

Kahalalide F: A Bioactive Depsipeptide from the Sacoglossan Mollusk *Elysia rufescens* and the Green Alga *Bryopsis* sp.¹

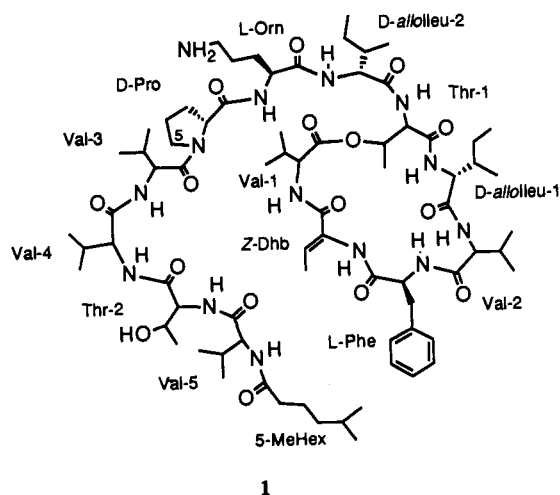
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Sacoglossans are herbivorous marine mollusks with the ability to sequester from their algal diet functioning chloroplasts,² which then may participate in the biosynthesis of secondary metabolites,³ frequently polypropionates.⁴ The sacoglossan genus *Elysia* is represented in Hawaii by several species,⁵ among them *Elysia degeneri* and *Elysia rufescens*. *E. degeneri* is known to feed on *Udotea* spp., a green alga from which antifeedant diterpene aldehydes have been isolated.⁶ Our interest in the chemistry of coral reef communities prompted us to study *E. rufescens*, for which no algal food source was described.⁵ In analogy with Paul's findings⁶ we expected to isolate diterpene aldehydes. Instead, we found that *E. rufescens*, which was observed feeding on the green alga *Bryopsis* sp., contains a series of difficultly separable depsipeptides ranging from a C₃₁ tripeptide to a C₇₅ tridecapeptide. While their component amino acids are, except for one, commonly occurring, each peptide contains a different relatively obscure fatty acid. In this paper we describe structure and properties of kahalalide F (1),⁷ the largest and most active of the peptides, which occurs in *E. rufescens* and in its diet, *Bryopsis* sp. Structures of the other peptides will be reported in a full paper.

Two hundred animals (300 g wet) were collected by snorkeling at low tide near Black Point, O'ahu, during Spring 1991. The ethanolic extract was chromatographed on a silica flash column, from which the peptide mixture was eluted with EtOAc/MeOH (1:1). Repeated HPLC on C18 reversed-phase columns (MeCN/H₂O/TFA, 55:45:0.1) yielded kahalalide F (1) as a white amorphous powder (40 mg, 0.01%), [α]_D -8° (c, 4.32 MeOH). Subsequently, the alga *Bryopsis* sp. was collected and kahalalide F (1) was isolated (10 mg, 0.003%) by the same separation scheme as for the mollusk.



