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Papillamide, a Novel Fatty Acid Amide from the Red Alga *Laurencia papillosa*

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Papillamide (**1**), a novel fatty acid amide that incorporates a cyclopropyl ring, was isolated from the Okinawan red alga *Laurencia papillosa*, which overgrows hermatypic corals. The structure of **1** was determined by spectroscopic analyses.

Red algae of the genus *Laurencia* are widely distributed throughout tropical and temperate zones, and are known to be a rich source of secondary metabolites. The major groups of these metabolites are terpenoides (sesquiterpenes,[1] diterpenes,[2] and triterpenes[3]) and polyketides (fatty acid[4] and C_{15}-acetogenins[5,6]), which frequently include halogen atoms. A number of these metabolites have been reported to possess various biological activities, including antibacterial,[5] antifungal,[7] and insecticidal[6] effects and cytotoxicity against tumor cells.[8]

Laurencia papillosa generally grows in the intertidal zone in tropical and subtropical seas.[9] Limited phytochemical studies of this species have been reported.[10] In our observation, *L. papillosa* overgrew hermatypic corals of the families *Acropridae* and *Poritidae* (Figures 1a and 1b). In our previous studies, biologically active molecules with unique structures were isolated from the marine organisms that overgrew corals, such as terpiodiene[11] and nakiterpiosin.[12] These compounds showed a strong cytotoxicity against mammal tumor cells and coral cells, and thus they may have a predative effect against corals. Based on these findings, *L. papillosa* was also thought to produce unique molecules, which we sought to identify. We report here the isolation and structural determination of the novel fatty acid amide papillamide (**1**) (Figure 1c) from *L. papillosa*.

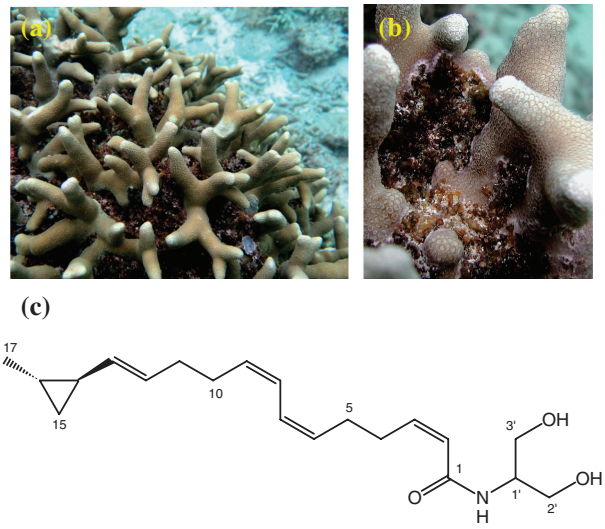


Figure 1. (a, b) Pictures of *L. papillosa* which overgrows corals, (c) the structure of compound **1**.

The red alga *L. papillosa* (15.0 kg, wet weight), collected at Ishigaki Island, Okinawa Prefecture, Japan, was extracted with 80% ethanol (10 L) for 14 days. The extract was filtered, concentrated, and partitioned between EtOAc and H_2O. The EtOAc-soluble material was further partitioned between 90% aqueous MeOH and hexane. The material obtained from the aqueous MeOH portion was subjected to fractionation using silica gel (EtOAc–hexane), ODS gel (60% aqueous MeOH to MeOH) and reversed-phase HPLC (Develosil ODS-MG-5, 45% aqueous acetonitrile) to give **1** as a colorless amorphous powder.[13]

Papillamide (**1**) has a molecular formula of $C_{20}H_{31}NO_3$ as suggested by HRESIMS at m/z 356.2177 $[M + Na]^+$ (calcd for $C_{20}H_{31}NO_3Na$, 356.2205). The ^1H and ^{13}C NMR data for **1** are summarized in Table 1. Further ^1H, ^{13}C, and HMQC NMR analyses in CD_3OD, $CDCl_3$, and CD_3CN revealed the presence of one sp^3-methyl group, four olefins, two equivalent hydroxy

Table 1. NMR data (δ) for **1** in CD_3OD

Position	^{13}C	^1H	Multi (J_H)
1	168.5 s[a,b]		
2	123.6 d	5.84[c]	d (11.0)[d]
3	145.7 d	5.99	m
4	29.6 t	2.70	q (7.4)
5	33.2 t	2.16	q (7.4)
6	132.5 d	5.53	q (7.4)
7	131.9 d	5.99	m
8	132.0 d	5.99	m
9	132.5 d	5.53	q (6.4)
10	34.0 t	2.06	q (6.4)
11	33.6 t	2.01	q (6.4)
12	127.8 d	5.39	dt (6.4, 15.5)
13	135.2 d	4.98	dd (8.2, 15.5)
14	23.5 d	0.98	m
15a	15.3 t	0.35	td (8.2, 3.7)
15b		0.44	td (8.2, 4.6)
16	15.5 d	0.63	m
17	18.8 q	1.01	d (5.5)
1′	62.1 d	3.93	m
2′ 3′	54.1 t	3.60	d (5.2)
NH[e]		6.38	
OH[e]		3.00	

[a]Recorded at 150 MHz. [b]Multiplicity was based on the HMQC spectrum. [c]Recorded at 800 MHz. [d]Coupling constants (Hz) are in parentheses. [e]Recorded in CD_3CN.

