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Discodermin E, a Cytotoxic and Antimicrobial Tetradecapeptide, from the Marine Sponge *Discodermia kiiensis* [1]

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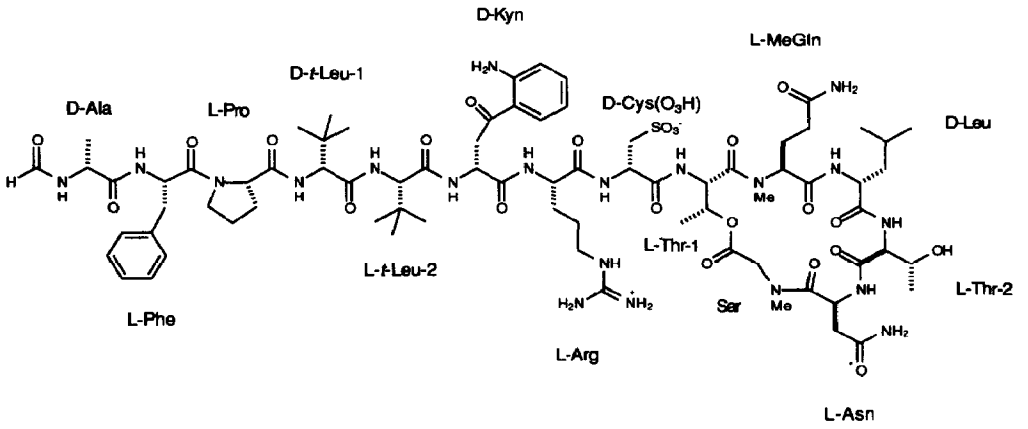
Abstract. A new cytotoxic and antimicrobial peptide, discodermin E (**1**), was isolated from the marine sponge *Discodermia kiiensis* together with the known discodermins A-D. The structure of discodermin E was determined by spectral and chemical methods.

Sponge peptides are a growing group of marine natural products.[2] Ten years ago we first isolated the bioactive sponge peptides, discodermins A-D from *Discodermia kiiensis*.[3-5] They were later found to be potent inhibitors of phospholipase A_2[6] and discodermin A inhibited the tumor promotion activity of okadaic acid.[7] We again examined *D. kiiensis* collected off Atami in the Gulf of Sagami, which was highly active in our antimicrobial and cytotoxic tests. Bioassay-guided fractionation of the extract of the sponge led to the isolation of discodermin E (**1**) together with the known discodermins A-D. Structural study showed that discodermin E had a D-kynurenine residue in place of D-Trp residue in discodermin A and a reversed sequence of the 12th and 13th residues from the *N*-terminus.[8] In this paper, we describe the isolation and structure elucidation of discodermin E.

The EtOH extract of the frozen sponge (5.0 kg)[9] was partitioned between H_2O and Et_2O; the aqueous phase was further extracted with *n*-BuOH. The *n*-BuOH layer was fractionated by ODS flash chromatography with aqueous MeOH. The active fraction eluted with 80% MeOH was gel-filtered on Sephadex LH-20 with MeOH and purified by reversed phase HPLC with MeCN/H_2O (33.5:66.5) containing 0.05% TFA to yield discodermin E (3.5 mg; 7.0×10^{-5} % yield, wet weight) along with discodermins A-D (A, 2.1 g, 0.42%; B, 55 mg, 1.1×10^{-3}%; C, 48 mg, 9.8×10^{-4}%; D, 32 mg, 6.4×10^{-4}%).

The molecular formula of discodermin E (**1**) was established to be $C_{76}H_{117}N_{20}O_{23}S$ on the basis of HRFABMS and NMR data.[10] The ^1H NMR spectrum was similar to that of discodermin A. Amino acid composition was determined by interpretation of the DQF-COSY, HOHAHA, and NOESY spectra; the NOESY technique was particularly useful for assignments of the residues containing non-protonated carbons. Thus, the presence of Asn, Thr (2 residues), Sar (*N*-methylglycine), MeGln (*N*-methylglutamine), Ala, Leu, Pro, Phe, Arg, Cys(O_3H), and *t*-Leu (2) residues and a formamide group was established (Table 1). These amino acids



