Zooxanthellatoxin-A, a Potent Vasoconstrictive 62-Membered Lactone from a Symbiotic Dinoflagellate

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Marine microalgae produce various types of compounds including nitrogenous neurotoxins, polyether sea food toxins, sulfonium compounds of dimethyl sulfide precursors, and antineoplastic macrolides.[1] Among them, dinoflagellate metabolites such as ciguatoxin and maitotoxin are unique and spectacular in terms of their complex structures, potent activities, and intricate biogenesis.[1-3] The fact that dinoflagellate toxins accumulate in higher organisms through the food chain and symbiotic relationships makes it difficult to identify the true origin of marine toxins.[1,2]

Zooxanthellatoxin-A (ZT-A, **1**, Figure 1) was isolated with a congener ZT-B from a symbiotic marine dinoflagellate *Symbiodinium* sp. (strain no. Y-6)[4] belonging to zooxanthellae, well-known symbionts distributed in a wide range of marine invertebrates.[5] ZTs exhibit potent vasoconstrictive activity like other marine toxins, such as palytoxin and maitotoxin.[6,7] Earlier degradation of ZT-A via periodate oxidation revealed partial structures having a sulfate ester, 11 nonconjugated olefinic double bonds including one exomethylene, two conjugated dienes, two tetrahydropyran rings, and a bisepoxide, accounting for about 80% of the full structure.[8] Extensive spectroscopic

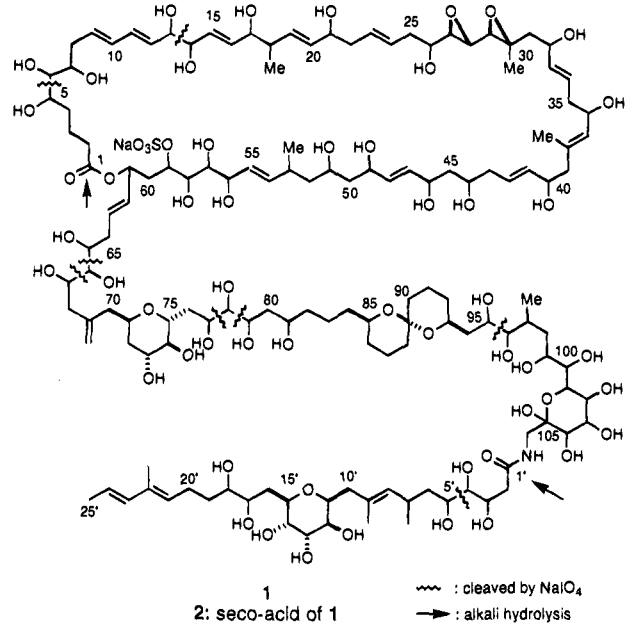

Figure 1. Structure of zooxanthellatoxin-A (**1**).

analyses and chemical degradation experiments of ZT-A now establish a novel 62-membered lactone structure for ZT-A.

ZT-A[9] exhibited a pseudomolecular ion at *m/z* 2872 $(M - Na)^-$ in the negative FAB-MS; ion chromatography of the hydrolysate and elemental analysis of **1** suggested the presence of one sulfate ester and one nitrogen atom per molecule, respectively.[10] The ^{13}C NMR spectrum of ZT-A in CD_3OD at 40 °C showed 30 sp^2 carbon, two carbonyl carbon, and two acetal carbon signals as well as many oxymethine and methylene signals. Degradation of ZT-A with $NaIO_4$ under various conditions revealed partial structures corresponding to C6−C13, C14−C65, C67−C77, and C5′−C25′, in which all of the olefinic carbons and eight of the nine methyls were found; however, one methyl, two carbonyl, and two acetal carbons were not located.

