Pergamon

Tetrahedron Letters 41 (2000) 1927–1930

TETRAHEDRON
LETTERS

Absolute configuration of the common terminal acid portion of zooxanthellatoxins from a symbiotic dinoflagellate *Symbiodinium* sp. established by the synthesis of its ozonolysis product

Hideshi Nakamura,[a,*] Kyoko Maruyama,[b] Kazuhiro Fujimaki [b] and Akio Murai [b]

[a]*Division of Biomodeling, Department of Applied Molecular Biosciences, Graduate School of Bioagricultural Sciences, Nagoya University, Chikusa, Nagoya 464-8601, Japan*

[b]*Division of Chemistry, Graduate School of Science, Hokkaido University, Sapporo 060-0810, Japan*

Received 4 October 1999; revised 3 December 1999; accepted 28 December 1999

Abstract

To determine the absolute configuration of the common C25 long chain acid part (L) of zooxanthellatoxins, the degradation product derived from the terminal carboxylic acid portion of L was synthesized from glucose and used to establish that the total absolute configuration of L was 3*R*,4*R*,5*S*,7*S*,11*S*,12*S*,13*R*,14*S*,15*R*,17*R*,18*R*. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: marine metabolite; polyketides; configuration; lactones.

Marine micro-algae produce various types of compounds including nitrogenous neurotoxins, polyether seafood toxins, sulfonium compounds as precursors of dimethylsulfide, and antineoplastic macrolides.[1–3] However, there are only a few examples of large polyoxygenated molecules with a known absolute configuration. Zooxanthellatoxin-A (ZT-A) and ZT-B have been isolated from a symbiotic marine dinoflagellate *Symbiodinium* sp. (strain No. Y-6) as vasoconstrictive components.[4] Their structures contain several common features such as a 62-membered lactone, a sulfate ester, an exomethylene and an amide linkage.[5] While the major long-chain amino acid portions including the 62-membered lactone vary with regard to the length of the carbon chain and the number of hydroxyl groups, the terminal acid portions (compound L, **1**) prepared from ZT-A and -B by basic hydrolysis are identical, including their absolute configuration.[5]

To elucidate the absolute structure of zooxanthellatoxins, we started by investigating the absolute configuration of the fragments obtained by degradation experiments.[6] Ozonolysis of L followed by treatment with HCl–MeOH and then acetic anhydride and pyridine gave two fragments which included the tetrahydrofuran ring portion (C9–C21) and the terminal carboxylic acid part (C1–C8), respectively.[6a]

* Corresponding author. Tel: 81-52-789-4284; fax: 81-52-789-4284; e-mail: hnak@agr.nagoya-u.ac.jp (H. Nakamura)

0040-4039/00/$ - see front matter © 2000 Elsevier Science Ltd. All rights reserved.
PII: S0040-4039(00)00062-9

We have previously reported the synthesis and absolute configuration of the tetrahydrofuran fragment.[6a] We report here the synthesis of the rest of the molecule, which fully characterizes compound L.

The relative configuration of the two tetrahydrofurans of **2** was difficult to elucidate by their NMR data because of the unreliability of the coupling constants and NOE in a five-membered ring system. The large influence of the side chain structure on the ^{1}H NMR spectral pattern of a γ-lactone prevented us from deducing the relative configuration at C3–C5 on the basis of comparison of the ^{1}H NMR data of either γ-lactone derivatives of L (**1a**) such as its heptaacetate **1b** or a mixture of acetals of the ozonolysis product (**2**) with those of the possible four diastereomeric γ-lactone models prepared from hexoses. Finally, we applied a newly developed *J*-based configuration analysis by considering a population of the possible rotamers and elucidated the relative configuration as shown in Fig. 1; i.e. an all-*syn* configuration of the triol and an *anti* configuration of the methyl group.[7] To confirm the reliability of the new method and to determine the absolute configuration, we started the synthesis of γ-lactones **2** from readily available glucose derivative **3** by a synthetic route applicable to the syntheses of other isomers, especially at C3 and C7 (Fig. 2).

