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Dinohydrazides A and B, Novel Hydrazides from a Symbiotic Marine Dinoflagellate

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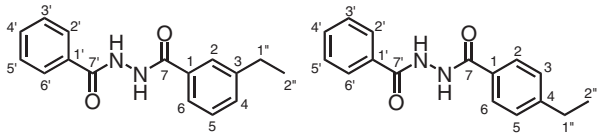
Dinohydrazides A (**1**) and B (**2**), novel naturally-occurring dibenzoylhydrazines, were isolated from a symbiotic marine dinoflagellate of Okinawan sponge. Their structures were determined by spectroscopic analyses and synthetic methods. They moderately inhibited the growth of mammalian cells.

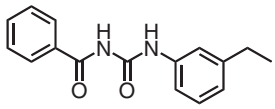
Marine organisms produce various molecules with remarkable physiological activities. It has been suggested that most of those metabolites are biosynthesized by marine microorganisms, i.e., bacteria, blue-green algae, and dinoflagellates that are in the food chain of, or in a symbiotic relationship with, their host animals. Among them, dinoflagellates are widely known to be a rich source of biologically active and structurally unique secondary metabolites.[1] Therefore, dinoflagellates that coexist with marine invertebrates have been isolated and cultured to search for useful compounds. In our recent studies, various biologically active metabolites have been isolated from symbiotic marine dinoflagellates, such as symbioimines,[2] symbiodinolide,[3] symbiospirols,[4] karatungiols,[5] and durinskiols.[6] In our continuing search for biologically active compounds, unique novel dibenzoylhydrazines, named dinohydrazides A (**1**) and B (**2**), were isolated from a symbiotic dinoflagellate of the Okinawan sponge. We describe here their isolation, structure elucidation, and biological activities.

An unidentified dinoflagellate, isolated from the marine sponge *Xeospongia* sp. which was collected at Bise, Okinawa Prefecture, Japan, was cultured for 60 days in 20 L of seawater medium enriched with 2% ES supplement.[7] After cultivation, the cells were harvested by centrifugation and extracted with 80% aqueous ethanol. The concentrated extract was partitioned with ethyl acetate and water, and the aqueous layer was chromatographed on TSK G-3000S polystyrene gel (water to EtOH), ODS gel (50% aqueous MeOH to MeOH) and reversed-phase HPLC (Develosil ODS-HG-5, 70% aqueous MeOH to MeOH), monitoring the growth inhibitory effect against HUVEC cells. Final purification was achieved by HPLC (Develosil RPAQUEOUS, 50% aqueous MeOH to 80% aqueous MeOH) to give dinohydrazides A (**1**) (1.1 mg) and B (**2**) (0.4 mg) as a white powder (Scheme 1).

Dinohydrazide A (**1**)[8] has a molecular formula of $C_{16}H_{16}N_2O_2$, as suggested by HRESIMS at *m*/*z* 291.1129 $[M + Na]^+$ (calcd for $C_{16}H_{16}N_2O_2Na$, 291.1109). The ^1H and ^{13}C NMR data for **1** are summarized in Table 1. Further ^1H, ^{13}C NMR and HMQC spectra in CD_3OD revealed the presence of one sp^3-methyl, one methylene, two amide groups, and two aromatic groups. A detailed analysis of the ^1H NMR and COSY spectra of **1** allowed us to elucidate three partial structures, C4–C6, C2′–C6′, and C1″–C2″. The HMBC correlations at H2/C1, H6/C1, H1″/C2, H1″/C3, H1″/C4, and H2″/C3 revealed the structure of a disubstituted benzene. The HMBC correlations at



dinohydrazide A (**1**) dinohydrazide B (**2**)



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Scheme 1.

H2′/C1′ and H2′/C7′ indicated connectivity between the amide group and one aromatic group. However, the connection around two amide bonds could not be confirmed by 2D NMR analyses.

Dinohydrazide B (**2**)[9] has the same molecular formula ($C_{16}H_{16}N_2O_2$) as **1**, as suggested by HRESIMS at *m*/*z* 291.1117 $[M + Na]^+$ (calcd for $C_{16}H_{16}N_2O_2Na$, 291.1109). The ^1H and ^{13}C NMR data for **2** are summarized in Table 1. On the basis of ^1H and ^{13}C NMR spectra, the structure of **2** was elucidated to be the same as that of **1** except for the ethyl residue. Therefore, we sought to establish the final structures of dinohydrazides by syntheses.

