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Thermoactinoamide A, an Antibiotic Lipophilic Cyclopeptide from the Icelandic Thermophilic Bacterium *Thermoactinomyces vulgaris*

Roberta Teta,[†] Viggó Thór Marteinsson,[‡,⊥] Arlette Longeon,[§] Alexandra M. Klonowski,[‡] René Groben,[‡] Marie-Lise Bourguet-Kondracki,[§] Valeria Costantino,[†] and Alfonso Mangoni*[,†]

[†]The NeaNat Group, Dipartimento di Farmacia, Università degli Studi di Napoli Federico II, Via D. Montesano 49, 80131 Napoli, Italy

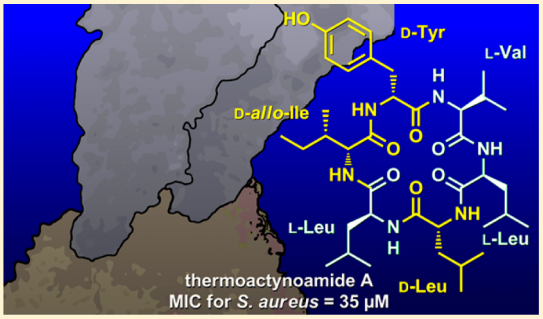
[‡]Matis ohf., Vinlandsleid 12, 113 Reykjavik, Iceland

[§]Laboratoire Molécules de Communication et Adaptation des Micro-organismes, UMR 7245 CNRS, Muséum National d'Histoire Naturelle, 57 Rue Cuvier (C.P. 54), 75005 Paris, France

[⊥]Faculty of Food Science and Nutrition, University of Iceland, Saemundargata 2, 101 Reykjavik, Iceland

Supporting Information

ABSTRACT: The thermophilic bacterium *Thermoactinomyces vulgaris* strain ISCAR 2354, isolated from a coastal hydrothermal vent in Iceland, was shown to contain thermoactinoamide A (**1**), a new cyclic hexapeptide composed of mixed D and L amino acids, along with five minor analogues (**2**–**6**). The structure of **1** was determined by one- and two-dimensional NMR spectroscopy, high-resolution tandem mass spectrometry, and advanced Marfey's analysis of **1** and of the products of its partial hydrolysis. Thermoactinoamide A inhibited the growth of *Staphylococcus aureus* ATCC 6538 with an MIC value of 35 μM. On the basis of literature data and this work, cyclic hexapeptides with mixed D/L configurations, one aromatic amino acid residue, and a prevalence of lipophilic residues can be seen as a starting point to define a new, easily accessible scaffold in the search for new antibiotic agents.



The global increase in the incidence of drug-resistant bacteria dictates novel inputs and efforts in antibacterial drug discovery. Multidrug-resistant bacteria are estimated to kill 23 000 people in the US and 25 000 in Europe per year.[1] At the same time, the development of new antibiotics is declining, and cross-resistance between newly introduced and currently used antibiotics is not uncommon. Therefore, not only new molecules but new scaffolds and new targets are needed.

When it comes to the discovery of new scaffolds and new targets, natural products have always played a major role. This is particularly true for antibiotics, where only three of the 20 classes of approved antibiotics are not based on the scaffold of a natural product (sulfa drugs, quinolones, and oxazolidinones).[2] In this respect, it has been observed that microorganisms living in specialized ecological niches may offer more chances to discover new scaffolds than widespread microorganisms.[3]

Examples of bacteria living in specialized ecological niches include the bacterial communities associated with many marine sponges. They are abundant, highly sponge-specific, and distinctly different from those in the surrounding water, so much so that marine sponges have been considered as natural microbial fermenters;[4] moreover, the distinctness of sponge-associated bacterial communities translates into a similar distinctness of their biosynthetic genes.[5,6] Not surprisingly, there is increasing evidence that many of the metabolites found in sponges are actually produced by microorganisms associated with them.[7,8]