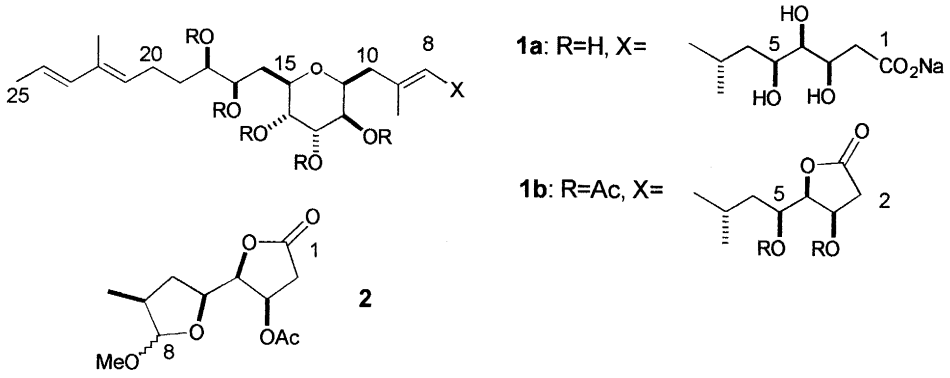

Fig. 1. Compound L (**1**) and related compounds

Benzylidene acetal **3** was brominated under conditions described in the literature and reductive opening of the pyranose ring gave an aldehyde[8] which was converted to α,β-unsaturated ester **4** by treating it with methyl (triphenylphoranylidene)acetate. 1,4-Reduction with CuH[9] followed by debenzoylation yielded an alcohol (**5**). Introduction of a methyl group was achieved by Evans' procedure[10,11] after protection of the hydroxyl group with benzyloxymethyl chloride (BOMCl) and diisopropylethylamine (DIPEA). The absolute configuration of the introduced methyl was confirmed by derivatizing to the amides of (*S*)- and (*R*)-phenylglycine methyl esters (PGME). The proton chemical shift of the methyl group of the (*R*)-PGME amide was smaller than that of (*S*)-PGME.[12] Reduction of the ester with lithium aluminum hydride afforded a primary alcohol (**7**). Treatment of the alcohol with benzyl bromide and *t*-BuOK gave a benzyl ether, which was transformed to **8** by five steps: (1) deprotection with HCl–MeOH; (2) protection with MOMCl; (3) hydroboration; (4) Swern oxidation; and (5) oxidation with $NaClO_2$.

A 1:1 mixture of the acetals **2** was obtained in four steps. The ^{1}H NMR spectra of the synthetic **2** in two different solvents were superimposable to those of the acetals derived from L, respectively [400 MHz in C_6D_6: two pairs of unassignable methoxy (δ 3.25, 3.19) and acetyl signals (δ 1.48, 1.50) and the signals for the isomer A; δ 0.88 (3H, d, *J*=7 Hz), 1.09 (ddd, *J*=12, 8, 6 Hz, H6), 1.81 (m, H6′), 2.14 (m, H7), 2.21 (dd, *J*=18, 7 Hz, H2), 2.49 (dd, *J*=18, 5 Hz, H2′), 3.92 (dd, *J*=6, 4 Hz, H4), 4.18 (ddd, *J*=8, 8, 4 Hz, H5), 4.51 (d, *J*=2 Hz, H8), 4.87 (ddd, *J*=7, 6, 5 Hz, H3): for isomer B; δ 0.98 (3H, d, *J*=7 Hz, Me), 1.37 (td, *J*=12, 10 Hz, H6), 1.53 (td, *J*=12, 7 Hz, H6′), 1.81 (m, H7), 2.09 (dd, *J*=18, 7 Hz, H2), 2.34 (dd, *J*=18, 3 Hz, H2′), 3.95 (dd, *J*=6, 5 Hz, H4), 4.22 (ddd, *J*=10, 7, 6 Hz, H5), 4.57 (d, *J*=5