High-resolution FABMS and detailed analysis of the ¹³C NMR spectrum provided a molecular formula of C₇₅H₁₂₄N₁₄O₁₆ (M⁺ + 1, m/z 1477.9408, 85%, Δ +1.0 mmu). Amino acid analysis by GC-MS on a Chirasil-Val column revealed 12 amino acids: D-alloisoleucine (2), L-ornithine, D-proline, L-threonine, D-allothreonine, D-valine (3), L-valine (2), and L-phenylalanine, accounting for C₆₄H₁₀₇N₁₃O₁₄. The absolute stereochemistry of the individual valines, and of threonine, is still under investigation. ¹H and ¹³C NMR spectra (Table I and supplementary material) permitted assignment of the remaining C₁₁H₁₇NO₂ aggregate and full structural elucidation of kahalalide F (1). The ¹H NMR spectrum revealed 14 NH protons when measured in DMF plus 1 equiv of TFA. One of the NH protons, a sharp singlet at δ 9.20, indicated an α,β-unsaturated amino acid. A broad 2H singlet at δ 8.13 was shown by a COSY experiment to be the terminal NH₂ of ornithine. Compound 1 was ninhydrin-positive, thus supporting the presence of the free amino group of ornithine. The remaining 11 NH protons were doublets; those that are part of the ring are sharp, while the NH protons of the linear portion of the molecule are split due to several conformations of the molecule. The β-proton of Thr-1 was shifted downfield to 5.07 ppm, suggesting an ester linkage, which was confirmed by an HMBC correlation between the β-proton of Thr-1 and the carbonyl carbon of Val-1. HMBC and ROESY experiments established the connectivity of the amino acids. Each NH proton showed a correlation to its α-carbon and to the carbonyl of the vicinal amino acid in the HMBC experiment, thereby establishing the sequence of all amino acids except proline. A ROESY experiment allowed connection of Pro to Val-3 through a correlation between the protons on C5 (3.75 and 3.68 ppm) of Pro and the α-proton of Val-3. A correlation between methyl and NH protons of dehydroaminobutyric acid (Dhb) in the ROESY experiment indicated Z stereochemistry of this uncommon amino acid. Linkage of the carbonyl in 5-methylhexanoic acid and Val-5 was secured by an HMBC experiment, which related α- and β-protons of the acid and the NH and α-protons of Val-5. Connection of the linear portion of kahalalide F (1) to the cyclic portion was determined by HMBC and ROESY experiments. The ROESY experiment showed a correlation between the NH proton of Thr-1 and the α-proton of Ileu-2, while the HMBC experiment links these two amino acids through the carbonyl by correlating the α-proton of Ileu-2 with the NH and α-proton of Thr-1.

Dehydroaminobutyric acid-containing peptides have been isolated from a terrestrial blue-green alga⁸ and from an herbivorous marine mollusk,^{9,10} but this appears to be the first such isolation from a macroalga. Our collections of *Bryopsis* sp. appeared uncontaminated by blue-greens.

Kahalalide F shows selectivity against solid tumor cell lines.¹¹ IC₅₀ values against A-549, HT-29, and LOVO are 2.5, 0.25, and <1.0 μg/mL, respectively; against P-388 and KB, 10 and >10 μg/mL. Kahalalide F is active against CV-1 cells with an IC₅₀ of 0.25 μg/mL. Antiviral activity at 0.5 μg/mL (95% reduction) was detected with HSV II using mink lung cells. The antifungal zones of inhibition for 50 μg/6 mm disk are as follows: *Aspergillus oryzae*, 19 mm; *Penicillium notatum*, 26 mm; *Trichophyton mentagrophytes*, 34 mm; *Saccharomyces cerevisiae*, negative; and *Candida albicans*, 16 mm. Additionally, this compound shows slight immunosuppressive activity in a mixed lymphocyte reaction

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(10) We are indebted to a referee, who mentioned this point.

(11) British Patent Appl. 9127225.2, Kahalalide F.

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Table I. ¹H and ¹³C NMR Data for Kahalalide F (1) in DMF/TFA