Submarine thermal vents are another example of such a specialized ecological niche, which in addition is virtually unexplored with respect to natural products discovery. Hydrothermal vents are inhabited by thermophilic bacteria, which have the ability to grow at very high temperatures. From the Icelandic Strain Collection and Records (ISCAR) a total of 150 bacterial strains that were originally isolated from various Icelandic marine environments were screened for biological activity. One of those strains showed antimicrobial activity and was chosen for further analysis. The isolate, ISCAR 2354, originated from a coastal hot spring on the Snæfellsness peninsula in West Iceland (64°57′52″ N, 23°5′21.53″ W). According to 16S rRNA gene sequencing (GenBank accession number M372920), the strain belongs to the species *Thermoactinomyces vulgaris* (Thermoactinomycetaceae, Bacillales, Firmicutes), showing 100% identity to other isolates of the species. From strain ISCAR 2354, a new antibacterial cyclic hexapeptide with mixed D and L amino acids, namely, thermoactinoamide A (**1**), was isolated, along with five minor analogues (thermoactinoamides B–F, **2**–**6**), whose structures were partly elucidated by mass spectrometry. In this

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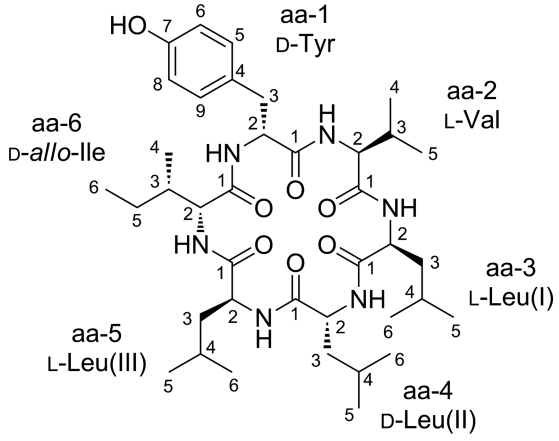
paper, we wish to report the isolation, structure elucidation, and antibacterial activity of thermoactinoamide A (**1**).



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RESULTS AND DISCUSSION

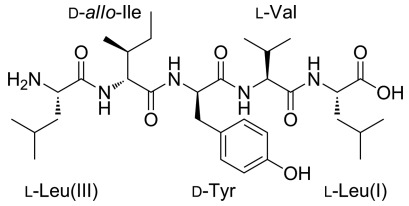
The thermophilic bacterium *T. vulgaris* strain ISCAR 2354 was grown at 60 °C in 166 medium[9] with 1% NaCl for 24 h. An organic extract of the culture was derived through sonication of the cells, followed by two extractions with H_2O-saturated BuOH and concentration of the organic phase. The organic extract was preliminarily analyzed by liquid chromatography electrospray high-resolution mass spectrometry (LC-ESI-HRMS) and was shown to contain six related compounds, which according to their MS/MS fragmentations appeared to be cyclic peptides. The most abundant compound (**1**) was isolated and analyzed by MS, MS/MS, and NMR spectroscopy.

The ESI-HRMS spectrum of compound **1** showed, in addition to the $[M + H]^+$ ion at m/z 715.4752 (base peak), an $[M + Na]^+$ ion at m/z 737.4570, a doubly charged $[M + Ca]^{2+}$ ion at m/z 377.2147, and an $[M + Ca + HCOO]^+$ ion at m/z 799.4277, all within 1 ppm of the calculated mass for the molecular formula $C_{38}H_{63}N_6O_7$. The fragmentation pattern observed in the tandem mass (MS/MS) spectrum of compound **1** was clearly suggestive of a peptide, with fragments originating from loss of one valine, one leucine or isoleucine, and one tyrosine residue. Consideration of the molecular formula led to the conclusion that the peptide **1** contains one tyrosine, one valine, and four leucine/isoleucine residues and that the peptide is cyclic. The overall fragmentation pattern (Figure S3) indicated that the tyrosine residue is flanked by a valine and a leucine/isoleucine residue.

