Brominated Metabolites from the Marine Red Alga *Laurencia similis*

Chun-Shun Li, Xiao-Ming Li, Chuan-Ming Cui, and Bin-Gui Wang

Key Laboratory of Experimental Marine Biology, Institute of Oceanology, Chinese Academy of Sciences, Nanhai Road 7, Qingdao 266071, P. R. China

Reprint requests to Prof. Dr. Bin-Gui Wang. Fax: +86-532-82880645.
E-mail: wangbg@ms.qdio.ac.cn

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One new sesquiterpenoid, aristolan-1α-bromo-9β,10β-epoxide (**1**), and one new indole alkaloid, 2,5-dibromo-*N*-methylindole (**2**), along with six sesquiterpenes and indoles were identified from the marine red alga *Laurencia similis*. Their structures were established on the basis of spectroscopic methods.

Key words: Sesquiterpenoid, Indole Alkaloid, *Laurencia similis*

Introduction

The marine red algae of the genus *Laurencia* (Rhodomelaceae, Ceramiales) are known to be a rich source of diverse halogenated secondary metabolites [1]. Previous chemical investigation of *Laurencia similis* collected from Borneo of Malaysia by the Suzuki group revealed the presence of two polybrominated indole alkaloids [2]. Recent studies on the species collected from Hainan island of China resulted in the isolation of several indole alkaloids and sesquiterpenoids [3 – 6]. In the course of our continuing studies on structurally interesting secondary metabolites from the genus *Laurencia* [5 – 11], the species of *L. similis* was re-investigated, and as a result one new sesquiterpenoid, aristolan-1α-bromo-9β,10β-epoxide (**1**), and one new indole alkaloid, 2,5-dibromo-*N*-methylindole (**2**), were identified. In addition, six known compounds including aristol-8(9)-en-1-one (**3**) [5], aristol-9(10)-en-8-one (**4**) [12], 9β-aristol-1(10)-en-9-ol (**5**) [13], 2,3,5-tribromo-1-methyl-1H-indole (**6**) [14], 2,3,5,6-tetrabromo-1H-indole (**7**) [14], and 3,5,6-tribromo-1H-indole (**8**) [5], were also isolated and identified. Herein, we report the isolation and structure elucidation of the new compounds.

Results and Discussion

Compound **1**, a colorless oil, was assigned the molecular formula $C_{15}H_{23}BrO$, as determined by HRESIMS ($[M+H]^+$ at *m/z* = 299.1063, calcd. for $C_{15}H_{24}BrO$, 299.1011), suggesting 4 degrees of unsaturation. The 1:1 ratio of the $[M+H]^+$ ion cluster at *m/z* = 299/301 in the mass spectrum revealed the presence of one Br atom in the molecule. Since all 15 carbons were sp^3 hybridized as indicated by ^{13}C NMR and DEPT spectra (Table 1), the 4 degrees of unsaturation indicated the presence of a tetracyclic ring system in **1**. The methine signal at δ_C = 65.2 and the quaternary carbon signal at δ_C = 66.5 were deduced to be those connected to the oxygen atom, and the methine signal resonating at δ_C = 57.2 was ascribed to be brominated. Detailed analysis of the NMR data indicated that compound **1** should be a sesquiterpene with an aristolane skeleton. Two structural fragments including **a** (C-1–C-4, C-4–C-14) and **b** (C-6–C-9) as drawn with bold lines in Fig. 1, were established by the ^1H-^1H COSY spectrum. The HMBC correlations from H-1 to C-9 and C-10, from H-14 to C-5, and from H-15 to C-4, C-5, and C-6, as well as from the protons of the

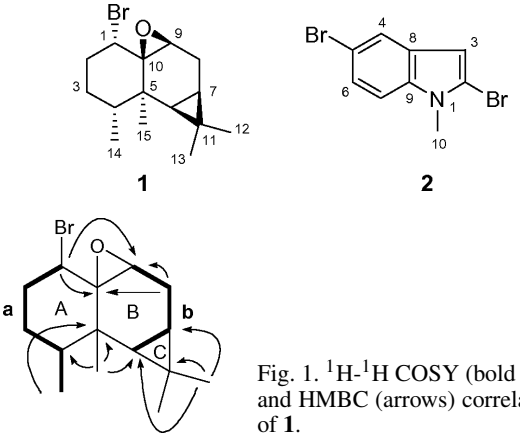

Fig. 1. ^1H-^1H COSY (bold lines) and HMBC (arrows) correlations of **1**.

