2D NMR spectra including ^{1}H—^{1}H gCOSY, gHSQC and gHMBC experiments of **3**. Thus, the structure of **3** was determined as (3*S*,3α*R*,8β*S*)-(−)-2,3,3α,8β-tetrahydro-3-hydroxy-3,3α,6,8β-tetramethyl-1*H*-cyclopenta[*b*]benzofuran, and named as 10-hydroxydebromoepiaplysin.

Compound **4** was obtained as colourless needles, mp 79–81°C, $[\alpha]_D^{20}$ −35.8 (*c* 0.12, CH_3OH), and showed IR absorption bands for the aromatic ring (1616 and 1504 cm^{-1}). The EI mass spectrum gave characteristic monobrominated molecular ion peaks at *m*/*z* 294/292 $[M]^+$, in combination with the NMR data (tables 1 and 2), the molecular formula of **4** was determined as $C_{15}H_{17}BrO$. The NMR data of **4** indicated that it is another analogue of **1** with the identical aromatic moiety. In the ^{1}H NMR spectrum of **4**, signals attributed to an olefinic proton at δ 5.47 (1H, brs, H-9) and a methylene at δ 2.49 and 2.68 (each 1H, br d, $J = 16$ Hz, H-8α and H-8β) replaced the signals of the two methylenes in **1**, demonstrating that **4** was a dehydrated product of **1** with a double bond between C-9 and C-10. This was unambiguously confirmed by the ^{13}C NMR and DEPT spectra of **4**, displaying signals for the double bond at δ 126.5 (d, C-9) and 142.1 (s, C-10) (table 2). Therefore, the structure of **4** was determined as (3α*R*,8β*S*)-(−)-3α,8β-dihydro-7-bromo-3,3α,6,8β-tetramethyl-1*H*-cyclopenta[*b*]benzofuran, named as aplysin-9-ene.

Compound **5** was obtained as colourless needles (MeOH), mp 172–174°C, $[\alpha]_D^{20}$ −66.4 (*c* 0.11, CH_3OH). The IR, EI-MS and NMR spectral features were similar to those of the co-occurring aplysinol (**7**). For comparison, the NMR data of **7** in acetone-d_6, unambiguously assigned by 2D NMR experiments, are listed in tables 1 and 2 since the reported data were obtained in $CDCl_3$. In the ^{1}H NMR spectrum of **5**, H-10 was at δ 2.18 (1H, m) distinctively different from that of **7** at δ 1.63 (1H, m), suggesting that **5** was a C-10 epimer of **7**. This was further confirmed by the NOE difference experiment of **5**. Irradiation of H_3-15 gave NOE enhancements of H-12a and H-12b. Therefore, the structure of **5** was determined as (3*R*,3α*S*,8β*S*)-(−)-2,3,3α,8β-tetrahydro-7-bromo-3α-hydroxymethyl-3,6,8β-trimethyl-1*H*-cyclopenta[*b*]benzofuran, designated as epiaplysinol.

Compound **6** was obtained as colourless needles (MeOH), mp 95–97°C, $[\alpha]_D^{20}$ −37.2 (*c* 0.12, CH_3OH). The NMR data of **6** were very similar to those of **5** except for the aromatic region that showed the presence of a 1,3,6-trisubstituted benzene moiety in **6** instead of the 1,3,4,6-tetra-substituted benzene moiety in **5**, suggesting that **6** was a debrominated derivative of **5**. This suggestion was confirmed by the EI-MS of **6** exhibiting a molecular ion peak at *m*/*z* 232, having a bromine atom less than that of **5**. Accordingly, the structure of **6** was determined as (3*R*,3α*S*,8β*S*)-(−)-2,3,3α,8β-tetrahydro-3α-hydroxymethyl-3,6,8β-trimethyl-1*H*-cyclopenta[*b*]benzofuran, named debromoepiaplysinol.

