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Durinskiol A: a long carbon-chain polyol compound from the symbiotic dinoflagellate *Durinskia* sp.

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Abstract—A long carbon-chain polyol compound with a molecular weight of 2128 mu, durinskiol A (**1**) was isolated from the cultured symbiotic dinoflagellate *Durinskia* sp. Its planar structure was elucidated based on 2D-NMR and MS/MS analysis. Durinskiol A (**1**) caused a short body length, abnormal pigment pattern, and pericardiac and yolk-sac edema in zebrafish.

Marine dinoflagellates are a rich source of various biologically and physiologically active secondary metabolites.[1] Among them, large polyol and polyether compounds composed of a long carbon backbone that is highly functionalized by oxygen, as we say 'super-carbon-chain compounds (SCC)',[1a] are some of the most unique and unusual compounds. Several kinds of SCC have been isolated from symbiotic dinoflagellates, such as karatungiols[2] and amphidinols[3] from *Amphidinium* sp. and symbiodinolide[4] and zooxanthellatoxins[5] from *Symbiodinium* sp., accompanying small-size bioactive compounds.[6] The true origin of secondary metabolites isolated from marine invertebrates such as sponges, ascidians, nudibranchs, has been suggested to be mostly microorganisms, which are accumulated in the host animals through a symbiotic relationship or a food chain. However, the true physiological functions or roles of SCC in the ecosystem or symbiotic relationship have rarely been clarified. Based on their structural, biological, and conformational diversity and uniqueness, various potential abilities of SCC can be considered, that is, chemical communication with host animals, defense materials, or nutrient sources. To establish their roles in symbiotic organisms, we have investigated polyol compounds from symbiotic dinoflagellates.

Recently, we found that polyol compounds with molecular weights of >1000 mu contained in dinoflagellates are easily detected as major ion peaks by MALDI-TOF mass spectrum analysis. Using this method, we identified a novel long carbon-chain polyol compound, named durinskiol A (**1**), in the symbiotic dinoflagellate *Durinskia* sp. We describe here the planar structure and biological activity of **1** (Fig. 1).

The cultivated dinoflagellate *Durinskia* sp. (191 g wet wt), isolated from the nudibranch *Chelidonura fulvipunctata*, was extracted with 80% aqueous EtOH. Purification using ODS and polystyrene gel column chromatography and reversed-phase HPLC guided by the mass spectrum analysis afforded **1** (20 mg). Durinskiol A (**1**) caused a short body length, abnormal pigment pattern, and pericardiac and yolk-sac edema in zebrafish

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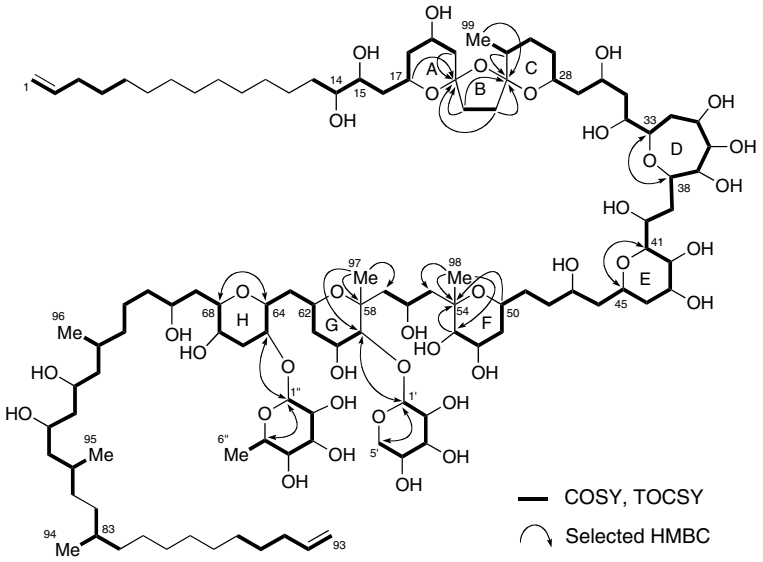


Figure 1. Planar structure of durinskiol A (**1**) determined by 2D-NMR spectroscopy.

