Two Acyclic Kahalalides from the Sacoglossan Mollusk *Elysia rufescens*[1]

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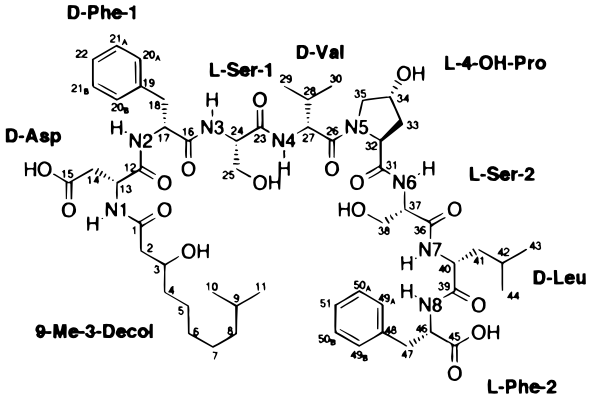
The sacoglossan mollusk *Elysia rufescens* elaborates six cyclic depsipeptides, kahalalides A−F. Its green algal diet (*Bryopsis* sp.) contains kahalalide G, which is an acyclic analogue of kahalalide F. Further analysis of the molluskan extract revealed two new acyclic peptides, kahalalide H (**1**) and kahalalide J (**2**), which share only four amino acids (leucine, phenylalanine, serine, and valine) with kahalalide F. Both contain aspartic acid and 4-hydroxyproline residues, and kahalalide J (**2**) also contains lysine. They have in common a β-hydroxy fatty acid, 3-hydroxy-9-methyldecanoic, previously encountered in kahalalide E. For kahalalide H (**1**) we succeeded in determining the sequential positions of the antipodal D- and L-phenylalanine. In common with the acyclic constituent of the alga, kahalalide G, the new compounds lack significant cytotoxicity.

We have recently reported[2,3] the isolation and structural determination of six cyclic depsipeptides, kahalalides A−F, from the sacoglossan mollusk *Elysia rufescens* Pease 1871 (Plakobranchidae). They range from a C_{31} tetrapeptide to a C_{75} octapeptide. Kahalalide G, which is the acyclic analogue of the octapeptide kahalalide F, was found in the diet of the animal, a green alga, *Bryopsis* sp. Only kahalalide F exhibited significant bioactivity against human colon and lung cancers.[4] Reisolation of kahalalide F for in vivo bioassays necessitated a major recollection, yielding two new acyclic peptides, which are the subject of this report.

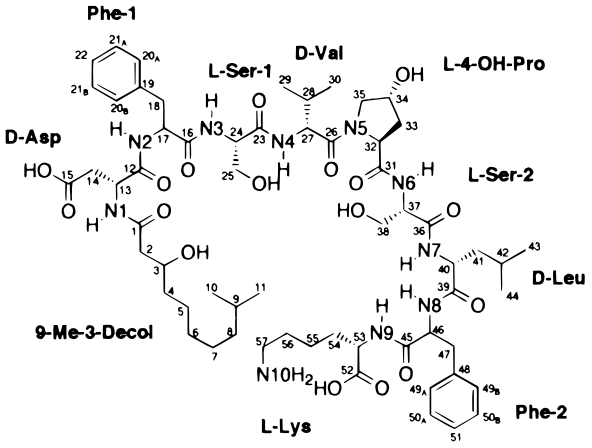
The new peptides, kahalalide H (**1**) and kahalalide J (**2**), are the first acyclic peptides found in the mollusk. They are octapeptides and nonapeptides, but they share only four amino acids—leucine, phenylalanine, serine, and valine—with the other kahalalides. Both contain aspartic acid and 4-hydroxyproline residues, and kahalalide J (**2**) also contains lysine. They have in common a β-hydroxy fatty acid, 3-hydroxy-9-methyldecanoic, previously encountered in kahalalide E.[3] This acid has previously been described as a constituent of an antibiotic, cerexin A, isolated from *Bacillus cereus* 60-6.[5] Except for the parallelism of kahalalides F and G, and now between H and J, these nine peptides exhibit considerable diversity in amino acid composition as well as sequence. As was true of the other acyclic member, kahalalide G, the new compounds also lack significant cytotoxicity. For kahalalide H (**1**) we succeeded in determining the sequential positions of the antipodal D- and L-phenylalanine.