amino acid	carbon	¹³ C, ppm ^a	mult	¹ H, ppm ^b	mult	amino acid	carbon	¹³ C, ppm ^a	mult	¹ H, ppm ^b	mult
Val-1	1	170.4	s	(NH) 7.11	d, J=8.9	Pro	1	172.6	s		
	2	60.3	d	4.16	t, J=9.0		2	60.2	d	4.42	m
	3	30.8	d	1.77	m		3	29.6	t	2.12, 1.97	m, m
	4	19.6	q	0.95	m		4	25.4	t	2.02, 1.88	m, m
	5	18.8	q	0.95	m		5	48.0	t	3.75, 3.68	m, m
Dhb	1	164.5	s	(NH) 9.20	s	Val-3	1	171.3	s	(NH) 7.90	d, J=7.2
	2	130.3	s				2	57.6	d	4.41	m
	3	131.3	d	6.48	q, J=6.9		3	30.5	d	2.12	m
	4	12.7	q	1.43	d, J=6.6		4	19.6	q	0.95	m
Phe	1	171.3	s	(NH) 8.62	d, J=6.6	Val-4	5	18.8	q	0.85	m
	2	56.3	d	4.68	q, J=6.6		1	171.8	s	(NH) 7.68	d, J=8.1
	3	36.8	t	3.23	dd, J=13.7, 7.2		2	59.1	d	4.34	m
	4	138.2	s	3.00	dd, J=13.7, 9.0		3	31.3	d	2.17	m
	5, 5'	129.9	d	7.32	d, J=7.2		4	19.5	q	0.95	m
	6, 6'	128.8	d	7.28	t, J=7.5		5	18.1	q	0.90	m
	7	127.0	d	7.21	t, J=7.2		Thr-2	1	171.0	s	(NH) 7.77
Val-2	1	172.9	s	(NH) 7.82	d, J=6.6	2		58.9	d	4.46	m
	2	58.6	d	4.36	m	3		67.4	d	4.21	dq, J=6.3, 3.6
	3	32.4	d	2.12	m	4	19.7	q	1.12	d, J=6.6	
	4	18.9	q	0.85	m	Val-5	1	172.7	s	(NH) 7.85,	d, J=8.1
	5	17.6	q	0.77	d, J=6.6		conf 2	(NH) 7.82	d, J=8.1		
Ileu-1	1	171.9	s	(NH) 8.38	d, J=9.6	2	59.6	d	4.32	m	
	2	57.5	d	4.53	m	3	30.7	d	2.14	m	
	3	38.8	d	1.98	m	4	19.6	q	0.95	m	
	4	14.6	q	0.92	d, J=6.6	5	18.4	q	0.90	m	
	5	26.8	t	1.40, 1.13	m, m	5-MetHex	1	173.8	s		
	6	11.7	q	0.88	t, J=7.2		2	36.3	t	2.26	m
Thr-1	1	169.7	s	(NH) 8.12	d, J=5.7		3	24.0	t	1.60	m
	2	57.4	d	4.63	t, J=9.3		4	39.0	t	1.20	m
	3	71.1	d	5.07	dq, J=9.6, 6.0		5	28.1	d	1.55	m
	4	17.3	q	1.18	d, J=6.3	6	22.5	q	0.87	d, J=7.2	
Ileu-2	1	171.9	s	(NH) 7.72	d, J=8.4	7	22.5	q	0.87	d, J=7.2	
	2	57.3	d	4.52	m	5-MetHex (second conformation)	1	174.1	s		
	3	38.0	d	1.88	m		2	33.9	t	2.29	m
	4	14.8	q	0.88	d, J=6.3		3	32.8	t	1.65, 1.40	m
	5	26.6	t	1.40, 1.13	m, m		4	29.8	t	1.13	m
	6	11.6	q	0.88	t, J=7.2		5	34.5	d	1.35	m
Orn	1	172.0	s	(NH) 7.92	d, J=7.8	6	19.5	q	0.90	m	
	2	52.9	d	4.48	m	7	11.2	q	0.90	m	
	3	29.6	t	1.76	m						
	4	24.4	t	1.83	m						
	5	40.1	t	3.10	p, 5.1						

^a At 127 MHz, DMF signal at 35.2 ppm. ^b At 500 MHz, DMF signal at 2.91 ppm.

assay (MLR) with an IC₅₀ of 3 μg/mL, and with lymphocyte viability (LcV) IC₅₀ of 23 μg/mL.

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Supplementary Material Available: 125-MHz ¹³C NMR and 500-MHz ¹H NMR spectra for 1 in DMF-*d*₇/TFA and DMSO-*d*₆, HMBC, COSY, and ROESY spectra for 1 in DMF-*d*₇/TFA, and amino acid analysis by GC-MS on a Chirasil-Val column (8 pages). Ordering information is given on any current masthead page.