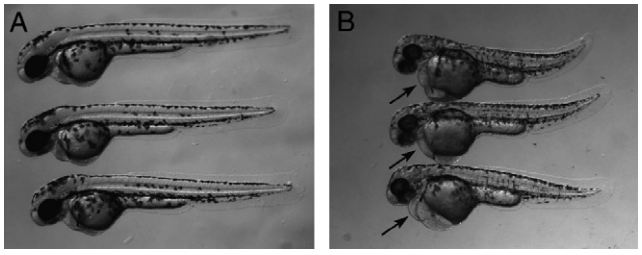


Figure 2. Bioassay using zebrafish embryo. (A), control (48 h post fertilization); (B), treatment with **1** (188 μM). Arrows in (B) indicate characteristic pericardiac and yolk-sac edema.

at 188 μM (Fig. 2). Meanwhile, compound **1** did not show significant vasoconstrictive activity against rat aortic rings even at 100 μM. Unlike palytoxin[7] or zooxanthellatoxins,[5a] known as vasoconstrictive polyol compounds, durinskiol A (**1**) lacks a terminal amino group, which may explain its lower activities.[1d,e]

Durinskiol A (**1**) was isolated as a colorless amorphous powder: $[\alpha]_D^{23}$ +13.5 (*c* 0.10, MeOH); IR (KBr) 3405, 1647, 1636, 1458, 1051, 985 cm^{-1}. The molecular formula of **1** was found to be $C_{110}H_{198}O_{38}$ [*m/z* 2151.3296, Δ +2.0 mmu for $(M+Na)^+$; *m/z* 1086.6630, Δ −4.8 mmu for $(M+2Na)^{2+}$] by positive HR–ESIMS. ^{13}C NMR spectra showed the presence of all carbon signals, including 7 methyls, 49 methylenes, 4 methines, 41 oxymethines, one oxymethylene, four acetal carbons, and a pair of terminal olefin carbons (Table 1). An extensive 2D-NMR analysis in CD_3OD allowed us to construct four partial structures of **1**: C1–C5; C13–C81 including four quaternary carbons and five methyl groups; C82–C84 and a methyl group; C89–C93 (Fig. 1). Deuterium shift analysis in CD_3OH/CD_3OD

Table 1. NMR data for durinskiol A (**1**) in CD_3OD

Position	δ_C (Multiplicity)[a]	δ_H (Multiplicity, *J* in Hz)[b]	Position	δ_C (Multiplicity)[a]	δ_H (Multiplicity, *J* in Hz)[b]
1 a	114.75 (t)	4.90 (br dd, 10.4, 2.0)	55 b		2.59 (m)
1 b		4.97 (br dd, 17.3, 2.0)	56	64.87 (d)	4.03 (m)
2	140.15 (d)	5.80 (ddt, 17.3, 10.4, 6.9)	57 a	43.16 (t)	1.62 (m)
3	34.91 (t)	2.03 (m, 2H)	57 b		2.59 (m)
4	30.15 (t)	1.38 (m, 2H)	58	77.88 (s)	
5	30.24[c] (t)	1.3–1.7 (m, 2H)	59	80.39 (d)	3.41 (m)
6	28.19[c] (t)	1.3–1.7 (m, 2H)	60	69.48 (d)	4.13 (ddd, 11.0, 6.3, 2.5)
7	26.01[c] (t)	1.3–1.7 (m, 2H)	61 a	35.17 (t)	1.40 (m)
8	20.40[c] (t)	1.3–1.7 (m, 2H)	61 b		1.76 (m)
9	23.76[c] (t)	1.3–1.7 (m, 2H)	62	65.79 (d)	3.97 (m)
10	30.26[c] (t)	1.3–1.7 (m, 2H)	63 a	39.82 (t)	1.25 (m)
11	30.66[c] (t)	1.3–1.7 (m, 2H)	63 b		2.30 (m)
12	30.80[c] (t)	1.3–1.7 (m, 2H)	64	76.61 (d)	3.38 (m)
13 a	39.25 (t)	1.30 (m)	65	74.70 (d)	3.46 (m)
13 b		1.70 (m)	66 a	39.25 (t)	1.30 (m)
14	77.89 (d)	3.13 (m)	66 b		2.50 (m)
15	68.77 (d)	4.06 (m)	67	70.38 (d)	3.18 (ddd, 11.3, 9.3, 4.3)
16 a	40.52 (t)	1.37 (m)	68	79.16 (d)	3.35 (m)
16 b		n.d.	69 a	40.75 (t)	1.40 (m)