Results and Discussion

Kahalalide H and kahalalide J were isolated from the EtOH and CH_2Cl_2 extracts of the frozen mollusk *Elysia rufescens* (5.4 kg). The residue was partitioned between CH_2Cl_2 and H_2O, and the organic layer was further separated by a modified Kupchan procedure[6] (without partitioning between 70% MeOH/CCl_4). The CH_2Cl_2 fraction was subjected to gel filtration followed by HPLC to yield kahalalide H (**1**; 15.3 mg, 2.83×10^{-4}% based on wet wt) and kahalalide J (**2**; 2.2 mg, 4.07×10^{-5}%).



Kahalalide H (**1**)



Kahalalide J (**2**)

Kahalalide H (**1**) gave rise to an $[M + Na]^+$ ion peak at *m/z* 1133.5685 upon HRFABMS, which suggested a molecular formula of $C_{55}H_{82}N_8O_{16} + Na$ (Δ −6.2 mmu), corroborated by ^{13}C-NMR data that showed 55 carbons (see Table 1). Examination of the ^{1}H-NMR spectrum immediately suggested a peptide with aromatic and aliphatic residues. A group of signals for α-protons at 4.2−4.6 ppm implied a peptide of 8−9 residues. Because a signal at 4.41 ppm (H-34) proved to be the signal for the γ-proton of OH-Pro, **1** was thought to be an

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Table 1. Correlated ^{13}C- and ^{1}H-Data and COSY for Kahalalide H[a]

