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Sesquiterpenes from Red Alga *Laurencia tristicha*

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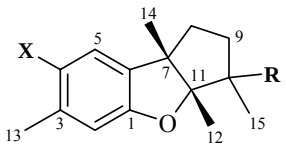
Abstract: Three naturally new sesquiterpenes named 10-hydroxyepiaplysin, 10-hydroxyaplysin and 10-hydroxybromoepiaplysin have been isolated from *Laurencia tristicha*. On the basis of the spectroscopic techniques their structures were elucidated as (3S, 3αR, 8βS)-(-)-2, 3, 3α, 8β-tetrahydro-7-bromo-3-hydroxy-3, 3α, 6,8β-tetramethyl-1H-cyclopenta[b]benzofuran, (3R, 3αR, 8βS)-(-)-2,3,3α, 8β-tetrahydro-7-bromo-3-hydroxy-3, 3α,6,8β-tetramethyl-1H-cyclopenta[b]benzofuran and (3S, 3αR, 8βS)-(-)-2, 3,3α,8β-tetrahydro-3-hydroxy-3,3α,6,8β-tetramethyl-1H-cyclopenta[b]-benzofuran, respectively.

Keywords: Red alga, *Laurencia tristicha*, sesquiterpenes.

Red algae of the genus *Laurencia* are known to produce a great variety of metabolites consisting mainly of sesquiterpenes, C_{15}-acetogenins and a few di- and triterpenes[1]. The majority of these secondary metabolites are characterized by their relatively high degree of halogenation[2]. As part of our program to assess systematically the chemical and biological diversity of seaweeds distributed in China Sea[3-5], the red alga *Laurencia tristicha* Tseng, Chang, E. Z. *et* B. M. Xia was collected from the Naozhou Island, Zhanjiang City, Guangdong Province, China. *L. tristicha* belongs to the Rhodomelaceae family and widely distributed along the coast of the South China Sea. No chemical constituent of this species has been investigated. We report here the isolation and structural elucidation of three naturally new sesquiterpenes (**1-3)** from this red alga.

Figure 1 The structures of compounds **1-3**



1 R = αOH, X = Br
2 R = βOH, X = Br
3 R = αOH, X = H

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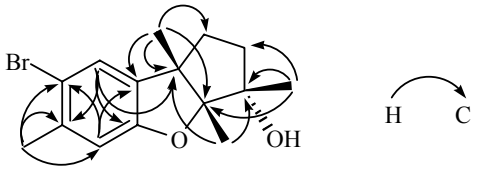
Figure 2 Key HMBC correlations of compound **1**



The air-dried alga material (10.5 Kg) was powdered and extracted with 95% EtOH at room temperature. The EtOH extract was concentrated under reduced pressure below 45℃ to give a residue. The residue was suspended in water and then partitioned with EtOAc. The EtOAc phase was concentrated and then chromatographed over silica gel using petroleum ether containing increasing proportions of EtOAc as eluent to yield eleven fractions on the basis of TLC analysis. The fraction eluted by 2% EtOAc in petroleum ether was separated by column chromatography over Sephadex LH-20 with petroleum ether-CH_3Cl- MeOH (5:5:1) as eluent, and further purified by preparative HPLC with 90% MeOH in H_2O as mobile phase to give compounds **1**-**3**.

Compound **1** was obtained as an oil, $[\alpha]_D^{20}$ −8.1 (*c* 0.06, MeOH). The IR spectrum of **1** showed the presence of hydroxy group (3564 cm^{-1}) and aromatic ring (1614, 1581 and 1485 cm^{-1}). The EI mass spectrum exhibited characteristic monobrominated molecular ion peaks at *m/z* 312/310 $[M]^+$ with an abundant ratio of 1:1. The molecular formula $C_{15}H_{19}BrO_2$ was established from the HREIMS at *m/z* 310.0575 $[M]^+$ (calcd. 310.0568). The ^{1}H NMR spectrum of **1** (see **Table 1**) displayed two singlets assignable to a 1,2,4,5-tetrasubstituted benzene moiety at δ_H 7.25 (s, H-5) and 6.64 (s, H-2) and four methyl singlets at δ_H 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_H 1.55 and 1.75. The ^{13}CNMR and DEPT spectra of **1** showed three quaternary carbon signals at δ_C 52.8 (C-7), 81.0 (C-10) and 100.9 (C-11) besides signals corresponding to the 1,2,4,5-tetrasubstituted benzene moiety, four methyls and two methylenes (see **Table 1**). These spectroscopic data suggested that **1** was a hydroxylated derivative of aplysin, which was firstly isolated from the sea hare *Aplysia kurodai* feeding on the seaweeds of the genus *Laurencia* containing related compounds[6]. 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 suggested that the hydroxy group was at C-10. 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 (see **Table 1**). In the gHMBC spectrum of **1** long range correlations (see **Figure 2**) 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 tetrasubstituted 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 connectionship 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 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 consideration the absolute configuration at C-7 was proposed to be identical to that of (-)-aplysin of which the absolute configuration has been determined[7,8]. Therefore, the structure of **1** was determined as (3S,3aR,8bS)-(-)-2,3,3a,8b-tetrahydro-7-bromo-3-hydroxy-3,3a,6,8b-tetramethyl-1H-cyclopenta[b]benzofuran, named as 10-hydroxyepiaplysin.

