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Short communication

Five new indole derivatives from the cyanobacterium *Moorea producens*

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ABSTRACT

Chemical analysis of the hydrophilic fraction from marine cyanobacterium *Moorea producens* extracts led to the isolation of five new indole derivatives (**1**-**5**). So far, 2-formyl-4,5,6,7-tetrahydroindole has been reported only for **6** from the nature, consequently compounds **1**-**5** were the second representatives of this class. Cytotoxicity, diatom growth inhibition, and antibacterial activity tests for compounds **1**-**5** showed no bioactivity at the concentration tested.

1. Introduction

The marine cyanobacterium *Lyngbya majuscula* (recently reclassified as *Moorea* spp.) produces many structurally diverse secondary metabolites with potent bioactive properties (Liu and Rein, 2010; Choi et al., 2012; Swain et al., 2015). Some of the compounds produced by *L. majuscula* are recognized as potential pharmaceutical leads (Singh et al., 2011; Tan 2013). On the other hand, *L. majuscula* can also be a nuisance from the human health perspective. Accidental contact with *L. majuscula* at the beach causes a form of contact dermatitis known as "swimmer's itch" (Moore, 1981; Werner et al., 2012). The causative agents of swimmer's itch were previously reported as aplysiatoxins and lyngbyatoxins produced by *L. majuscula* (Mynderse et al., 1977; Cardellina et al., 1979). Furthermore, food contaminated with *L. majuscula* was tainted with aplysiatoxins, which led to food poisoning (Nagai et al., 1996). In these studies, most of the isolated compounds were lipophilic ones. Many biologically important compounds, such as dysiherbaine (Sakai et al., 1997), domoic acid (Wri ght et al., 1989), palytoxin (Uemura et al., 1981), maitotoxin, tetrodotoxins, and saxitoxins (Yasumoto and Murata, 1993) have been found in the hydrophilic fraction from marine organisms. However, the hydrophilic fraction from *L. majuscula* has hardly been studied thus far. Therefore, we initiated a chemical study of the hydrophilic fraction of the cyanobacterium *Moorea producens* (formerly *Lyngbya majuscula*). In this study, five novel indole-based compounds (**1**-**5**, see Fig. 1) were isolated from the Okinawan collection of *M. producens*. The isolation, structure elucidation, and bioactivity of compounds **1**-**5** will be discussed in this paper.

2. Results and discussion

Compounds **1**-**5** were isolated as light brown powders. The tabulated Nuclear Magnetic Resonance (NMR) spectral data of the five isolated compounds are shown in Tables 1 and 2. The HR-ESI–MS analysis of compound **1** revealed a sodium adduct ion peak at *m/z* 232.0944 $[M + Na]^+$ (calcd. for $C_{11}H_{15}NO_3Na$, 232.0950). The molecular formula was determined to be $C_{11}H_{15}NO_3$, indicating five degrees of unsaturation. The ^1H and ^{13}C NMR spectral data were assigned by analyzing with ^1H–^1H COSY, ^1H–^{13}C HSQC, ^1H–^{13}C HMBC, and ^1H–^1H NOESY spectra. In the 1D NMR spectra, the alkene carbon chemical shifts of δ_C 136.1 (C-2), δ_C 121.2 (C-3), δ_C 117.1 (C-3a), and δ_C 137.6 (C-7a) indicated the existence of a pyrrole ring. The peaks at δ_C 188.3 (C-9) together with δ_H 9.50 (H-9) indicated the presence of an aldehyde functional group. The ^1H–^1H COSY spectrum revealed the carbon sequences of C-4 (δ_C 61.7), C-5 (δ_C 33.8), C-6 (δ_C 66.0), C-7 (δ_C 31.4), and C-10 (δ_C 11.3). ^1H–^{13}C HMBC correlations were observed from H-5α (δ_H 2.11) to C-3a (δ_C 117.1), C-4 (δ_C 61.7), C-6 (δ_C 66.0), and C-7 (δ_C 31.4) and from H-10 (δ_H 1.08) to C-6 (δ_C 66.0), C-7 (δ_C 31.4), and C-7a (δ_C 137.6); these suggested the existence of a 6-membered cyclic ring and determined its connection to the pyrrole ring. HMBC correlations from H-8 (3.64) to C-2 (δ_C 136.1), and from H-9 (9.50, s) to C-2 (δ_C 136.1), C-3 (δ_C 121.2), and C-3a (δ_C 117.1) were also observed. These results indicated that compound **1** was planar in structure. Moreover, the NOESY spectra showed key NOE correlations between H-5β and H-6 and between H-6 and H-7. However, the NOE correlation between H-4 and H-6 was not detected. These observations suggested that compound **1** had the relative stereochemistry of 4*R**, 6*R**, 7*S**.

