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Structures of Periodate Oxidation Products with a Conjugated Diene or an Exomethylene from Zooxanthellatoxin-A

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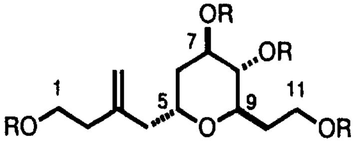
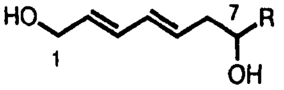
Abstract: Partial structures of zooxanthellatoxin-A were established on the basis of spectroscopic analyses of degradation products prepared by periodate oxidation followed by reduction with $NaBH_4$.

Zooxanthellatoxins are potent vasoconstrictive polyenepolyols isolated from a symbiotic dinoflagellate (zooxanthellae) *Symbiodinium* sp.[1] These compounds are large molecules with a molecular weight of about 2900 and contain characteristic functionalities such as a diepoxide, an exomethylene, a sulfate ester, two carbonyl, two acetal, and two conjugated diene groups. We recently reported the structures of major fragments with a sulfate ester, a diepoxide, and olefin alcohols prepared by sodium borohydride reduction of periodate oxidation products of zooxanthellatoxin-A (ZT-A).[2] Here we wish to report structures of periodate oxidation products with a conjugated diene or an exomethylene units from ZT-A.



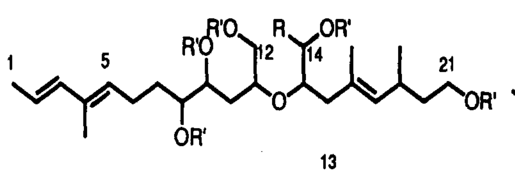
1 : R=H
1a : R=Ac



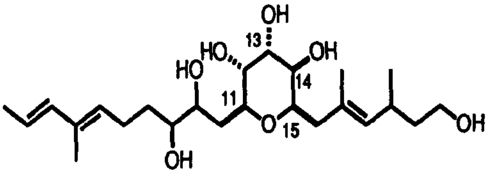
2 : R=H
3 : R=CH_2OH
8

Sodium borohydride reduction of periodate oxidation (25 eq.) products of ZT-A (50 mg) yielded a complex mixture of alcohols, which was fractionated on a polystyrene column with H_2O-EtOH of increasing EtOH content (0-100%). The 20% EtOH fraction was purified on a silica gel column (5:1 CH_2Cl_2-MeOH) to afford crude exomethylene 1 (3.7 mg), and conjugated dienes 2 (1.1 mg) and 3 (1.2 mg). The 60% EtOH portion was separated on a silica gel column (5:1 CH_2Cl_2-MeOH) to give conjugated dienes 4 (3.5 mg) and 5 (3.0 mg).

Upon treatment with Ac_2O/Py, **1** afforded a tetraacetate (**1a**, 0.8 mg) after chromatography on a silica gel column. FDMS spectrum of **1a** showed a pseudomolecular ion at *m/z* 415 $(M+H)^+$. The ^1H NMR spectrum of **1a** in C_6D_6 contained signals for four acetyls (δ1.71, 1.72, 1.74, 1.75), one exomethylene (δ4.79, 4.81), two acetoxymethylenes [δ4.10 (2H, t, *J*=7 Hz, H-1), 4.21 (2H, AB center, H-11)] and two acetoxymethines [δ5.33 (1H, ddd, *J*=9, 8, 5 Hz, H-7), 4.98 (1H, t, *J*=8 Hz, H-8)] at lower field than 4 ppm, and signals for two allylic methylenes [δ2.17 (2H, t, *J*=7 Hz, H-2), 1.89 (1H, dd, *J*=15, 6 Hz, H-4), 2.34 (1H, dd, *J*=15, 9 Hz, H-4)], two methylenes [δ1.67 (1H, m, H-6) and1.78 (1H, m, H-6) and 1.88-1.94 (2H, m, H-10)], and two oxymethines [δ3.92 (1H, m, H-5), 3.79 (1H, dt, *J*=4, 8 Hz, H-9)]. In the ^1H NMR spectrum of crude **1** in CD_3OD, the signals for H-5 and H-9 were found to have the similar chemical shifts [δ4.19 (1H, m, H-5), 3.57 (1H, m, H-9)], suggesting that **1** is a terahydropyran tetraol with an exomethylene. The structure of **1a** was determined by homo-spin decoupling experiments. The relative stereochemistry at C-7, C-8 and C-9 was deduced on the basis of a large coupling constant of 8 Hz whereas one at C-5 was determined on the basis of small coupling constants of H-5 with H-6α and H-6β (~4 Hz) analyzed by the decoupling difference experiments.