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were also detected by standard amino acid analysis.[11] Amide protons in the side chain carboxyl groups of both Asn and MeGln were observed as two pairs of exchangeable protons which gave rise to NOESY cross peaks with methylene protons linked to carbonyl carbon. Two *N*-methyl groups were assigned to MeGln and Sar residues on the basis of intra-residual cross peaks in the NOESY and HMBC spectra. There was one unusual residue which replaced the Trp residue in discodermin A, and it exhibited signals for mutually coupled four aromatic protons [δ 7.71 (d, *J*=8.0 Hz), 7.22(t, 8.0), 6.73 (d, 8.0), 6.53 (t, 8.0)] and an NH-CH-CH_2 unit [δ 8.34 brs, 4.83 (q, 6.0), and 3.34 (2H, br m)]. HMBC cross peaks were observed between the protons at δ 7.71 and 3.34 and a ketone (δ 198.2), which connected the two partial structures. ^{13}C NMR data for the aromatic ring (δ 116.2, 150.9, 116.4, 134.0, 114.3, and 131.0) were reminiscent of kynurenine (Kyn),[12] which was confirmed by HPLC analysis of the acid hydrolysate.

The amino acid sequence of discodermin E was deduced by interpretation of inter-residual cross peaks in the NOESY spectrum which exhibited the following cross peaks: CHO/Ala NH; Ala Hα/Phe NH; Phe NH, Hα/Pro $H_2δ$; Pro $H_2β$/*t*-Leu-1 NH; *t*-Leu-1 NH, Hα/*t*-Leu-2 NH; *t*-Leu-2 Hα/Kyn NH; Kyn NH, Hα/Arg NH; Arg NH, Hα/Cys(O_3H) NH; Cys(O_3H) NH, Hα/Thr-1 NH; Thr-1 Hα/MeGln NMe; MeGln Hα/Leu NH; Leu Hα/Thr-2 NH; Thr-2 NH, Hα/Asn NH; Asn NH, Hα/Sar NMe. The only connection left unassigned was the macrocyclic lactone between the carboxyl group of the C-terminal Sar residue and one of the hydroxyl groups of the two Thr residues. The β-proton of Thr-1 resonated at 5.17 ppm, thereby revealing participation of the hydroxyl group of this residue in the lactone formation. This was confirmed by an HMBC cross peak between Thr-1 Hβ and Sar C-1.

Absolute stereochemistry of amino acid residues in **1** was determined by HPLC analysis of the acid hydrolysate derivatized with Marfey's reagent.[13] All amino acids except Kyn had identical absolute configuration as was the case with discodermin A; Kyn was in the D-form. Configuration of the two *t*-Leu residues was determined as follows:[4] deformylated discodermin E was subjected to four cycles of the Edman degradation

followed by Marfey analysis of the acid hydrolysate, which resulted in L- and D-*t*-Leu in a ratio of 5:1. Therefore, 4th and 5th residues from the *N*-terminus were D-*t*-Leu and L-*t*-Leu, respectively.

Table 1. ^{1}H and ^{13}C NMR signal assignments of discodermin E (1) in DMSO-d_6*

