ORGANIC LETTERS

2002
Vol. 4, No. 18
3039−3042

Nagahamide A, an Antibacterial Depsipeptide from the Marine Sponge *Theonella swinhoei*[1]

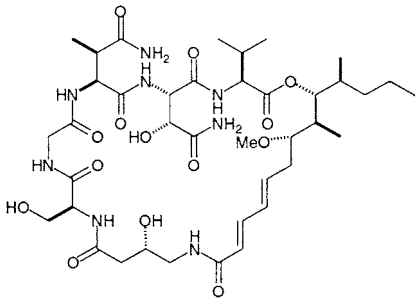
Yumika Okada,[†] Shigeki Matsunaga,[†] Rob W. M. van Soest,[‡] and Nobuhiro Fusetani*,[†]

Laboratory of Aquatic Natural Products Chemistry, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Bunkyo-ku, Tokyo 113-8657, Japan, and Institute for Systematics and Ecology, The University of Amsterdam, P.O. Box 94766, 1090 GT Amsterdam, The Netherlands

anobu@mail.ecc.u-tokyo.ac.jp

Received June 2, 2002

ABSTRACT



An antibacterial depsipeptide, nagahamide A (1), has been isolated from the marine sponge *Theonella swinhoei*. Its structure was determined on the basis of spectral and chemical methods. Absolute configuration of amino acid residues was determined by Marfey's analysis, and relative stereochemistry of the polyketide moiety was assigned by comparison of NMR data with those of a model compound.

Marine sponges of the genus *Theonella* are a rich source of unusual peptides possessing such biological activities as cytotoxic, enzyme inhibitory, antifungal, and anti-HIV.[2] In our continuing program on discovery of potential drug leads from Japanese marine invertebrates, we detected considerable antifungal activity of the hydrophobic extract of the marine sponge *Theonella swinhoei*[3] collected in southern Japan. Bioassay-guided fractionation furnished an active compound that turned out to be an unusual depsipeptide. This paper describes the isolation and structure elucidation of the peptide.

The frozen sponge (100 g) was homogenized and extracted with MeOH. The combined extracts were concentrated and partitioned between H_2O and ether; the polar layer was partitioned between *n*-BuOH and H_2O. The antifungal *n*-BuOH layer was fractionated by ODS flash chromatography with aqueous MeOH, while insoluble materials obtained during H_2O/*n*-BuOH partitioning were similarly processed. Antifungal fractions were combined and separated by ODS HPLC with *n*-PrOH/H_2O (32:68) containing 100 mM $NaClO_4$ to afford theonellamides,[4] together with a peak exhibiting antibacterial activity, which was further purified by ODS HPLC with *n*-PrOH/H_2O (24:76) containing 100 mM $NaClO_4$ to afford nagahamide A (**1**) as a colorless powder (0.0027% based on wet weight).

Nagahamide A (**1**)[5] had a molecular formula of $C_{39}H_{64}N_8O_{14}$, which was determined by HRFABMS in conjunction with NMR data (Table 1). Its peptidic nature was evident

[†] The University of Tokyo.
[‡] The University of Amsterdam.

(1) Bioactive Marine Metabolites. 114. Part 113: Fujita, M.; Nakao, Y.; Matsunaga, S.; Nishikawa, T.; Fusetani, N. Submitted for publication.

(2) Fusetani, N.; Matsunaga, S. *Chem. Rev.* **1993**, *93*, 1793−1806.

(3) The sponge was collected at depths of 3−5 m near Nagahama, Kamikoshiki-jima Island (31° 39.00′ N, 129° 44.30′ E) in October 1994.

(4) Matsunaga, S.; Fusetani, N. *J. Org. Chem.* **1995**, *60*, 1177−1181.

(5) White powder; $[\alpha]_D$ +26.6° [*c* 0.1, *n*-PrOH/H_2O (4:1)], UV [*n*-PrOH/H_2O (4:1)] (λ_{max} 260 nm (ϵ = 7800); HRFABMS *m*/*z* 869.4642 (M + H)$^+$ ($C_{39}H_{64}N_8O_{14}$, +2.2 mmu); ^{1}H and ^{13}C NMR data, see Table 1.