1, **2**, and their derivative **3** were synthesized according to a previous report.[10] Synthesis of compound **1** started with 3-bromobenzoyl chloride, which was stirred in THF at 0 °C, followed by addition of benzoylhydrazine and sodium carbonate in aqueous THF. After the mixture was stirred for 3 h at 0 °C, the resulting white precipitate of 1-benzoyl-2-(3-bromobenzoyl)hydrazine in the reaction mixture was filtered. An ethyl group was then introduced to the 3-bromobenzoyl moiety by the Suzuki–Miyaura cross-coupling. The hydrazide, triethylborane, cesium carbonate, and Pd(dppf)Cl_2 catalyst were stirred in THF under argon atmosphere at reflux for 3 h to give **1** as a white amorphous powder (83% yield in 2 steps). Synthesis of compound **2** was carried out following the same protocol as that of the first step in the preparation of **1**, which utilized 4-ethylbenzoyl chloride as substrate (91% yield). The synthesis of compound **3** began with benzoyl isothiocyanate, which was dissolved in acetonitrile and treated with 3-ethylaniline for 3 h at 40 °C to give a coupled product of thiourea as a white precipitate. The thiocarbonyl unit was then oxidized to a carbonyl by sodium metaperiodate in DMF/water for 15 min to give **3** as a white amorphous powder (31% yield in 2 steps). The NMR spectra of natural dinohydrazide A was not superimposable with that of **3**.[11] Meanwhile, the NMR spectrum of synthetic **1** and **2** were identical to those of natural dinohydrazides A and B. Thus, the structures of dinohydrazides A and B were confirmed to be as proposed.

Table 1. NMR assignments

	Dinohydrazide A (**1**)			Dinohydrazide B (**2**)		
	^{13}C NMR(δ)	^{1}H NMR(δ)	HMBC (H → C)		^{13}C NMR(δ)	^{1}H NMR(δ)
1	133.8 s[a,b]			1	136.2 s	
2	128.5 d	7.85 s[c]	C-1, 4	2, 6	128.8 d	7.94 d (8.6)
3	145.6 s			3, 5	128.7 d	7.39 d (8.6)
4	135.0 d	7.52 d (7.4)[d]	C-2, 6	4	150.2 s	
5	130.0 d	7.45 t (7.4)	C-3, 4			
6	126.4 d	7.81 d (7.4)	C-1, 2			
7	169.7 s			7	169.2 s	
1′	129.3 s			1′	133.2 s	
2′, 6′	130.7 d	8.10 d (7.4)	C-1′, 2′, 3′, 5′, 6′, 7′	2′, 6′	130.5 d	8.09 d (8.4)
3′, 5′	129.6 d	7.51 t (7.4)	C-1′, 2′, 6′	3′, 5′	129.6 d	7.51 t (8.4)
4′	134.5 d	7.62 t (7.4)	C-2′, 3′, 5′, 6′	4′	133.2 d	7.63 t (8.4)
7′	169.5 s			7′	169.2 s	
1″	30.7 t	2.74 q (8.2)	C-2, 3, 4, 2″	1″	29.8 t	2.60 q (8.2)
2″	16.0 q	1.26 t (8.2)	C-3, 1″	2″	15.8 q	1.25 t (8.2)
NH[e]		9.46 br		NH[e]		9.39 br
NH[e]		9.54 br		NH[e]		9.43 br

[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 $CDCl_3$.

Table 2. Growth-inhibitory activity of the isolated compounds toward mammalian cells

Compounds	IC_{50}/μg mL^{-1}		
	HUVEC endothelial cells	HL60 leukemia	B16 melanoma
1	—	22.1	>30
2	44.5	12.8	55.0
3	44.0	2.8	14.5

Next, **1**, **2**, and **3** were examined for their growth-inhibitory activities against human umbilical vein endothelial cells (HUVEC) and mammalian cancer cell lines (HL60 and B16) using the MTT assay. After 72 h of incubation, each compound showed moderate growth-inhibitory activity toward these cells (Table 2). The results showed that **1** and **2** might function as defensive compounds toward the host animals. Other biological activities of **1** and **2** are now being investigated.

In conclusion, dinohydrazides A (**1**) and B (**2**), novel naturally-occurring hydrazides, were isolated from a symbiotic marine dinoflagellate of Okinawan sponge. Their structures were elucidated by spectroscopic analyses, and were confirmed by comparing the spectroscopic data of natural and synthetic compounds. **1** and **2** showed growth-inhibitory activity toward mammalian cells.

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8 Selected spectroscopic data of **1**: UV (MeOH): 204, 231 nm; IR (KBr): 3206, 1631, 1579, 1533, 1288 cm^{-1}.

9 Selected spectroscopic data of **2**: UV (MeOH): 201, 237 nm; IR (KBr): 3209, 1631, 1540, 1286 cm^{-1}.

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11 Selected spectroscopic data of **3**: UV (MeOH): 203, 234, 267 nm; IR (KBr): 3245, 1704, 1671, 1610, 1563 cm^{-1}; ^{1}H NMR (300 MHz, CD_3OD): δ 1.24 (t, $J = 7.5$ Hz, 3H), 2.65 (q, $J = 7.5$ Hz, 2H), 6.98 (d, $J = 8.0$ Hz, 1H), 7.25 (t, $J = 8.0$ Hz, 1H), 7.37 (d, $J = 8.0$ Hz, 1H), 7.42 (s), 7.53 (t, $J = 7.7$ Hz, 2H), 7.63 (t, $J = 7.7$ Hz, 1H), 7.97 (d, $J = 7.7$ Hz, 2H); ^{13}C NMR (75 MHz, CD_3OD): δ 16.1 (q), 29.9 (t), 118.8 (d), 120.9 (d), 124.9 (d), 129.1 (d), 129.8 (d), 129.9 (d), 130.0 (d), 134.2 (s), 141.1 (s), 144.6 (s), 165.4 (s), 165.9 (s); ESIMS m/z 269.1 $[M + H]^+$.