Table 1. NMR spectral data for compounds **1** and **2**[a].

	— **1** —		— **2** —	
No.	δ_C	δ_H	δ_C	δ_H
1	57.2, d	3.47, d, J = 2.2		
2	25.7, t	2.31, qd, J = 16.4, 9.9, 6.4, 2.2 2.02, dq, J = 16.4, 4.3, 2.7, 1.4	115.0, s	
3	25.7, t	1.63, td, J = 17.2, 12.7, 4.7 1.37, m	103.4, d	6.52, s
4	37.4, d	1.54, m	122.1, d	7.65, d, J = 1.8
5	36.3, s		113.4, s	
6	34.7, d	0.74, d, J = 9.2	124.6, d	7.26, dd, J = 8.7, 1.8
7	18.3, d	0.83, dd, J = 9.2, 4.5	110.8, d	7.13, d, J = 8.7
8	24.2, t	1.05, m 1.33, m	129.4, s	
9	65.2, d	3.96, dd, J = 12.6, 6.5	135.7, s	
10	66.5, s		31.4, q	3.73, s
11	17.8, s			
12	18.7, q	1.02, s		
13	29.3, q	1.09, s		
14	16.0, q	0.87, d, J = 6.9		
15	19.7, q	1.08, s		

[a] Measured in $CDCl_3$ (at 125 MHz for ^{13}C and 500 MHz for ^{1}H). Chemical shift values δ in ppm, coupling constants J in Hz.

geminal methyls H-12 and H-13 to C-6, C-7 and C-11, verified the existence of rings A, B, and C (Fig. 1), which constitute the basic ring system of the aristolane skeleton. Since compound **1** comprised four rings, an epoxide was deduced to be present between C-9 and C-10, which was supported by the observed correlations from H-8 to the oxygenated carbons C-9 (δ = 65.2) and C-10 (δ = 66.5). Thus, a flat structure of **1** was established, as shown in Fig. 1.

The relative stereochemistry of **1** was determined by an analysis of the coupling constants and by NOESY experiments. The NOE correlations of the two methyl signals H-14 and H-15 with H-6 indicated that H-6 and the two methyls (C-14 and C-15) are on the same face of the ring system. The coupling constant between H-6 and H-7 (J = 9.2 Hz) implied the *cis*-orientation of H-6 and H-7 [15, 16], which was also verified by the NOESY experiment. The small coupling constant for H-1 (J = 2.2 Hz) clearly established the Br functionality at C-1 to be in the α position. In addition, the relative configuration of the epoxide was determined as β-oriented by the coupling constant of H-9 (J = 12.6, 6.5 Hz) with H_2-8. Based on the above evidences, the structure of **1** was established as aristolan-1α-bromo-9β, 10β-epoxide. Previous investigations have revealed that all the aristolane-type sesquiterpenes isolated from the marine red alga species *L. similis* were not halogenated [5]. Our first finding of the brominated aristolane-type sesquiterpene from this species enriches the chemical diversity of the algal genus *Laurencia*.

