Corallinafuran and Corallinaether, Novel Toxic Compounds from Crustose Coralline Red Algae

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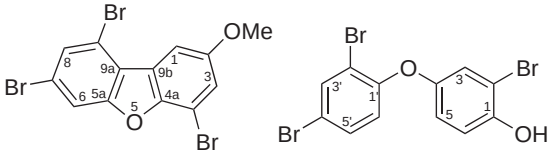
Two new brominated toxic compounds, corallinafuran (**1**) and corallinaether (**2**), have been isolated from crustose coralline red algae (CCA). Their structures were elucidated based on a detailed analysis of spectral data. Compounds **1** and **2** showed toxicity against larvae of the scleractinian coral *Pseudosiderastrea tayamai*, with LD_{99} values of 1.9 and 0.14 μg/mL, respectively.

Crustose coralline red algae (CCA) are plants that deposit a particularly hard and geologically resistant form of calcium carbonate. These algae cement together large quantities of sand, dead coral, and debris to create a stable substrate. CCA play an important role in reef-building processes.[1] In addition, their metabolites exhibit interesting biological activities. For example, the CCA *Lithothamnion*, *Lithophyllum*, and *Hildenbrandia* induced the preferential settlement and metamorphosis of abalone larvae.[2] The aqueous extract of *Lithothamnium californicum* induced larval settlement and metamorphosis in *Haliotis rufescens*.[3] Nonpolar substances of *Lithophyllum* sp. showed growth-inhibitory activity against the dinoflagellate *Heterosigma akashiwo* and zoospore *Laminaria religiosa*.[4] In our continuing search for bioactive compounds from marine organisms,[5] we focused on the relationship between CCA and larvae of invertebrates mediated by their metabolites. Biological phenomena were investigated by a simple bioassay using larvae of the scleractinian coral *Pseudosiderastrea tayamai*.[6] The MeOH extract of CCA showed toxic activity against the coral larvae. Bioassay-guided isolation of the MeOH extract afforded two new brominated compounds, a dibenzofuran and a diphenyl ether. This paper describes the isolation, structural elucidation, and biological activities of these two compounds.

The MeOH extract of CCA (15 kg, wet weight) collected off Yomitan, Okinawa, Japan, was partitioned between water and EtOAc. The EtOAc layer was further partitioned between 90% aqueous MeOH and hexane. The 90% aqueous MeOH layer, which showed toxic activity against the larvae of *P. tayamai*, was subjected to silica gel column chromatography (benzene–MeOH) and reversed-phase HPLC (MeOH–water) to afford corallinafuran (**1**) (2.2 mg) as a white powder and corallinaether (**2**) (2.4 mg) as a colorless oil (Scheme 1).



1 **2**

Scheme 1.

