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Two polyketides: Biosynthetic transitional of Aplysiatoxin from a marine cyanobacterium *Moorea producens*

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Abstract
Aplysiatoxin and its derivatives have been isolated from marine cyanobacteria. The biosynthesis of their characteristic bicyclic skeletons from a linear polyketide intermediate has been proposed. Two polyketides, aplysiadione (1) and aplysiaenal (2), were isolated from the marine cyanobacterium *Moorea producens* (formerly *Lyngbya majuscula*) collected in the Sambalpur coastal area. The polyketide chemical structures were elucidated from HR ESI-MS and NMR spectra. Aplysiadione (1) corresponds to a decarboxylated analog of the proposed biosynthetic intermediate of Aplysiatoxin. Aplysiaenal (2) is a truncated analog of the linear intermediate. The isolation of aplysiadione (1) strongly supports the proposed ring formation mechanisms for the Aplysiatoxin, and this compound is a rare example of a biosynthetic intermediate of polyketide secondary metabolites from marine cyanobacteria.

Keywords: Aplysiatoxin, biosynthetic intermediates, polyketides, cyanobacteria

Introduction
Cyanobacteria are well known to produce a number of bioactive compounds. Aplysiatoxin (Fig. 1) and its derivatives are produced by marine cyanobacteria [1-8] and cause severe contact dermatitis [9] and food poisoning via the ingestion of red algae [10]. Aplysiatoxin have been shown to act as protein kinase C activators and potent tumor promoters [11-14]. In addition to these activities, Aplysiatoxin show potassium channel inhibition activity [4-8]. In 2010, a bloom of the cyanobacterium *Moorea producens* occurred in the Sambalpur Prefecture. We have studied the isolation of aplysiatoxin and its derivatives from *M. producens* and have elucidated the structures of new aplysiatoxin derivatives [15-18].
The structures of aplysiatoxin and its derivatives are characterized by three types of bicyclic structure containing a six-membered ether ring [15]. These different ring systems are proposed to be biosynthesized from a common polyketide intermediate (Fig. 1). The biosynthetic intermediates have not been obtained, but some probable biosynthetically related compounds, the nhatrangins, have been isolated from *Lyngbya majuscula* [3]. The nhatrangins consist of a C_7 polyketide chain and a phenol, which have the same structure as the C-10 to C-21 portion of aplysiatoxin. Recently, we isolated two monocyclic aplysiatoxin derivatives, oscillatoxin I and its debrominated analog, which possess the cyclohexenone from the C-2 to C-7 portion but do not have an ether ring [16, 18]. The structure of oscillatoxin I enabled us to explain the biosynthesis of the cyclic structures in Aplysiatoxin from a linear polyketide intermediate.
Our continued efforts have led to the isolation of two polyketides, aplysiadione (1) and aplysiaenal (2), from *M. producens* (Fig. 1). In this communication, we report the isolation and structural elucidation of the aplysiatoxin-related compounds aplysiadione (1) and aplysiaenal (2), which correspond to linear biosynthetic intermediates of the Aplysiatoxin.

Results and discussion
The cyanobacterium *M. producens* was collected at Sambalpur, India, in 2010. A frozen sample of *M. producens* was extracted with EtOH once, MeOH five times, and then acetone. The combined extracts were partitioned between 80% MeOH and hexane, and then EtOAc and water. The EtOAc layer was purified with a reversed-phase column. Final purification was performed with an HPLC method.

Aplysiadione (1) was isolated as a colorless solid ($[\alpha]_D$ [18] − 5.0 (c 0.01, MeOH)). The UV maxima observed at 224 nm (ε = 6780), 275 nm (ε = 2050), and 313 nm (ε = 530) indicated the presence of an aromatic group.