Compound **2** was obtained as a colorless powder, and its molecular formula was determined to be $C_9H_7Br_2N$ by HRESIMS (m/z = 287.8976, $[M+H]^+$, calcd. for $C_9H_8Br_2N$, 287.9023). The dominant $[M+H]^+$ ion cluster at m/z = 287/289/291 in a 1:2:1 ratio in the mass spectrum suggested the existence of two bromine substituents. Detailed analysis of the 1D NMR data revealed that **2** should be a dibromo-substituted indole alkaloid. The ^1H NMR (Table 1) spectrum showed four aromatic signals at δ_H = 7.65 (d, J = 1.8 Hz, H-4), 7.26 (dd, J = 8.7, 1.8 Hz, H-6), 7.13 (d, J = 8.7 Hz, H-7), and 6.52 (s, H-3). The ^{13}C NMR and DEPT charts exhibited the presence of nine carbons, including one methyl (δ = 31.4, MeN), four methines, and four quaternary carbons. The positions of the bromine substituents were established by the exhaustive analysis of the mode of proton splitting as well as by 2D NMR experiments. The ^1H-^1H COSY plot exhibited one structural fragment comprising C-6–C-7, which was also verified by the coupling constant (J = 8.7 Hz) among them. In the HMBC spectrum, correlations from the signals of the methyl group at δ_H = 3.73 to two quaternary carbons at δ_C = 115.0 (C-2) and 135.7 (C-9) suggested that C-2 was brominated. The observed cross peaks from H-4, H-6, and H-7 to C-5 (δ = 113.4) determined the position of the other Br atom. The structure of compound **2** was thus established as 2,5-dibromo-1-methylindole.

Thus far, 29 polybromoindoles have been found only in three species of the genus *Laurencia* including *L. similis* [2, 3, 5], *L. decumbens* [11], and *L. brongniartii* [14, 17–20]. Most of these indole alkaloids usually have at least three bromine substituention in the molecules. The present paper is the second report on dibromoindole alkaloids from *L. similis* [3].

Experimental Section

General experimental procedures

Optical rotation was measured on a Jasco P-1020 digital polarimeter. The IR spectra were obtained on a Nicolet Nexue 470 infrared spectrophotometer with KBr pellets. The UV spectrum was recorded on a PuXi TU-1810 UV spectrophotometer. The ESI-MS and high resolution mass spectra were measured by a Finnigan MAT 90 instrument and a VG Auto Spec-3000 spectrometer, respectively. NMR spec-

tra were recorded on a Bruker Avance 500 spectrometer. Column chromatography (CC) was carried out on commercial silica gel (Qingdao Haiyang Chemical Group Co.; 200 – 300 mesh) and Sephadex LH-20 (Sigma).

Algal material

The marine red alga *Laurencia similis* was collected offcoast of Hainan Islands, P. R. China, in May 2007, and was identified by Dr. L. P. Ding at the Institute of Oceanology, Chinese Academy of Sciences (IOCAS). A voucher specimen (HZ0705L) was deposited in the Key Laboratory of Experimental Marine Biology of IOCAS.

Extraction and isolation

The dried and powdered alga material (0.73 kg) was extracted with $CHCl_3$/MeOH (1:1). The concentrated extracts were partitioned between H_2O and EtOAc. The EtOAc-soluble fraction was subjected to a silica gel CC (petroleum ether/EtOAc, 1:0 → 0:1) to get six fractions (Frs. I – VI). Fr. II was further purified by CC (silica gel and Sephadex LH-20) and by preparative TLC to obtain compounds **1** (9 mg) and **2** (21 mg).

Identification

*Aristolan-1α-bromo-9β,10β-epoxide (**1**):* Colorless oil. – $[\alpha]_D^{25} = -20.78$ (c = 0.17, $CHCl_3$). – IR: ν = 2928, 1450, 1378, 1267, 1030, 958, 842 cm^{-1}. – ^1H and ^{13}C NMR data: see Table 1. – HRESIMS: *m/z* = 299.1063 (calcd. 299.1011 for $C_{15}H_{24}{}^{79}BrO$, $[M+H]^+$).

*2,5-Dibromo-N-methylindole (**2**):* Colorless powder. – IR: ν = 3676, 1604, 1573, 1493, 1461, 1436, 1321, 1265, 971, 903, 874 cm^{-1}. – UV ($CHCl_3$): λ_{max} (log ε_{max}) = 226 nm (4.38). – ^1H and ^{13}C NMR data: see Table 1. – HRESIMS: *m/z* = 287.8976 (calcd. 287.9023 for $C_9H_8{}^{79}Br_2N$, $[M+H]^+$).

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