Durinskiol B: A New Durinskiol Congener from the Symbiotic Marine Dinoflagellate *Durinskia* sp.

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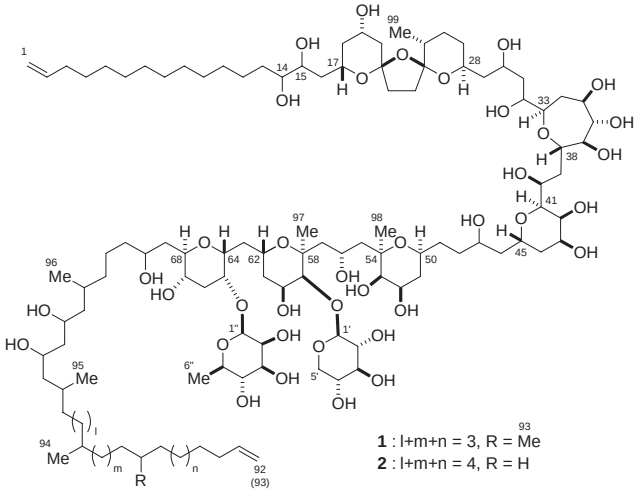
Durinskiol B (**1**) was isolated from the symbiotic marine dinoflagellate *Durinskia* sp., along with durinskiol A (**2**). The planar structure of **1** was determined by detailed NMR analysis. Durinskiol B (**1**) has structure similarity with durinskiol A (**2**) except for the existence of one methyl excess in the side chain of durinskiol B.

Symbiotic marine dinoflagellates have been known as sources of various molecules with remarkable biological activities. In particular, large polyol compounds which called as "super-carbon-chain compounds"[1] have been isolated from these microorganisms such as symbiodinolide,[2] karatungiols,[3] zooxanthellatoxins,[4] and zooxanthelamides.[5] In our continuing search for polyol compounds from symbiotic marine dinoflagellates, we examined the constituents of culture of *Durinskia* sp. isolated from Okinawan nudibranch *Chelidonura fulvipunctata*. Recently, we reported the isolation and structure determination of a long carbon-chain polyol compound with a molecular weight of 2128 mu, durinskiol A (**2**). It caused a short body length, abnormal pigment pattern, pericardiac and yolk-sac edema in zebrafish at 188 μM.[6] We describe here another congener of durinskiol A (**2**) which is named durinskiol B (**1**) (Scheme 1).

The harvested cells (191 g wet weight from 400 L of culture) were extracted with 80% ethanol. Ethanol extracts were initially partitioned between EtOAc and water. Water-soluble material was chromatographed by using a polystyrene gel TSK G-3000S and ODS column chromatography, followed by reversed-phase HPLC to furnish durinskiol B (**1**, 0.0009% of wet weight)[7] and durinskiol A (**2**, 0.004%). Interestingly, **1** and **2** did not show cytotoxicity against P388 murine leukemia cells or B16 melanoma cells even at concentration 100 μg/mL.



Scheme 1.

1D NMR data of **1** in all regions were almost identical to those of **2**, however, **1** displayed one more doublet methyl and the corresponding carbon than **2** (Figure 1). Molecular weight of **1** was also consistent with that of **2**. ^{1}H and ^{13}C NMR data revealed that **1** consists of eight methyls, 49 methylenes, four methines, 41 oxymethines, one oxymethylene, four acetal carbons, and a pair of terminal olefin carbons (see Supporting Information). On the basis of the ^{1}H–^{1}H COSY, TOCSY, and HMBC correlations, the planar structure of **1** has been elucidated except for the positions of two methyl groups (C93 and C94) (Figure 2). Durinskiol B (**1**) contained a 6,5,6-bisspiroacetal ring, a seven-membered ether ring, four six-membered ether rings, and two sugar moieties (rhamnose and xylose) similar to **2**.

To confirm the entire planar structure of **1**, degradation reaction was carried out. $NaIO_4$ oxidation of **1** followed by $NaBH_4$ reduction afforded the C1–C14 and the C15–C92 fragments. On the basis of the ^{1}H NMR and mass spectra, structure of the C1–C14 fragment was established to be 13-tetradecenal,[8] which suggested that both of the C93 and C94 methyl groups were located on the other linear carbon-chain terminus. Despite the detail 2D NMR analysis, however, the number of methylene carbons between the spin systems including the isolated methyl groups and those of the other part in **1** could not be established. As in the case of durinskiol A (**2**),[6] the C93 and C94 methyl groups can be placed between C83 and C87 in **1**, thus tentatively connected to C86 and C83, respectively (Figure 2). The stereochemistry of **1** could be suggested as the same as **2** due to the identical spectroscopic data on both compounds especially in the vicinal coupling constants.