Table 1 ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) data of compound **1** and compound **2** (CD_3COCD_3, TMS, δ ppm)

No.	**1**		**2**		**3**	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1		158.2 s		158.5 s		158.6 s
2	6.64 s	112.1 d	6.61 s	111.9 d	6.48 (s)	110.3 d
3		137.6 s		137.5 s		138.5 s
4		114.9 s		114.6 s	6.65d, (7.5)	122.1 d
5	7.25 s	127.2 d	7.24 s	127.3 d	6.96 d, (7.5)	123.3 d
6		138.3 s		138.1 s		135.0 s
7		52.8 s		54.7 s		52.5 s
8α	1.63 m	40.4 t	1.68 dd (12.0, 7.0)	41.6 t	1.61 m	40.4 t
8β	1.72 m		2.07 ddd (12.0, 12.0, 7.0)		1.69 m	
9α	1.56 m	38.0 t	1.50 ddd (12.0, 12.0, 7.0)	37.7 t	1.56 m	38.2 t
9β	1.67 m		1.61 dd (12.0, 7.0)		1.63 m	
10		81.0 s		82.3 s		81.2 s
11		100.9 s		102.5 s		99.9 s
12	1.21 s	15.9 q	1.25 s	15.2 q	1.19 s	16.0 q
13	2.27 s	23.1 q	2.26 s	23.1 q	2.23 s	21.4 q
14	1.37 s	23.5 q	1.38 s	23.5 q	1.34 s	23.7 q
15	1.16 s	23.0 q	1.33 s	22.3 q	1.16 s	22.9 q
OH	3.40 brs		3.65 s		3.30 s	

Compound **2** was obtained as colorless needles (MeOH), m.p. 48-50 °C, $[\alpha]_D^{20}$−50.1 (*c* 0.81, MeOH). Its IR and EIMS 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 it possessed the planar structure completely identical to that of **1** though the ^{1}H NMR spectrum of **2** (see **Table 1**) was distinctively different from that of **1**, especially the partially overlapped and unresolved H_2-8 and H_2-9 in the ^{1}H NMR spectrum of **1** were clearly separated 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 $\Delta\delta_H$ 0.35, 0.04 and 0.17 ppm, 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 showing that C-12 and C-15 of **2** upfield shifted by $\Delta\delta_C$ 0.7 ppm, respectively. 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 (3R, 3αR, 8βS)-(-)-2,3,3α,8β-tetrahydro-7-bromo-3-hydroxy-3, 3α, 6,8β-tetramethyl-1*H*-cyclopenta[b]benzofuran, designated as 10-hydroxyaplysin.

Compound **3** was obtained as an oil, $[\alpha]_D^{20}$–19.7 (*c* 0.10, MeOH). The IR spectrum of **3** showed absorption bands for hydroxy group (3566 cm^{-1}) and aromatic ring (1620, 1593 and 1500 cm^{-1}). The EI mass spectrum gave a molecular ion peak at *m/z* 232 $[M]^+$, and in combination with the NMR data (see **Table 1**) its molecular formula was determined as $C_{15}H_{20}O_2$ that was less a bromine atom than **1** or **3**. The ^1H NMR spectrum of **3** was very similar to that of **1** except for that the signals attributed to the 1,2,4,5-tetrasubstituted aromatic moiety of **1** were replaced by signals attributed to a 1,2,4-trisubstituted aromatic moiety at δ 6.96 (d, 1H, *J*=7.5 Hz, H-5), 6.65 (d, 1H, *J*=7.5 Hz, H-4) and 6.47 (s, 1H, H-2). These data demonstrated that **3** was a debrominated product of **1**, which was supported by the ^{13}C NMR data of **3** (see **Table 1**) and further confirmed by the comprehensive analysis of 2D NMR spectra of **3** including ^1H-^1H gCOSY, gHSQC and gHMBC experiments. Thus, the structure of **3** was determined as (3S,3aR,8bS)-(-)-2,3,3a,8b-tetrahydro-3-hydroxy-3,3a,6,8b-tetramethyl-1*H*-cyclopenta[b]benzofuran, and named as 10-hydroxydebromoepiaplysin.

The identical planar structure to that of **1** and **2** appeared as an intermediate in the total synthesis of racemic (±)-aplysin and (±)-debromoaplysin[9], but without any chemical and physical data. Compound **3** was reported as an intermediate in the enantio-controlled synthesis of (–)-aplysin and (–)-debromoaplysin[10], but it was obtained as a natural product for the first time.

By using standard MTT method the three compounds were tested for cytotoxicity against several human tumor cell lines HCT-8, Bel-7402, BGC-823, A549 and HELA, but found that they all are inactive (IC_{50}>10μg/mL).

Acknowledgments

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