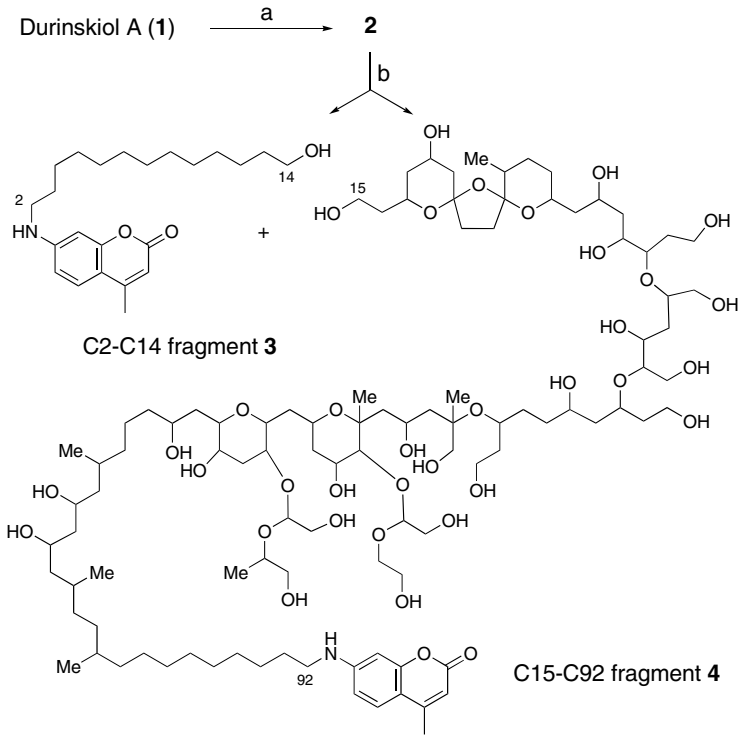
Table 1 (*continued*)

