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Karatungiols A and B, two novel antimicrobial polyol compounds, from the symbiotic marine dinoflagellate *Amphidinium* sp.

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Abstract—Karatungiols A (**1**) and B (**2**), two novel antimicrobial polyol compounds, were isolated from the cultivated symbiotic marine dinoflagellate *Amphidinium* sp. Their structures were elucidated based on spectroscopic analysis and degradation reactions. Karatungiols A (**1**) and B (**2**) consisted of a C69-linear chain with a ketone moiety, 24 or 25 hydroxyl groups, and two tetrahydropyran rings. Karatungiol A (**1**) exhibited antifungal activity against *Aspergillus niger* at 12 μg/disc and antiprotozoan activity against *Trichomonas foetus* at 1 μg/ml.

Marine organisms produce various secondary metabolites that show remarkable biological activities and chemical structures. In particular, huge polyol and polyether compounds, such as palytoxin, halichondrin, and maitotoxin, are some of the most attractive molecules in natural products chemistry.[1] These compounds are composed of a long carbon backbone functionalized by oxygen atoms, and have been called 'super-carbon-chain compounds'.[1a] Also, it has been proposed that these compounds are accumulated in higher organisms through a symbiotic relationship or a food chain, and that these compounds actually originate in microorganisms; for example, bacteria, cyanobacteria, and symbiotic microalgae. Marine dinoflagellates are unicellular phytoplankton and are a rich source of such various bioactive compounds, that is, symbioimines,[2] amphidinols,[3] luteophanols,[4] lingshuiols,[5] colopsinols,[6] and zooxanthellatoxins.[7]

In our continuing search for bioactive metabolites from marine dinoflagellates, we have isolated two novel antimicrobial polyol compounds, karatungiols A (**1**) and B (**2**) (Fig. 1). We describe here the isolation, structure elucidation, and biological activities of **1** and **2**.

The symbiotic dinoflagellate *Amphidinium* sp. was isolated from an unidentified marine acoel flatworm, which was collected at Karatung Island, Indonesia. The dinoflagellate was cultured for 40 days at 25 °C in seawater medium enriched with 2% ES supplement.[8] The cultured cells were harvested by centrifugation and extracted with 80% aqueous EtOH. The concentrated extract was partitioned with EtOAc and H_2O, and the aqueous layer was chromatographed on TSK G-3000S polystyrene gel with aqueous EtOH (0–100%) and on Sephadex LH-20 gel with MeOH. Final purification was achieved by reversed-phase HPLC (Develosil TMS-UG5) with aqueous CH_3CN (20–50%) to give karatungiols A (**1**) (4.3 mg) and B (**2**) (1.5 mg). Karatungiol A (**1**) showed antifungal activity against NBRC4407 *Aspergillus niger* (12 μg/disc) and antiprotozoan activity against *Trichomonas foetus* (1 μg/ml).[9]

Karatungiol A (**1**) was isolated as a pale yellow amorphous solid: $[\alpha]_D^{17}$ +13.6 (*c* 0.10, MeOH); IR (KBr) 3404, 1675 cm^{-1}. The molecular formula of **1** was found to be $C_{73}H_{132}O_{28}$ [$(M+Na)^+$, *m*/*z* 1479.8766, Δ −3.5 mmu] by HR-ESIMS. The ^1H and

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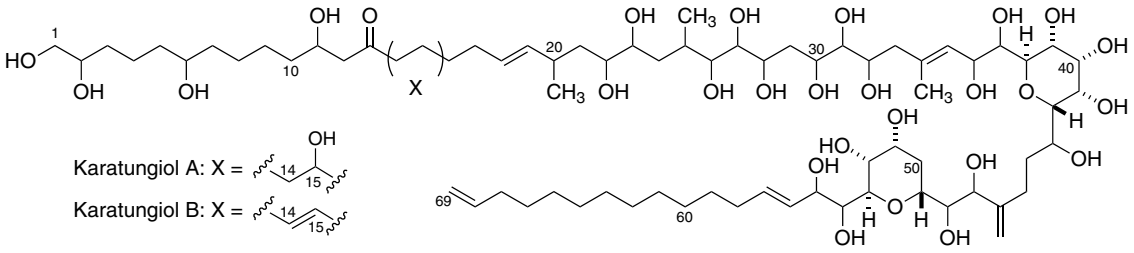

Figure 1. Structures of karatungiols A (**1**) and B (**2**).

