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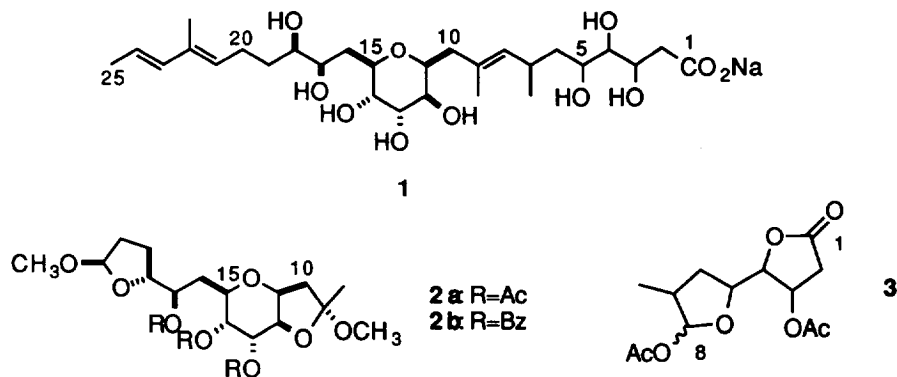
## Synthetic Studies on the Common C25 Long Chain Acid Portion of Zooxanthellatoxins from a Symbiotic Dinoflagellate *Symbiodinium* sp.

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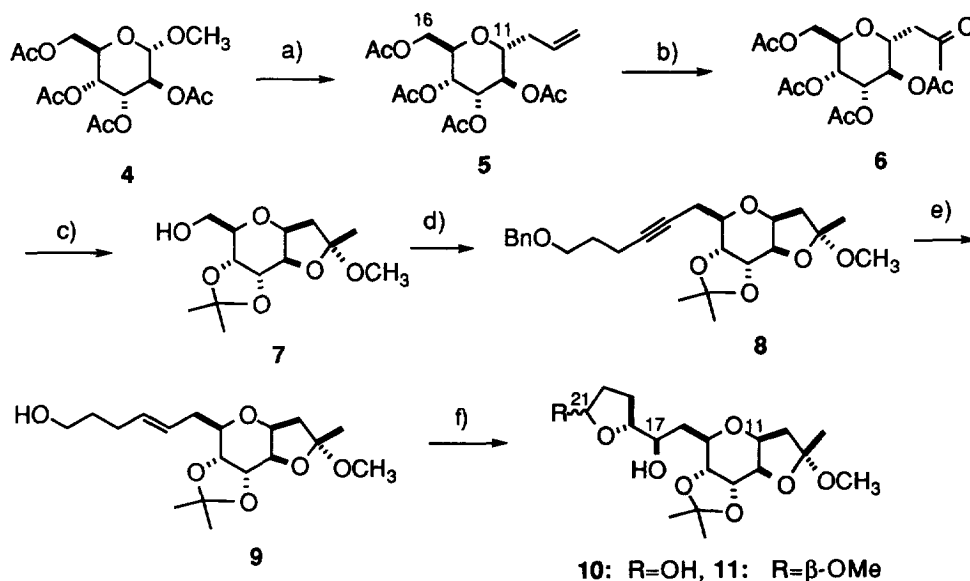
**Abstract:** The absolute stereochemistry of a portion from C11 to C18 of the common C25 long chain acid part of zooxanthellatoxins was determined by synthesizing the degradation product **2a** to be 11*S*, 12*R*, 13*S*, 14*R*, 15*R*, 17*R*, and 18*R*. Copyright © 1996 Elsevier Science Ltd

Marine micro-algae produce various types of compounds including nitrogenous neurotoxins, polyether sea food toxins, sulfonium compounds of dimethylsulfide precursors, and antineoplastic macrolides.<sup>1-3</sup> Zooxanthellatoxin-A (ZT-A) and ZT-B have been isolated from a symbiotic marine dinoflagellate *Symbiodinium* sp. (strain No. Y-6).<sup>4</sup> The structures contained several common structural units and consisted with two portions connected through an amide linkage, *i.e.*, the major amino acid portion forming a 62-membered lactone and the common terminal acid with C25 chain length (**1**).<sup>5</sup> ZT-A and ZT-B showed similar biological activities such as vasoconstrictive and platelet aggregation activities.<sup>3,6</sup>



In order to elucidate the mechanisms of biological activities of non-peptidic large molecules, we started to study on the structure-activity relationships and the absolute stereochemistry of zooxanthellatoxins by synthesizing their fragments obtained during the degradation experiments. Here, we report the synthesis and the absolute stereochemistry of the fragment containing C9-C21 portion of the common C25 long chain acid (1) in zooxanthellatoxins.