HR-ESI–MS analysis of compound **2** showed an $[M + Na]^+$ ion peak

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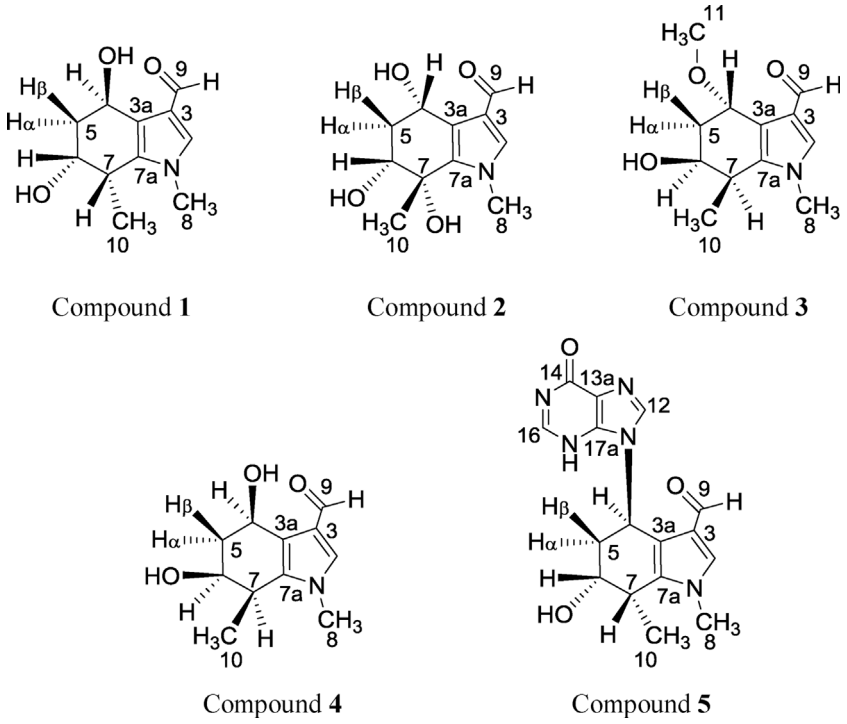

Fig. 1. Chemical structures of five new compounds isolated from *Moorea producens* in this study.

at *m/z* 248.0923 (calcd. for $C_{11}H_{15}NO_4Na$, 248.0899) indicating a molecular formula of $C_{11}H_{15}NO_4$ with five degrees of unsaturation. The ^1H and ^{13}C NMR spectra of compound **2** were almost the same as those of compound **1**, indicating that compound **2** should have a structure similar to compound **1**. There were some differences between the two structures. Instead of the tertiary carbon in compound **1,** the carbon signal of a quaternary carbon atom C-7 (δ_C 69.7) was observed. One oxygen atom should be attached to C-7. A careful analysis of the COSY, HSQC, and HMBC spectra led us to conclude that compound **2** was also planar in structure. In addition, the key NOE correlations were observed between H-4 and H-6 and between H-6 and H-10, revealing that the relative stereochemistry of compound **2** is 4*S**, 6*R**, 7*S**.

HR-ESI–MS of compound **3** showed a molecular ion peak at *m/z* 246.1113 $[M + Na]^+$ (calcd. for $C_{12}H_{17}NO_3Na$, 246.1106), consistent with a molecular formula of $C_{12}H_{17}NO_3$, indicating one more methyl or methylene moiety compared to compound **1**. The planar structure was determined by 1D and 2D NMR spectral analyses. Using 1D NMR spectra, the additional methyl moiety was confirmed by the carbon chemical shift of δ_C 55.8 (C-11) and proton chemical shift of δ_H 3.30 (H-11). Moreover, the ^1H–^{13}C HMBC correlations observed from H-4 (δ_H 4.52) to C-11 (δ_C 55.8) and from H-11 (δ_H 3.30) to C-4 (δ_C 71.3), revealed that the methoxy group was connected to C-4 of the 6-membered cyclic ring. Since ^1H–^1H NOESY correlations were observed between H-4 and H-5β, between H-6 and H-7, between H-5β and H-10, and between H-5α and H-11, the relative stereochemistry of **3** was deduced to be 4*S**, 6*S**, 7*R**.