The racemic form of **1** and **2** without any chemical and physical data, as well as the racemic form of **4** with the limited IR and ^{1}H NMR data reported, were synthesised as intermediates in the total synthesis of racemic (±)-aplysin and (±)-debromoaplysin [36]. Compound **3** was reported as an intermediate in the enantiocontrolled synthesis of (−)-aplysin and (−)-debromoaplysin [15].

By using standard MTT method all purified compounds were tested for cytotoxicity against several human tumour cell lines including lung adenocarcinoma (A549), stomach cancer (BGC-823), hepatoma (Bel 7402), colon cancer (HCT-8) and HeLa cell lines. Compound **14** was toxic against all tested cell lines with IC_{50} values of 16.8, 5.1, 0.5, 0.5, and 0.3 μM, respectively, and compound **6** showed selective cytotoxicity against HeLa cell line with IC_{50} 15.5 μM. Other compounds were inactive ($IC_{50} > 10$ μg/ml).

3. Experimental

3.1 General experimental procedures

Melting points were determined on an XT-4 micro melting point apparatus and are uncorrected. Optical rotations were measured on a Rudolph Research Autopol III automatic polarimeter. IR spectra were recorded as KBr disks on a Nicolet Impact 400 FT-IR Spectrophotometer. 1D and 2D NMR spectra were obtained at 500 and 125 MHz for ^{1}H and ^{13}C, respectively, on an Inova 500 MHz spectrometer in acetone-d_6 with solvent peaks as references. EI-MS and HREI-MS data were measured with a Micromass Autospec-Ultima ETOF spectrometer. Column chromatography was performed with silica gel (200–300 mesh), and Sephadex LH-20. TLC was carried out with glass precoated silica gel GF_{254} plates (Qingdao Marine Chemical Factory, China). Spots were visualised under UV light or by spraying with 7% sulphuric acid in EtOH followed by heating. HPLC was performed using an Alltima C18 10 μm preparative column (22 × 250 mm).

3.2 Plant material

The red alga *Laurencia tristicha* was collected on the coast of Naozhou Island, Zhanjiang City, China in April 2003, and identified by Dr Lan-Ping Ding (Institute of Oceanology, Chinese Academy of Sciences, Qingdao 266071, China). A voucher specimen (No. 2003052) is deposited at the Herbarium of Institute of Oceanology.

3.3 Extraction and isolation

The air-dried alga *Laurencia tristicha* (10.5 kg) were extracted with EtOH at room temperature for 3 × 72 h. After the solvent was removed under reduced pressure at $< 40°C$, a dark residue (896 g) was obtained. The residue was suspended in water, and then partitioned with EtOAc. The EtOAc fraction (550 g) was chromatographed over silica gel column (1200 g) eluting with a gradient increasing EtOAc (0–100%) in light petroleum, and separated into 11 fractions (I–XI) on the basis of TLC analysis. Fraction I eluted by light petroleum was recrystallised with the same solvent to give a large amount of **8** (45.7 g). Fraction II eluted by 2% EtOAc in light petroleum was recrystallised with light petroleum to yield **7** (8.3 g), and the residue of the mother solution was subjected to chromatography over Sephadex LH-20 with petroleum ether/CH_3Cl/MeOH (5:5:1) as eluent to give three subfractions. The third subfraction was further purified by preparative HPLC with 90% MeOH in H_2O as mobile phase to give compound **11** (31 mg). Fraction III eluted by 3% EtOAc in light petroleum was separated by column chromatography over Sephadex LH-20 with petroleum ether/CH_3Cl/MeOH (5:5:1) as eluent to give three subfractions, and the last subfraction was further purified by preparative HPLC with 90% MeOH in H_2O as mobile phase to give compounds **1** (56 mg), **3** (48 mg), and **5** (3 mg). Fraction IV eluted by 5% EtOAc in light petroleum was separated by column chromatography over Sephadex LH-20 with petroleum ether/CH_3Cl/MeOH (5:5:1) as eluent to give two subfractions, the second subfraction was purified by preparative HPLC with 85% MeOH in H_2O as mobile phase to give compounds **2** (21 mg) and **6** (11 mg). Fraction V eluted by 10% EtOAc in light petroleum was separated by column chromatography over Sephadex LH-20 with petroleum ether/CH_3Cl/MeOH (5:5:1) as eluent to give two subfractions, the second subfraction was