amino acid	no.	^{13}C	^{1}H	mult	*J* (Hz)	HMBC	COSY
9Me3Decol	1	175.03					
	2_A	44.12	2.25	dd	9.9, 1.8	C: 1, 3, 4	H: 2_B, 3
	2_B		2.34	dd	3.5, 13.8	C: 1, 3, 4	H: 2_A, 3
	3	70.05	3.95	m		C: 1	H: 2_A, 2_B, 4
	4_A	38.51	1.44	m		C: 3, 5	H: 3
	4_B		1.44	m		C: 3, 5	H: 3
	5_A	26.39	1.29	m		C: 4, 6	H: 5_B
	5_B		1.40	m		C: 6	H: 5_A
	6_A	30.73	1.29	m		C: 5, 7, 8	H: 8
	6_B		1.29	m		C: 5, 7, 8	H: 8
	7_A	28.25	1.37	m		C: 6,9	H: 8
	7_B		1.37	m		C: 6,9	H: 8
	8_A	39.84	1.16	m		C: 6, 7, 9, 10, 11	H: 6, 7, 9
	8_B		1.16	m		C: 6, 7, 9, 10, 11	H: 6, 7, 9
	9	28.86	1.50	m		C: 7, 8	H: 8, 10, 11
	10	23.04	0.85	d	6.6	C: 8, 9, 11	H: 9
	11	23.04	0.85	d	6.6	C: 8, 9, 10	H: 9
D-Asp	12	172.51					
	13	51.68	4.55	dt	5.3, 7.4	C: 12, 14, 15, 1	H: N_1, 14_A, 14_B
	14_A	37.97	2.50	dd	7.8, 17.1	C: 12, 13, 15	H: 13, 14_B
	14_B		2.65	dd	5.2, 17.1	C: 12, 13, 15	H: 13, 14_A
	15	173.24					
	N_1		7.68	d	7.3	C: 13, 14, 1	H: 13
D-Phe-1	16	173.17					
	17	56.41	4.49	dt	5.5, 8.5	C: 16, 18, 19, 12	H: N_2, 18_A, 18_B
	18_A	37.99	2.82	dd	9.6, 13.8	C: 16, 17, 19, 20	H: 17, 18_B
	18_B		3.17	t	5.6	C: 16, 17, 19, 20	H: 17, 18_A
	19	138.40					
	20_A	130.34	7.19	m		C: 19, 20_B, 22	H: 21_A
	20_B	130.34	7.19	m		C: 19, 20_A, 22	H: 21_B
	21_A	129.45	7.25	m		C: 19, 21_B, 22	H: 20_A, 22
	21_B	129.45	7.25	m		C: 19, 21_A, 22	H: 20_B, 22
	22	127.77	7.20	m		C: 20_A, 20_B, 21_A, 21_B	H: 21_A, 21_B
	N_2		7.72	d	7.6	C: 12	H: 17
L-Ser-1	23	172.24					
	24	57.18	4.24	m	4.5, 11.5	C: 23, 25, 16	H: N_3, 25_A, 25_B
	25_A	62.98	3.65	dd	4.5, 11.5	C: 23, 24	H: 24, 25_B
	25_B		3.83	m	4.5	C: 23, 24	H: 24, 25_A
	N_3		7.51	dd	6.8	C: 23, 24, 25, 16	H: 24
D-Val	26	173.08					
	27	58.16	4.34	t	8.8	C: 23, 26, 28, 29, 30	H: N_4, 28
	28	30.83	2.08	m	6.6	C: 26, 27, 29, 30	H: 27, 29, 30
	29	18.96	0.91	d	6.6	C: 27, 28, 30	H: 28
	30	19.93	0.91	d	6.6	C: 27, 28, 29	H: 28
	N_4		8.08	d	8.3	C: 26, 27, 23	H: 27
L-OH-Pro	31	173.24					
	32	61.49	4.36	t	9.8	C: 31, 33, 34	H: 33_A, 33_B
	33_A	38.65	2.00	m	4.4, 9.3	C: 31, 32, 34, 35	H: 32, 33_B, 34
	33_B		2.22	m		C: 34, 35	H: 32, 33_A, 34
	34	70.33	4.41	m, sl			H: 33_A, 33_B, 35_A, 35_B
	35_A	56.98	3.61	m		C: 32, 33, 34, 26	H: 34, 35_B
	35_B		3.85	dd	4.3, 11.3	C: 33	H: 34, 35_A
L-Ser-2	36	171.97					
	37	51.68	4.22	m	11.3	C: 36, 38	H: N_6, 38_A, 38_B
	38_A	62.76	3.75	dd	3.8, 11.3	C: 36, 37	H: 37, 38_B
	38_B		3.80	m	3.8	C: 37	H: 37, 38_A
	N_6		7.28	dd	7.6	C: 37, 38, 31	H: 37
D-Leu	39	174.21					
	40	52.74	4.29	m	4.6, 10.7	C: 39, 41, 42, 36	H: N_7, 41_A, 41_B
	41_A	41.03	1.32	m	4.6	C: 39, 40, 42, 43, 44	H: 40, 41_B
	41_B		1.60	dd	4.6, 10.7	C: 39, 40, 42, 43, 44	H: 40, 41_A
	42	25.32	1.50	m	6.6	C: 41, 44	H: 41_A, 41_B, 43, 44
	43	21.71	0.79	d	6.6	C: 41, 42, 44	H: 42, 44
	44	23.50	0.81	d	6.6	C: 41, 42, 43	H: 42, 43
	N_7		7.59	d	8.3	C: 40, 36	H: 40
L-Phe-2	45	174.30					
	46	54.89	4.60	dt	5.2, 8.8	C: 45, 47, 48	H: N_8, 47_A, 47_B
	47_A	38.27	2.89	dd	9.3, 13.9	C: 45, 46, 48, 49	H: 46, 47_B
	47_B		3.15	t	5.5	C: 45, 46, 48, 49	H: 46, 47_A
	48	138.27					
	49_A	130.43	7.19	m		C: 48, 51, 49_B	H: 50_A
	49_B	130.43	7.19	m		C: 48, 51, 49_A	H: 50_B
	50_A	129.51	7.26	m		C: 48, 51, 50_B	H: 49_A, 51
	50_B	129.51	7.26	m		C: 48, 51, 50_A	H: 49_B, 51
	51	127.80	7.21	m		C: 49_A, 49_B, 50_A, 50_B	H: 50_A, 50_B
	N_8		7.60	d	8.3	C: 39	H: 46

[a] Measured in CD_3CN/CD_3OH.

octapeptide. Another group of signals for methylene groups between 1.1 and 1.6 ppm suggested the likely presence of a fatty acid. Detailed analysis of the NMR data, including COSY, NOESY, HMQC, and HMBC spectra,[7] allowed us to identify and sequence one each Val, Asp, Leu, and OH-Pro, two each Phe, and Ser residues, as well as the 9-Me-3-Decol.