10.1021/ol0262791 CCC: $22.00
Published on Web 08/09/2002

Table 1. NMR Data for 1 in DMSO-d_6

amino acid		δ^a ^{13}C	δ^a ^{1}H (mult, J in Hz)	HMBC
Val	1	171.0		
	2	57.9	4.14 (t, 6.9)	C-1,3,4,5, β-OH Asn 1
	3	29.8	2.05 (m)	C-1,2,4,5
	4	18.2	0.91 (m)	C-2,3
	5	19.3	0.92 (m)	C-2,3
	NH		7.65 (d, 8.5)	C-2,3, β-OH Asn 1
β-OH-Asn	1	168.4		
	2	55.2	4.73 (dd, 4.2, 8.5)	C-1,3,4, β-Me Asn 1
	3	72.0	4.06 (dd, 4.2, 6.2)	C-1,2,4
	4	173.3		
	β-OH		5.48 (d, 6.2)	
	NH		7.83 (d, 8.5)	C-2, β-Me Asn 1
	NH2		7.06 (br s)	
			7.18 (br s)	
β-Me-Asn	1	169.7		
	2	54.9	4.37 (dd, 5.0, 8.1)	C-1,3, β-Me
	3	40.1	2.81 (m)	C-1,2,4, β-Me
	4	176.2		
	β-Me	14.4	1.01 (d, 7.3)	C-2,3,4
	NH		7.78 (d, 8.1)	C-2,3, Gly 1
	NH2		6.93 (br s)	
			7.46 (br s)	
Gly	1	169.2		
	2	42.9	3.66 (m)	C-1, Ser 1
			3.82 (dd, 5.8, 16.5)	C-1, Ser 1
	NH		8.30 (t, 5.8)	C-2, Ser 1
Ser	1	171.1		
	2	55.6	4.21 (dd, 5.0, 12.0)	C-1,3
	3	61.2	3.64 (m)	C-1,2
			3.68 (m)	C-1,2
	NH		7.96 (d, 7.3)	C-1,2,3
	OH		4.82 (t, 5.8)	
AHBA[b]	1	170.9		
	2	40.1	2.21 (dd, 6.5, 14.6)	C-1,3,4
			2.35 (dd, 6.5, 14.6)	C-1,3,4
	3	66.9	3.99 (m)	C-1,2
	4	43.6	3.12 (t, 5.6)	C-2,3, DHMT 1
			3.32 (m)	
	NH		8.01 (t, 5.6)	
	OH		4.90 (d, 4.6)	
DHMDA[c]	1	166.4		
	2	123.2	6.01 (d, 15.2)	C-1,4
	3	139.9	7.09 (dd, 11.0, 15.2)	C-1,2,4,5
	4	130.2	6.24 (dd, 11.0, 15.0)	C-2,3,5,6
	5	139.2	6.09 (td, 7.3, 15.0)	C-3,4,6,7
	6	34.9	2.12 (td, 7.3, 13.5)	C-4,5,7,8
			2.45 (td, 7.3, 13.5)	C-4,5,7,8
	7	78.7	3.06 (br t, 6.7)	C-5,8,9, 7-OMe, 8-Me
	8	37.9	1.68 (m)	
	9	78.9	4.85 (dd, 2.1, 9.8)	C-7,8,10,11, 10-Me, Val 1
	10	32.9	1.69 (m)	C-9, 8-Me
	11	30.5	1.05 (m)	C-12
			1.28 (m)	C-12
	12	19.8	1.13 (m)	C-11,13
			1.38 (m)	C-11,13
	13	14.0	0.86 (t, 7.3)	C-11,12
	7-OMe	57.7	3.14 (s)	C-7
	8-Me	8.8	0.75 (d, 6.9)	C-8
	10-Me	16.8	0.77 (d, 7.3)	C-10,11

[a] Referenced to residual solvent DMSO-d_6 and measured at 40 °C, 600 MHz for ^{1}H and 150 MHz for ^{13}C. [b] γ-Amino β-hydroxy butyric acid. [c] 8,10-Dimethyl-9-hydroxy-7-methoxytridecadienoic acid.

from the presence of ten amide and six α-protons in the ^{1}H NMR spectrum, along with eight amide carbons and six α-carbons in the ^{13}C NMR spectrum. In fact, amino acid analysis of the acid hydrolysate resulted in 1 mol each of Gly, Val, and Ser, together with three unusual amino acids.[6]