The acid portion 1 was obtained in a good yield from both ZT-A and ZT-B by alkaline hydrolysis. The relative stereochemistry at C11-C15 was deduced on the basis of the  $^3J_{\text{H-H}}$  coupling constants of H11 to H15 protons.<sup>5d</sup> Stereochemistry in the acyclic portion was unable to be clarified by spectroscopic methods. Ozonolysis of 1 followed by reduction with  $\text{Me}_2\text{S}$ , acetalization with  $\text{HCl-MeOH}$ , and acetylation afforded diacetate 2a containing the C9-C21 portion and a 1:1 mixture of methyl acetals of a  $\gamma$ -lactone (3) derived from the C1-C8 unit of zooxanthellatoxins. The structure 2a was confirmed by the synthesis started from known methyl altroside tetraacetate (4) by a sequence of the following reactions.<sup>7</sup>



**Reagents and conditions:** a) allylsilane,  $\text{BF}_3 \cdot \text{OEt}_2$ , TMSOTf,  $\text{CH}_3\text{CN}$ ,  $-10^\circ\text{C}$ – $23^\circ\text{C}$ , 24 h, 71%. b)  $\text{O}_2$ ,  $\text{PdCl}_2$ ,  $\text{CuCl}$ ,  $\text{DMF-H}_2\text{O}$ , rt, 45 h, 92%. c)  $\text{MeONa}$ ,  $\text{MeOH}$ ,  $0^\circ\text{C}$ , 2h;  $t\text{-BuOK}$ ,  $t\text{-BuOH}$ ,  $27^\circ\text{C}$ , 19 h; 1:1 dimethoxypropane- $\text{MeOH}$ , CSA,  $23^\circ\text{C}$ , 8 h, 44% from 6. d)  $\text{Tf}_2\text{O}$ ,  $\text{Et}_3\text{N}$ ,  $\text{CH}_2\text{Cl}_2$ ,  $-78^\circ\text{C}$ , 20 min;  $\text{BnOCH}_2\text{CH}_2\text{CH}_2\text{C}\equiv\text{CLi}$ ,  $\text{THF}$ ,  $-78^\circ\text{C}$ , 1 h, 61%. e)  $\text{Li}$ ,  $\text{NH}_3$ ,  $\text{THF}$ ,  $-78^\circ\text{C}$ , 7 h, 90%. f) Swern oxidation, 89%; AD-mix- $\beta$ ,  $\text{MeSO}_2\text{NH}_2$ , 1:1  $\text{H}_2\text{O}$ - $t\text{-BuOH}$ ,  $0^\circ\text{C}$ , 25 h, 96%.

The glycoside 4 prepared from methyl D-glucoside<sup>8</sup> was treated with allyltrimethylsilane to afford C-glycoside 5 having the opposite stereochemistry at C11. Wacker oxidation of 5 gave methyl ketone 6<sup>9</sup> and the stereochemistry at C11 was adjusted by a base treatment after deacetylation of 6. Acetalization of the reaction

mixture with 1:1 MeOH-dimethoxypropane gave methyl acetal **7**<sup>10</sup> in which the hydroxyl groups at C-13 and 14 and the methyl ketone were protected with an acetonide and a methyl acetal groups, respectively.

