Pergamon

Tetrahedron Letters 42 (2001) 5265–5267

TETRAHEDRON LETTERS

Zamamistatin, a significant antibacterial bromotyrosine derivative, from the Okinawan sponge *Pseudoceratina purpurea*

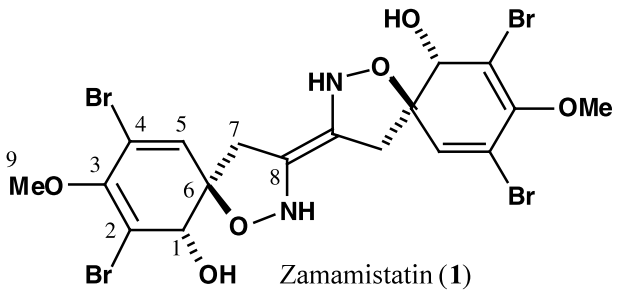
Noboru Takada,[a] Reiko Watanabe,[a] Kiyotake Suenaga,[b] Kaoru Yamada,[a] Katsuhiro Ueda,[c] Masaki Kita[a] and Daisuke Uemura[a,*]

[a]*Department of Chemistry, Graduate School of Science, Nagoya University, Chikusa, Nagoya 464-8602, Japan*
[b]*Research Center for Materials Science, Nagoya University, Chikusa, Nagoya 464-8602, Japan*
[c]*Department of Chemistry, Biology and Marine Science, University of the Ryukyus, Nishihara-cho, Okinawa 903-0213, Japan*

Received 24 April 2001; accepted 8 June 2001

Abstract—Zamamistatin was isolated from the Okinawan sponge *Pseudoceratina purpurea*. It was determined to be a novel bromotyrosine derivative by careful analysis of 2D NMR spectra and comparison of its ^{1}H NMR spectrum with those of structurally related compounds. The absolute stereostructure of zamamistatin was determined by the modified Mosher's method. Zamamistatin exhibited significant antibacterial activity against *Rhodospirillum salexigens*, which has adhering properties. © 2001 Elsevier Science Ltd. All rights reserved.

Biofouling causes serious problems in the shipping business, in aquaculture and in the cooling systems of power stations. Metallic compounds, such as copper(I) oxide and bis(tributyltin)oxide (TBTO), have previously been used as antifouling agents. Today, however, the use of TBTO in antifouling paints is restricted to prevent environmental pollution. Therefore, the development of environmentally acceptable antifouling agents is essential to resolve this global problem.[1] The progress of biofouling on a newly immersed unprotected surface in sea water is well documented.[2] The surface rapidly adsorbs organic material, which may influence the settlement of micro-organisms.



Zamamistatin (**1**)

Bacteria and diatoms are present soon after immersion, resulting in a biofilm that covers the surface. The establishment of this microfouling biofilm layer is rapidly followed by macrofouling. We have focused on the formation of this microbial biofilm, and have searched for compounds to prevent microfouling, which would consequently prevent such macrofouling by barnacles, mussels and algae. Antibacterial activity against the marine bacteria *Rhodospirillum salexigens* SCRC 113 strain which has adhering properties was selected as a bioassay to identify such compounds. In our continuing search for such compounds, untenines, kasarin, nakijinols, and so on have been isolated.[3] We describe here the isolation and structural determination of zamamistatin, which exhibits significant antibacterial activity.

The EtOAc extract of the Okinawan sponge *Pseudoceratina purpurea* collected off Zamami Island in Okinawa, Japan, was partitioned between aqueous 90% MeOH and hexane. The aqueous MeOH-soluble fraction was subjected to fractionation guided by antibacterial activity against *R. salexigens* using column chromatography (SiO_2) and HPLC (SiO_2 and ODS). Final purification was achieved by reversed-phase HPLC (ODS, MeOH–H_2O) to afford zamamistatin (**1**).[4] Zamamistatin (**1**) exhibited significant antibacterial activity against *R. salexigens* (21 mm, 1.6 μg/disk).