Interpretation of the COSY and HOHAHA spectra indicated the presence of spin systems for one residue each of Gly, Val, and Ser. Further analysis of COSY data led to two β-substituted asparagine residues, one of which was assigned as β-hydroxyasparagine (β-OH-Asn) on the basis of the presence of the β-methine proton (δ 4.06) attached to a carbon at δ 72.0. This proton showed NOESY cross-peaks with a pair of amide protons (δ 7.06 and 7.18). The other asparagine derivative was assigned as β-methylasparagine (β-Me-Asn); the α-proton was coupled to the β-proton (δ 2.81), which was in turn correlated with a methyl signal (δ 1.01). The β-proton exhibited NOESY cross-peaks with a pair of amide protons (δ 6.93 and 7.46). The remaining unusual amino acid residue was a γ-amino acid; connectivities of NH-CH_2-CH(OH)-CH_2CO were readily derived by COSY and HOHAHA data, thereby establishing the 4-amino-3-hydroxybutanoic acid (AHBA) residue. The structures of these unusual amino acid residues were also supported by HMQC and HMBC data.

The remaining ^{1}H NMR signals consisted of four olefinic protons, four methines including two oxymethines, three methylenes, and four methyls including one oxymethyl. Interpretation of COSY data led to connectivities from H-2 to H-6; H-6 protons were coupled to the oxymethine (H-7), which showed no correlation with other protons. The other oxymethine proton (H-9) was coupled to a two-proton multiplet centering at 1.68 ppm (H-8 and H-10), which was further coupled to two methyl signals at δ 0.75 (Me-8) and 0.77 (Me-10). The HOHAHA spectrum led to connectivities from H-10 to H-13, while HMBC cross-peaks not only connected C-7 and C-8 but also placed a methoxyl group on C-7, thus constructing 8,10-dimethyl-9-hydroxy-7-methoxytrideca- 2,4-dienoic acid (DHMDA).

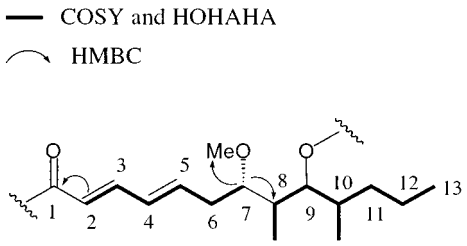


Figure 1. COSY and HMBC correlations in DHMDA.

The sequence of the above residues was established by HMBC cross-peaks: Val-NH/OH-Asn-CO; OH-Asn-NH/Me-Asn-CO; Me-Asn-NH/Gly-CO; Gly-NH/Ser-CO; Ser-

(6) Retention times of standard samples in the amino acid analysis (min): 3.05 (*threo*-β-OH-Asp), 4.50 (*erythro*-β-OH-Asp), 9.57 (*erythro*-β-Me-Asp), 10.90 (*threo*-β-Me-Asp), Ser (16.18), Gly (32.64), and Val (39.30). Retention times of the amino acids in acid hydrolysate of nagahamide A: 4.48 (*erythro*-β-OH-Asp), 9.60 (*erythro*-β-Me-Asp), 16.74 (Ser), 33.44 (Gly), 39.65 (Val), 54.18 (AHBA). Amino acid analysis was carried out with a Hitachi L8500A amino acid analyzer. *threo*-β-OH-Asp, *threo*-β-Me-Asp, and a diastereomeric mixture of *threo*- and *erythro*-β-Me-Asp are commercially available. *erythro*-β-OH-L-Asp was prepared from the acid hydrolyzate of theonellamide F.[4]

NH/AHBA-CO; AHBA-NH/DHMDA-CO; DHMDA-H9/Val-CO. The gross structure thus obtained was supported by NOESY data, as indicated below.

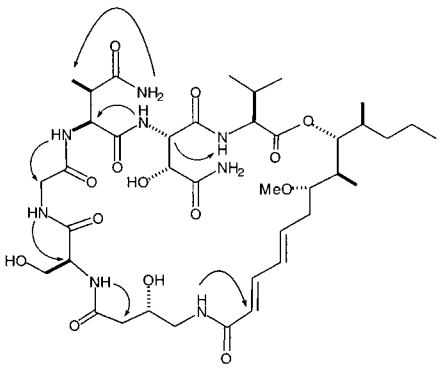
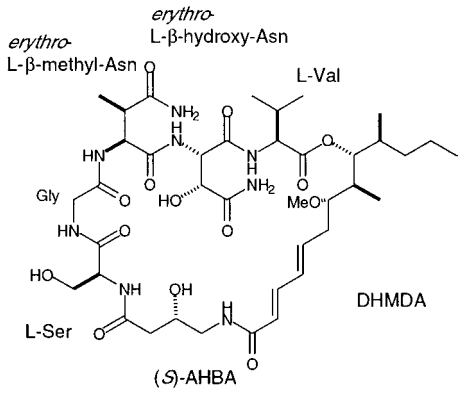