A methyl moiety excess in **1** might be explained by the

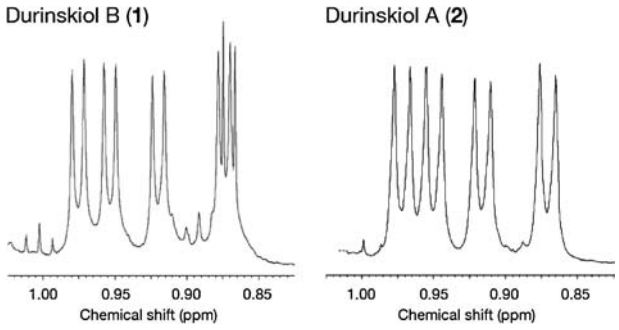


Figure 1. Comparison of the doublet methyl signals between durinskiols A and B in ^{1}H NMR spectra (800 MHz for **1** and 600 MHz for **2**).

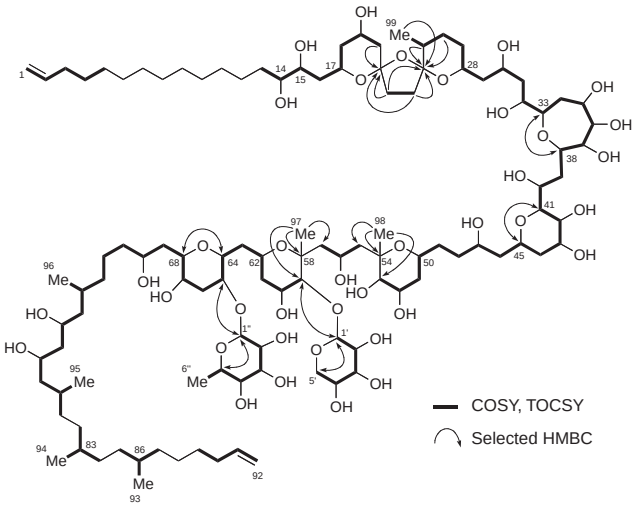
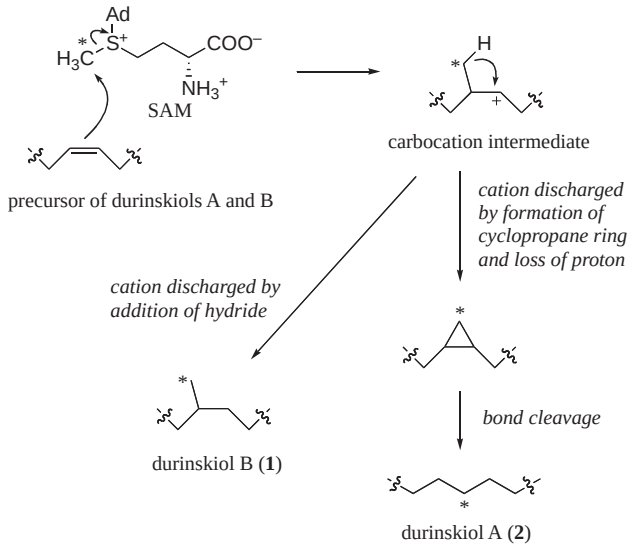


Figure 2. Planar structure of **1** determined by 2D NMR spectroscopy.



Scheme 2. Plausible biogenetic pathway of the methyl excess in **1**. Ad, adenosyl.

plausible biogenetic pathway shown in Scheme 2. It is assumed that the aliphatic chains on **1** and **2** are derived from an unsaturated fatty acid chain. Cyclopropane ring formation on the double bond are formed from a carbocation intermediate provided by *S*-adenosylmethionine (SAM), followed by bond cleavage to construct the methylene bond in **2**. On the other hand, the methylation mechanism from carbocation intermediate can be postulated by accepting hydride from some reducing agents, such as NADPH, giving **1**.[9]

In summary, a new durinskiol congener, durinskiol B (**1**) was isolated from *Durinskia* sp. The planar structure of **1** including partial relative stereochemistry was almost identical with **2**, except for the existence of one more methyl excess in side chain of **1**. Considering of biogenetic pathway, **1** might be produced from the same precursor with **2**. Further studies on other durinskiol congeners are in progress.

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References and Notes

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7 Colorless amorphous powder, $[\alpha]_D^{23}$ +8.5 (*c* 0.10, MeOH); IR (KBr) 3400, 1650, 1636, 1458, 1051, 985 cm^{-1}; HR-ESIMS [m/z $(M + 2Na)^{2+}$; found 1086.6692, calcd for $C_{55}H_{99}NaO_{19}$ (Δ +1.4 mmu)].

8 ^{1}H NMR ($CDCl_3$, 600 MHz) δ 9.74 (1H, t, J = 1.8 Hz), 5.79 (1H, m), 4.97 (1H, dt, J = 17.0, 1.2 Hz), 4.90 (1H, dt, J = 10.0, 1.2 Hz), 2.39 (2H, td, J = 7.2, 1.8 Hz), 2.01 (2H, q, J = 7.2 Hz), 1.60 (2H, pent., J = 7.2 Hz), 1.34 (2H, m), 1.24 (14H, m); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 202.9 d, 139.2 d, 114.1 t, 43.9 t, 33.8 t, 29.5 t, 29.5 t, 29.4 t, 29.3 t, 29.1 t (×3), 28.9 t, 22.1 t; HRESIMS [m/z $(M + Na)^+$; found 233.1906, calcd for $C_{14}H_{26}NaO$ (Δ +2.5 mmu)].

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