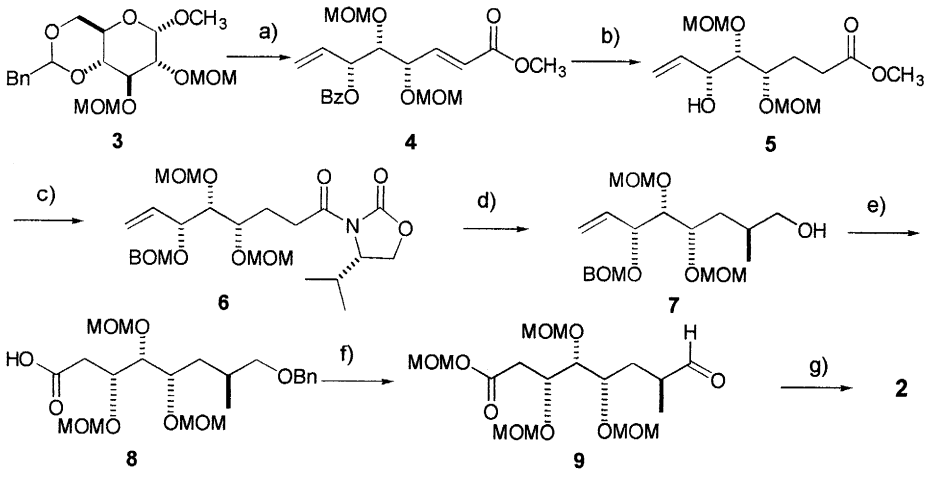

Fig. 2. Synthesis of a γ-lactone (**2**). Reagents and conditions: (a) (1) NBS, AIBN, $BaCO_3$, CCl_4, reflux, 1 h; (2) Zn, EtOH–H_2O, reflux, 3 h; (3) $Ph_3P{=}CHCOOCH_3$, benzene, 23°C, 23 h, 77% for three steps; (b) (1) $NaBH_4$, $CuCl_2$, MeOH, 23°C, 3 h; (2) NaOMe, MeOH, 23°C, 7 h, 46% for two steps; (c) (1) BOMCl, DIPEA, CH_2Cl_2, 23°C, 2 days, 88%; (2) NaOH, MeOH–H_2O, 0°C, 18 h, 92%; (3) PivCl, Et_3N, THF, LiCl, (4*S*)-4-isopropyl-2-oxazolidinone, −20–23°C, 6.5 h, 97%; (d) (1) LDA, MeI, THF, −78–−40°C, 22 h, 77%; (2) LAH, THF, 0°C, 35 min; (e) (1) BnBr, *t*-BuOK, THF, 0°C, 5.5 h, 92%; (2) 4% HCl–MeOH, 23°C, 3 days, 78%; (3) MOMCl, DIPEA, 23°C, 2 days, 100%; (4) BH_3–THF, THF, 0°C, 10 h, then H_2O_2, NaOH, MeOH–H_2O, 0–23°C, 2.5 h, 64%; (5) $(COCl)_2$, DMSO, CH_2Cl_2, −78°C for 1 h, −45°C for 1 h, then Et_3N, 0°C, 35 min, 89%; (6) $NaClO_2$, $NaHPO_4$, 2-methyl-2-butene, *t*-BuOH–H_2O, 23°C, 1.5 h, 71%; (f) (1) MOMCl, DIPEA, THF, 23°C, 3.5 h, 100%; (2) 5% Pd/C, H_2, MeOH, 23°C, 2 days, 78%; (3) $(COCl)_2$, DMSO, CH_2Cl_2, −78°C, 1 h, then Et_3N, 0°C, 40 min, 81%; (g) 1% HCl–MeOH, 23°C, 3 days; (2) Ac_2O, pyridine, 23°C, 4 h, 35% for two steps

Hz, H8), 4.86 (ddd, *J*=7, 5, 3 Hz, H3)], which confirmed the relative configuration deduced by *J*-based configuration analysis. The $[\alpha]_D$ data of the degradation product **2** [+34 (MeOH, *c* 0.02) at 20°C] and the synthetic product [+32 (MeOH, *c* 0.03) at 17°C] suggested that the absolute configuration of **2** from L is the same as that of the synthetic product. The above results, together with those of our previous paper, indicate that the absolute configuration of L is 3*R*,4*R*,5*S*,7*S*,11*S*,12*S*,13*R*,14*S*,15*R*,17*R*,18*R*. There are few examples of large polyols from marine organisms; however, several common structural features are found, such as 1,2-*syn*-diol structures in a plausible acetate unit, which may be one of the characteristics in the biogenesis of these polyoxygenated marine metabolites including palytoxins.

