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Tetrahedron Letters 47 (2006) 155–158
Tetrahedron Letters

Revised structure of zamamistatin

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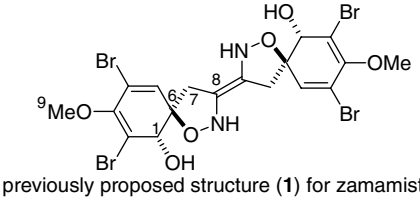
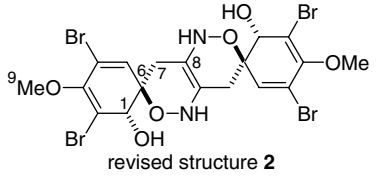
Received 6 October 2005; revised 24 October 2005; accepted 31 October 2005
Available online 17 November 2005

Abstract—The structure of zamamistatin, a novel bromotyrosine derivative from the Okinawan sponge *Pseudoceratina* sp. was revised by comparison of the ^{13}C NMR data of zamamistatin with those of synthetic model compounds.

Zamamistatin, isolated from Okinawan sponge *Pseudoceratina purpurea* by Uemura and co-workers,[1] exhibits significant antibacterial activity against the marine bacteria *Rhodospirillum salecigens* SCRC 113 strain. The structure was elucidated as shown in **1**, an *exo*-type dimer of the azaoxa-spiro[6.5] unit consisting of a cyclohexadienyl moiety and an isoxazolidine ring. In our continuing search for new substances from marine organisms, we investigated the constituents of the marine sponge *Pseudoceratina* sp. collected at Okuma, Okinawa, Japan, and isolated zamamistatin (Fig. 1). The spectral analysis of zamamistatin let us reconsider its structure. We report here the revised structure of zamamistatin, **2**, by comparing the ^{13}C NMR data for zamamistatin with those of synthetic model compounds.



previously proposed structure (**1**) for zamamistatin



revised structure **2**

Figure 1.

The marine sponge *Pseudoceratina* sp. (115 g), collected at Okuma, Okinawa, Japan, in August 2005, was extracted with MeOH (300 mL) for 7 days. The extract was filtered, concentrated, and partitioned between EtOAc and H_2O. The EtOAc-soluble material was further partitioned between 90% aqueous MeOH and hexane. The material obtained from the aqueous MeOH portion was subjected to fractionation with column chromatography (ODS silica gel, MeOH–H_2O) and reversed-phase HPLC (Develosil ODS-HG-5, MeOH–H_2O) to give zamamistatin as a colorless oil (35.0 mg).

The ^1H and ^{13}C NMR data of isolated zamamistatin in $CDCl_3$ are identical with the reported data.[1] Table 1

Table 1. NMR data for zamamistatin in CD_3OD and acetone-d_6[a]

Position	^1H		^{13}C	
	500 MHz CD_3OD	270 MHz acetone-d_6	125 MHz CD_3OD	67.8 MHz acetone-d_6
1	4.10 d (1.1)	4.23 d (7.3)	78.8	78.6
2			114.2	113.8
3			148.9	148.2
4			121.5	120.7
5	6.33 d (1.1)	6.41 d (1.1)	133.5	133.6
6			74.5	74.3
7a	2.78 d (16.7)	2.91 br s	27.0	26.7
7b	2.83 d (16.7)			
8			118.4	117.8
9	3.70 s	3.70 s	60.2	60.0
–OH		5.33 d (7.3)		
–NH		5.33 br s		

[a] Coupling constants (Hz) are given in parentheses.

Keywords: Zamamistatin; Revised structure; *endo*-Type dimer of azaoxa-spiro[6.6] unit.
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doi:10.1016/j.tetlet.2005.10.157

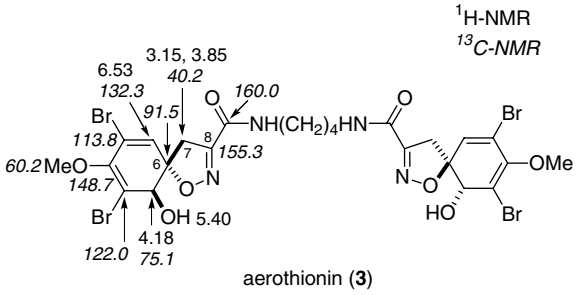


Figure 2. Selected NMR data of aerothionin (**3**) in acetone-d_6.

summarizes the NMR data in different solvents. The chemical shifts in acetone-d_6 of C1–C5 and C9 in zamamistatin closely resembled those of aerothionin (Fig. 2).[2c] However, the chemical shifts of C6 (δ_{C6} 74.3) and C7 (δ_{C7} 26.7; δ_{H7} 2.91) in zamamistatin were apparently different from those of aerothionin (δ_{C6} 91.5, δ_{C7} 40.2; δ_{H7} 3.15, 3.85) (Fig. 2). Therefore, zamamistatin was thought to have a different ring system from an isoxazolidine ring. Considering the molecular formula of zamamistatin, we proposed structure **2**, possessing a dihydro-1,2-oxazine ring for zamamistatin.