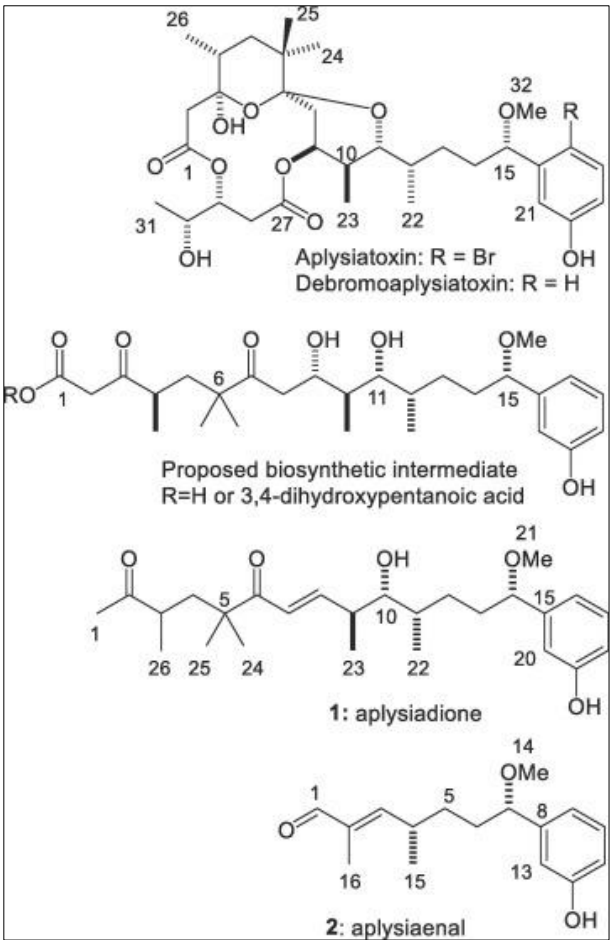

Fig 1: Structures of aplysiatoxin, debromoaplysiatoxin, proposed biosynthetic intermediate, aplysiadione (1), and aplysiaenal (2).

HR ESI-MS analysis of 1 showed an $[M-H]^-$ ion peak at *m/z* 431.2847 (calcd for $C_{26}H_{39}O_5$: 431.2792), indicating a molecular formula of $C_{26}H_{40}O_5$ with seven degrees of unsaturation. The ^{13}C NMR and HSQC spectra of **1** showed seven methyl groups (three singlets, three doublets, and a methoxy), three methylenes, three methines bonded to methyl groups, two oxygenated methines, two olefinic methines, four aromatic protons, and five quaternary (one aliphatic, two aromatic in the phenol, and two ketone) carbon atoms (Table 1).

The unsaturation in 1 was explained by a disubstituted aromatic ring, a double bond, and two ketones. The MS, UV, and NMR spectra suggested that 1 contained a conjugated ketone and two hydroxy groups with one of them being a phenolic chromophore. The carbon signal at δ_C = 211.6 and an HMBC correlation from CH_3-1 (δ_H = 2.08) to a ketone suggested that 1 had an acetyl structure. The proton chemical shift of CH_3-1 measured in acetone-d_6 was very close to that of acetone. The presence of an acetyl methyl group was confirmed by NMR experiments measured in methanol-d_4. Connectivity between the H-3 (CH_3-26) and H_2-4 protons was assigned from the ^1H–^1H COSY spectrum (Fig. 2). HMBC correlations were observed from H_3-26 to C-2, from H-4b to a ketone carbon atom (C-6) at δ_C = 203.2, and from H_3-24 and H_3-25 to C-4 and C-6. A partial structure from H-7 to H-14 was also assigned from analysis of the ^1H–^1H COSY and TOCSY spectra. The hydroxy proton observed at δ_H = 3.56 was coupled to H-10 in the ^1H–^1H COSY spectrum, apparently indicating that a hydroxy group was situated on C-10. The methoxy (CH_3O-21) protons at δ_H = 3.15 were confirmed to bond to C-14 by the HMBC experiment. The proton chemical shifts for H-7 and H-8 were observed downfield at δ_H = 6.58 and δ_H = 6.92, respectively, suggesting that these protons were involved in a conjugated system.

Table 1: NMR data for aplysiadione (**1**) (600 MHz for ^1H, 150 MHz for ^{13}C; δ_H = 2.05 and δC = 206.26 for acetone-d_6).