Compound **4** was determined to have a molecular formula of $C_{11}H_{15}NO_3$, as per the HR-ESI–MS $[M + Na]^+$ ion peak at *m/z* 232.0991 (calcd. for $C_{11}H_{15}NO_3Na$, 232.0950), which is the same as that for compound **1**. Moreover, 1D and 2D NMR spectral analyses showed that the planar structure of compound **4** was identical to that of compound **1**. However, compound **4** showed levorotatory optical rotation ($[\alpha]_D^{14}$ −1.2 (*c* 0.010, H_2O)), while compound **1** showed dextrorotatory optical rotation ($[\alpha]_D^{14}$ +14.5 (*c* 0.020, H_2O)), indicating that the compounds are diastereomers. Analysis of the ^1H–^1H NOESY spectrum revealed correlations between H-4 and H-5α, between H-6 and H-7, and between H-5β and H-10. These results indicated that the relative stereochemistry of compound **4** was 4*R**, 6*S**, 7*R**.

HR-ESI–MS analysis of compound **5** showed an $[M + Na]^+$ ion peak at *m/z* 350.1248 (calcd. for $C_{16}H_{17}N_5O_3Na$, 350.1229) consistent with a molecular formula of $C_{16}H_{17}N_5O_3$, possessing eleven degrees of unsaturation. The 1D and 2D NMR data suggested that the partial structure was the same as compound **1**. The presence of a purine ring was revealed due to one C_5N_4 moiety when compared to compound **1** ($C_{11}H_{15}NO_3$), as well as the characteristic carbon chemical shifts of δ_C 140.9 (C-12), δ_C 123.4 (C-13a), δ_C 158.9 (C-14), δ_C 145.6 (C-16), and δ_C 148.3 (C-17a). Furthermore, the ^1H–^{13}C HMBC correlations observed from H-12 (δ_H 7.41) to C-13a and C-17a, and from H-16 (δ_H 8.11) to C-14 and C-17a were used to confirm the presence of a purine ring. The correlation observed from H-4 (δ_H 5.89) to C-12 (δ_C 140.9) and C-17a (δ_C 148.3) in the HMBC spectrum contributed to the structural elucidation of compound **5**, as shown in Fig. 1. Observations of NOE correlations between H-5α and H-10, between H-5β and H-6, between H-6 and H-7, and between H-6 and H-12 in the NOESY spectrum revealed that the relative stereochemistry was 4*R**, 6*R**, 7*S**.

The ^1H–^1H NOESY correlations between H-2 and H-9 supported the s-*trans* configuration on 5-membered ring of compounds **1**-**5**.

Two indole derivatives **6** and **7** isolated from cyanobacterium *Moorea producens* (formerly *Lyngbya majuscula*) have previously been reported (Todd and Gerwick, 1995; Nogle and Gerwick, 2003). Compound **6** was obtained from a Puerto Rican collection of *L. majuscula* (Nogle and Gerwick, 2003). Compound **6** was purified on ODS HPLC with 75% methanol elution (Nogle and Gerwick, 2003). Therefore, **6** was much non-polar than compounds **1**–**5**. Prior to this study, compound **6** was the only reported 2-formyl-4,5,6,7-tetrahydroindole. Thus, compounds **1**-**5** were only the second batch of representatives reported for this class of compounds. Compound **7** was previously isolated from an Okinawan collection of *L. majuscula* (Todd and Gerwick, 1995). Furthermore, **7** has been reported as a widespread minor metabolite of *L. majuscula* (Todd and Gerwick, 1995). In this study, compound **7** was also isolated and identified from a fraction that is less polar than compounds **1**-**5**. Compound **7** might be produced from 2-formyl-4,5,6,7-tetrahydroindoles such as **1**, **2**, and **4** through the biosynthetic pathway of *M. producens*. Otherwise, compounds **1** to **5** might be derivatized from compound **7**, which is derived from tryptophan (Fig. 2).