assignment	$\delta^{13}C$	$\delta^{1}H$ (mult.)	*J* (Hz)	assignment	$\delta^{13}C$	$\delta^{1}H$ (mult.)	*J* (Hz)
CHO	160.3	7.90 s		γ	23.3	1.48 m	
Ala				δ	40.0	3.02 m	
α	46.1	4.33 m		NH		7.83 d	7.5
β	18.3	0.93 d	7.0	δ-NH		7.38 t	4.6
NH		8.07 d	7.9	Cys(O_3H)			
Phe				α	50.5	4.59 q	6.9
α	51.7	4.71 m		β	52.0	2.92 dd	13.7, 7.6
β	36.8	2.75 dd	14.1, 10.5			2.97 dd	13.7, 5.2
		3.01 m		NH		8.29 d	7.3
1'	137.3			Thr-1			
2'/6'	129.1	7.27 d	7.2	α	51.3	4.89 d	8.7
3'/5'	127.8	7.24 t	7.3	β	69.2	5.17 q	6.7
4'	126.1	7.17 t	7.2	γ	16.9	1.19 d	6.7
NH		8.31 d	8.7	NH		7.97 d	9.1
Pro				MeGln			
α	59.3	4.52 m		α	54.5	5.02 m	
β	29.2	1.86 m		β	24.3	1.79 m	
		2.12 m				1.93 m	
γ	24.0	1.86 m		γ	31.4	1.96 m	
δ	46.6	3.61 m		NMe	30.7	3.06 s	
		3.66 m		γ-NH_2		6.68 brs	
t-Leu-1						7.06 brs	
α	59.2	4.51 d	9.5	Leu			
β	34.7			α	51.1	4.46 m	
γ	26.3	0.91 s		β	41.6	1.46 m	
NH		7.65 d	9.5			1.55 m	
t-Leu-2				γ	23.6	1.55 m	
α	61.0	4.06 d	7.5	δ	22.2	0.91 d	7.5
β	33.2			NH		7.57 d	6.8
γ	26.3	0.91 s		Thr-2			
NH		7.80 d	7.5	α	61.0	3.95 dd	8.3, 4.2
Kyn				β	65.4	4.03 m	
α	49.1	4.83 q	6.0	γ	20.0	1.05 d	6.3
β	40.0	3.34 br m		NH		7.88 d	8.3
γ	198.2			Asn			
1'	116.2			α	45.8	5.01 m	
2'	150.9			β	36.9	2.11 dd	15.2, 4.2
3'	116.4	6.73 d	8.0			2.67 dd	15.2, 9.4
4'	134.0	7.22 t	8.0	NH		7.51 d	9.4
5'	114.3	6.53 t	8.0	β-NH_2		6.61 brs	
6'	131.0	7.71 d	8.0			7.06 brs	
NH		8.34 brs		Sar			
Arg				α	49.3	3.49 d	17.5
α	52.4	4.31 m				4.45 d	17.5
β	28.4	1.69 m		NMe	35.2	2.77 s	
		1.79 m					

* ^{13}C chemical shifts were determined by tracing HMQC and HMBC. Chemical shifts of amide carbons were not assigned.

To our best knowledge discodermin E is the first marine peptide containing kynurenine as an amino acid residue.[14] It is of interest to check whether *D. kiiensis* from Shikine-jima Island, from which we first isolated discodermins A-D, contains discodermin E or not.

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8. The structure of discodermin E prompted us to reexamine structures of discodermins A-D by modern NMR spectroscopy and gas-phase protein sequencer (manuscipt in preparation).
9. The sponge was collected at depths of 15-25 m off Atami, 90 km southwest of Tokyo. The voucher specimen was deposited at Laboratory of Marine Biochemistry, University of Tokyo.
10. **1**: colorless solid; $[\alpha]_D^{23}$ -7.1° (*c* 0.01, MeOH); FABMS (pos.) *m/z* 1747 $(M+K)^+$, 1731 $(M+Na)^+$, 1709 $(M+H)^+$, 959, 344; HRFABMS (pos.) *m/z* 1709.8215 [$(M+H)^+$, $C_{76}H_{117}N_{20}O_{23}S$, Δ -10.6 mmu]; UV (MeOH) λ_{max} 368 nm (ε 5,040), 258 (7,160), 232 (26,720); IR (film) ν_{max} 3320, 3050, 2950, 1735, 1640, 1530, 1420, and 1180 cm^{-1}. Discodermin E inhibited the growth of *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonus aeruginosa* at 2.5 μg/disk, and *Mortierella ramannianus*, *Candida albicans*, and *Penicillium chrysogenum* at 25 μg/disk. It was cytotoxic against P388 leukemia cells at IC_{50}, 0.02 μg/mL and inhibited the development of starfish (*Asterina pectinifera*) embryo at 5 μg/mL.
11. Assignment of the Thr residues was ambiguous at this moment, because *a*Thr and Thr residue give the same ^1H NMR spin systems and retention times in amino acid analysis. They were differentiated by Marfey analysis of the acid hydrolysate.
12. ^{13}C NMR data of authentic kynurenine purchased from Nacalai Tesque Inc. in DMSO-d_6: δ 115.4, 151.4, 117.1, 134.9, 114.6, and 131.1.
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