Table 1. ^{1}H (800 MHz) and ^{13}C NMR (150 MHz) data for karatungiols A (**1**) and B (**2**)

Atom	**1**			**2**		Atom	**1**			**2**	
	In CD_3OD		In CD_3OD/C_5D_5N (2/1)	In CD_3OD			In CD_3OD		In CD_3OD/C_5D_5N (2/1)	In CD_3OD	
	δ_C	δ_H	δ_H	δ_C	δ_H		δ_C	δ_H	δ_H	δ_C	δ_H
1	67.6	3.41, 3.46	3.54	67.6	3.42, 3.46	37	72.4	3.65	3.88	72.5	3.65
2	73.4	3.56	3.70	73.4	3.56	38	80.1	4.03	4.40	80.1	4.04
3	34.6	1.37, 1.48	1.43	34.6	1.37, 1.46	39	71.0	4.13	4.48	71.0	4.13
4	26.9	1.45	1.37	26.9	1.50	40	68.3	3.79	4.01	68.3	3.79
5	27.0	1.37, 1.48	1.40	27.0	1.37, 1.48	41	70.9	4.05	4.34	70.9	4.05
6	72.5	3.53	3.54	72.4	3.53	42	78.3	3.29	3.51	78.3	3.29
7	30.3–30.6	1.38	1.40	30.3–30.6	1.38	43	72.0	3.87	4.13	72.0	3.87
8	30.3–30.6	1.38	1.48	30.3–30.6	1.38	44	33.8	1.54, 2.20	1.74, 2.40	33.7	1.54, 2.20
9	23.0	1.37, 1.58	1.40, 1.67	23.0	1.37, 1.58	45	27.8	2.14, 2.46	2.32, 2.73	27.8	2.15, 2.45
10	38.4	1.47, 1.58	1.48	38.5	1.37, 1.46	46	151.5			151.5	
11	68.8	4.05	4.17	69.3	4.04	47	76.9	4.20	4.45	76.9	4.19
12	52.0	2.59	2.62, 2.69	48.4	2.67, 2.74	48	75.2	3.36	3.56	75.2	3.36
13	211.8			202.1		49	70.5	4.02	4.26	70.6	4.02
14	51.9	2.59	2.62, 2.69	132.3	6.15	50	31.6	1.54, 2.06	1.65, 2.39	31.6	1.54, 2.06
15	68.1	4.05	4.17	149.9	6.93	51	67.4	4.02	4.21	67.4	4.02
16	38.5	1.43, 1.50	1.41, 1.52	33.8	2.32	52	68.7	4.03	4.38	68.7	4.03
17	29.8	2.05, 2.09	2.05, 2.14	32.3	2.20	53	80.7	3.72	4.05	80.7	3.73
18	130.3	5.46	5.44	129.4	5.46	54	72.0	3.97	4.24	72.0	3.97
19	137.5	5.25	5.22	138.3	5.28	55	74.4	4.36	4.67	74.4	4.36
20	35.0	2.37	2.44	34.9	2.37	56	128.5	5.58	5.78	128.5	5.58
21	41.7	1.37, 1.47	1.41, 1.52	41.7	1.34, 1.46	57	136.2	5.78	5.82	136.2	5.78
22	73.6	3.44	3.54	73.7	3.42	58	33.8	2.08	1.93	33.8	2.07
23	73.2	3.52	3.70	73.2	3.52	59	30.3–30.6	1.40	1.23	30.3–30.6	1.39
24	38.5	1.37, 1.68	1.56, 1.89	38.6	1.37, 1.68	60	30.8–30.9	1.28	1.16	30.8–30.9	1.28
25	31.4	2.17	2.45	31.4	2.17	61	30.8–30.9	1.28	1.10	30.8–30.9	1.28
26	72.9	3.71	4.03	72.9	3.72	62	30.8–30.9	1.28	1.10	30.8–30.9	1.28
27	75.2	3.31	3.54	75.2	3.31	63	30.8–30.9	1.28	1.10	30.8–30.9	1.28
28	68.9	4.18	4.52	68.9	4.18	64	30.8–30.9	1.28	1.10	30.8–30.9	1.28
29	39.0	1.50, 2.09	1.77, 2.38	39.0	1.48, 2.07	65	30.8–30.9	1.28	1.16	30.8–30.9	1.28
30	70.6	3.92	4.22	70.6	3.92	66	30.3–30.6	1.38	1.26	30.3–30.6	1.37
31	76.5	3.26	3.47	76.5	3.26	67	35.1	2.04	1.93	35.0	2.03
32	69.6	4.07	4.28	69.6	4.06	68	140.3	5.80	5.74	140.3	5.80
33	45.4	2.31	2.40	45.4	2.32	69	114.9	4.90, 4.97	4.88, 4.94	114.9	4.90, 4.97
34	136.9			136.9		70	22.7	1.00	0.92	22.7	1.00
35	128.8	5.52	5.78	128.8	5.53	71	13.6	0.90	1.02	13.7	0.92
36	68.2	4.55	4.81	68.2	4.56	72	17.7	1.79	1.81	17.7	1.79
						73	113.3	5.00, 5.06	5.03, 5.13	113.3	5.00, 5.07