No.	δ_H, multiplicity (*J* in Hz)	δ_C	HMBC
1	2.08, s	28.5, CH_3	2, 3
2	–	211.6, C	
3	2.48, m	44.0, CH	
4a	1.48, dd (4.1, 14.4)	42.2, CH_2	
4b	2.14, dd (7.1, 14.4)		2, 3, 5, 6
5	–	46.8, C	
6	–	203.2, C	
7	6.58, d (15.3)	125.3, CH	6, 9
8	6.92, dd (8.7, 15.3)	150.7, CH	6, 23
9	2.51, m	41.1, CH	7, 8, 10, 23
10	3.27, m	77.9, CH	
11	1.51, m	36.7, CH	
12	1.40, m	30.6, CH_2	11, 13, 21
13a	1.58, m	36.7, CH_2	12, 14
13b	1.73, m		
14	4.03, dd (5.3, 7.6)	84.8, CH	13, 16, 20, 21
15	–	145.6, C	
16	6.76, d (7.7)	118.7, CH	14, 18, 20
17	7.16, dd (7.8, 7.8)	130.1, CH	15, 19
18	6.74, d (8.0)	115.1, CH	16, 20
19	–	158.4, C	
20	6.79, s	114.2, CH	14, 16, 18
21	3.15, s	56.7, CH_3	14
22	0.88, d (6.7)	14.3, CH_3	10, 11, 12
23	1.03, d (6.8)	17.5, CH_3	8, 9, 10
24	1.04, s	24.3, CH_3	4, 5, 6, 25
25	1.09, s	25.3, CH_3	4, 5, 6, 24
26	0.99, d (7.1)	19.3, CH_3	2, 3, 4
10-OH	3.56, m		
19-OH	8.33, s		

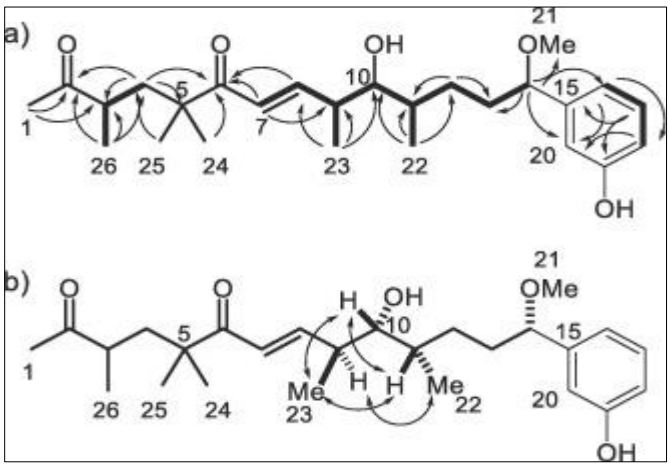

Fig 2: NMR interpretation of aplysiadione: a) bold lines: COSY correlations; arrows: HMBC correlations; b) NOE correlations and deduced configuration.

The HMBC correlation from H-7 to the ketone carbon (C-6) atom confirmed the presence of the conjugated ketone in the molecule. The structure of a *meta*-substituted phenol group

was determined by proton chemical shifts and proton couplings, with a double doublet signal for H-17 and a singlet signal for H-20, respectively, and a COSY correlation from H-16 to H-18. The HMBC correlations from H-14 to C-16, C-20, and C-21 confirmed the position of the methoxy group and the phenol substituent. Thus, the planar structure of aplysiadione (1) was elucidated (Table 2).

The large proton coupling constant (J = 15.3 Hz) indicated an *E* configuration of Δ^7. The limited amount of compound 1 prevented the measurement of $^{2,3}J_{C\text{-}H}$ for application of the *J*-based conformation analysis method to elucidate the stereo structure of compound 1. The proton chemical shifts of H-9 (δ_H = 2.51), H-10 (δ_H = 3.27), and H-11 (δ_H = 1.51) in **1** are similar to those of the corresponding protons H-10 (δ_H = 2.49), H-11 (δ_H = 3.25), and H-12 (δ_H = 1.61) in oscillatoxin I. The H_3-23/H-10 and H-10/H-11 NOE correlations indicated the stereo structure from C-9 to C-11. The proton chemical shift and coupling constants (J = 5.3, 7.6 Hz) of H-14 were identical with those of the debromo Aplysiatoxin [15]. Therefore, aplysiadione 1 was deduced to have the same stereo structure as the Aplysiatoxin, except at C-3. These observations allowed us to elucidate the structure of compound 1 as shown in Fig. 1.