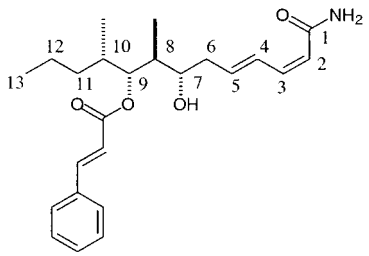
Figure 2. Key NOESY correlation of **1**.

The absolute configuration of Val, Ser, and AHBA was determined to be L by Marfey's analysis[7,8] of the acid hydrolysate. The retention times of β-Me-Asn and β-OH-Asn in the amino acid analysis indicated that they were both *erythro* isomers,[6] whereas Marfey's analysis showed their L-form.[8] The Marfey's analysis also showed 3*S*-stereochemistry for the AHBA residue.

^{1}H,^{1}H coupling constants between methine protons in the four contiguous stereogenic centers of the DHMDA residue were as follows: $J_{7,8} = 0$ Hz, $J_{8,9} = 9.8$ Hz, and $J_{9,10} = 2.1$ Hz. Qualitative analysis of HMBC data (optimized for $J = 8.3$ Hz) demonstrated that $^3J_{H9,10-CH_3} > {}^3J_{H9,8-CH_3}$ and $^3J_{H7,8-CH_3} > {}^3J_{H9,8-CH_3}$. The NOESY spectrum did not give conclusive evidence as to the relative stereochemistry of this portion of the molecule. The limited amount of the sample and overlapped signals (H-8 and H-10; C-7 and C-9) hampered the determination of accurate ^{1}H,^{13}C long-range coupling constant values. At this moment, we noticed that the gross structure of DHMDA residue was identical with that of the des-cinnamoyl derivative of YM-47522 (**2**), an antifungal metabolite of *Bacillus* sp., except for the geometry of one double bond; the stereochemistry of **2** was determined to be 7*S*,8*S*,9*R*,10*S* by synthesis.[9a−c]



Nagahamide A (**1**)



YM47522(**2**)

To assign the relative stereochemistry of the DHMDA residue, we attempted to prepare a common derivative from nagahamide A (**1**) and YM-47522 (**2**). After several unsuccessful attempts, **2** was hydrogenated, hydrolyzed, and O-methylated with NaH/MeI to afford a mixture of 7-*O*-Me, 9-*O*-Me, and 7,9-di-*O*-Me derivatives, whose NMR parameters were obtained by extensive 2D NMR analysis of the mixture.[10] Similarly, **1** was hydrogenated and hydrolyzed. Despite the lability of the DHMDA residue even after hydrogenation, we were able to detect the ^{1}H NMR signals of the 7-*O*-Me derivative in the hydrolysate mixture by the HOHAHA spectrum. Although we were not able to determine the chemical shift values of H-10 and 10-Me because of a small coupling constant between H9 and H10, chemical shift values for H-7, H-8, 8-Me, and H-9[10] were identical with those for the corresponding protons in the 7-*O*-methyl derivative prepared from **2**. Therefore, we concluded that the relative stereochemistry of the DHMDA residue was identical with that of YM-47522. All attempts at reaction of methoxy (trifluoromethyl)-phenylacetyl chloride (MTPA-Cl)

(7) Marfey, P. *Carlsberg Res. Commun.* **1984**, *49*, 591−596.