^{13}C NMR (Table 1) and HMQC spectra of **1** in CD_3OD revealed that **1** contained a ketone group (δ_C 211.8), 2 sp^2 quaternary carbons, 6 sp^2 methines, 2 sp^2 methylenes, 28 oxymethines, 1 oxymethylene, 2 sp^3 methines, 3 methyl groups, and 28 sp^3 methylenes. The number of hydroxyl groups was established by deuterium shift analysis of the oxymethine and oxymethylene carbon signals in the ^{13}C NMR spectra in CD_3OD and CD_3OH (1:1, v/v). Of the 29 signals observed for oxymethine and oxymethylene carbons (δ_C 67.4–80.7), four signals (δ_C 70.5, 78.3, 80.1, 80.7) did not show deuterium shifts. These results suggested that **1** contained two ether rings.

A detailed analysis of the ^{1}H–^{1}H COSY and TOCSY spectra of **1** allowed us to elucidate the following seven partial structures: C-1–C-4, C-10–C-12, C-14–C-33 containing two methyl groups, C-35–C-45, C-47–C-49, C-52–C-59, and C-66–C-69 (Fig. 2). HMBC correlations

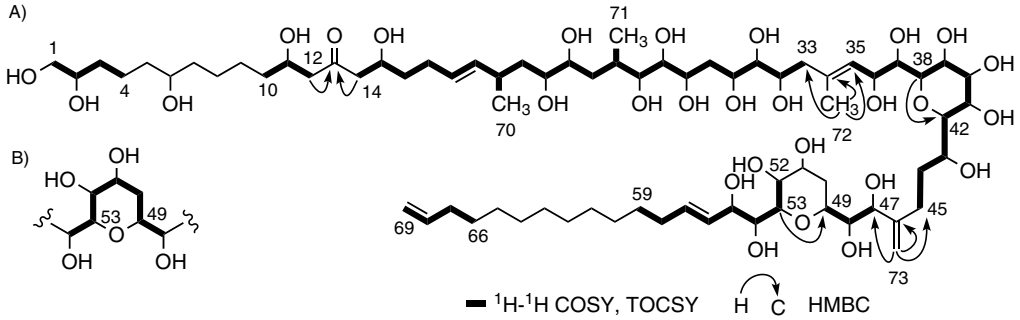

Figure 2. Gross structure of karatungiol A (**1**) based on 2D NMR data: (A) in CD_3OD and (B) in $CD_3OD/C_5D_5N = 2/1$.

CH_2-12/C-13 and CH_2-14/C-13 suggested that both C-12 and C-14 were directly connected to a C-13 ketone group. Similarly, HMBC correlations CH_3-72/C-33, C-34, and C-35 indicated that C-33 and C-35 were connected to a quaternary carbon C-34. HMBC correlations CH_2-73/C-45, C-46, and C-47 suggested that both C-45 and C-47 were connected to the *exo*-olefin carbon C-46. As mentioned above, the presence of two tetrahydropyran rings was confirmed by HMBC correlations H-38/C-42 and H-53/C-49. Due to signal overlap in the ^{1}H NMR spectrum in CD_3OD, connectivities from C-49 to C-53 could not be confirmed. Based on an analysis of ^{1}H–^{1}H COSY and TOCSY spectra of **1** in CD_3OD/C_5D_5N (2/1), however, all of the proton and carbon signals from C-49 to C53 were assigned unequivocally (Fig. 2B and Table 1).