A complete set of 2D NMR spectra of compound **1** was recorded. The proton spectrum contained six distinct amide NH signals (thus showing that no *N*-methylated residue is present in the molecule) and six distinct α-proton signals (Table 1). Analysis of the TOCSY spectrum (Figure S2) showed correlation of all the protons of the six side chains with the corresponding α-proton and/or amide NH signals (Table 1). This information, combined with the analysis of the COSY spectrum, led to the identification of a tyrosine, a valine, an isoleucine, and three leucine residues. The sequence of the six amino acids in the peptide was determined on the basis of the NOESY spectrum, which contained a clear correlation peak between each α-proton and the amide NH proton of the subsequent amino acid residue in the sequence (Table 1). The amino acid sequence determined in this way was cyclo(Tyr-Val-Leu(I)-Leu(II)-Leu(III)-Ile).

The D/L configuration of the six amino acid residues was determined using an advanced Marfey's method, adapted to the use with the Orbitrap high-resolution MS detector[10] and further modified on account of the Ile residue present in **1**. In fact, in our experience, a C18 HPLC column cannot resolve the FDAA derivatives of D/L-Ile from those of D/L-*allo*-Ile. However, the use of a C3 column has been proposed to overcome difficulties with reversed-phase separations of this compound class.[11] We found that a pentafluorophenyl (PFP) bonded-phase column can provide baseline separation of six isobaric Leu, Ile, and *allo*-Ile derivatives and is suited for use with an MS-selective detector (Figure 1A). A small amount of compound **1** (12 μg) was hydrolyzed by treating it with 6 N HCl/AcOH (1:1) at 120 °C for 12 h. The hydrolysate was reacted with 100 μL of 1% 1-fluoro-2,4-dinitrophenyl-5-D-alaninamide (D-FDAA), the resulting D-FDAA derivatives of Tyr, Val, Leu, and Ile were analyzed by HPLC-ESI-HRMS, and their retention times were compared with authentic standards prepared by reaction of L- and D-FDAA with L-Tyr, L-Val, L-Leu, L- Ile, and D-*allo*-Ile. Analysis of the resulting chromatograms (Figure 1) revealed that the peptide **1** contains 2 × L-Leu, 1 × D-Leu, 1 × D-*allo*-Ile, 1 × L-Val, and 1 × D-Tyr.

The location of the D-Leu residue in compound **1** could not be determined from the above data. Therefore, compound **1** was subjected to partial hydrolysis, which produced a complex mixture of linear peptides ranging from two to six amino acids. HPLC separation of the mixture yielded two pure peptides (**7** and **8**). The peptide **7** showed an $[M + H]^+$ ion in the ESIMS spectrum at m/z 620.4018, corresponding to the molecular formula $C_{32}H_{54}N_5O_7$, in accordance with a linear pentapeptide originating from the loss of one leucine or isoleucine residue from compound **1**. The sequence of the peptide was determined as H-Leu(III)-Ile-Tyr-Val-Leu(I)-OH by analysis of the fragmentation pattern observed in its MS/MS spectrum (Figure S4), showing that the lost amino acid residue was Leu(II) (aa-4 in structure **1**). Hydrolysis and Marfey's analysis of this fragment as described above revealed the presence of D-*allo*-Ile and L-Leu, but not D-Leu. This showed that the lost amino acid residue, i.e., Leu(II), was indeed D-Leu.



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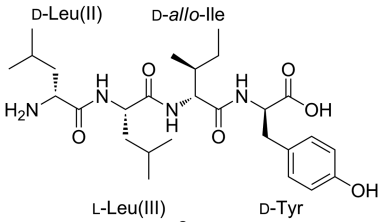

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The peptide **8** showed an $[M + H]^+$ ion in the ESIMS spectrum at m/z 521.3333, corresponding to the molecular formula $C_{27}H_{44}N_4O_6$, in accordance with a linear tetrapeptide originating from the loss of one valine and one leucine or isoleucine residue from compound **1**. There is only one such tetrapeptide that can be obtained from compound **1**, i.e., H-Leu(II)-Leu(III)-Ile-Tyr-OH, and this sequence was confirmed by the MS/MS fragmentation pattern (Figure S5). Hydrolysis and Marfey analysis of this fragment showed the presence of both