ESIMS of **1** showed the $[M + H]^+$ ion cluster at m/z 432, 434, 436, and 438 in a ratio of 1:3:3:1, indicating the presence of three bromine atoms in the molecule. The molecular formula of **1** was found to be $C_{13}H_7Br_3O_2$ (m/z 435.7986, calcd for $C_{13}H_8{}^{79}Br^{81}Br_2O_2$ $[M + H]^+$ 435.7956) by HR-FABMS.[7] The ^1H and ^{13}C NMR and HMQC spectra of **1** (Table 1) showed the presence of four aromatic methine signals (δ_H 6.81, 7.18, 7.34, and 7.45; δ_C 119.0, 120.2, 127.6, and 131.0, respectively), eight aromatic quaternary carbon signals, three of them oxygenated carbons (δ_C 146.1, 150.6, and 150.8), and one methoxy group (δ_H 4.03; δ_C 61.7). The presence of 12 aromatic carbons, one residual oxygen atom, and 9 degrees of unsaturation was reminiscent of a tetrasubstituted dibenzofuran structure. The substitution pattern of this molecule was elucidated by NOE and 2D NMR experiments. One of the benzene rings contained one methoxy group and two meta-protons (δ_H 6.81 and 7.45, each d, $J = 2.4$ Hz). Both HMBC cross-peaks (H-1/C-2, C-3; H-3/C-1, C-2, C-4, C-4a; OMe/C-2) and NOEs (H-1/OMe; H-3/OMe) suggested that the methoxy group at C-2 was flanked by two aromatic protons at δ_H 6.81 (H-3) and 7.45 (H-1). Consequently, the bromine atom was placed at C-4. The other benzene ring also contained two meta-protons (δ_H 7.18 and 7.34, each d, $J = 2.4$ Hz). They could be placed at either C-6 and C-8, or C-7 and C-9. The observation of HMBC cross-peaks from the proton signal at δ_H 7.18 to the carbon signals at δ_C 150.8 (C-5a) and δ_C 120.0 (C-9a) suggested that the proton should be assigned at C-6 or C-9. In the case of dibenzofuran, the chemical shift of H-9 (δ_H 7.84) was significantly low-field compared to the other proton signals.[8] The characteristic proton signal was not observed in compound **1**. This result suggested that the proton at δ_H 7.18 was located at C-6. Furthermore, if the proton was located at C-9, an NOE correlation could be expected between H-9/H-1. However, the absence of an NOE correlation suggested that C-9 was substituted by a bromine atom. Therefore, the meta-position of the aromatic protons at δ_H 7.18 (H-6) and 7.34 (H-8) was secured at C-6 and C-8, respectively. Thus, the structure of corallinafuran (**1**) was established as 4,7,9-tribromo-2-methoxydibenzofuran.

ESIMS of **2** showed the $[M + H]^+$ ion cluster at m/z 420,

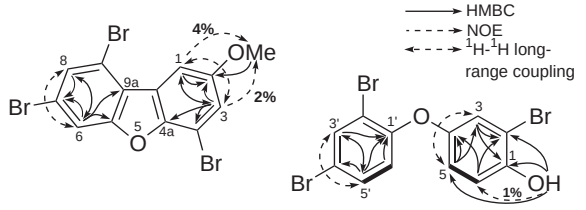


Figure 1. 1H–1H long-range coupling correlations, NOE and HMBC correlations of **1** and **2**.

Table 1. NMR data for **1** and **2** in $CDCl_3$

1				**2**			
Position	^1H /ppm[a]	^{13}C /ppm[b]	HMBC $^1H \rightarrow {}^{13}C$	Position	^1H /ppm[a]	^{13}C /ppm[b]	HMBC $^1H \rightarrow {}^{13}C$
1	7.45 d (2.4)	131.0 d	C-2, 3	1		149.1 s	
2		146.1 s		2		110.2 s	
3	6.81 d (2.4)	119.0 d	C-1, 2, 4, 4a	3	7.12 d (2.4)	122.2 d	C-1, 2
4		117.4 s		4		149.7 s	
4a		150.6 s		5	6.90 dd (9.0, 2.4)	120.0 d	C-3, 4
5a		150.8 s		6	7.01 d (9.0)	116.6 d	C-2, 4
6	7.18 d (2.4)	120.2 d	C-5a, 7, 8, 9a	1′		153.6 s	
7		139.1 s		2′		114.9 s	
8	7.34 d (2.4)	127.6 d	C-6, 7	3′	7.76 d (2.4)	136.1 d	C-1′, 5′
9		117.5 s[c]		4′		116.3 s	
9a		120.0 s		5′	7.35 dd (8.4, 2.4)	131.6 d	C-1′, 3′, 4′
9b		119.2 s[c]		6′	6.75 d (8.4)	120.3 d	C-1′
OCH_3	4.03 s	61.7 q		OH	5.36 s		C-1, 2, 5

[a]Recorded at 600 MHz. Coupling constants (Hz) are in parentheses. [b]Recorded at 150 MHz. Multiplicity was based on the HMQC spectrum. [c]Signals may be interchanged.