To confirm the carbon–carbon connectivities at C-4–C-10 and C-59–C-66, degradation reactions of **1** were carried out. Karatungiol A (**1**) was treated with $NaIO_4$ and then reduced with $NaBH_4$. Purification of the reaction mixture by TSK G-3000S polystyrene gel column chromatography with aqueous EtOH (0–100%) gave a segment **3** (Fig. 3). Other fractions were assembled and treated with acetic anhydride and pyridine. Purification of these acetylated products by silica gel column chromatography gave a segment **4**. The molecular formula of **3** was confirmed by ESIMS analysis [*m*/*z* 225 $(M+H)^+$]. An analysis of 2D NMR spectra suggested that **3** contained an allylic alcohol and a terminal olefin group, which were derived from C-56 and C-68 olefin moieties in **1**.[10] Therefore, compound **3** was assigned to be the C-55–C-69 fragment of **1**. As with **3**, the planar structure of compound **4** was established by 2D NMR data. Compound **4** contained six acetyl groups, corresponding to four hydroxyl groups at C-2, 11, 15, and 22, a ketone moiety at C-13,[11] and an unidentified hydroxyl group in **1**.[12] Thus, an analysis of ^{1}H–^{1}H COSY and TOCSY spectra revealed that compound **4** was assigned to be a C-2–C-22 fragment of **1**, and the remaining hydroxyl group in **1** was connected at C-6. Therefore, the gross structure of karatungiol A was determined to be as shown in **1**.

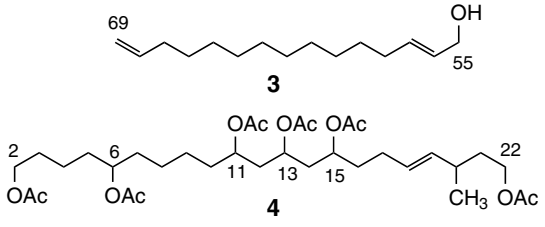

Figure 3. Structures of degradation products **3** and **4**.

The relative stereochemistries of the two tetrahydropyran rings (C-38–C-42 and C-49–C-53) in **1** were determined by ^{1}H–^{1}H coupling constants and ROESY correlations (Fig. 4). The ROE correlations H-37/H-40, H-40/H-42, and H-37/H-42 suggested that the tetrahydropyran ring C-38–C-42 had a chair conformation and H-40 and H-42 were in a 1,3-diaxial orientation with each other. Meanwhile, the small coupling constants ($J_{H39–H40} = 3.2$ Hz, $J_{H40–H41} = 2.7$ Hz, and $J_{H41–H42} = \sim 0$ Hz) suggested that H-39 and H-41 were oriented in an equatorial conformation. The tetrahydropyran ring C-49–C-53 was also suggested to exhibit a chair conformation based on the ROE correlations of H-49/H-55, the large coupling constants ($J_{H49–H50b} = 7.4$ Hz, $J_{H50b–H51} = 11.4$ Hz), and small coupling constants ($J_{H49–H50a} = \sim 0$ Hz, $J_{H50a–H51} = 4.6$ Hz, $J_{H51–H52} = 3.2$ Hz, and $J_{H52–H53} = 1.6$ Hz). The geometry of a trisubstituted double bond (C-34–C-35) was confirmed to be *E* from the ROESY correlation CH_2-33/H-35. Meanwhile, the geometries of both disubstituted olefins (C-18–C-19 and C-56–C-57) were also determined to be *E* based on ^{1}H–^{1}H coupling constants ($J_{H18–H19} = 15.0$ Hz and $J_{H56–H57} = 15.2$ Hz). The relative stereochemistries of two tetrahydropyrans of **1** were identical to those of amphidinol and its analogs.[3–5]

Karatungiol B (**2**) was isolated as a pale yellow amorphous solid: $[\alpha]_D^{15}$ +24.0 (*c* 0.1, MeOH); UV_{max} 228 nm (ε 16,300); IR (KBr) 3417, 1630 cm^{-1}. The molecular weight of **2** was determined by ESIMS (1461.7 $[M+Na]^+$, 742.4 $[M+2Na]^{2+}$). The molecular formula of **2** was found to be $C_{73}H_{130}O_{27}$ [$(M+2Na)^{2+}$, *m*/*z* 742.4317, Δ +2.0 mmu, calcd for $1/2 \times C_{73}H_{130}O_{27}Na_2$ 742.4297] by HR-ESIMS.

A detailed analysis of 2D NMR data including ^{1}H–^{1}H COSY, TOCSY, HMQC, and HMBC spectra revealed that the partial structures C-1–C-13 and C-16–C-73 of **2** were identical to those of **1**. The IR data (1630 cm^{-1}) and ^{1}H NMR data (δ_H 6.15 and 6.93) suggested the presence of an α,β-unsaturated ketone moiety in **2**.