The lactone structure of **1** was evident from the formation of the corresponding seco acid (**2**, negative FAB-MS, *m/z* 2890) upon treatment of **1** with 0.1 M KOH in 10:1 MeOH−H_2O at 22 °C for 2 h. The TOCSY spectrum of **2** in CD_3OD established that the lactone ring is formed between oxymethine C61 (δ_H 5.43 in **1** and δ_H 4.35 in **2**) found in the partial structure C14−C65 and the carboxyl terminus (δ_C 175.28 in **1** and δ_C 182.81 in **2**). The field gradient HMBC (FG-HMBC, 500 MHz) spectrum of **1** revealed the long-range C−H spin couplings between C1 and H2 (δ_H 2.36). This makes it possible to follow proton connectivity from the C terminus H2 to H6, leading into the partial structure C6−C13. By using a combination of different concentrations and different mixing times (30−100 ms) at 40 °C, the TOCSY spectrum of **1** in 1:1 CD_3OD−Py-d_5 established the connection of C65 to C67 through C66 oxymethine (δ_H 3.23). The connection between C13 and C14 could not be established by spectroscopic methods because of the similarity of ^1H and ^{13}C NMR chemical shifts of H13 and H14 (δ_H 3.94) and C13 and C14 (δ_C 76.81). However, treatment of

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(9) ZT-A (**1**) was isolated from 70% EtOH extracts of the cultured alga by four steps: BuOH extraction, polystyrene colum chromatography (40% EtOH), DEAE−Sephadex column (1/30 M phosphate buffer pH 6.5), and HPLC on an ODS column (0.2 M NaCl in 70% EtOH as described previously.[4] **1**: amorphous solids; mp 125−127 °C; $[\alpha]^{24}_D$ +10.0° (*c* 0.10, MeOH); λ_{max} (MeOH) 233 nm (ϵ 29 000); ν_{max} (KBr) 3396, 2920, 1714, 1646, 880 cm^{-1}. Anal. Calcd for $C_{140}H_{232}O_{57}NSNa\cdot 18H_2O$: C, 52.20; H, 8.39; N, 0.43. Found: C, 52.42; H, 8.24; N, 0.47.
(10) 6 M HCl hydroysate of ZT-A (96 °C for 18 h) was analyzed by ion chromatography with an electroconductivity detector.

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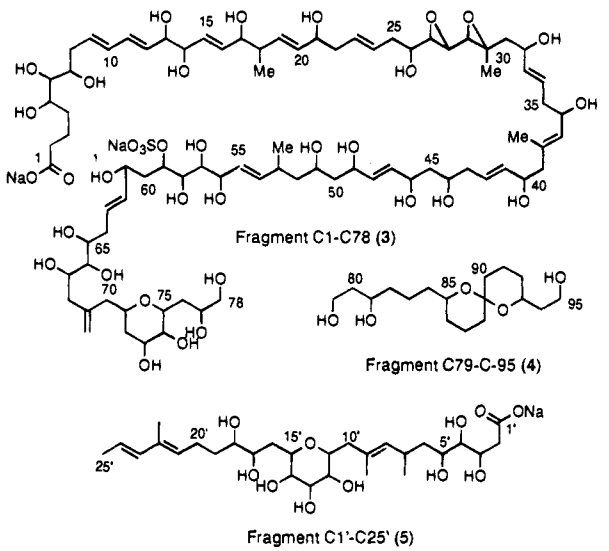

Figure 2. Structures of fragments C1−C78 (**3**) and C79−C95 (**4**) and of carboxylic acid **5**.

ZT-A with 3 equiv of $NaIO_4$ for 10 min at 0 °C followed by $NaBH_4$ reduction gave a carboxylic acid containing C1−C78 carbons, such as a conjugated diene (δ_H 5.78, 6.13, 6.25, 5.60), a bisepoxide (δ_H 2.98, 2.94, 2.66), a sulfate ester (δ_H 4.76), two singlet (δ_H 1.45, 1.70) and two doublet (δ_H 0.95, 1.01) methyls, and an *exo*-methylene (δ_H 4.96, 4.97) [**3**, negative FAB-MS, *m/z* 1724, (M-Na), structure shown in Figure 2], revealing the presence of a 62-membered lactone in **1**.