The α-proton of Asp (H-13; δ 4.55) showed HMBC correlations to three carbonyls, C-12, C-15, and C-1, which could be further correlated to the remaining signals of 9-Me-3-Decol. The carbon chemical shift of C-14 (δ 37.97) is typical for an Asp methylene group. The α-proton of OH-Pro (H-32; δ 4.36) showed HMBC correlation to one carbonyl (C-31), one methylene (C-33), and one carbon bearing a hydroxy group (C-34); C-33 could further be correlated to H-34 and to both methylene protons of C-35. Crucial COSY correlations were observed between H-32/H-33/H-34/H-35, confirming the hydroxyproline structure. Particularly helpful were the correlations observed previously between the carbons and protons of Phe, Leu, Ser, and Val in the other kahalalides.[2,3] Comparison with these data firmly confirmed the presence of these amino acids in kahalalide H.

Connectivity between neighboring amino acids could be demonstrated by a combination of NOE experiments and an HMBC spectrum. Sequential HMBC correlations from NH protons to neighboring carbonyls were seen between Asp/9-Me-3-Decol (NH-*1*/C-1), Phe-1/Asp (NH-*2*/C-12), Ser-1/Phe-1 (NH-*3*/C-16), Val/Ser-1 (NH-*4*/C-23), Ser-2/OH-Pro (NH-*6*/C-31), Leu/Ser-2 (NH-*7*/C-36), and Phe-2/Leu (NH-*8*/C-39). The correlations from the α-proton to carbonyl carbon of the neighboring amino acid were also observed between Asp/9-Me-3-Decol (H-13/C-1), Phe-1/Asp (H-17/C-12), Ser-1/Phe-1 (H-24/C-16), Val/Ser-1 (H-27/C-23), and Leu/Ser-2 (H-40/C-36). An HMBC correlation between OH-Pro/Val (H-35_A/C-26), and an NOE between the α-proton of Val and the δ-proton of OH-Pro (H-27/H-35_A), suggested a *trans*-amide bond.[8] No correlations were observed between any methylene group bearing hydroxyl and any carboxylic group not involved in an amide bond, leading to an acyclic structure. The chemical shift of H-3 is 3.95 ppm, whereas the chemical shifts of the protons on the carbons bearing oxygens involved in an ester link of the fatty acid of kahalalide D and E are 5.03 and 5.11 ppm, respectively. The fact that those spectra were taken in deuterated DMSO as opposed to kahalalide H taken in CD_3CN/CD_3OH would not explain this variation, even if kahalalide H were cyclic. Another fact strongly suggested that kahalalide H was acyclic: the HR-FABMS showed an $[M + Na]^+$ ion peak at *m/z* 1133.5685 (Δ −6.2 mmu) as well as an $[M - H + Na_2]^+$ ion peak at *m/z* 1155.5497 (Δ −6.9 mmu) and an $[M - H_2 + Na_3]^+$ ion peak at *m/z* 1177.5385 (Δ −0.1 mmu); these latter two peaks revealed the presence of two acidic functions, the γ-COOH of Asp and the C-terminal COOH of Phe-2.

The relative stereochemistry of the OH-proline unit could be determined from the clear NOESY correlations between the α-proton H-32 and both H-33_B and H-35_A, while the proton H-34 only shows correlations to H-33_A and H-35_B. This relative stereochemistry is *trans* because C-32 is *S* and C-34 is *R* (see Figure 1).

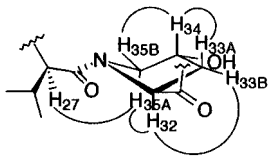

Figure 1. NOE correlations of *trans*-4-OH-proline.

The absolute stereochemistry of the amino acids of kahalalide H was determined by Marfey's method,[9] which showed Asp, Leu, and Val to be D and Ser and *trans*-4-OH-Pro to be L. The Phe analysis proved that both enantiomers were present in kahalalide H. Therefore, kahalalide H was hydrazinolyzed[10] to provide the free unmodified carboxy terminal amino-acid Phe-2, which was then analyzed by Marfey's method showing the L configuration. Thus, Phe-1 must have the D configuration.