Zooxanthella (strain Y-6) was shown to produce another type of nitrogen-containing compound, zooxanthellamine.[13] Zooxanthellamine and zooxanthellatoxins are polyketide-derived molecules whose chain-elongation reactions are believed to start from glycine. Similar polyketide metabolites are found in other *Symbiodinium* species.[14] Studies on the details of biogenesis and the absolute configuration of these polyketide amino acids are in progress at our laboratory.

Acknowledgements

The authors acknowledge Dr. Matsumori and Prof. Murata at Tokyo University for their help in determining the relative configuration of L, and Prof. Kusumi at Tokushima University for his generous gift of (*S*)- and (*R*)-phenylglycine methyl esters. This work was supported in part by a Grant-in-Aid from the Ministry of Education, Science, Sports and Culture, Japan. We are also thankful for financial support from the Naito Foundation, Fujisawa Foundation, and Takeda Foundation.

References

1. Faulkner, D. J. *Nat. Prod. Rep.* **1999**, *16*, 155–198, and references cited therein.
2. Shimizu, Y. *Chem. Rev*. **1993**, *93*, 1685–1698.
3. Yasumoto, T.; Murata, M. *Chem. Rev*. **1993**, *93*, 1897–1909.
4. Nakamura, H.; Asari, T.; Matsuoka, S.; Ohizumi, Y.; Kobayashi, J.; Yamasu, T.; Murai, A. *Toxicon* **1993**, *31*, 371–376.
5. (a) Nakamura, H.; Asari, T.; Fujimaki, K.; Maruyama, K.; Murai, A.; Ohizumi, Y.; Kan, Y. *Tetrahedron Lett.* **1995**, *36*, 7255–7258. (b) Nakamura, H.; Asari, T.; Murai, A.; Kan, Y.; Kondo, T.; Yoshida, K.; Ohizumi, Y. *J. Am. Chem. Soc*. **1995**, *117*, 550–551. (c) Nakamura, H.; Asari, T.; Murai, A.; Kondo, T.; Yoshida, K.; Ohizumi, Y. *J. Org. Chem*. **1993**, *58*, 313–314. (d) Asari, T.; Nakamura, H.; Murai, A.; Kan, Y. *Tetrahedron Lett.* **1993**, *34*, 4059–4062.
6. (a) Nakamura, H.; Fujimaki, K.; Murai, A. *Tetrahedron Lett*. **1996**, *37*, 3153–3156. The absolute configuration at C12–C14 was incorrectly described as 12*R*,13*S*,14*R*. (b) Nakamura, H.; Sato, K.; Murai, A. *Tetrahedron Lett*. **1996**, *37*, 7267–7270. (c) Nakamura, H.; Takahashi, H.; Murai, A. *Tetrahedron: Asymmetry* **1998**, *9*, 2571–2574.
7. Matsumori, N.; Kaneno, D.; Murata, M.; Nakamura, H.; Tachibana, K. *J. Org. Chem.* **1999**, 64, 866–876.
8. Bernet, B.; Vasella, A. *Helv. Chim. Acta* **1979**, 51, 12229–12238.
9. Narisada, M.; Horibe, I.; Watanabe, F.; Takeda, K. *J. Org. Chem.* **1989**, *54*, 5308–5313.
10. Evans, D. A.; Bender, S. L.; Morris, J. *J. Am. Chem. Soc*. **1988**, *110*, 2506–2526.
11. Ho, G. -J.; Mathre, D. J. *J. Org. Chem.* **1995**, *60*, 2271–2273.
12. Nagai, Y.; Kusumi, T. *Tetrahedron Lett.* **1995**, *36*, 1853–1856.
13. Nakamura, H.; Kawase, Y.; Maruyama, K.; Murai, A. *Bull. Chem. Soc. Jpn.* **1998**, *71*, 781–787.
14. Shimizu, Y. *1998 Annual Meeting of the Japanese Society of Bioscience, Biotechnology and Agrochemistry*, Nagoya, April, 1998; S-5-4; Abstract, p. 466.