Chain elongation at C16 was achieved by the successive three step reactions, triflation, coupling reaction with an alkynyl lithium, and reduction under Birch conditions to yield *trans*-olefin alcohol **9**. Introduction of two new chiral centers was conducted after oxidation of the terminal hydroxyl group with Sharpless asymmetric dihydroxylation reagents (AD-mix)<sup>11</sup> to give a mixture of diol **10**. The diols were converted to dimethylacetal triacetate **2a**<sup>12</sup> in 37% yield by the following reactions; 1) removal of an acetonide in 80% AcOH at 23 °C for 24 h, 2) acetalization with HCl-MeOH at 23 °C for 30 min, and 3) acetylation with Ac<sub>2</sub>O-Py at 23 °C for 16 h. The synthetic **2a** was identical with one from natural products whereas a diacetate from **9** with AD-mix- $\alpha$  and the isomers prepared from *cis*-olefin alcohol with AD-mix- $\alpha$  were not identical.<sup>13</sup> The stereochemistry at C17 position was established to be *R* by applying advanced Mosher method<sup>14</sup> to a major dimethyl acetal **11**<sup>15</sup> prepared from **10**. Configurations at C9 and C21 of **2a** were determined by the NOESY spectrum (from each two MeO signals to H-12 and H-18 signals, respectively). Thus the stereochemistry of the synthetic **2a** was proved as shown in the figure.

Absolute stereochemistry of **2a** from zooxanthellatoxins was confirmed to be the same to that of synthetic one by comparing their  $[\alpha]_D$  and the CD spectra of their benzoate derivative **2b**.<sup>16</sup> The stereochemistry in C11 to C18 of the common C25 long chain acid **1** was established chemically to be 11*S*, 12*R*, 13*S*, 14*R*, 15*R*, 17*R*, and 18*R*.

The C25 acid is the common terminal structure of ZT-A and ZT-B although their main chain structures are different each other in terms of their chain length and their oxidation state. It may be suggested that the C25 acid might be derived biogenetically before amidation with the main chains. Studies on the stereochemistry of C1-C8 portion and total synthesis of the C25 acid **1** are in progress at our laboratory.

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7. All new compounds gave satisfactory spectral data.