The absolute stereochemistry of 3-hydroxy-9-methyldecanoic acid remains under investigation and will require isolation of additional material for hydrolysis and preparation of Mosher's esters as modified by Ohtani et *al.*[11]

Kahalalide J (**2**) gave rise to an $[M + H]^+$ ion peak at *m/z* 1239.6971 upon HRFABMS, which suggested a molecular formula of $C_{61}H_{94}N_{10}O_{17}$ (Δ +9.5 mmu), corroborated by ^{13}C-NMR data that showed 61 carbons (see Table 2). Examination of the ^{1}H-NMR spectrum immediately suggested a peptide bearing essentially the same amino acids as kahalalide H with a notable exception, a signal at 8.02 ppm integrating for two protons. Hydrolysis followed by amino acid analysis of **2** confirmed nine amino acids, 1 mol each of Lys, Leu, OH-Pro, Val, and Asp and 2 mol each of Phe and Ser, the same basic constituents as kahalalide H, in addition to Lys. Detailed analysis of the NMR data, including COSY, ROESY, HMQC, and HMBC spectra, allowed us to identify and sequence these amino acids, as well as 9-Me-3-Decol.

The connectivities between neighboring amino acids could be demonstrated by a combination of ROESY and HMBC spectra. Sequential ROESY correlations from NH protons to neighboring α protons were seen between Asp/9-Me-3-Decol (NH-*1*/H-2), Phe-1/Asp (NH-*2*/H-13), Ser-1/Phe-1 (NH-*3*/H-17), Val/Ser-1 (NH-*4*/H-24), Ser-2/OH-Pro (NH-*6*/H-32), Leu/Ser-2 (NH-*7*/H-37), Phe-2/Leu (NH-*8*/H-40), and Lys/Phe-2 (NH-*9*/H-46). Sequential HMBC correlations from NH protons to neighboring carbonyls were also seen between Asp/9-Me-3-Decol (NH-*1*/C-1), Phe-1/Asp (NH-*2*/C-12), Ser-1/Phe-1 (NH-*3*/C-16), Val/Ser-1 (NH-*4*/C-23), Ser-2/OH-Pro (NH-*6*/C-31), Leu/Ser-2 (NH-*7*/C-36), Phe-2/Leu (NH-*8*/C-39), and Lys/Phe-2 (NH-*9*/C-45). The α-proton of Val and the δ-proton of OH-Pro (H-27/H-35_A) were correlated by ROESY. As for **1**, no correlations were observed between any methylene group bearing hydroxyl and any carboxylic group not involved in a amide bond, thus indicating a linear sequence. Furthermore, the chemical shift of H-3 parallels the corresponding value in **1**: 4.04 vs. 3.95 ppm.

The absolute stereochemistry of the amino acids of kahalalide J was determined by Marfey's method,[9] which proved that Asp, Leu, and Val are D, while Ser, Lys, and *trans*-4-OH-Pro are L. The Phe analysis proved that both enantiomers were present in kahalalide H. Because of stronger conditions some racemization oc-

Table 2. Correlated ^{13}C- and ^{1}H-Data and ROESY for Kahalalide J[a]