The spiroacetal structure of **1** was indicated by two sets of a series of high-field proton resonances with similar coupling patterns, together with C−H long-range couplings of C89 (δ_C 98.01) with one H88 and one H90 (δ_H 1.57). This structure was unambiguously established by the formation of spiroacetal **4** (Figure 2) upon treatment with a large excess of $NaIO_4$ (100 equiv in MeOH−H_2O at 0 °C for 2 h) followed by $NaBH_4$ reduction. The relative stereochemistry[11] of the spiro ring was verified by coupling constants $J_{85-86} = J_{92-93} = 12$ Hz) and NOESY (H85 ↔ H93) experiments (C_6D_6 at 25 °C) on the corresponding triacetate of **4**.

The HMQC spectrum of **1** in CD_3OD suggested the presence of only one methylene bearing a heteroatom. The methylene protons at C106 of **1** resonated at δ_H 3.28 and 3.68, which were correlated by the HMQC spectrum with a carbon signal at δ_C 46.53; this suggests the presence of an amide structure C1′-(=O)−NH−C106H_2−. The FG-HMBC spectrum of **1** verified the C−H long-range coupling of H106 to C1′ (δ_C 175.65) as well as to an acetal carbon (C105, δ_C 100.05). The carbonyl of the amide structure was chemically validated by production of a carboxylic acid [**5** (Figure 2), negative FAB-MS, *m/z* 559.3137, calcd for $C_{28}H_{47}O_{11}$, 559.3118, $[\alpha]^{19}_D$ +29.0° (*c* 0.42, MeOH)] upon treatment with 1.5 M LiOH in 2:1 H_2O−MeOH at 25 °C for 4.5 h.

The TOCSY spectrum in CD_3OD of **1** showed cross peaks at oxymethine signals, δ_H 3.83 (H99), 3.62 (H100), 4.04 (H95), and 3.12 (H96), with a previously unassigned methyl doublet C97Me (δ_H 1.04, d, $J = 7$ Hz) which reveals connection from C97Me to C97 and thence to C95 and C99 by separate paths. The DQF-COSY spectrum of **1** in CD_3OD at 23 °C clearly showed cross peaks among a series of six oxymethines, C99−C104 (δ_H 3.83, 3.62, 3.95, 3.81, 3.89, 3.72). On the basis of these data and the mass number obtained by the negative FAB-MS of **1**, the molecular formula of $C_{140}H_{232}NO_{57}SNa$ was assigned for **1**. From the chemical shift of C105, we propose the tetrahydropyran ring formation at the C101−C105 portion;[12] thus, the structure **1** was concluded for ZT-A.

Zooxanthellatoxin-A is shown to belong to a new class of macrolides containing the largest 62-membered ring in natural products.[13] Although various types of polyols and polyethers have been isolated from marine organisms, nitrogeneous polyols like palytoxin are rare.[2] Further studies on the detailed structure of zooxanthellatoxin-A and its congeners, including stereochemistry and structure−activity relationship, are in progress in our laboratory.

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Supplementary Material Available: Table of ^1H and ^{13}C NMR assignments of zooxanthellatoxin-A (**1**) in CD_3OD and CD_3OD−Py-d_5; comparison of NMR data of ZT-A (**1**), seco acid (**2**), fragment C1−C78 (**3**), C6−C13, C14−C65, C67−C77, C79−C95 (**4**), and C1′−C25′ (**5**); negative FAB-MS, ^1H and ^{13}C NMR, and 2D HMQC, HMBC, DQF-COSY, and TOCSY spectra of **1** with partial assignment; and 1D and 2D ^1H and ^{13}C NMR and negative FAB-MS spectra of **2**, **3**, **4**, and **5** (60 pages). This material is contained in many 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.

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(11) The relative stereochemistry on the ring portion of the fragments was determined as shown in the structure, but the relative stereochemistry between ring-containing structures is not know.

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