Position	δ_C (Multiplicity)[a]	δ_H (Multiplicity, J in Hz)[b]	Position	δ_C (Multiplicity)[a]	δ_H (Multiplicity, J in Hz)[b]
17	66.58 (d)	4.10 (m)	69 b		1.92 (m)
18 a	42.14 (t)	1.22 (m)	70	68.06 (d)	3.80 (m)
18 b		1.88 (m)	71 a	35.10 (t)	1.50 (m)
19	66.36 (d)	4.01 (m)	71 b		1.60 (m)
20 a	43.41 (t)	1.45 (m)	72 a	27.25 (t)	1.31 (m)
20 b		1.94 (m)	72 b		1.49 (m)
21	108.43 (s)		73 a	39.53 (t)	1.20 (m)
22 a	39.13 (t)	1.70 (m)	73 b		n.d.
22 b		1.95 (m)	74	30.10 (d)	1.74 (m)
23 a	32.77 (t)	2.01 (m)	75 a	44.60 (t)	1.29 (m)
23 b		2.29 (m)	75 b		1.37 (m)
24	118.22 (s)		76	66.85 (d)	3.75 (m)
25	41.55 (d)	2.08 (m)	77 a	38.61 (t)	1.35 (m)
26 a	39.09 (t)	1.72 (m)	77 b		2.18 (m)
26 b		2.03 (m)	78	66.41 (d)	3.97 (m)
27 a	41.55 (t)	1.92 (m)	79 a	47.02 (t)	1.38 (m)
27 b		1.30 (m)	79 b		1.50 (m)
28	74.29 (d)	4.37 (br tt, 9.6, 3.0)	80	30.68 (d)	1.62 (m)
29 a	47.13 (t)	1.30 (m)	81 a	38.45 (t)	1.05 (m)
29 b		1.50 (m)	81 b		1.45 (m)
30	65.62 (d)	4.08 (m)	82 a	39.53 (t)	1.20 (m)
31 a	42.90 (t)	1.48 (m)	82 b		n.d.
31 b		1.62 (m)	83	34.00 (d)	1.46 (m)
32	72.94 (d)	3.76 (m)	84 a	39.45 (t)	1.10 (m)
33	80.89 (d)	3.55 (m)	84 b		1.20 (m)
34 a	37.23 (t)	1.88 (m)	85	31.04[c] (t)	1.3–1.7 (m, 2H)
34 b		2.12 (ddd, 14.0, 5.7, 3.7)	86	30.83[c] (t)	1.3–1.7 (m, 2H)
35	69.88 (d)	3.86 (ddd, 10.4, 8.8, 3.7)	87	30.81[c] (t)	1.3–1.7 (m, 2H)
36	81.46 (d)	3.31 (dd, 9.2, 8.8)	88	30.89[c] (t)	1.3–1.7 (m, 2H)
37	78.10 (d)	3.15 (t, 9.2)	89	30.97[c] (t)	1.3–1.7 (m, 2H)
38	78.28 (d)	3.54 (dt, 2.2, 9.2)	90	30.15 (t)	1.38 (m, 2H)
39 a	42.38 (t)	1.47 (m)	91	34.91 (t)	2.03 (m, 2H)
39 b		1.88 (m)	92	140.15 (d)	5.80 (ddt, 17.3, 10.4, 6.9)
40	71.43 (d)	3.85 (ddd, 8.7, 4.4, 2.2)	93 a	114.75 (t)	4.90 (br dd, 10.4, 2.0)
41	77.71 (d)	3.95 (m)	93 b		4.97 (br dd, 17.3, 2.0)
42	71.87 (d)	3.63 (br t, 2.0)	94	19.76 (q)	0.87 (d, 6.8, 3H)
43	67.06 (d)	4.12 (ddd, 6.2, 3.2, 2.0)	95	20.93 (q)	0.92 (d, 6.4, 3H)
44 a	35.89 (t)	1.50 (m)	96	20.00 (q)	0.95 (d, 6.4, 3H)
44 b		1.72 (m)	97	24.37 (q)	1.36 (s, 3H)
45	62.93 (d)	4.06 (m)	98	17.92 (q)	1.43 (s, 3H)
46 a	40.42 (t)	1.45 (m)	99	13.14 (q)	0.97 (d, 6.4, 3H)
46 b		n.d.	1′	106.45 (d)	4.27 (d, 7.8)
47	68.35 (d)	3.88 (m)	2′	75.45 (d)	3.21 (dd, 10.0, 7.8)
48 a	43.99 (t)	1.35 (m)	3′	77.83 (d)	3.29 (t, 10.0)
48 b		1.52 (m)	4′	71.21 (d)	3.46 (ddd, 10.5, 10.0, 5.3)
49 a	46.04 (t)	1.52 (m)	5′ a	67.00 (t)	3.16 (dd, 14.0, 10.5)
49 b		1.62 (m)	5′ b		3.82 (dd, 14.0, 5.3)
50	62.83 (d)	4.17 (m)	1″	97.57 (d)	4.59 (d, 1.2)
51 a	40.08 (t)	1.52 (m)	2″	73.00 (d)	3.78 (dd, 2.8, 1.2)
51 b		1.72 (m)	3″	75.01 (d)	3.40 (dd, 9.2, 2.8)
52	69.48 (d)	4.03 (m)	4″	73.96 (d)	3.32 (t, 9.2)
53	74.77 (d)	3.23 (d, 3.6)	5″	73.55 (d)	3.21 (dq, 9.2, 6.0)
54	78.68 (s)		6″	18.16 (q)	1.30 (d, 6.0, 3H)
55 a	43.20 (t)	1.70 (m)			

[a] Recorded at 150 MHz. Multiplicity was determined based on HMQC spectrum.
[b] Recorded at 800 MHz. Higher-field methylene proton signals are labeled 'a' and lower-field signals are 'b'.
[c] Signals may be interchanged. n.d., not determined.

and mass data for the acetylated product (MALDI-TOF MS m/z 3242.6 $[M(C_{162}H_{250}O_{64})+Na]^+$) suggested the presence of 26 hydroxyl groups in **1**. Four key HMBC correlations (H59/C1′, H65/C1″, H1′/C59, and H1″/C65) confirmed the linked positions of two sugar moieties. Other carbon–carbon connectivities through oxygen atoms were also confirmed by HMBC correlations. Combining these data, a C_{93} carbon-chain poly-oxygenated skeleton was established, which included a 6,5,6-bis-spiroacetal ring, a seven-membered ether ring, four six-membered ether rings, and two sugar moieties.