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9. **6**:  $[\alpha]_{\text{D}}^{20} +5.3^{\circ}$  (c 3.15,  $\text{CHCl}_3$ ); IR (film) 1748, 1374, 1228, and  $1176\text{ cm}^{-1}$ ;  $^1\text{H NMR}$  (270 MHz,  $\text{CDCl}_3$ )  $\delta$  2.05 (3H, s), 2.08 (3H, s), 2.11 (3H, s), 2.13 (3H, s), 2.19 (3H, s), 2.57 (1H, dd,  $J=16$ , 4 Hz), 2.87 (1H, dd,  $J=16$ , 9 Hz), 4.10-4.20 (2H, m), 4.30 (1H, td,  $J=9$ , 4 Hz), 4.49 (1H, dd,  $J=11$ , 6 Hz), 5.04 (1H, t,  $J=9$  Hz), and 5.26 (2H, m); HR-FABMS  $m/z$  389.1425 (M+H)<sup>+</sup>. Calcd for  $\text{C}_{17}\text{H}_{25}\text{O}_{10}$  389.1448.
10. **7**:  $[\alpha]_{\text{D}}^{22} -20.9^{\circ}$  (c 8.54, MeOH);  $^1\text{H NMR}$  (400 MHz,  $\text{CDCl}_3$ )  $\delta$  1.36 (3H, s), 1.46 (6H, s), 1.97 (1H, br s, exchangeable), 2.06 (1H, d,  $J=15$  Hz), 2.32 (1H, dd,  $J=15$ , 6 Hz), 3.23 (1H, m), 3.24 (3H, s), 3.58 (1H, br d,  $J=11$ , 6 Hz), 3.84 (1H, br d,  $J=11$  Hz), 3.95 (1H, dd,  $J=10$ , 6 Hz), 4.16 (1H, dd,  $J=3$ , 1 Hz), 4.19 (1H, dd,  $J=6$ , 3 Hz), and 4.44 (1H, br d,  $J=6$  Hz); HR-EIMS  $m/z$  274.1396 Calcd for  $\text{C}_{13}\text{H}_{22}\text{O}_6$  274.1417.
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12. Synthetic **2a**:  $[\alpha]_{\text{D}}^{21} -32.9^{\circ}$  (c 1.18,  $\text{CHCl}_3$ ); IR (film) 2956, 1750, 1376, 1242, and  $1100\text{ cm}^{-1}$ ;  $^1\text{H NMR}$  (400 MHz,  $\text{CDCl}_3$ )  $\delta$  1.48 (3H, s, H8), 1.56 (1H, m, H19), 1.65 (1H, m, H16), 1.83 (1H, m, H20), 1.92 (1H, m, H16), 1.97 (1H, m, H20), 2.01 (1H, m, H19), 2.01 (1H, m, H10), 2.03 (3H, s), 2.10 (3H, s), 2.11 (3H, s), 2.21 (1H, dd,  $J=14$ , 6 Hz, H10), 3.23 (3H, s), 3.32 (3H, s), 3.59 (1H, ddd,  $J=10$ , 9, 2, H15), 3.92 (1H, dd,  $J=4$ , 3 Hz, H12), 4.11 (1H, dt,  $J=7$ , 3 Hz, H18), 4.18 (1H, dd,  $J=4$ , 2 Hz, H11), 4.94 (1H, dd,  $J=9$ , 3 Hz, H14), 5.19 (1H, d,  $J=5$  Hz, H21), 5.21 (1H, ddd,  $J=10$ , 4, 3 Hz, H17), and 5.40 (1H, t,  $J=3$  Hz, H13); HR-FABMS  $m/z$  473.1998 (M-H)<sup>+</sup>. Calcd for  $\text{C}_{22}\text{H}_{33}\text{O}_{11}$  473.2023. **2a** from ZT;  $[\alpha]_{\text{D}}^{23} -38^{\circ}$  (c 0.01,  $\text{CHCl}_3$ ).
13. Dihydroxylation of the cis-olefin alcohol with AD-mix- $\alpha$  was not diastereoselective and gave a mixture of possible diol isomers.
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15. Treatment of **10** with HCl-MeOH for 10 min at  $23^{\circ}\text{C}$  gave **11** after HPLC separation. **11**:  $^1\text{H NMR}$  (400 MHz,  $\text{CDCl}_3$ )  $\delta$  1.37 (3H, s), 1.46 (3H, s), 1.47 (3H, s), 1.53 (1H, m), 1.70 (1H, m), 1.80-2.05 (4H, m), 2.02 (1H, d,  $J=15$  Hz), 2.32 (1H, dd,  $J=15$ , 6 Hz), 2.55 (1H, d,  $J=6$  Hz), 3.24 (3H, s), 3.34 (3H, s), 3.42 (1H, dt,  $J=10$ , 3 Hz), 3.73 (1H, m), 3.86 (1H, dd,  $J=10$ , 6 Hz), 3.98 (1H, dt,  $J=7$ , 5 Hz), 4.17 (2H, m), 4.43 (1H, d,  $J=6$  Hz), 5.05 (1H, dd,  $J=5$ , 1 Hz).
16. Deacetylation of **2a** followed by benzylation in  $\text{CH}_2\text{Cl}_2$  with benzoic acid, DCC, and DMAP gave **2b**:  $^1\text{H NMR}$  (400 MHz,  $\text{CDCl}_3$ )  $\delta$  1.25 (3H, s), 1.50-1.72 (2H, m), 1.76-1.96 (3H, m), 2.12 (1H, d,  $J=15$  Hz), 2.07-2.17 (1H, m), 2.23 (1H, dd,  $J=15$ , 6 Hz), 3.26 (3H, s), 3.28 (3H, s), 3.98 (1H, t,  $J=9$  Hz), 4.11 (1H, t,  $J=3$  Hz), 4.24 (1H, m), 4.32 (1H, m), 5.03 (1H, d,  $J=5$  Hz), 5.34 (1H, dd,  $J=4$ , 3 Hz), 5.54 (1H, m), 5.79 (1H, t,  $J=3$  Hz), 7.23 (2H, t,  $J=8$  Hz), 7.32 (2H, t,  $J=8$  Hz), 7.45 (2H, t,  $J=8$  Hz), 7.48 (1H, t,  $J=8$  Hz), 7.49 (1H, t,  $J=8$  Hz), 7.60 (1H, t,  $J=8$  Hz), 7.82 (2H, d,  $J=8$  Hz), 7.84 (2H, d,  $J=8$  Hz), 8.08 (2H, d,  $J=8$  Hz); CD (2-propanol) 237.5 nm ( $\Delta\epsilon +5.9$ ) and 224.0 nm ( $-3.0$ ). **2b** from **1**: CD (2-propanol) 237.5 nm (+3.3) and 224.0 nm (-2.2).

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