4 : R=H, R'=H **5** : R=CH_2OH, R'=H
4a : R=H, R'=Ac **5a** : R=CH_2OAc, R'=Ac



6

In contrast to the simple structure of conjugated dienes **2**[3] and **3**[4], both the trisubstituted conjugated dienes **4** and **5** contained several characteristic functionalities such as four methyls, three hydroxylmethylenes, and trisubstituted olefin groups, indicating that **4** and **5** have a homologous structure originated from the same structural unit of ZT-A. DQF-COSY spectra of **4** and **5** in CD_3OD revealed that they contained the same substructure from C-1 to C-12.

Negative FABMS spectra of **4**, *m/z* 413.2887 $(M-H)^-$, and **5**, *m/z* 443.3009 $(M-H)^-$ established the molecular formula of $C_{23}H_{42}O_6$ (Δ -1.6 mmu) and $C_{24}H_{44}O_7$ (Δ +0.7 mmu) for **4** and **5**, respectively. DQF COSY spectra of **4** and **5** confirmed carbon connectivities from C-14 to C-21 and one additional oxymethine was shown to exist between C-15 and C-13 in **5**.[5] Configuration of the double bond at C-2 in **5** was determined on the basis of a large coupling constant and those at C-4 and C-17 were deduced by NOE experiments (H-3→H-5 and H-18→H-16). Although deuterium effects on chemical shifts of ^{13}C NMR signals of **4** failed to establish the position of an ethereal linkage, acetylation of **4** confirmed the ethereal linkage between C-11 and C-15 (Table 1). Periodate oxidation of ZT-A with 3 eq. of $NaIO_4$ in $MeOH-H_2O$ at 0 °C followed by $NaBH_4$ reduction gave the corresponding tetrahydropyran compound **6**, $[\alpha]_D^{24}$ +23 ° (*c* 0.04, MeOH), (0.8 mg from 21.0 mg of ZT-A).

Positive FABMS spectrum of **6** confirmed the molecular formula of $C_{24}H_{42}O_7$ [*m/z* 443.2993, $(M+H)^+$ Δ 0.7 mmu]. ^1H NMR spectrum of **6** in CD_3OD displayed proton signals for H-1~H-9 and

Table 1. ^{1}H NMR and ^{13}C NMR data for the conjugated dienes **4**, **4a**, **5**, and **6**.[a]