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Table 1. NMR Data of Thermoactinoamide A (1) (^{1}H 700 MHz, ^{13}C 175 MHz, CD_3SOCD_3)

amino acid	pos.	δ_C, type		δ_H, mult (J in Hz)	NOESY[a]	HMBC[b]
D-Tyr	NH			7.49, d (5.7)	Ile-NH, Ile-2, Ile-3	Ile-1
	1	171.2, C				
	2	54.8, CH		4.64, ddd (8.6, 5.7, 5.4)	Val-NH, Tyr-5/9	Tyr-1
	3	37.6, CH_2	a	2.92, dd (13.6, 5.4)	Tyr-5/9	Tyr-1
			b	2.75, dd (13.6, 8.6)	Tyr-5/9	Tyr-1
	4	126.6, C				
	5/9	129.9, CH		6.91,d (8.3)	Tyr-2, Tyr-3a, Tyr-3b	
	6/8	114.7, CH		6.63, d (8.3)		
	7	155.8, C				
L-Val	NH			7.91, d (6.2)	Leu(I)-NH, Tyr-2	Tyr-1
	1	170.5, C				
	2	60.0, CH		3.62, t (5.8)	Leu(I)-NH	Tyr-1, Val-1
	3	29.0, CH		1.86, octet (6.6)		
	4	17.4, CH_3		0.68, d (6.8)		
	5	18.6, CH_3		0.66, d (6.8)		
L-Leu(I)	NH			7.75, d (8.1)	Val-NH, Val-2, Leu(II)-NH	Val-1
	1	170.7, C				
	2	50.6, CH		4.23, ddd (10.5, 8.1, 5.2)	Leu(II)-NH	Leu(I)-1
	3	39.2, CH_2	a	1.59, m		
			b	1.54, m		
	4	24.0, CH		1.46, m		
	5	22.8, CH_3		0.85, d (6.8)		
	6	20.9, CH_3		0.78, d (6.8)		
D-Leu(II)	NH			7.53, d (8.1)	Leu(I)-NH, Leu(I)-2, Leu(III)-NH	Leu(I)-1
	1	171.4, C				
	2	50.5, CH		4.33, q (7.4)	Leu(III)-NH	Leu(II)-1
	3	40.8, CH_2	a	1.48, m		
			b	1.48, m		
	4	24.2, CH		1.47, m		
	5	22.5, CH_3		0.87, d (6.8)		
	6	21.8, CH_3		0.82, d (6.8)		
L-Leu(III)	NH			8.13, d (7.1)	Leu(II)-NH, Leu(II)-2, Ile-NH	Leu(II)-1
	1	171.7, C				
	2	51.1, CH		4.34, q (7.1)	Ile-NH	Leu(III)-1
	3	39.3, CH_2	a	1.46, m		
			b	1.36, m		
	4	24.0, CH		1.50, m		
	5	22.1, CH_3		0.89, d (6.8)		
	6	22.0, CH_3		0.83, d (6.8)		
D-*allo*-Ile	NH			8.26, d (9.0)	Leu-III-NH, Leu(III)-2, Tyr-NH	Leu(III)-1
	1	170.8, C				
	2	55.8, CH		4.25, dd (9.0, 5.0)	Tyr-NH	Leu(III)-1, Ile-1
	3	35.9, CH		1.97, m	Tyr-NH	
	4	14.3, CH_3		0.78, d (7.0)		
	5	25.5, CH_2	a	1.23, m		
			b	1.11, m		
	6	11.2, CH_3		0.81, t (7.3)		

[a]Mixing time 300 ms. Correlations also present in the TOCSY spectrum are not shown. [b]Selected HMBC correlations from proton stated to the indicated carbon.

L-Leu and D-Leu in the peptide, further confirming the location of the D-Leu residue.

