Structure of Periodate Oxidation Products with Characteristic Partial Structures of Zooxanthellatoxin-A, a Potent Vasoconstrictive Polyol from a Symbiotic Dinoflagellate

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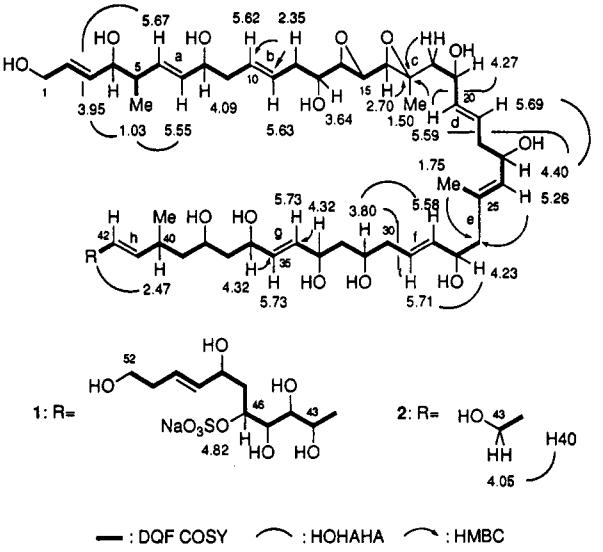
Summary: Reduction of the periodate oxidation products of zooxanthellatoxin-A with $NaBH_4$ gave the fragment 2 ($C_{47}H_{76}O_{14}$) and its elongated fragment 1 ($C_{56}H_{91}O_{22}SNa$) to which the polyhydroxylated polyene structure (1), which possesses a 1,3-diepoxide and a sulfate ester functionality, was assigned on the basis of spectral data.

Zooxanthellae are typical symbiotic dinoflagellates associated with a wide range of marine invertebrates, and they play important roles, such as CO_2 fixation and production of organic molecules, in the maintenance of the ecological balance in marine environments.[1-3] Since marine microorganisms have been shown to produce not only toxins but also various bioactive substances,[4] they are thought to be the actual producers of some of the marine natural products that are isolated from marine animals such as marine sponges and soft corals. While screening symbiotic microorganisms from marine invertebrates for bioactive compounds,[5] we discovered the potent vasoconstrictive polyols named zooxanthellatoxin-A (ZT-A) and ZT-B taken from a cultured zooxanthella, *Symbiodinium* sp. (strain number Y-6).[6,7] Zooxanthellatoxins are sulfate esters of highly unsaturated polyols with a molecular weight of ca. 2900.[7] In this paper, we report the structural elucidation of their periodate oxidation products which contain the characteristic partial structures of a diepoxide and a sulfate ester found in zooxanthellatoxin-A.

ZT-A (50.1 mg) was carefully treated with an excess amount of $NaIO_4$ in MeOH–H_2O (1:1) (0 °C, 10 min), and this was followed by reduction with $NaBH_4$ (0 °C, 10 min). Purification of the products on a polystyrene column, eluted first with H_2O and then with H_2O–EtOH of increasing EtOH content, gave two fragments 1[8] (20% EtOH elution, 8.2 mg) and 2[9] (40% EtOH elution, 8.1 mg) after purification on a silica gel column (5:1 CH_2Cl_2–MeOH).



The negative HR-FABMS spectrum of the fragment 2 dictated a molecular formula of $C_{47}H_{76}O_{14}$, m/z 863.5162 $(M - H)^-$ (calcd for M – H, 863.5157). The structures of the segments C1–C5(C5Me)–C6, C7–C10, C11–C16, C18–20, C21–C25(C25Me), C26–C28, C29–C34, C35–C40-(C40Me)–C41, and C42–C43 were elucidated by a detailed analysis of the double quantum filtered (DQF) COSY spectrum.[10] The seriously overlapping olefinic proton signals were partly resolved by 1D and 2D HOHAHA experiments,[10] by which the connectivities C6–C7 (a, C5Me/H4 and C5Me/H7), C20–C21 (d, H23/H20, H23/H21, H19/H20, and H19/H21), C28–C29 (f, H27/H28, H27/H29, H31/H28, and H31//H29), and C41–C42 (h, H40/H43) were established. An HMBC experiment (J_{C-H} = 8.2 Hz)[10] clarified the connectivities between C10 and C11