purified by preparative HPLC with 80% MeOH in H_2O as mobile phase to give compounds **4** (7 mg) and **12** (16 mg). Fraction VI eluted by 20% EtOAc in light petroleum was separated by repeated column chromatography over silica gel with petroleum ether/acetone (10:1) as eluent to yield compounds **13** (820 mg), **14** (68 mg) and **15** (11 mg). Fraction VII eluted by 30% EtOAc in light petroleum was separated by column chromatography over Sephadex LH-20 with petroleum ether/CH_3Cl/MeOH (5:5:1) as eluent to give three subfractions, the third subfraction was purified by preparative HPLC with 80% MeOH in H_2O as mobile phase to give compounds **16** (39 mg) and **17** (44 mg). Fraction VIII eluted by 40% EtOAc in light petroleum was separated by column chromatography over silica gel with petroleum ether/acetone (10:1) as eluent to yield a mixture which was further separated by preparative HPLC with 80% MeOH in H_2O as mobile phase to give compounds **9** (12 mg) and **10** (5 mg). Fraction XI eluted by 100% EtOAc was separated by column chromatography over silica gel eluting with $CHCl_3$-acetone (3:1) to yield compounds **18** (123 mg) and **19** (92 mg).

3.4 10-Hydroxylepiaplysin (1)

A colourless oil, $[\alpha]_D^{20}$ −8.1 (*c* 0.06, CH_3OH); IR (film) ν_{max} 3564, 3474, 2954, 2870, 1614, 1581, 1485, 1471, 1385, 1352, 1265, 1230, 1182, 1138, 1097, 1065, 1032, 974, 912, 874, 849 cm^{-1}; ^1H NMR (acetone-d_6, 500 MHz) and ^{13}C NMR (acetone-d_6, 125 MHz) data, see tables 1 and 2; EI-MS *m/z* (%) 312/310 (28:30) $[M]^+$, 241 (64), 239 (80), 161 (11), 160 (100), 159 (12), 149 (12), 145 (6), 115 (8), 85 (13), 83 (18), 58 (36); HREI-MS *m/z* 310.0575 $[M]^+$ (calcd. for $C_{15}H_{19}BrO_2$, 310.0568).

3.5 10-Hydroxylaplysin (2)

Colourless needles (MeOH), mp 48–50°C, $[\alpha]_D^{20}$ −50.1 (*c* 0.81, CH_3OH); IR (KBr) ν_{max} 3487, 3307, 2958, 2868, 1616, 1579, 1501, 1487, 1394, 1377, 1258, 1252, 1234, 1152, 1158, 1090, 1062, 1038, 978, 924, 872, 854, 825 cm^{-1}; ^1H NMR (acetone-d_6, 500 MHz) and ^{13}C NMR (acetone-d_6, 125 MHz) data, see tables 1 and 2; EI-MS *m/z* (%) 312/310 (24:25) $[M]^+$, 241 (46), 239 (65), 232 (16), 215 (62), 201 (34), 199 (24), 185 (11), 173 (16), 167 (33), 161 (22), 160 (76), 159 (65), 149 (100), 145 (41), 115 (18), 105 (21), 85 (13), 83 (21), 71 (45), 57 (78), 55(56); HREI-MS *m/z* 310.0579 $[M]^+$ (calcd. for $C_{15}H_{19}BrO_2$, 310.0568).