The minor cyclic hexapeptides thermoactinoamides B–F (**2**–**6**) were present in much smaller amounts in the extract of *T. vulgaris*. They were not isolated, but only partially characterized by analysis of the respective MS/MS spectra. Putative structures for compounds **2**–**6** are shown in Table 2. They were obtained by analogy with compound **1**, under the assumptions that (i) differences between compounds **2**–**6** and compound **1** are confined to a single amino acid and (ii) the configurations of the corresponding amino acids are the same as in **1** (see also Figures S6–S10).

Several hexapeptides have been isolated from natural sources, but most of them (such as the iron-binding ferrichromes or the cyanobacterial oxazole- or thiazole-containing peptides) are unrelated to the thermoactinoamides. A few more similar peptides (i.e., peptides made of unmodified, mostly lipophilic proteogenic amino acids with mixed D/L configurations) have been isolated from actinomycetes (it must be noted that, in spite of the name, the genus *Thermoactinomyces* is phylogenetically far

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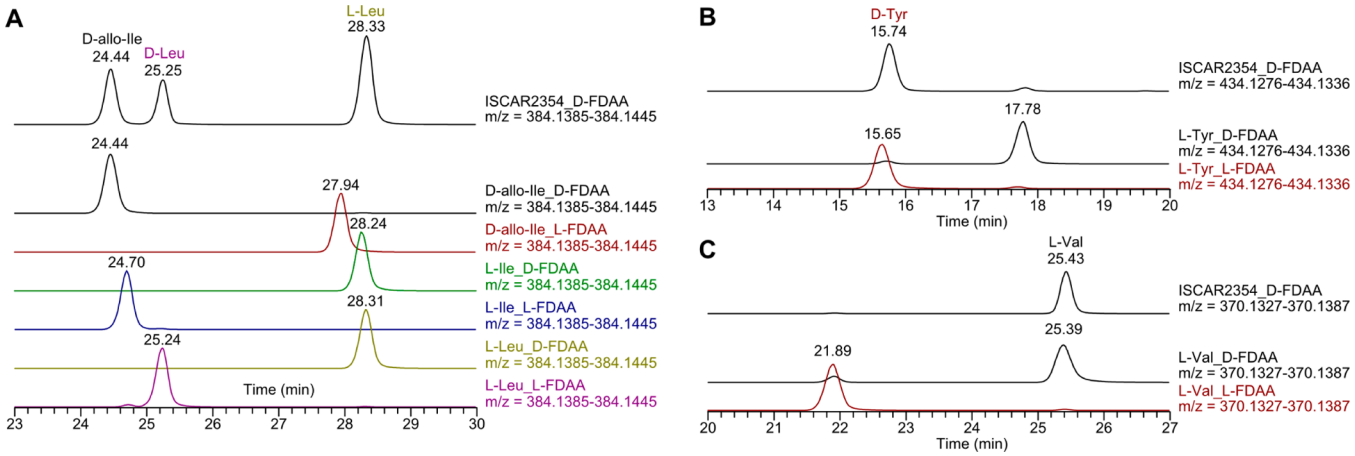

Figure 1. Advanced Marfey's analysis of compound **1** using a pentafluorophenyl (PFP) bonded-phase column. (A) Extracted-ion chromatograms at m/z 384.1415 of the D-FDAA derivatives from the hydrolysis of **1** and of D- and L-FDAA derivatives L-Leu, L-Ile, and D-*allo*-Ile. (B) Extracted-ion chromatograms at m/z 434.1306 of the D-FDAA derivatives from the hydrolysis of **1** and of D- and L-FDAA derivatives L-Tyr. (C) Extracted-ion chromatograms at m/z 370.1357 of the D-FDAA derivatives from the hydrolysis of **1** and of D- and L-FDAA derivatives L-Val.