In its ESIMS spectrum, zamamistatin (**1**) showed 1:4:6:4:1 quintet ion peaks at *m*/*z* 697, 699, 701, 703, and 705, indicative of the presence of four bromine atoms. The molecular formula of **1** was determined to be $C_{18}H_{18}Br_4N_2O_6$ by ESIMS (*m*/*z* 696.7766, calcd for

Keywords: antifouling agent; antibacterial activity; isolation; structural determination; absolute stereochemistry.

* Corresponding author. E-mail: uemura@chem3.chem.nagoya-u.ac.jp

PII: S0040-4039(01)00993-5

Table 1. NMR data for zamamistatin (**1**) in $CDCl_3$

Atom	^{13}C[a]	^{1}H[b]	HMBC[c]
1	77.4 d	4.44 d (5.1) 1H	H-5
2	112.3 s		H-1, 5
3	148.1 s		H-1, 5, 9
4	121.3 s		H-5
5	131.6 d	6.42 s 1H	H-1, 7
6	74.3 s		H-1, 7
7a	25.5 t	2.81 d (16.0) 1H	H-5
7b		2.85 d (16.0) 1H	
8	116.6 s		H-7
9	60.3 q	3.77 s 3H	
OH		2.45 d (5.1) 1H	
NH		2.52 br s 1H	

[a] Recorded at 200 MHz. Multiplicity was based on HMQC spectrum.
[b] Recorded at 800 MHz. Coupling constant (Hz) are in parenthesis.
[c] Based on the correlation from each carbon atom.

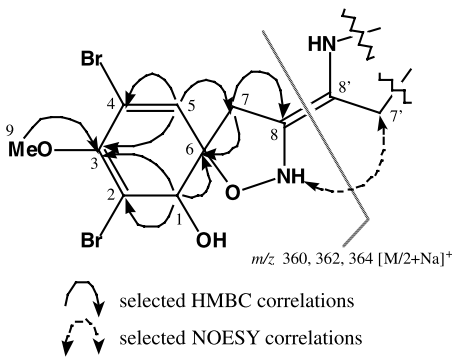


Figure 1. Partial structure of zamamistatin (**1**) based on 2D NMR correlations.

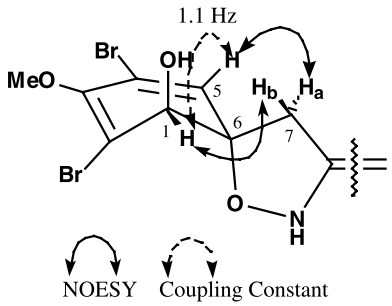


Figure 2. Relative stereochemistry of zamamistatin (**1**).

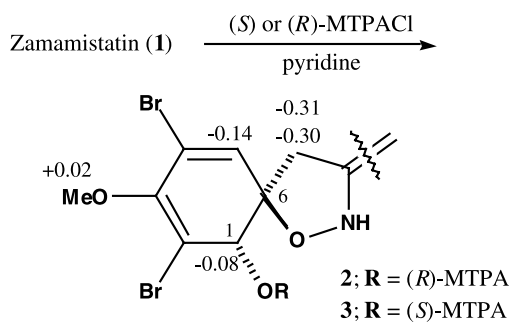


Figure 3. $\Delta\delta$ values (δ_S–δ_R) for the MTPA esters **2** and **3** in ppm.

