Miraziridine A, a Novel Cysteine Protease Inhibitor from the Marine Sponge *Theonella* aff. *mirabilis*[1]

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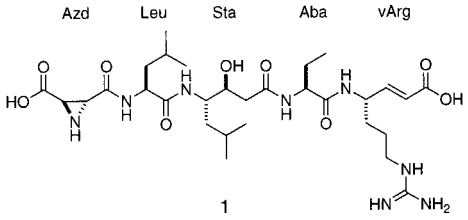
The marine sponge *Theonella mirabilis* was reported to contain unusual depsipeptides, papuamides A−D, which showed HIV-inhibitory and cytotoxic activities.[2] During the collection cruise on R/V *Toyoshio-maru* of Hiroshima University to the Amami and Tokara Islands, we encountered a blue sponge closely related to *T. mirabilis*,[3] which exhibited a variety of bioactivities such as antifungal and protease inhibitory. We have already reported the isolation and structure determination of tokaramide A, a cathepsin B inhibitory linear peptide.[3] Further examination of the extract afforded another cathepsin B inhibitor, miraziridine A (**1**).[4] This work describes isolation and structure elucidation of miraziridine A (**1**).

A Sephadex LH-20 fraction obtained during isolation of tokaramide A was repeatedly purified by ODS HPLC to furnish 7.7 mg of miraziridine A (**1**; 6.7×10^{-5} % yield based on wet weight). Miraziridine A (**1**) had a molecular formula of $C_{30}H_{52}N_8O_9$ as determined by HR-FABMS [$(M + H)^+$ *m/z* 669.3961 (Δ +2.5 mmu)] and NMR data. Its peptidic nature was evident from four amide protons in the ^{1}H NMR spectrum, together with six carbon signals between 170 and 175 ppm in the ^{13}C NMR spectrum.



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The presence of a vinylogous arginine (vArg) was indicated by two olefinic protons at 6.82 and 5.90 ppm (J = 15.8 Hz each), which showed connectivity to the spin system of an arginine residue as revealed by the COSY and HOHAHA[5] spectra. This was fully supported by the ^{13}C chemical shifts. Further analysis of the HOHAHA spectrum indicated the presence of α-aminobutanoyl (Aba) and Leu residues. There was another spin system that contained two methyl doublets at 0.90 and 0.85 ppm, both of which were coupled to a methine at 1.57 ppm. This methine proton was further correlated with methylene protons (1.58 and 1.30 ppm), which were in turn coupled to a methine proton at 3.89 ppm. The latter methine proton was also coupled to an oxygenated methine proton at 4.01 ppm which was correlated with another pair of methylene protons (2.36 and 2.27 ppm). This spin system indicated the presence of a statin (Sta) residue, which was substantiated by HMBC data[6] (Table 1).

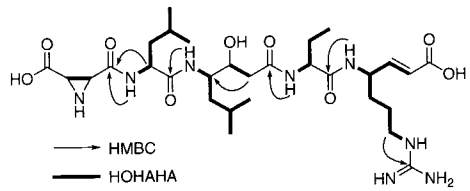

Figure 1. Key correlations in HMBC and HOHAHA spectra.

The remaining portion of composition $C_4H_4NO_3$ contained two carbonyl groups (175.1 and 171.8 ppm). The HMQC spectrum[7] exhibited two methine protons (δ 2.82 and 2.63) attached to carbons at δ 38.0 and 37.4, respectively. Large one bond C−H coupling constants ($^1J_{CH}$ = 175 Hz) for both methines were remniscent of a three-membered ring. These data were consistent with an aziridine-2,3-dicarboxylate half amide.[8]

The sequencing of the amino acid residues was done by HMBC experiments, which resulted in the following sequence: vArg (NH)/Aba (CO), Aba (NH)/Sta (CO), Sta (NH)/Leu (CO), and Leu (NH)/Azd (CO) (Figure 1).

Absolute stereochemistry of each residue was determined by a combination of Marfey analysis[9] and NMR data. The acid hydrolysate of **1** was separated by ODS HPLC to afford Leu, Sta, Aba, and hydroxy aspartic acid (hAsp). The *S*-configuration for both Leu and Aba was determined by Marfey analysis of the isolated amino acids.

The ^{1}H NMR spectrum of the isolated Sta was identical with that of commercially available 3*S*,4*S*-Sta, thereby indicating their identical relative stereochemistry. The absolute stereochemistry of Sta was determined by Marfey analysis. Because 3*R*,4*R*-Sta was not commercially available, a chromatographical equivalent was prepared by reaction with D-FDAA instead of L-FDAA.[10] Sta from **1** coeluted with the standard reacted with L-FDAA, thus disclosing the 3*S*,4*S*-stereochemistry.

Absolute stereochemistry of the vArg unit was determined by Marfey analysis after conversion to Arg. Miraziridin A (**1**) was ozonized followed by oxidative workup to furnish the product which was hydrolyzed with acid; the product was passed through an anion exchange column (Dowex 1−X8). The effluent, which was enriched with Arg, was subjected to Marfey analysis, demonstrating the presence of L-Arg, which was consistent with the *S*-configuration of the vArg residue.