422, 424, and 426 in a ratio of 1:3:3:1, indicating the presence of three bromine atoms in the molecule. The molecular formula of **2** was found to be $C_{12}H_7Br_3O_2$ (m/z 421.7952, calcd for $C_{12}H_8{}^{79}Br_2{}^{81}BrO_2$ $[M+H]^+$ 421.7976) by HR-FABMS. The IR spectrum of **2** showed absorption of a hydroxyl group (3520 cm^{-1}).[9] The ^1H and ^{13}C NMR and HMQC spectra of **2** (Table 1) showed the presence of six aromatic methine signals (δ_H 6.75, 6.90, 7.01, 7.12, 7.35, and 7.76; δ_C 120.3, 120.0, 116.6, 122.2, 131.6, and 136.1, respectively), six aromatic quaternary carbons, including three oxygenated carbons (δ_C 149.1, 149.7, and 153.6), and a hydroxyl group (δ_H 5.36). These data together with MS analysis suggested that the molecule might be a trisubstituted diphenylether derivative. Analysis of the 2D NMR spectra revealed the gross structure of **2**. The proton–proton coupling pattern (dd, $J = 8.4$ or 9.0, 2.5 Hz) and HMBC cross-peaks indicated the presence of a pair of 1,2,4-trisubstituted benzenes. The hydroxyl group was assigned at C-1 on the basis of HMBC cross-peaks (OH/C-1, C-2, and C-5). This was further supported by an NOE correlation (OH/H-6). Accordingly, a 2-bromo-4-phenoxyphenol moiety was assigned. Similarly, another part was determined to be a 2,4-dibromophenol. Thus, **2** was established as 2-bromo-4-(2′,4′-dibromophenoxy)phenol.

In conclusion, two new brominated compounds **1** and **2** have been isolated from a CCA. These compounds exhibited toxic activity against *P. tayamai* larvae, with LD_{99} values of 1.9 and 0.14 μg/mL for **1** and **2**, respectively. Various dibenzofuran derivatives are known to be metabolites of terrestrial organisms.[10] However, this is the first example of a dibenzofuran derivative from a marine organism. Antimicrobial, antifungal, and cytotoxic activities of dibenzofuran derivatives and polybrominated dibenzofuran have been reported,[11,12] but their toxicities against larvae of coelenterata are unknown. These compounds from CCA may be used as part of a chemical defense mechanism for coral. This finding provides insight into the relationship between CCA and coral. Further studies on chemical interaction between CCA and coral larvae are in progress.

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6 Bioassay was performed as follows. Samples dissolved in MeOH were applied to glass petri dishes. After the solvent was removed, 10 mL of artificial seawater and 6 planula larvae of the scleractinian coral *P. tayamai* (10–20 days old) were added to each dish, and the dishes were incubated for 24 h at 25–28 °C in the dark. The number of dead larvae was then counted under an optical microscope.
7 Compound **1**: ^1H and ^{13}C NMR: (Table 1); ESIMS: m/z 434 (33), 436 (97), 438 (100), 440 (35); HRFABMS (positive, glycerol matrix): m/z 435.7986 (calcd for $C_{13}H_8{}^{79}Br^{81}Br_2O_2$ $[M+H]^+$ 435.7956).
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9 Compound **2**: IR ν_{max} ($CHCl_3$): 3520, 1490, 1470, 1260, 1220 cm^{-1}; UV λ_{max} (MeOH): 285 nm (ε 2100); ^1H and ^{13}C NMR: (Table 1); ESIMS: m/z 420 (37), 422 (100), 424 (89), 426 (32); HRFABMS (positive, glycerol matrix): m/z 421.7952 (calcd for $C_{12}H_8{}^{79}Br_2{}^{81}BrO_2$ $[M+H]^+$ 421.7976).
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