amino acid	no.	^{13}C	^{1}H	mult	*J* (Hz)	HMBC	ROESY
9Me3Decol	1	174.0					
	2	44.1	2.38	m, dl		C: 1, 3, 4	H: N_1, 3, 4, 5_A, 5_B, 6
	3	69.3	4.04	m		C: 1	H: 2, 4, 5_A, 5_B, 6
	4	38.2	1.47	m		C: 5	H: 2, 3, 5_A, 5_B
	5_A	26.0	1.46	m		C: 3	H: 2, 3, 4, 5_A
	5_B		1.31	m		C: 3, 6	H: 2, 3, 4, 5_B
	6	30.2	1.26	m		C: 8	H: 2, 3, 5_A, 5_B
	7	27.9	1.28	m		C: 6	H: 8, 9, 41, 11
	8	39.4	1.16	m		C: 6, 7, 9, 41, 11	H: 7, 9, 41, 11
	9	28.4	1.51	m		C: 41, 11	H: 7, 8, 41, 11
	41	22.7	0.85	d	6.6	C: 8, 9, 11	H: 7, 8, 9, 11
	11	22.7	0.85	d	6.6	C: 8, 9, 41	H: 7, 8, 9, 41
D-Asp	12	171.8					
	13	51.3	4.67	dt	5.0, 7.8	C: 12, 1	H: N_2, N_1, 14_A, 14_B
	14_A	36.0	2.57	m		C: 12, 13, 15	H: N_1, 13, 14_B
	14_B		2.75	m		C: 12, 13, 15	H: 13, 14_A
	15	172.6					
	N_1		8.61	d	6.9	C: 1	H: 13, 14_A, 2
Phe-1	16	172.4					
	17	55.4	4.61	m, dtl		C: 16, 18	H: N_3, N_2, 18_A, 18_B, 20_A, 20_B
	18_A	37.9	2.89	m		C: 16, 20, 21	H: N_2, 17, 18_B, 20_A, 20_B
	18_B		3.21	dd	4.8, 13.8	C: 16, 20, 21	H: 17, 18_A, 20_A, 20_B
	19	138.4					
	20_A	129.9	7.28	m		C: 19, 20_B, 22	H: 24, 17, 18_A, 18_B, 21_A
	20_B	129.9	7.28	m		C: 19, 20_A, 22	H: 24, 17, 18_A, 18_B, 21_A
	21_A	128.7	7.26	m		C: 19, 21_B, 22	H: 20_A, 22
	21_B	128.7	7.26	m		C: 19, 21_A, 22	H: 20_B, 22
	22	126.9	7.20	m		C: 21_A, 21_B	H: 21_A, 21_B
	N_2		8.07	d	7.8	C: 12	H: 17, 18_A, 13
L-Ser-1	23	171.5					
	24	56.7	4.45	m		C: 23, 25, 16	H: N_4, N_3, 25_A, 25_B, 20
	25_A	62.8	3.76	dd	4.8, 11.1	C: 23	H: N_3, 24, 25_B
	25_B		3.88	dd	5.1, 11.4	C: 23	H: N_3, 24, 25_A
	N_3		8.04			C: 23, 16	H: 24 25_A, 25_B, 17
D-Val	26	172.0					
	27	57.5	4.37	m		C: 26, 23, 29, 30	H: N_4, 27_A, 27_B, 28, 29, 30
	28	30.1	2.15	m		C: 27, 29, 30	H: N_4, 27, 29, 30
	29	18.7	0.95	d	5.7	C: 27, 28, 30, 25	H: N_4, 27, 28
	30	19.9	0.95	d	5.7	C: 27, 28, 29, 25	H: N_4, 27, 28
	N_4		8.37	d	8.4	C: 23	H: 27, 28, 29, 30, 24
L-OH-Pro	31	172.5					
	32	61.0	4.46	m	9.8	C: 31	H: N_6, 33_A, 33_B, 35_A
	33_A	38.5	2.08	m	4.4, 9.3	C: 31, 32	H: 32, 33_B, 34
	33_B		2.24	m		C: 34, 35	H: 32, 33_A, 34
	34	69.7	4.47	m		C: 33	H: 33_A, 33_B, 35_A, 35_B
	35_A	56.6	3.68	d	11.1	C: 32, 34	H: 32, 34, 35_B, 27
	35_B		3.90	dd	3.9, 10.5	C: 33, 26	H: 34, 35_A, 27
L-Ser-2	36	171.4					
	37	56.8	4.33	m		C: 36	H: N_7, N_6, 38
	38	62.3	3.82	d	5.4	C: 36, 37	H: N_6, 37
	N_6		7.65	d	7.8	C: 31	H: 37, 38, 32
D-Leu	39	173.8					
	40	52.8	4.29	m		C: 41, 44, 36	H: N_8, N_7, 41_A, 41_B, 42, 43, 44
	41_A	40.9	1.32	m			H: 40, 41_B
	41_B		1.58	m		C: 42, 43	H: N_7, 40, 41_A
	42	24.8	1.48	m		C: 43, 44	H: N_7, 40,41_A, 41_B, 43, 44
	43	21.7	0.76	d	6.6	C: 41, 42, 44	H: 40, 42, 44
	44	23.2	0.81	d	6.3	C: 41, 42, 43	H: 40, 42, 43
	N_7		7.83	d	7.5	C: 36	H: 40, 41_B, 42, 37
Phe-2	45	172.1					
	46	55.1	4.72	m		C: 45, 47	H: N_9, N_8, 47_A, 49_A, 49_B
	47_A	37.8	3.24	dd	3.9, 14.1	C: 45, 46, 48, 49, 50	H: 46, 49_A, 49_B
	47_B		2.92	m		C: 45, 46, 48, 49, 50	H: N_8, 49_A, 49_B
	48	138.8					
	49_A	130.0	7.28	m		C: 48, 51, 49_B	H: 46, 47_A, 47_B
	49_B	130.0	7.28	m		C: 48, 51, 49_A	H: 46, 47_A, 47_B
	50_A	128.8	7.26	m		C: 48, 51, 50_B	H: 49_A, 51
	50_B	128.8	7.26	m		C: 48, 51, 50_A	H: 49_B, 51
	51	127.0	7.20	m		C: 50_A, 50_B	H: 50_A, 50_B
	N_8		8.43	d	8.4	C: 39	H: 46, 47_B, 40
Lys	52	173.7					
	53	52.7	4.39	m		C: 52, 57, 45	H: N_9, 54_A, 54_B
	54_A	31.1	1.78	m			H: 53
	54_A		1.88	m			H: 53, 55
	55	22.7	1.48	m			H: 54_B
	56	27.4	1.76	m		C: 57	H: N_{10}, 57
	57	40.3	3.09	m, ql			H: N_{10}, 56
	N_9		8.15	d	8.1	C: 45	H: 53, 46
	N_{10}		8.02	s			H: 56, 57