Table 2. Putative Structures of the Minor Cyclic Hexapeptides 2–6 from *T. vulgaris*

	name	molecular formula	aa-1	aa-2	aa-3	aa-4	aa-5	aa-6
1	thermoactinoamide A	$C_{38}H_{62}N_6O_7$	D-Tyr	L-Val	L-Leu	D-Leu	L-Leu	D-*allo*-Ile
2	thermoactinoamide B	$C_{37}H_{60}N_6O_7$	D-Tyr	L-Val	L-Leu	D-Leu	L-Leu	D-Val
3	thermoactinoamide C	$C_{39}H_{64}N_6O_7$	D-Tyr	L-Leu/L-Ile	L-Leu	D-Leu	L-Leu	D-*allo*-Ile
4	thermoactinoamide D	$C_{41}H_{60}N_6O_8$	D-Tyr	L-Val	L-Leu	D-Leu	L-Tyr	D-*allo*-Ile
5	thermoactinoamide E	$C_{35}H_{64}N_6O_6$	D-Leu/D-*allo*-Ile	L-Val	L-Leu	D-Leu	L-Leu	D-*allo*-Ile
6	thermoactinoamide F	$C_{38}H_{62}N_6O_6$	D-Phe	L-Val	L-Leu	D-Leu	L-Leu	D-*allo*-Ile

from Actinomycetales).[12] Among them are phepropeptins A–D from *Streptomyces* sp.,[13] desotamides from *Streptomyces scopuliridis* SCSIO ZJ46,[14] wollamides from *Streptomyces scopuliridis* SCSIO ZJ46,[15] and nocardiamides A and B from the *Nocardiopsis* sp. CNX037[16] (see Figure S1 for the structures of some of these compounds). In particular, nocardiamides appear very similar to thermoactinoamide A (**1**) in amino acid composition, but show a different pattern of D/L configurations for the amino acid residues, suggesting a different, possibly unrelated biosynthetic origin.

Antibacterial Activity of Thermoactinoamide A (1). The cyclic hexapeptide **1** was tested against a panel of three Gram-positive (*Staphylococcus aureus* ATCC 6538, *Enterococcus faecalis* ATCC 29212, *Bacillus cereus* ATCC 14579) and three Gram-negative (*Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 13388, *Klebsiella pneumoniae* ATCC 11296) bacterial strains. It exhibited a significant and selective growth inhibition against *S. aureus* with a minimum inhibitory concentration (MIC) value of 35 μM. It was inactive against the other strains up to 140 μM.

Thermoactinoamide A (**1**), which revealed a significant and selective activity against *S. aureus* ATCC 6538, may be a promising antibacterial compound. A comparably narrow antibiotic activity against *S. aureus* ATCC 29213 with an MIC value of 23 μM was previously reported for the related cyclic hexapeptides desotamides A and B.[14] While it is probably premature to draw conclusions about the exact structural requirements for the antibiotic activity of cyclic hexapeptides, the common structural features of thermoactinoamides and desotamides (cyclic hexapeptides with mixed D/L configurations, one aromatic amino acid residue, prevalence of lipophilic residues) can be seen as a starting point to define a new, easily accessible scaffold for antibiotic drugs. Future research to develop thermoactinoamides along these lines will involve evaluation of their activity against MRSA strains, definition of their structure–activity relationship, and identification of their mechanism of action.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured using a Jasco P-2000 polarimeter at the sodium D line. Electronic circular dichroism (ECD) spectra were recorded using a Jasco-715 spectropolarimeter. NMR spectra were determined on Varian Unity Inova spectrometers at 700 MHz; chemical shifts were referenced to the residual solvent signal (CD_3SOCD_3: δ_H 2.50, δ_C 39.52). For an accurate measurement of the coupling constants, the one-dimensional ^{1}H NMR spectra were transformed at 64 K points (digital resolution: 0.09 Hz). Through-space ^{1}H connectivities were evidenced using a NOESY experiment with a mixing time of 300 ms. The HSQC spectra were optimized for $^1J_{CH}$ = 145 Hz, and the HMBC experiments for $^{2,3}J_{CH}$ = 8 Hz. High-resolution ESIMS and HR-ESI-HPLC experiments were performed on a Thermo LTQ Orbitrap XL mass spectrometer coupled to a Thermo U3000 HPLC system. High-performance liquid chromatography (HPLC) separations were achieved on an Agilent 1260 Infinity Quaternary LC apparatus equipped with a diode-array detector .