3.6 10-Hydroxyldebromoepiaplysin (3)

A colourless oil, $[\alpha]_D^{20}$ −19.7 (*c* 0.10, CH_3OH); IR (film) ν_{max} 3566, 3478, 2954, 2870, 1620, 1593, 1500, 1444, 1383, 1352, 1269, 1184, 1139, 1107, 1062, 1030, 974, 947, 910, 802 cm^{-1}; ^1H NMR (acetone-d_6, 500 MHz) and ^{13}C NMR (acetone-d_6, 125 MHz) data, see tables 1 and 2; EI-MS *m/z* (%) 232 (58) $[M]^+$, 199 (4), 189 (5), 173 (9), 162 (29), 161 (100), 160 (52), 159 (42), 149 (12), 145 (15), 133 (13), 115 (6), 105 (5), 91 (5), 57 (5); HREI-MS *m/z* 232.1473 $[M]^+$ (calcd. for $C_{15}H_{20}O_2$, 232.1463).

3.7 Aplysin-9-ene (4)

Colourless needles (CH_3OH) mp 79–81°C, $[\alpha]_D^{20}$ −35.8 (*c* 0.12, CH_3OH); IR (KBr) ν_{max} 2922, 2870, 1651, 1616, 1576, 1504, 1485, 1471, 1449, 1438, 1377, 1306, 1267, 1234, 1165, 1068, 1039, 974, 910, 874, 850 cm^{-1}; ^1H NMR (acetone-d_6, 500 MHz) and ^{13}C NMR (acetone-d_6, 125 MHz) data, see tables 1 and 2; EI-MS *m/z* (%) 294/292 (7:8) $[M]^+$, 198 (6),

154 (4), 140 (4), 126 (10), 114 (6), 83 (12), 72 (66), 59 (100); HREI-MS *m/z* 292.0476 $[M]^+$ (calcd. for $C_{15}H_{17}BrO$, 292.0463).

3.8 Epiaplysinol (5)

Colourless needles (CH_3OH) mp 172–174°C, $[\alpha]_D^{20}$ −66.4 (*c* 0.11, CH_3OH); IR (KBr) ν_{max} 2960, 2931, 2873, 1593, 1552, 1485, 1377, 1263, 1159, 1101, 1057, 997, 949, 879, 843, 804; ^1H NMR (acetone-d_6, 500 MHz) and ^{13}C NMR (acetone-d_6, 125 MHz) data, see tables 1 and 2; EI-MS *m/z* (%) 312/310 (97:92) $[M]^+$, 280 (23),278 (42), 252 (12), 250 (10), 238 (53), 236 (55), 232 (60), 201 (49), 199 (30), 173 (22), 160 (36), 159 (79), 115 (23), 91 (13), 77 (10), 57 (18); HREI-MS *m/z* 310.0564 $[M]^+$ (calcd. for $C_{15}H_{19}BrO_2$, 310.0568).

3.9 Debromoepiaplysinol (6)

Colourless needles (CH_3OH), mp 95–97°C, $[\alpha]_D^{20}$ −37.2 (*c* 0.12, CH_3OH); IR (KBr) ν_{max} 3253, 2933, 2875, 1618, 1593, 1500, 1458, 1375, 1325, 1281, 1263, 1161, 1101, 1057, 997, 949, 843, 804 cm^{-1}; ^1H NMR (acetone-d_6, 500 MHz) and ^{13}C NMR (acetone-d_6, 125 MHz) data, see tables 1 and 2; EI-MS *m/z* (%) 232 (82) $[M]^+$, 217 (6), 214 (8), 201 (63), 199 (38). 187 (8), 173 (30), 161 (21), 160 (23), 159 (100), 149 (10), 145 (12), 135 (10), 115 (15), 91 (14), 77 (8), 58 (35); HREI-MS *m/z* 232.1455 $[M]^+$ (calcd. for $C_{15}H_{20}O_2$, 232.1463).

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