Table 2: NMR data for aplysiaenal (2) (800 MHz for ^{1}H, 200 MHz for ^{13}C; δ_H = 3.31 and δ_C = 49.0 for methanol-d_4).

No.	δ_H, multiplicity (J in Hz)	δ_C	HMBC
1	9.33, s	197.4, CHO	2, 16
2	–	139.0, C	
3	6.32, dd (1.4, 10.0)	162.0, CH	
4	2.72, m	34.7, CH	1, 15
5	1.30, m	30.1, CH_2	
6a	1.57, m	36.8, CH_2	
6b	1.74, m		
7	4.04, dd (5.8, 7.1)	85.2, CH	6, 9, 13, 14
8	–	144.7, C	
9	6.72, d (7.5)	119.1, CH	7, 11, 13
10	7.15, dd (7.5, 7.5)	130.5, CH	8, 12
11	6.69, d (7.6)	115.6, CH	9, 13
12	–	158.8, C	
13	6.70, s	114.3, CH	7, 9, 12
14	3.17	56.8, CH_3	
15	1.04, d (6.7)	20.1, CH_3	3, 4
16	1.70, s	9.4, CH_3	1, 2, 3

Aplysiaenal (2) was isolated as a white solid ($[\alpha]_D^{18}$ − 7.14 (c 0.007, MeOH), UV maxima at 220 nm (ε = 5680) and 270 nm (ε = 1680)). The molecular formula of $C_{16}H_{22}O_3$ was elucidated by HR ESI-MS ($[C_{16}H_{21}O_3]^-$ at *m/z* 261.1522) and showed six degrees of unsaturation. Proton signals observed at δ_H = 6.69, 6.70, 6.72, and 7.15 indicated the presence of a mono substituted phenol in the molecule. The observed aldehyde signal at δ_H = 9.33 and an olefinic methine at δ_H = 6.32 are typical for an α, β -unsaturated aldehyde. The proton connectivity from H-3 to H-7 was elucidated by ^{1}H–^{1}H COSY. HMBC correlations from H-1 to C-2, from H_3-16 to C-1 and C-2, and from H-7 to C-9 allowed us to elucidate the planar structure of aplysiaenal. The H-1/H-3 and H_3-16/H-4 NOE correlations and the carbon chemical shift at δ_C = 9.4 indicated an *E* configuration of the double bond. The proton chemical shifts of 2 in $CDCl_3$ agreed well those of a reaction product of aplysiatoxin with KOH in MeOH[1] and the negative optical rotation of 2, which was identical with that of reported synthetic side chains, suggested that 2 had the same stereo structure at C-4 and C-7 as the Aplysiatoxin [19, 20]. Aplysiaenal (2) has the similar structure as a truncated biosynthetic intermediate of aplysiatoxin containing the C_7 polyketide chain with the phenol substituent (Fig. 1). Aplysiaenal is supposed not to be a degradation product of Aplysiatoxin because the isolation of 2 was done under neutral conditions and mild temperature. Aplysiaenal is corresponded to a reduction and dehydrated compound of nhatrangins A and B [3].

Aplysiadione corresponds to a decarboxylated biosynthetic intermediate of the Aplysiatoxin, and aplysiaenal is a shorter analog [15, 16]. Therefore, the isolation of aplysiadione provides evidence for the proposals about the structure of the biosynthetic intermediate and the ring formation of Aplysiatoxin [15]. The biosynthetic genes of curacin A and jamaicamide produced by cyanobacteria have been identified [21]. The biosynthetic gene sequences of a marine alkaloid, saxitoxin, produced by cyanobacteria have been also elucidated [22], and biosynthetic intermediates deduced from the gene sequence were detected by LC-MS methods [23]. The biosynthetic genes of Aplysiatoxin have not yet been unveiled; however, to our knowledge, the isolation of biosynthetic intermediates of polyketide secondary metabolites from marine microalgae is quite rare [24]. The reported results will accelerate the biosynthetic study of secondary metabolites produced by marine cyanobacteria.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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