	4		**4a**	**5**	**6**
No.	δ_C[b]	δ_H[c]	δ_H[d]	δ_H[c]	δ_H[c]
1	18.6	1.78 (d, 7)	1.70 (d, 6)	1.78 (d, 7)	1.79 (d, 7)
2	122.9	5.61 (dq, 16, 7)	5.55 (dq, 16, 6)	5.61 (dq, 16, 7)	5.61 (dq, 16, 7)
3	137.7	6.10 (br d, 16)	6.18 (br d, 16)	6.09 (br d, 16)	6.09 (br d, 16)
4	134.7	-			
5	130.8	5.39 (t, 7)	5.39 (t, 7)	5.40 (t, 7)	5.39 (t, 7)
6	25.8	2.25 (m)	2.23 (q, 7)	2.25 (dt, 14, 7)	2.24 (m)
		2.33 (m)		2.34 (dt, 14, 7)	2.31 (m)
7	34.3	1.52-1.62 (m)	1.60-1.80 (m)	1.52-1.69 (m)	1.51-1.67 (m)
8	75.7	3.42 (dt, 9, 4)	5.26 (dt, 8, 4)	3.43 (dt, 9, 4)	3.44 (dt, 9, 4)
9	72.0	3.70 (m)	5.55(m)	3.69 (m)	3.75 (m)
10	37.5	1.57 (m)	1.80-1.91 (m)	1.56 (m)	1.51-1.63 (m)
		1.65 (m)	1.63 (m)		2.01 (ddd, 14, 10, 2)
11	78.2	3.84 (m)	3.80 (dq, 7, 5)	3.78 (m)	3.77 (dt, 3, 7)
12	66.6	3.56 (m)	4.14 (dd, 12, 5)	3.56 (dd, 12, 6)	3.56 (dd, 10, 4)
		3.72 (m)	4.22 (dd, 12, 5)	3.71 (dd, 12, 4)	
13				3.55-3.62 (m)	3.93 (t, 4)
				3.67-3.73 (m)	
14	65.6	3.45 (dd, 12, 7)	4.15 (12, 7)	3.63 (m)	3.60 (br d, 4)
		3.67 (m)	4.35 (dd, 12, 3)		
15	80.1	3.76 (m)	3.96 (m)	3.83 (ddd, 9, 5, 2)	3.95 (br t, 7)
16	43.8	2.12 (dd, 13, 9)	2.30 (dd, 13, 9)	2.39 (dd, 14, 5)	2.22 (dd, 13, 7)
		2.41 (dd, 13, 5)	2.58 (dd, 13, 5)	2.45 (dd, 14, 9)	2.30 (dd, 13, 7)
17	132.2				
18	135.5	5.01 (d, 10)	4.99 (d, 10)	5.10 (d, 9)	5.05 (d, 10)
19	30.5	2.58 (m)	2.45 (m)	2.59 (m)	2.59 (m)
20	41.7	1.46 (m)	1.41 (m)	1.47 (m)	1.47 (m)
		1.60 (m)	1.59 (m)	1.59 (m)	1.62 (m)
21	61.6	3.50-3.62 (m)	4.02-4.15 (m)	3.51-3.61 (m)	3.50-3.59 (m)
4Me	12.9	1.77 (s)	1.76 (s)	1.77 (s)	1.77 (s)
17Me	17.2	1.73 (s)	1.74 (s)	1.73 (s)	1.72 (s)
19Me	21.8	0.99 (d, 7)	0.91 (d, 6)	0.99 (d, 6)	0.99 (d, 7)
OAc			1.76, 1.77, 1.79		
			1.79, 1.87		

a: δ in ppm (multiplicity, coupling constant in Hz).

b: 100 MHz in CD_3OD at 40 °C. CD_3OD was used as an internal standard (δ=49.3). Assignment was based on HMQC, HMBC, and DEPT experiments.

c: 400 MHz or 500 MHz in CD_3OD at 25-27 °C. CHD_2OD was used as an internal standard (δ=3.35). Assingment was based on DQF-COSY spectra and homo-spin decoupling.

d: 400 MHz in C_6D_6 at 25 °C. C_6HD_6 was used as an internal standard (δ=7.20).

H-16~H-21 similar to those of **4** and **5**. DQF-COSY spectrum of **6** in CD$_3$OD allowed to follow the proton sequences, C-4Me to C-14 and the connectivity between C-14 and C-15 was established by homo-spin decoupling experiments. Relative stereochemistry on the tetrahydropyran ring was deduced on the basis of coupling constants and NOE experiments (H-11→H-15).