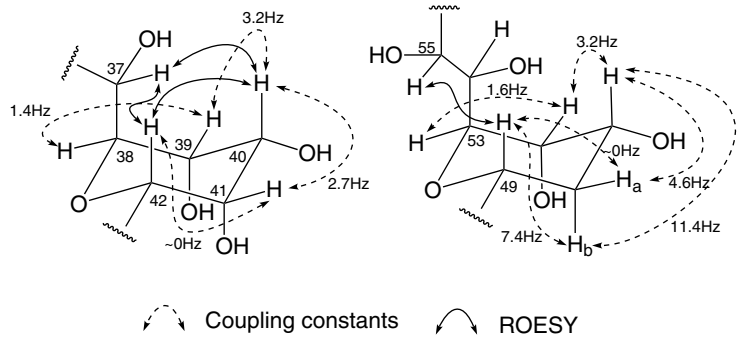

Figure 4. Relative stereochemistries of two tetrahydropyran rings of karatungiol A (**1**) in $CD_3OD/C_5D_5N = 2/1$. The relative stereochemistries of C-37, C-54, and C-55 were not assigned.

In fact, HMBC correlations (H-14/C-13, H-15/C-13, H-12/C-13, H-14/C-16, and H-15/C-16) confirmed the carbon–carbon connectivities between C-13 and C-16. The geometry of the C-14 conjugated olefin was confirmed to be *E* based on the large coupling constant ($J_{H14–H15} = 16.0$ Hz). Thus, the planar structure of **2** was elucidated to be a dehydroxy derivative of karatungiol A (**1**), as shown in Figure 1.

In summary, we isolated karatungiols A (**1**) and B (**2**) from the cultured marine dinoflagellate *Amphidinium* sp. Using spectroscopic analysis and degradation reactions, the structures of **1** and **2** were determined to be novel polyol compounds. Karatungiols were structurally similar to lingshuiol,[5a] and were amphidinol analogs with a ketone moiety and a terminal saturated alkyl chain moiety, as in lingshuiol. Karatungiol A (**1**) exhibited potent antifungal activity against NBRC4407 *A. niger* and antiprotozoan activity against *T. foetus*. This is the first report that Karatungiol A (**1**) showed antiprotozoan activity against *Trichomonas* sp. as an amphidinol derivative. Further studies on the structures of karatungiols as well as their structure–activity relationships are in progress.

Acknowledgments

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9. The MIC of metronidazole, a positive control for anti-protozoan activity against *T. foetus*, was 3 μg/ml.
10. Spectroscopic data for **3**; ^{1}H NMR (CD_3OD, 800 MHz) δ 3.96 (H-55), 5.56 (H-56), 5.65 (H-57), 2.04 (H-58), 1.38 (H-59), 1.30 (H-60–H65), 1.38 (H-66), 2.04 (H-67), 5.78 (H-68), 4.88 (H-69_a), 4.98 (H-69_b); ^{13}C NMR (CD_3OD, 150 MHz) δ 63.8 (C-55), 130.3 (C-56), 133.5 (C-57), 30.1–30.8 (C-59–C-66, 8C), 33.3 and 34.9 (C-58 and C-67), 139.8 (C-68), 114.7 (C-69); ESIMS: *m/z* 225 $[M+H]^+$.
11. Two diastereomers (ca. 4:1–3:1) of the secondary alcohol at C-13 were obtained by $NaBH_4$ reduction of the ketone moiety in **1**.
12. Spectroscopic data for **4**; ^{1}H NMR (major isomer, C_6D_6, 800 MHz) δ 3.96 (H-2), 1.38 (H-3), 1.11 (H-4_a), 1.16 (H-4_b), 1.20 (H-5_a), 1.33 (H-5_b), 5.00 (H-6), 1.43 (H-7_a), 1.48 (H-7_b), 1.54 (H-8_a), 1.59 (H-8_b), 1.34 (H-9_a), 1.43 (H-9_b), 1.56 (H-10_a), 1.64 (H-10_b), 5.16 (H-11), 1.78 (H-12_a), 1.93 (H-12_b), 5.23 (H-13), 1.73 (H-14_a), 1.91 (H-14_b), 5.13 (H-15), 1.56 (H-16_a), 1.62 (H-16_b), 1.98 (H-17), 5.32 (H-18), 5.19 (H-19), 2.11 (H-20), 1.43 (H-21_a), 1.48 (H-21_b), 4.06 (H-22), 0.91 (CH_3-20), 1.71–1.80 ($6\times$ O*Ac*); HR-ESIMS: *m/z* 679.3658 $[M+Na]^+$, calcd for $C_{34}H_{56}O_{12}Na$, 679.3669 (Δ −1.1 mmu).