$C_{18}H_{18}Br_4N_2NaO_6$ $[M+Na]^+$ 696.7795). The NMR data for **1** are summarized in Table 1. The observation of only 9 carbons by ^{13}C NMR and the specific rotation, $[\alpha]_D^{29}=+248°$ (*c* 0.012, $CHCl_3$), suggested that zamamistatin (**1**) was an optically active dimer with a symmetrical structure. ^{1}H NMR, ^{13}C NMR, and HMQC spectra showed the presence of a methyl carbon (δ_C 60.3) connected to an oxygen atom, a methylene carbon, an oxygenated methine carbon (δ_C 77.4), an oxygenated quaternary carbon (δ_C 74.3) and five olefinic carbons (δ_C 112.3, 116.6, 121.2, 131.6, and 148.1). Although zamamistatin (**1**) showed little ^{1}H–^{1}H spin coupling, the HMBC correlations H-1/C-2, H-1/C-3, H-1/C-5, H-1/C-6, H-5/C-1, H-5/C-2, H-5/C-3, H-5/C-4, H-5/C-7, H-7/C-5, H-7/C-6, H-7/C-8, and H-9/C-3 enabled us to elucidate the entire carbon framework, C-1 to C-8 including C-9 (Fig. 1). Furthermore, comparison of the ^{13}C NMR data for **1** with those of aerothionin-related compounds, which was previously reported bromotyrosine derivatives, suggested the presence of a spirohexadienyl moiety.[5] However, the chemical shifts of C-7 (δ_H 2.81, 2.85; δ_C 25.5) and C-8 (δ_C 116.6) in **1** were different from those (δ_{7H} 3.15, 3.85; δ_{7C} 40.2; δ_{8C} 155.3) of aerothionin. Therefore, zamamistatin (**1**) was thought to have an isooxazolidine ring, rather than an isooxazoline ring like aerothionin. Based on the molecular formula, C-8 must form a double bond with C-8′. Finally, the geometry of the C-8 olefin was elucidated by a NOESY H-7′/NH correlation to be *trans*.[6] Thus, the gross structure of zamamistatin was determined to be as shown in **1**.[7]

The relative stereochemistry in **1** was determined as follows (Fig. 2). The equatorial orientation of H-1 was clarified by the observation of W coupling (1.1 Hz, in CD_3OD) between H-1 and H-5. Furthermore, analysis of NOESY correlations H-1/H-7b and H-5/H-7a suggested that the relative stereochemistry in **1** was 1*S**, 6*R**. This stereochemistry in **1** was supported by comparison of the ^{1}H NMR spectrum of **1** (δ_{1H} 4.11) in CD_3OD with those of related compounds; the ^{1}H chemical shift of the *trans* spiroisooxazoline moiety, which has a *trans* vicinal relationship between a hydroxyl group and an oxygen atom, is δ_{1H} 4.08, in contrast to that of a *cis* spiroisooxazoline moiety (δ_{1H} 4.40) reported by Yamamura group.[5e] This stereochemistry in **1** also coincided with the biogenesis of the spiroisooxazolidine ring, which involves a pathway through an oximino epoxide.

Since zamamistatin (**1**) was an optically active dimer, the absolute stereochemistry was determined using the modified Mosher's method.[8] Treatment of **1** with (*S*)- or (*R*)-MTPACl gave (*R*)- or (*S*)-MTPA esters **2** and **3**, respectively, the ^{1}H NMR signals of which were assigned based on the 2D NMR spectra. The calculated $\Delta\delta$ values (δ_S–δ_R, ppm) (Fig. 3) suggested that the absolute stereochemistry of C-1 was 1*S*. Thus, the absolute stereochemistry of zamamistatin was determined to be as shown in **1**.

In conclusion, zamamistatin was isolated from the Okinawan sponge *P. purpurea*. It was determined to be a bromotyrosine derivative, which was an optically active

dimer with a C_2 symmetrical structure, as shown in **1** by detailed analysis by 2D NMR and comparison of its ^{1}H NMR spectrum with those of structurally related compounds. The dimer structure of zamamistatin may be biologically synthesized by reductive dimerization of the spiroisooxazoline moiety in a bromotyrosine precursor followed by decarboxylation. Zamamistatin exhibited significant antibacterial activity against *R. salexigens* which has adhering properties, and may be a valuable candidate for novel antifouling agents.

Acknowledgements

This work was supported in part by Grants-in-Aid for Scientific Research (No. 11558079) and Scientific Research on Priority Areas (A) (No. 12045235) from the Ministry of Education, Culture, Sports, Science, and Technology, Japan. The authors are indebted to Wako Pure Chemical Industries Ltd. and Banyu Pharmaceutical Co. Ltd. for their financial support. They like to thank Professor P. R. Bergquist of the University of Auckland for identifying the sponge. They would also like to thank Professors R. Noyori and M. Kitamura (COE, Nagoya University) for allowing them to use the NMR instruments.

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