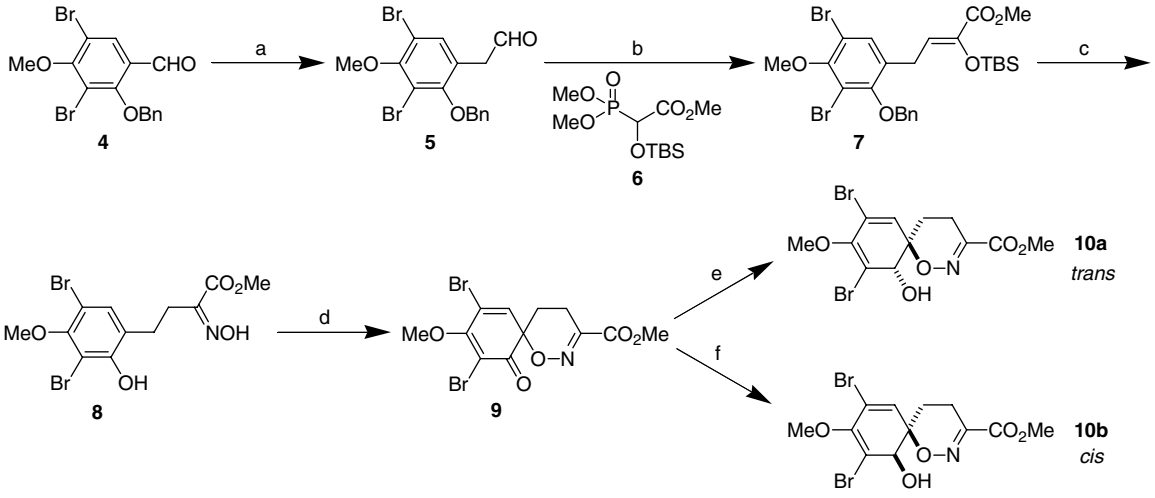
To confirm the proposed structure, we planned to compare the ^{13}C NMR data of zamamistatin with those of dihydro-1,2-oxazine methyl ester **10a** and isoxazoline methyl ester **11a**.[3] Although NMR data of isoxazoline methyl ester **11a** were reported by Hoshino,[3c] dihydro-1,2-oxazine methyl ester **10a** has not been prepared. We therefore synthesized dihydro-1,2-oxazine methyl ester **10a**.

Our synthetic plan was based on reported procedures[3] (Scheme 1). The Wittig-type reaction of **4** and subsequent hydrolysis gave aldehyde **5** in a good yield. Aldehyde **5** was subjected to Horner–Wadsworth–Emmons olefination with phosphonate **6**[4] to give silyl enol ether **7**. Treatment of silyl enol ether **7** with HF·pyr in MeOH, followed immediately by the addition of $NH_2OH{\cdot}HCl$, yielded an oxime, the hydrogenolysis of which gave oxime ester **8**. In the oxidative cyclization of **8**, we used 2,4,4,6-tetrabromo-2,5-cyclohexadienone in acetonitrile.[5] This reaction took place readily to yield **9** in a 92% yield. According to Yamamura's method,[6] reduction of **9** with $Zn(BH_4)_2$[7] gave *trans* dihydro-1,2-oxazine methyl ester **10a**. On the other hand, **9** was reduced with $NaBH_4$[5a] to give *cis* dihydro-1,2-oxazine methyl ester **10b**.[8]

Stereochemistry of **10a** and **10b** was determined by comparison of the ^{1}H NMR spectra of **10a** (δ_{H1} 4.13, δ_{OH} 5.30) and **10b** (δ_{H1} 4.36, δ_{OH} 4.89) in acetone-d_6 with those of related compounds;[6] the ^{1}H chemical shifts of the *trans* isoxazoline methyl ester **11a**, which has a *trans* vicinal relationship between a hydroxy group and an oxime oxygen atom, were δ_{H1} 4.22 and δ_{OH} 5.38, while those of *cis* isoxazoline methyl ester **11b** were δ_{H1} 4.53 and δ_{OH} 4.98 (Fig. 3).[6] Thus, the stereochemistry of compounds **10a** and **10b** was found to be *trans* and *cis*, respectively.