Collection, Cultivation, and Extraction. The thermophilic bacterial strain *Thermoactinomyces vulgaris* ISCAR 2354, used for the isolation and characterization of thermoactinoamide A, originated from a coastal hot spring in Icelandic marine waters (64°57′52″ N, 23°5′21.53″ W). A second strain of the same species (ISCAR 2850) that was isolated from a deep sea hydrothermal vent sediment core at 400 m depth (66°36′51″ N, 17°39′09″ W), was also found to contain thermoactinoamide A. Both strains were identified based on their 16S rRNA gene sequences (GenBank accession numbers M372920 and M372921, respectively) with 100% identity to other *T. vulgaris* sequences in GenBank. However, phylogenetic analysis based on 16S rDNA sequences of *Thermoactinomyces* species suggested that the genus

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should be taxonomically re-evaluated using other useful taxonomic markers.[12]

The strains were grown at 60 °C in 250 mL culture flasks in medium 166 containing 1% NaCl for 24 h.[9] The bacterial culture was sonicated for 3 min using a Branson Sonifier 250 with the duty cycle on constant-output control on 4 and the output at 20 to break up the cells. The whole culture fluid was transferred to a 1 L centrifuge flask and mixed with 100 mL of H_2O-saturated BuOH with 10% MeOH before centrifugation (10 min, 10000*g*, 4 °C). The upper, organic phase was transferred into a new flask, and the BuOH extraction steps were repeated. The organic extract was filtered through a 0.45 μm PTFE filter and then concentrated on a rotary evaporator at 40 °C to obtain an extract for the analyses.

Isolation of Thermoactinoamide A. The organic extract of ISCAR 2354 (739 mg) was analyzed by LC-ESI-HRMS on a Thermo LTQ Orbitrap XL using a 5 μm Kinetex C18 column (50 × 2.10 mm) maintained at 25 °C and a gradient system [eluent A: 0.1% HCOOH in H_2O; eluent B: CH_3CN; gradient program: 30% B 5 min, 30% → 99% B over 17 min; flow rate 200 μL min^{-1}]. The extract was shown to contain six related compounds (compounds **1**–**6**), which accounted for most of the peaks in the total ion current chromatogram. An MS/MS spectrum was recorded for each compound. Analysis of the fragmentation pattern suggested them to be cyclic peptides. The most abundant compound, **1**, was isolated by reversed-phase HPLC using a 10 μm Kinetex C18 column (250 × 10 mm) [eluent A: 0.1% HCOOH in H_2O; eluent B: MeOH; gradient program: 60% B 5 min, 60% → 100% B over 17 min, 100% B 13 min; flow rate 5 mL min^{-1}, wavelength 280 nm], thus obtaining pure thermoactinoamide A (**1**, 1.2 mg, t_R 18.7 min).

*Thermoactinoamide A (**1**):* colorless, amorphous solid; $[\alpha]^{25}_D$ +80 (*c* 0.5, MeOH); ECD (412 μM, MeOH) λ_{max} ($\Delta\varepsilon$) 280 (+0.5); ^{1}H and ^{13}C NMR data, Table 1; HRESIMS (positive ion mode, 0.1% HCOOH in MeOH) *m*/*z* 715.4752 $[M + H]^+$ (calcd for $C_{38}H_{62}N_6O_7$, 737.4753), *m*/*z* 737.4570 $[M + Na]^+$ (calcd for $C_{38}H_{62}N_6O_7Na^+$, 737.4572), 377.2147 $[M + Ca]^{2+}$ (calcd for $C_{38}H_{62}N_6O_7Ca^{2+}$, 377.2147), 799.4277 $[M + Ca + HCOO]^+$ (calcd for $C_{39}H_{63}N_6O_9Ca^+$, 799.4277).