These degradation products and the previously reported fragments revealed location of the characteristic functionalities in ZT-A including all of double bonds, a diepoxide, an exomethylene, two conjugated dienes, and a sulfate ester. These data as well as pharmacological properties of zooxanthellatoxins suggested that zooxanthellatoxins were new type of bioactive marine natural products.[6]

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2. Nakamura, H., Asari. T., Murai, A., Kondo, T., Yoshida, K., and Ohizumi, Y., *J. Org. Chem.*, **1993**, *58*, 313-314.
3. **2**: UV (MeOH) λmax 229 nm (ε20,000); EIMS *m/z* 128 (M$^+$), 110 (M-H$_2$O)$^+$; HR-EIMS *m/z* 110.0072 [(M-H$_2$O)$^+$, Δ -0.7 mmu]; 400 MHz ^{1}H NMR (CD$_3$OD, 25 °C) δ2.34 (2H, br q, *J*=7 Hz, H-6), 3.62 (2H, t, *J*=7Hz, H-7), 4.11 (2H, d, *J*=6 Hz, H-1), 5.74 (1H, dt, *J*=15, 7 Hz, H-5), 5.75 (1H, dt, *J*=15, 7 Hz, H-2), 6.17 (1H, dd, *J*=15, 10 Hz, H-4), 6.26 (1H, dd, *J*=15, 10 Hz, H-3).
4. **3**: HR-EIMS *m/z* 140.0857 [(M-H$_2$O)$^+$, Δ 2.0 mmu]; 400 MHz ^{1}H NMR (CD$_3$OD, 25 °C) δ2.23 (1H, dt, *J*=14, 8 Hz, H-6), 2.36 (1H, *J*=14, 7 Hz, H-6), 3.47 (1H, dd, *J*=11, 6 Hz, H-8), 3.53 (1H, dd, *J*=11, 5 Hz, H-8), 3.67 (1H, m, H-7), 4.12 (2H, d, J=6 Hz, H-1), 5.74 (1H, dt, *J*=15, 6 Hz, H-5), 5.78 (1H, dt, *J*=15, 6 Hz, H-2), 6.17 (1H, dd, *J*=15, 12 Hz, H-4), and 6.27 (1H, dd, *J*=15, 12 Hz, H-3).
5. The conjugated diene (**5**) gave a hexaacetate (**5a**) by acetylation with Ac$_2$O/Py. **5a**: 400 MHz ^{1}HNMR (C$_6$D$_6$, 25 °C) δ0.96 (3H, d, *J*=7 Hz, C-19Me), 1.57 (1H, m, H-20), 1.61-1.79 (2H, m, H-7), 1.70 (3H, d, *J*=7 Hz, H-1), 1.71 (3H, s, C-17Me), 1.76 (3H, s, C-4Me), 1.75 (3H, s, Ac), 1.80 (3H, s, Ac), 1.81 (3H, s, Ac), 1.82 (3H, s, Ac), 1.83 (3H, s, Ac), 1.85 (3H, s, Ac), 1.79-1.92 (1H, m, H-10), 2.23 (2H, br q, H-6), 2.43 (2H, AB center, H-16), 2.50 (2H, m, H-19), 3.85 (1H, m, H-11), 3.98 (1H, ddd, *J*=8, 6, 3 Hz, H-15), 4.09 (1H, dd, *J*=12, 5 Hz, H-12), 4.15 (1H, dd, *J*=12, 4 Hz, H-12), 4.17 (2H, t, *J*=6 Hz, H-21), 4.44 (1H, dd, *J*=12, 8 Hz, H-13), 4.57 (1H, dd, J=12, 5 Hz, H-14), 5.11 (1H, d, *J*=10 Hz, H-18), 5.23 (1H, dt, *J*=8, 4 Hz, H-8), 5.40 (1H, t, *J*=7 Hz, H-5), 5.50 (1H, dt, *J*=9, 4 Hz, H-9), 5.52 (1H, m, H-14), 5.56 (1H, dq, *J*=16, 7 Hz, H-2), 6.18 (1H, br d, *J*=16 Hz, H-3).
6. Faulkner, D. J., *J. Nat. Prod. Rep.*, **1984**, *1*, 251, 551; **1986**, *3*, 1; **1987**, *4*, 539; **1988**, *5*, 613; **1990**, *7*, 269; **1991**, *8*, 97.

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