The ^{13}C NMR data in acetone-d_6 of the synthetic **10a** and **10b** were similar to those of zamamistatin rather than aerothionin-related compounds.[9] In the synthetic compound **10a**, carbon signal due to C6 appeared at δ_C 79.9, while the carbon signal in zamamistatin appeared at δ_C 74.3. On the other hand, the carbon signal due to C6 for **11a** appeared at δ_C 92.4 (Fig. 4).[3c] These observations indicated that zamamistatin has a dihydro-1,2-oxazine ring rather than an isoxazolidine ring. Based on these results, it was concluded that the structure of natural zamamistatin, previously proposed as **1**, should be revised to structure **2**.

This novel structure of zamamistatin, an *endo*-type dimer of the azaoxa-spiro[6.6] unit, can be explained by a plausible biogenetic pathway shown in Figure 5. Reductive dimerization of the isoxazoline derivative **12**,[10] followed by oxidative decarboxylation, yielded an



Scheme 1. Reagents and conditions: (a) (i) (methoxymethyl)triphenylphosphonium chloride, *t*-BuOK, THF, rt; (ii) 2 M HCl, THF, reflux, 97% in two steps; (b) **6**, LHMDS, THF, −78 °C, 98%; (c) (i) HF·pyr., MeOH, rt, then $NH_2OH{\cdot}HCl$, rt; (ii) H_2, Pd/C, 1,4-dioxane-AcOH, rt, 98% in two steps; (d) 2,4,4,6-tetrabromo-2,5-cyclohexadienone, MeCN, rt, 92%; (e) $Zn(BH_4)_2$, CH_2Cl_2, rt, 9%; (f) $NaBH_4$, MeOH, rt, 19%.

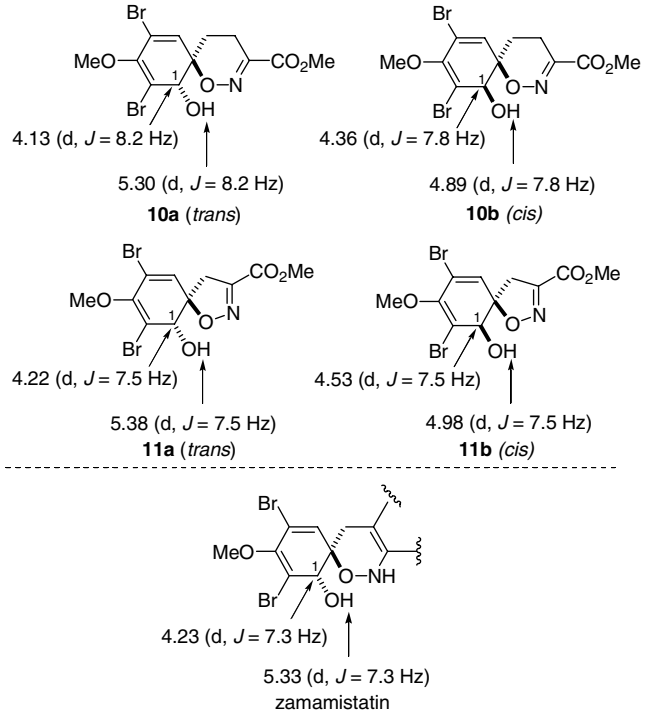


Figure 3. Selected ^{1}H NMR data of zamamistatin and compounds **10a**, **10b**, **11a**, and **11b** in acetone-d_6.

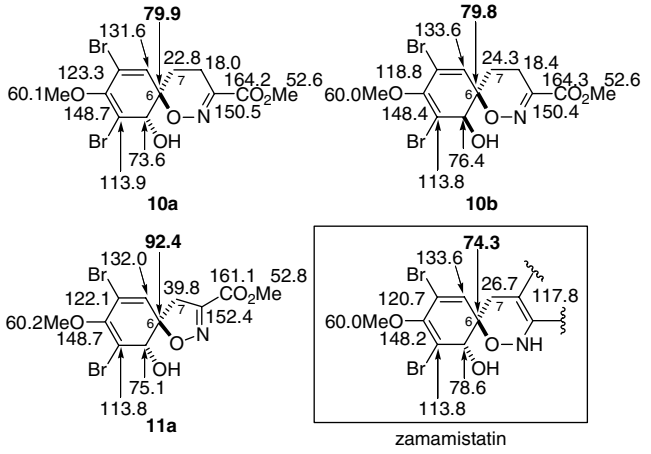


Figure 4. ^{13}C NMR data of zamamistatin and compounds **10a**, **10b**, and **11a** in acetone-d_6.

exo-type dimer of azaoxa-spiro[6.5] unit **1**. Hydrolysis and recyclization of **1** gave the *endo*-type dimer of azaoxa-spiro[6.6] unit **2**. This isomerization from **1** to **2** was supported by computational calculation, in which structure **2** is more stable than structure **1** (52.7 kcal/mol for **1**, 49.1 kcal/mol for **2**: MacroModel 6.0, AMBER*).