Advanced Marfey's Analysis. Compound **1** (12 μg) was hydrolyzed with 6 N HCl/AcOH (1:1) at 120 °C for 12 h. The residual HCl fumes were removed under N_2 stream. The hydrolysate of **1** was dissolved in TEA/acetone (2:3, 100 μL), and the solution was treated with 100 μL of 1% 1-fluoro-2,4-dinitrophenyl-5-D-alaninamide (D-FDAA) in CH_3CN/acetone (1:2). The vial was heated at 50 °C for 1.5 h. The mixture was dried, and the resulting D-FDAA derivatives of Tyr, Val, Leu, and Ile were redissolved in MeOH (100 μL) for subsequent analysis. Authentic standards of L-Tyr, L-Val, L-Leu, L-Ile, and D-*allo*-Ile were treated with L-FDAA and D-FDAA as described above and yielded the L-FDAA and D-FDAA standards. Marfey's derivatives of **1** were analyzed by HPLC-ESI-HRMS, and their retention times were compared with those from the authentic standard derivatives. A 2.6 μm Kinetex PFP column (100 × 4.6 mm) maintained at 25 °C was eluted at 200 μL min^{-1} with 0.1% HCOOH in H_2O and MeOH. The gradient program was as follows: 60% MeOH 5 min, 60% → 100% MeOH over 30 min, 100% MeOH 15 min. Mass spectra were acquired in positive ion detection mode, and the data were analyzed using the Xcalibur suite of programs.

Partial Hydrolysis. An aliquot (34 μg) of compound **1** was treated with a mixture of 6 N HCl/AcOH (1:1) at 100 °C for 2 h. The hydrolysate was concentrated under a N_2 stream, resuspended in MeOH (50 μL), and purified by HPLC using the same conditions as above. For HPLC separation, the high-resolution mass spectrometer was used as detector, splitting the flow before the ion source with 10% going to the mass spectrometer. This afforded the two pure linear peptides **7** and **8** (t_R 23.8 and 24.9 min, respectively), which were subjected to total hydrolysis and derivatization with D-FDAA as previously described.

Antibacterial Activity of Thermoactinoamide A (1). The antibacterial activity was assayed against the Gram-positive bacterial strains *S. aureus* ATCC 6538, *E. faecalis* ATCC 29212, and *B. cereus* ATCC 14579 and Gram-negative *E. coli* ATCC 8739, *P. aeruginosa* ATCC 13388, and *K. pneumoniae* ATCC 11296 by the liquid growth inhibition in 96-well microplates. A preculture of 5 mL of Luria Bertoni was prepared by inoculating a colony of each bacterial strain and was incubated at 37 °C with stirring overnight. The concentration of the preculture was assessed by measuring the optical density (OD) at 620 nm and was adjusted by dilution in order to obtain a suspension of 0.03 OD. An aliquot of 200 μL of the bacterial suspension was distributed in each well containing 2-fold serial dilutions of the peptide. The 96-well microplates were incubated at 34 °C overnight with shaking (450 rpm). The optical density of the wells was measured at 620 nm with a microplate reader, and MICs, performed in triplicate, were defined as the lowest concentration of drug that completely inhibits bacterial growth. MIC values of positive controls: ampicillin [*S. aureus* (0.11 μM), *E. faecalis* (4.59 μM)], gentamicin [*B. cereus* (17.2 μM), *P. aeruginosa* (10.5 μM)], and chloramphenicol [*E. coli* (19.3 μM), *K. pneumoniae* (3.17 μM)].

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jnatprod.7b00560.

Structures of some known cyclic hexapeptides from actinomycetes, 1D sections of the TOCSY spectrum of **1**, MS/MS spectra of **1**–**8**, and copies of the MS and one- and two-dimensional NMR spectra of **1** (PDF)

■ AUTHOR INFORMATION

Corresponding Author

*Phone: +39-081678532. Fax: +39-081678552. E-mail: alfonso.mangoni@unina.it.

ORCID

Valeria Costantino: 0000-0001-8723-9505

Alfonso Mangoni: 0000-0003-3910-6518

Notes

The authors declare no competing financial interest.

A patent application about these results has been filed to the Icelandic patent office, Patent Number 9079.

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