[a] Measured in DMF-d_7/CF_3COOH.

curred during hydrolysis, as we were able to detect significant amounts of D-Lys, L-Val, and D-*cis*-4-OH-Pro. Assignment of the D- and L-Phe therefore could not be unambiguously established, but because the other amino acids possess the same configuration as in **1**, it is highly probable that Phe-1 is D and Phe-2 is L.

Experimental Section

General Experimental Procedures. All NMR spectra were recorded on a General Electric GN Omega 500 MHz NMR spectrometer. The IR spectra were measured on a Perkin-Elmer 1420 spectrophotometer in $CHCl_3$. UV spectra were measured with a Hewlett-Packard 8452A diode array spectrometer. Optical rotations were determined with a JASCO DIP-370 digital polarimeter. FABMS were measured on a JEOL JMX-SX102/SX102 tandem mass spectrometer using NBA + NaCl for kahalalide H and glycerol as matrix for kahalalide J.

Extraction and Isolation. About 4000 animals (5.4 kg wet wt) were collected by snorkeling at low tide near Black Point, O'ahu, during February 1995. The animals were identified by Drs. Marilyn Dunlap and Alison Kay. A voucher specimen is deposited at the Bernice P. Bishop Museum, Honolulu, BPBM 247679. The EtOH (5 × 3 L) and CH_2Cl_2 (2 L) extracts were partitioned between CH_2Cl_2 and H_2O. The aqueous layer was extracted with *n*-BuOH, which was combined with the CH_2Cl_2 layer, concentrated, and partitioned between hexane and MeOH−H_2O (9:1). The MeOH layer was collected, and H_2O was added to adjust the MeOH concentration to 60%. Extraction with CH_2Cl_2 and concentration yielded a fraction that was subjected twice to ODS flash column chromatography, using a stepwise aqueous MeOH gradient, then a stepwise aqueous MeCN gradient. The peptide-containing fractions (monitored by TLC) were further purified on an ODS column using a stepwise aqueous MeCN gradient solvent system (61, 62, 70% aqueous MeCN, and 100% MeOH). The desired fraction was passed through an ODS BondElut short column. A reversed-phase HPLC separation [ULTRACARB 10 ODS PO; MeOH−H_2O−TFA, 65:35:0.05] led to several fractions containing peptides.