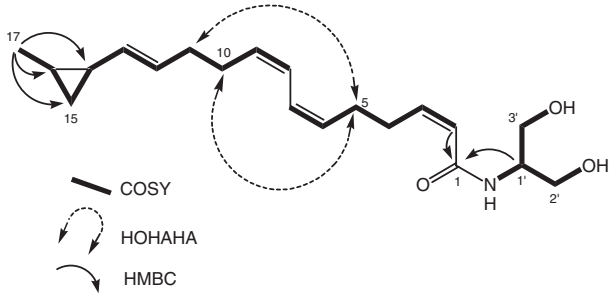


Figure 2. Partial structures of **1**, based on 2D NMR correlations.

groups, one amino group, and one carbonyl group. A detailed analysis of the ^{1}H NMR and COSY spectra of **1** allowed us to elucidate three partial structures; C2–C7, C8–C17, and C1′–C3′ (Figure 2). The HMBC correlations at H17/C14, H17/C15, and H17/C16 indicated the connectivity of the cyclopropyl ring. The HMBC correlations at H2/C1 and H1′/C1 suggested a C1–C1′ amide bond. The proton and/or carbon chemical shifts of consecutive double bonds at H6/C6 and H9/C9, and H7/C7 and H8/C8 highly overlap, and a connection could not be confirmed by COSY and HMBC spectral analyses. Meanwhile, IR (neat) and UV spectra showed absorption bands for a diene moiety (1654 cm^{-1}, 228 nm). In addition, the HOHAHA correlations of H5/H10 and H5/H11 strongly suggested the connectivity of C5–C11.

The geometries of olefins were determined to be one *E*-olefin at C12–C13 and three *Z*-olefins at C2–C3, C6–C7, and C8–C9 based on the coupling constants ($J_{2,3} = 11.0$ Hz, $J_{6,7} = 7.4$ Hz, $J_{8,9} = 6.4$ Hz, $J_{12,13} = 15.5$ Hz) and the NOE correlations of H2/H3, H6/H7, and H8/H9. The relative configuration of the cyclopropyl ring was determined by the NOE correlations (Figure 3). The NOE correlations observed in the NOESY spectrum at H13/H15b, H14/H15a, H15b/H16, and H15a/H17 revealed the *trans* relationship of the cyclopropyl substituent.

Compound **1** was examined with regard to cytotoxicity against P388 mouse leukemia cells and B16 mouse melanoma cells. After incubation for 96 h, **1** did not show definite cytotoxic activity ($IC_{50} > 100\ \mu g\ mL^{-1}$) toward these cell lines. This result shows **1** may have other biological activities that are not related to a toxic or predative effect. Other biological activities are now being investigated.

The biosynthetic mechanism of the cyclopropyl ring might be explained by methylation and cyclization on the unsaturated hexadecanoic acid (Scheme 1).[14] After the double bond of fatty acid had been exposed to *S*-adenosyl methionine (SAM) methylation, a carbocation intermediate might be formed. The cation was then discharged by formation of a cyclopropyl ring to give the precursor of **1**. Another mechanism of cyclopropanation on unsaturated fatty acids was also proposed,[15] and thus, the exact biosynthetic mechanism of **1** remains under discussion.

In conclusion, the novel fatty acid amide papillamide (**1**) was isolated from the Okinawan red alga *Laurencia papillosa*. The structure of **1** was determined by spectroscopic analyses. **1** possesses a unique structure, which may be produced via unusual biosynthetic pathways. **1** did not show significant cytotoxicity against mouse tumor cells. Further studies on other biological activities of **1** are in progress.

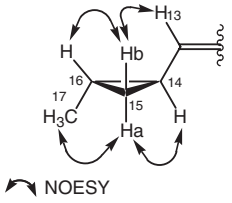
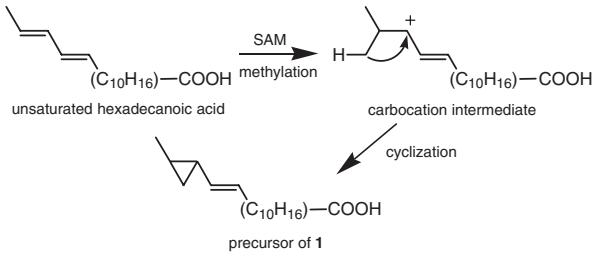


Figure 3. Relative stereochemistry of cyclopropyl ring, based on NOESY correlations.



Scheme 1. Possible biosynthetic mechanism of the cyclopropyl ring.

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