(8) HPLC retention times of standard amino acids after derivatization with L-FDAA (min): 30.9 (*erythro*-β-OH-L-Asp), 33.0 (*erythro*-β-OH-D-Asp), 40.6 (*erythro*-β-Me-L-Asp), 42.3 (*erythro*-β-Me-D-Asp), 41.2 (L-Ser), 43.7 (D-Ser), 46.4 (*R*-AHBA), 47.1 (*S*-AHBA), 49.8 (L-Val), 55.2 (L-Val). Retention times of each component amino acid of **1** were 31.3 min (β-OH-L-Asp), 39.9 min (β-Me-L-Asp), 41.3 min (L-Ser), 46.9 min (*S*-AHBA), 49.2 min (L-Val). A chromatographic equivalent of *erythro*-β-OH-D-Asp was prepared by reacting *erythro*-β-OH-L-Asp with D-FDAA.[4] Both isomers of *erythro*-β-Me-Asp were prepared as follows. A commercially available diastereomeric mixture of *threo*- and *erythro*-β-Me-Asp was converted to a mixture of *N*-Cbz-di(*p*-bromophenacyl) ester derivatives, which was separated by silica gel HPLC to afford *N*-Cbz-di(*p*-bromophenacyl) ester derivative of *erythro*-β-Me-DL-Asp. The enatiomeric mixture was separated by HPLC on Chiralcel OJ (EtOH) followed by acid hydrolysis to afford *erythro*-β-Me-D-Asp and *erythro*-β-Me-L-Asp, whose configurations were assigned by Marfey's analysis using the D-isomer obtained from the acid hydrolysate of microsystin LR as a standard. AHBA was obtained by acid hydrolysis of (3*S*)-3-hydroxypyrrolidine-2-one.

(9) (a) Shibazaki, M.; Sugawara, T.; Nagai, K.; Shimizu, Y.; Yamaguchi, H.; Suzuki, K. *J Antibiot.* **1996**, *49*, 340−344. (b) Sugawara, T.; Shibazaki, M.; Nakahara, H.; Suzuki, K. *J. Antibiot.* **1996**, *49*, 345−348. (c) Ermolenko, M. S. *Tetrahedron Lett.* **1996**, *37*, 6711−6712.

(10) The site of O-methylation was assigned on the basis of 2D NMR data including HOHAHA, HMQC, and HMBC spectra. 7-*O*-Methyl derivative: δ_C 85.7 (C-7), 38.0 (C-8), 77.5 (C-9), 34.5 (C-10), 57.0 (OMe-7), 12.2 (Me-8), 11.9 (Me-10); δ_H 3.24 (H-7), 1.76 (H-8), 3.35 (H-9), 1.53 (H-10), 3.28 (OMe-7), 0.67(Me-8), 0.77 (Me-10). 9-*O*-Methyl derivative: δ_C 74.0 (C-7), 41.8 (C-8), 91.0 (C-9), 36.0 (C-10), 14.4 (Me-8), 61.5 (OMe-9), 13.4 (Me-10); δ_H 3, 56 (H-7), 1.63 (H-8), 2.90 (H-9), 1.58 (H-10), 0.72 (Me-8), 3.40 (OMe-9), 0.81 (Me-10). ^{1}H NMR data for the fragment prepared from **1**: δ_H 3.24 (H-7), 1.76 (H-8), 3.35 (H-9), and 0.67 (Me-8).

or phenyl ethyl isocyanate (PEI) with 9-OH were unsuccessful, probably as a result of steric hindrance. Therefore, absolute stereochemistry of the DHMDA residue in **1** remains to be elucidated.

Nagahamide A (**1**) was weakly antibacterial against *Escherichia coli* and *Staphylococcus aureus* but not antifugal.[11] It is a seven-residue depsipeptide containing three unusual amino acids and a polyketide acid. Particularly interesting is the presence of the DHMDA residue, which is closely related to YM-47522 (**2**), a polyketide metabolite produced by *Bacillus* sp. The other notable residue is AHBA, possibly a condensation product of a glycine and an acetate, which was also found in microsclerodermins, antifungal peptides isolated from lithisitid sponges.[12]

(11) An inhibitory zone of 7 mm was observed when 50 μg of **1** was applied to a 6-mm ϕ paper disk on an agar plate inoculated with either *E. coli* or *S. aureus*. No inhibition was observed against *Saccharomyces cerevisiae* and *Mortierella ramanniana* at the same dose.

(12) Bewley, C. A.; Debitus, C.; Faulkner, D. J. *J. Am. Chem. Soc.* **1994**, *116*, 7631−7636.

Acknowledgment. We thank Dr. K. Suzuki, Yamanouchi Pharmaceutical Co., Ltd., for a generous supply of culture broth of YL-03709B, Takasago International Company for a gift of (3*S*)-3-hydroxypyrrolidine-2-one, and Dr. M. Murakami, Department of Aquatic Bioscience of The University of Tokyo, for a gift of microcystin LR.

Supporting Information Available: ^{1}H NMR, COSY, HOHAHA, HMQC, HMBC, and NOESY spectra of **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

OL0262791