To confirm the entire planar structure of **1**, we developed a fluorescent label method to enhance the sensitivities of degraded fragments. Ozonolysis of **1** followed by direct reductive amination using $NaBH_3CN$ afforded bis-7-amino-4-methylcoumarin (AMC) derivative **2** quantitatively (Scheme 1). An ionic charge located at both terminals of the amino group in **2** facilitated typical charge-remote fragmentations derived from the ether rings and polyol moieties (Fig. 3). Thus, tandem FAB-MS/MS analysis of **2** observed well-defined fragmenta-



Scheme 1. Reagents and conditions: (a) O_3, MeOH, −78 °C, 1 min; Me_2S, −78 °C to rt for 6 h; AMC (50 equiv), DMF–AcOH (9:1), rt, 1 h; $NaBH_3CN$ (200 equiv), rt, 24 h, 97%; (b) $NaIO_4$ (xs), aq MeOH, rt, 3 h; $NaBH_4$ (xs), 0 °C, 1 h, 26% for **3**.

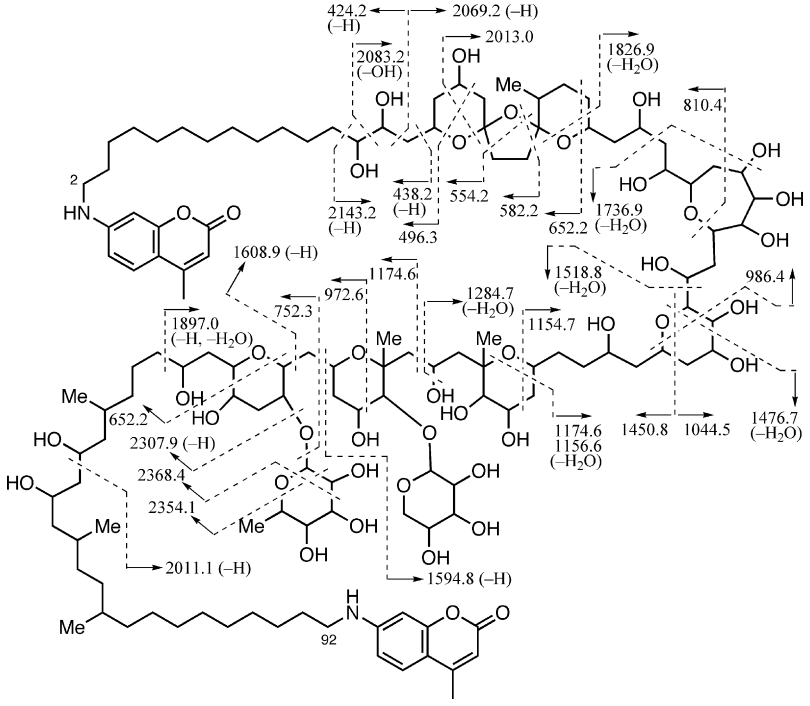


Figure 3. MS/MS fragmentation pattern in bis-AMC derivative **2**. The mass units indicated are those of Na-adduct ions. Precursor ion: 2472.5 $[M+Na]^+$.

tion ion peaks, all of which were unambiguously assigned in the structure of **2**.

Furthermore, $NaIO_4$ oxidation of **2** followed by $NaBH_4$ reduction gave C2–C14 fragment **3** and C15–C92 fragment **4**, molecular ions of which were detected on LC–ESIMS analysis [**3**: *m*/*z* 396.2532 (Δ +1.7 mmu for $[M+Na]^+$); **4**: *m*/*z* 1022.1 for $[M+2Na]^{2+}$]. Typical charge-remote fragmentation of **4** on tandem FAB-MS/MS analysis also supported its planar structure as with **2** (data not shown). Thus, the position of a 1,2-diol moiety on the linear carbon-chain in **1** was elucidated to be C14, and the planar structure of **1** was confirmed as shown in Figure 1.[8]

In summary, a novel polyol compound with a molecular weight of 2128 mu, durinskiol A (**1**), was isolated from the dinoflagellate *Durinskia* sp., and its planar structure was elucidated. Further studies on the biological activity of **1** are in progress. In a subsequent paper, we report our efforts to establish the relative stereochemistry of durinskiol A (**1**).[9]

Acknowledgments

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