Acknowledgements

We express our gratitude to Professor Daisuke Uemura (Nagoya University) for his helpful discussion. This work was supported in part by the 21st COE program and Grant-in-Aid for Scientific Research on Priority Areas (No. 16073204, 'Creation of Biologically Functional Molecules') from the Ministry of Education, Culture, Sports, Science, and Technology, Japan and University of Tsukuba Projects. The authors are grateful to the Chemical Analysis Center, University of Tsukuba, for NMR experiments.

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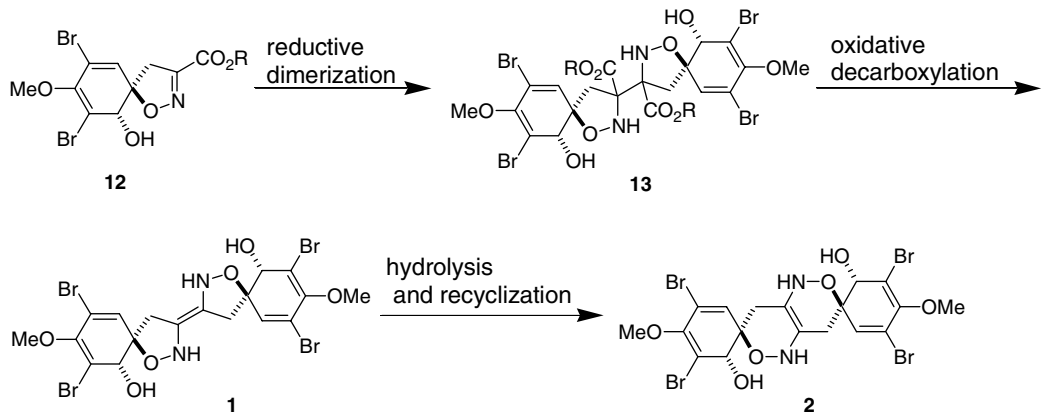

Figure 5. Plausible biogenetic pathway for zamamistatin.

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9. **10a**: ^{1}H NMR (500 MHz, acetone-d_6) δ1.93 (1H, ddd, $J = 6.6$, 8.9, 13.8 Hz), 2.38 (1H, ddd, $J = 5.0$, 7.0, 13.8 Hz), 2.51 (1H, ddd, $J = 7.0$, 8.9, 19.6 Hz), 2.64 (1H, ddd, $J = 5.0$, 6.6, 19.6 Hz), 3.71 (3H, s), 3.78 (3H, s), 4.13 (1H, d, $J = 8.2$ Hz), 5.30 (1H, d, $J = 8.2$ Hz), 6.32 (1H, d, $J = 1.0$ Hz); ^{13}C NMR (125 MHz, acetone-d_6) δ 18.0, 22.8, 52.6, 60.1, 73.6, 79.9, 113.9, 123.3, 131.6, 148.7, 150.5, 164.2; ESIMS m/z 431.9074, calcd for $C_{12}H_{13}Br_2NNaO_5$ $[M+Na]^+$ 431.9058 **10b**: ^{1}H NMR (500 MHz, acetone-d_6) δ 2.05 (1H, ddd, $J = 6.7$, 9.2, 13.8 Hz), 2.15 (1H, ddd, $J = 4.8$, 7.3, 13.8 Hz), 2.53 (1H, ddd, $J = 7.3$, 9.2, 19.5 Hz), 2.65 (1H, ddd, $J = 4.8$, 6.7, 19.5 Hz), 3.70 (3H, s), 3.78 (3H, s), 4.36 (1H, d, $J = 7.8$ Hz), 4.89 (1H, d, $J = 7.8$ Hz), 6.45 (1H, d, $J = 0.8$ Hz); ^{13}C NMR (125 MHz, acetone-d_6) δ 18.4, 24.3, 52.6, 60.0, 76.4, 79.8, 113.8, 118.8, 133.6, 148.4, 150.4, 164.3; ESIMS m/z 431.9031, calcd for $C_{12}H_{13}Br_2NNaO_5$ $[M+Na]^+$ 431.9058.
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