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(7) Cultured algae (164 g wet weight) were extracted with 70% EtOH, and the extracts were partitioned with H_2O–EtOAc and then H_2O–*n*-BuOH. The *n*-BuOH-soluble portion was purified by monitoring the vasoconstrictive activities. HPLC separation of the crude materials on an ODS column (0.2 M NaCl in 8:2 MeOH–H_2O) after chromatographies on a polystyrene column (40% EtOH) and a Sephadex DEAE column (1/30 M phosphate buffer) gave ZT-A (35.8 mg) and ZT-B (19.6 mg) after being desalted with a polystyrene column.[6]
(8) $[\alpha]_D$ −6.9° (*c* 0.26, MeOH).
(9) $[\alpha]_D$ −4.9° (*c* 0.39, MeOH).
(10) All ^1H and ^{13}C NMR spectra of 1 and 2 were recorded in CD_3OD on a JEOL GX-400, EX-400 (400 MHz for ^1H and 100 MHz for ^{13}C), or GX-500 spectrometer (500 MHz for ^1H and 125 MHz for ^{13}C). DQF-COSY, phase-sensitive 2D HOHAHA, and 1D HOHAHA were measured at 500 MHz, and HMBC and HMQC spectra of 2 were obtained on GX-500 and EX-400, respectively.

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(b, H12/C10 and H12/C11) and C24 and C25 (e, C25Me/C26 and H24/C26). Although the ^1H and ^{13}C NMR signals of C33 and C34 could not be distinguished from those of C36 and C35, respectively, the connectivity of the sp^2 carbons C34 and C35 was confirmed. The symmetrically substituted C34=C35 double bond assignment was further supported by the fact that in the HMBC spectrum of 2 the C34 (and/or C35) carbon showed a cross peak only with H36 (and/or H34). The geometries of the double bonds were assigned the *E*-configuration on the basis of the coupling constants of 15–17 Hz (H2/H3, H6/H7, H10/H11, H20/H21, H28/H29, and H41/H42) obtained by homo-spin decoupling, decoupling difference, and NOE (C25Me/H23) experiments. The geometry of the C34=C35 double bond was tentatively assigned by comparing the chemical shifts of C33 and C36 (δ 71.98) with those of other allylic alcohol carbons (δ 69.11 for C23 and δ 70.48–77.24 for C4, C8, C19, C27, and C48).

The molecular formula of 2 suggested the presence of two ethereal linkages which were established by the fact that the four oxygenated carbon signals (C14, C15, C16, and C17) were not affected by deuterium exchange with CD_3OD and CD_3OH. The high-field resonances in both the ^1H [δ 3.01 (H14), 2.99 (H15), and 2.70 (H16)] and ^{13}C [δ 59.7 (C14), 55.36 (C15), 65.16 (C16), and 61.54 (C17)] NMR spectra suggested the presence of a 1,3-diepoxide structure rather than a bisoxetane or a 2,3-epoxytetrahydrofuran structure. The connectivity around the quaternary carbon C17 was determined by an HMBC experiment (H16/C17, C17Me/C17, and H18/C17). The relative stereochemistry of the epoxides was found to be the trans-configuration by NOE experiments (C17Me/H15 and H16/H14).

The fragment 1 was characterized as a sodium salt of an acidic functionality and was established to be the salt of a sulfate ester by HR-FABMS, *m/z* 1193.5518 $(M+Na)^+$ (calcd for $C_{56}H_{91}O_{22}SNa_2$, 1193.5520), and an ion analysis of the HCl hydrolysate of 1.[11,12] Comparison of the ^1H and ^{13}C NMR spectra of the fragment 1 with those of 2 revealed that most of 1's signals are almost superimposable on those of the C1–C40 protons of 2, and the carbon connectivities from C40 to C52 were assigned by detailed analysis of the DQF-COSY and HOHAHA spectra. The low-field proton signal due to H46 (δ 4.82) clearly suggested the location of the sulfate ester because the other hydroxylated proton resonances occurred at higher field than δ 4.40. The configuration at C49 was deduced on the basis of a coupling constant obtained by the decoupling difference spectrum upon irradiation of H-48.

From marine dinoflagellates, bioactive polyethers and polyols[13] have been isolated. However, zooxanthellatoxins are the first examples of potent bioactive substances isolated from zooxanthellae. Although the pharmacological properties of zooxanthellatoxins are similar to other marine toxins with large molecular weights, such as palytoxin[14] and maitotoxin,[15] zooxanthellatoxins are unique in terms of their large number of double bonds and their small number of ethereal rings. Studies on the structure of the rest of the molecules are in progress in our laboratory.

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Supplementary Material Available: All ^1H and ^{13}C NMR assignments of 2 and 1, ^1H NMR, ^{13}C NMR, DQF-COSY, HOHAHA, and FABMS spectra of 1 and 2, HMBC and HMQC spectra of 2, ^{13}C NMR spectra of 2 in CD_3OD and in CD_3OH, and spin decoupling and NOE experiments (21 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

(11) As well as ZT-A and ZT-B, the fragment 1 in water was retained on a DEAE-Sephadex column and recovered by eluting with 1 M NaCl aqueous solution. Negative FABMS spectrum of 1 gave an ion peak at *m/z* 1147 $(M-Na)^-$, and a sulfate ion was analyzed by a use of a C_{18} HPLC column.[12]

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