The stereochemistry of Azd unit was deduced by Marfey analysis of hAsp which was derived by hydrolytic opening of the aziridine ring. It was reported that the acid-catalyzed hydrolysis of 2*S*,3*S*-aziridine-2,3-dicarboxylic acid afforded 2*S*,3*R*-2-amino-3-hydroxybutanedioic acid (L-*erythro*-hAsp).[8] The relative stereochemistry of hAsp was assigned as *erythro* on the basis of amino acid analysis,[11] in which the *erythro*-isomer had a longer retention time than the *threo*-isomer. The absolute stereochemistry of hAsp from **4** was assigned to be D-*erythro* by Marfey analysis using L- and D-FDAA and an authentic L-*erythro*-isomer isolated

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(4) Miraziridine A (**1**): $[\alpha]^{20}_D$ −74° (*c* 0.085, MeOH); UV (MeOH) 205 nm (ϵ 14 000).

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(11) *threo*- And *erythro*-hAsp could be separated during amino acid analysis (retention time for *threo* 3.08 min, for *erythro* 4.50 min.). Authentic D,L-*threo*-hAsp was purchased from Sigma-Aldrich Japan K. K. (Tokyo), and L-*erythro*-hAsp was obtained from the acid hydrolysate of theonellamide F.

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Table 1. NMR Data for Miraziridine A (**1**) in CD_3OH

		δ_C	δ_H (mult., Hz)	HMBC	ROESY
Azd	α-CO	171.8			
	α	37.4	2.82 d 2.1	175.1, 171.8, 38.0	8.47
	β	38.0	2.63 d 2.1	175.1, 171.8, 37.4	
	β-CO	175.1			
Leu	CO	174.8			
	α	54.8	4.29 ddd 9.6, 6.9, 5.6	174.8, 171.8, 41.8, 26.0	8.47, 7.46, 0.91
	β	41.8	1.64	174.8, 54.8, 23.4, 21.9	
	γ	26.0	1.71	23.4, 21.9	
	δ	21.9	0.91 d 6.5	41.8, 26.0, 23.4	4.29
	δ′	23.4	0.96 d 6.5	41.8, 26.0, 21.9	
	NH		8.47 d 6.9	171.8, 54.8, 41.8	7.46, 2.82
Sta	CO	174.2			
	α	41.4	2.36 dd 14.5, 7.2	174.2, 71.4, 52.1	8.09, 4.01, 3.89
			2.27 dd 14.5, 6.7	174.2, 71.4, 52.1	8.09, 4.01, 3.89
	β	71.4	4.01 ddd 7.2, 6.7, 2.2	174.2, 41.4	2.36, 2.27, 0.85
	γ	52.1	3.89 ddt 2.2, 9.4, 4.6	41.4	7.46, 2.36, 2.27, 0.85
	δ	41.4	1.58	26.0, 23.7, 22.5	
			1.30	26.0, 23.7	
	ϵ	26.0	1.57	52.1, 41.4	
	ζ	22.5	0.85 d 6.2	41.4, 26.0, 23.7	4.01, 3.89
	ζ′	23.7	0.90 d 6.2	41.4, 26.0, 22.5	
	NH		7.46 d 9.4	174.8, 52.1	8.47, 4.29, 3.89
Aba	CO	174.7			
	α	57.1	4.17 ddd 8.5, 6.9, 5.4	174.7, 26.2, 10.9	8.36, 8.09, 0.99
	β	26.2	1.86 ddq 13.8, 5.0, 7.5	174.7, 57.1, 10.9	
			1.70	174.7, 57.1, 10.9	
	γ	10.9	0.99 t 7.5	57.1, 26.2	8.09, 4.17
	NH		8.09 d 6.5	174.2, 57.1, 26.2	8.36, 4.17, 2.36, 2.27, 0.99
vArg	CO	169.9			
	α	122.9	5.90 dd 15.4, 1.9	169.9, 148.6, 51.0	8.36
	β	148.6	6.82 dd 15.4, 5.4	169.9, 122.9, 51.0, 31.8	8.36, 4.57
	γ	51.0	4.57 dtt 1.9, 8.8, 5.6	148.6, 122.9, 31.8	8.36, 6.82, 3.21, 3.16
	δ	31.8	1.67	148.6, 51.0, 42.0	
	ϵ	26.4	1.64	31.8	
	ζ	42.0	3.21	158.8, 31.8, 26.4	7.56, 4.57
			3.16	158.8, 31.8, 26.4	7.56, 4.57
	C═N	158.8			
	δ-NH		8.36 d 8.8	174.7, 51.0, 31.8	
	ζ-NH		7.56 t 5.2		

from the acid hydrolysate of theonellamide F.[12] Therefore, the absolute stereochemistry of Azd was deduced as *2R*,*3R*.

Miraziridine A (**1**) inhibited cathepsin B with an IC_{50} value of 1.4 *μ*g/mL. Aziridine-2,3-dicarboxylic acid is a rare natural product, reported only once from a *Streptomyces*,[8] and vArg has never before reported as a natural product. However, some synthetic cysteine protease inhibitors incorporate these residues.[13]

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Supporting Information Available: Table of NMR data and NMR spectra for **1** (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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