One fraction, after two reversed-phase HPLC columns [COSMOSIL 5C_{18}-AR, MeCN−H_2O−TFA, 65:35:0.05; COSMOSIL 5C_{18}-AR, MeCN−H_2O−TFA, 55:45:0.05], yielded 15.3 mg (2.83×10^{-4}% based on wet wt) of pure kahalalide H.

Another fraction, after two reversed-phase HPLC columns [ULTRACARB 10 ODS PO, MeCN−H_2O−TFA, 46:54:0.05; ULTRACARB 10 ODS PO, MeCN−H_2O−TFA, 47:53:0.05], yielded 2.2 mg (4.07×10^{-5}% based on wet wt) of pure kahalalide J.

Kahalalide H (1): colorless amorphous solid; $[\alpha]^{20}_D$ +38.8° (*c* 4.05, MeOH); UV (MeOH) λ_{max} (log ϵ) 209 (4.43), 212 (4.41), 259 (2.65) nm; IR ($CHCl_3$) ν_{max} 3308, 2954, 2933, 2869, 1728, 1655, 1533 cm^{-1}; ^1H and ^{13}C NMR (CD_3CN−CD_3OH), see Table 1; HRFABMS, see text.

Kahalalide J (2): colorless amorphous solid; $[\alpha]^{20}_D$ +102.9° (*c* 1.75, MeOH) ; UV (MeOH) λ_{max} (log ϵ) 208 (4.10), 282 (2.32) nm; IR ($CHCl_3$) ν_{max} 3013, 1661 cm^{-1}; ^1H and ^{13}C NMR (DMF-d_7/CF_3COOH), see Table 2; HRFABMS, see text.

Marfey Analysis of 1 and 2. Kahalalide H and kahalalide J (100 μg each) were respectively dissolved in 5 and 6 N HCl (500 μL) and degassed under vacuum for 1 min, then hydrolyzed at 105 °C for 17 h. The acid hydrolysate was dried under N_2, and to it was added 50 μL of 0.1% FDAA solution in Me_2CO and 100 μL of 0.1 N $NaHCO_3$, followed by heating at 80 °C for 3 min. After being cooled to room temperature, the reaction mixture was neutralized with 50 μL of 0.2 N HCl and diluted with 100 μL of 50% MeCN containing 0.05% TFA.

This solution was analyzed by reversed-phase HPLC with three isocratic solvent systems (COSMOSIL 5C_{18}-AR, solvent I: 42% MeCN + 0.05% TFA; solvent II: 33% MeCN + 50 mM NH_4OAc; solvent III: 15% MeCN + 50 mM NH_4OAc).

Analysis with solvent I showed that Val, Leu, and Phe had the D-configuration, but the presence of L-Phe could not be excluded. Analysis was thus carried out with solvent II, which showed that Phe was present in both D and L forms. Analysis of the Asp, Ser, Lys, and OH-Pro residues with solvent III indicated that Ser and Lys were L, and both Asp and OH-Pro were D. All these results were confirmed by coinjection with authentic standard derivatives.

Hydrazinolyzis of Kahalalide H. Kahalalide H (1 mg) was added under argon to 9 mg of dry Amberlite GC50, followed by 400 μL of freshly distilled hydrazine. The reaction mixture was then heated at 80 °C for 120 h. After cooling to room temperature, the reaction mixture was frozen and lyophilyzed. The residue was suspended in H_2O (1.2 mL) and filtered. One-tenth of this solution (120 μL) was freeze-dried, and to it was added 50 μL of 0.1% FDAA solution in Me_2CO and 100 μL of 0.1 N $NaHCO_3$, followed by heating at 80 °C for 3 min. After being cooled to room temperature, the reaction mixture was neutralized with 50 μL of 0.2 N HCl and diluted with 100 μL of 50% MeCN containing 0.05% TFA. This solution was analyzed by reversed-phase HPLC (COSMOSIL 5C_{18}-AR, 42% MeCN + 0.05% TFA). The only unmodified residue, Phe-2, was analyzed to show L-configuration (result confirmed by coinjection with authentic D- and L-Phe derivatives).

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Supporting Information Available: ^1H, ^{13}C, COSY, HMBC, HMQC NMR spectra of **1** and **2**, NOESY NMR spectra of **1** and ROESY NMR spectra of **2** (29 pages). Ordering information is given on any current masthead page.

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