The Kapakahines, Cyclic Peptides from the Marine Sponge *Cribrochalina olemda*

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Four cyclic peptides, kapakahines A−D, were isolated from the marine sponge *Cribrochalina olemda*. Their structures including complete stereochemistry were elucidated by spectral analysis and chemical degradation. The unique structural feature of these peptides is the lack of an amide linkage between two tryptophan residues. Instead the ring is closed by a bond from the indole nitrogen of Trp-1 to the β-carbon of Trp-2.

Introduction

The kapakahines are cyclic peptides isolated from a Pohnpei sponge, *Cribrochalina olemda*. They share a unique structural feature: two tryptophan residues (Trp-1 and -2) are not linked by an amide bond but by a N−C bond from the indole nitrogen of Trp-1 to the β-indole carbon of Trp-2. We have previously reported the structure of kapakahine B (**2**),[3] which is a hexapeptide and is the smallest member of the group. In this paper we describe the structures of the three octapeptides, kapakahines A (**1**), C (**3**), and D (**4**) and the full stereochemistry of all four compounds (Chart 1).

The three octapeptides kapakahines A (**1**), C (**3**), and D (**4**) have identical eastern amino acid patterns, symmetrical sequence of Ile-1, Pro-1, Val, Pro-2, Ile-2, but they differ in their western moieties.[4] Kapakahines C (**3**) and D (**4**) differ from A (**1**) by the addition of the elements of H_2O to the indole double bond of Trp-1 together with the formation of a bond between C-4 and N-1; this formal hydration apparently takes place with antipodal stereochemistry, which is α in kapakahine C (**3**) and β in D (**4**) and with concomitant formation of a new pyrrole fused with indole.

Results and Discussion

The kapakahines were isolated from the EtOH extract of the frozen sponge *C. olemda* (840 g). The resulting residue was partitioned between EtOAc and H_2O, and the organic layer was further separated by the Kupchan procedure.[5] The CH_2Cl_2 fraction was gel-filtered, followed by HPLC to yield kapakahine A (**1**; 5.8 mg, 6.9×10^{-4}% based on wet weight), B (**2**; 0.3 mg, 3.6×10^{-5}%), C (**3**; 2.6 mg, 3.1×10^{-4}%), and D (**4**; 1.8 mg, 2.1×10^{-4}%). Combined with isolates from a subsequent collection (4.0 kg), totals of 14.4 mg of **1** and 2.0 mg of **2** were obtained.

Kapakahine A. Kapakahine A (**1**) gave rise to an $(M + H)^+$ ion peak at m/z 1053.5562 upon HR-FABMS, which suggested a molecular formula of $C_{58}H_{72}N_{10}O_9$ (Δ 0.0 mmu), corroborated by ^{13}C NMR data that showed 58 carbons (see Table 1).

Examination of the ^1H NMR spectrum immediately suggested a peptide with aromatic and aliphatic residues. A group of signals for α-protons at 4.0−5.1 ppm implied a peptide of 8−9 residues. Since a signal at 4.08 ppm (H-32) proved to be part of a diastereotopic methylene group of Pro-2, **1** was thought to be an octapeptide. Hydrolysis followed by amino acid analysis of **1** confirmed six amino acids, 1 mol each of Val and Tyr and 2 mol each of Pro and Ile. Detailed analysis of the NMR data, including COSY, NOESY, HMQC,[6] and HMBC[7] spectra, allowed us to sequence one each Val, Tyr, and Trp and two each Pro and Ile residues. A seventh amino acid residue appeared to be related to a dihydrotryptophan, which was familiar from kapakhine B (**2**).[3] This residue forms a fused tetracycle (fragment **a**) incorporating a Tyr instead of a Phe residue as in kapakahine B (**2**).

Fragment a. The α-proton of Tyr (H-51; δ 4.93) showed HMBC correlation to C-39 carbonyl, which could further be correlated to the remaining signals of Trp-2. The carbon chemical shift of C-42 (δ 82.5) is typical for a diaminomethine derivative.[8] Crucial HMBC correlations were observed between H-42/C-39, 41, 43, and 49; H-41a/C-39, 40, 42, and 43; and H-41b/C-44. Particularly helpful was the correlation between H-45/C-43, which firmly established the dihydrotryptophan unit.

The α-proton of Tyr (H-51) also showed an HMBC correlation to C-50, which showed no other correlations. This carbonyl carbon was believed to form an amide bond with a nitrogen atom on the dihydroindole of Trp-2, but in the Dreiding model the dihedral angle between the CH bond at C-42 and the N−CO bond was nearly 90°, thus resulting in weak coupling between H-42 and C-50. The nitrogen on the dihydroindole ring of Trp-2 is the only possible atom to form an amide bond with C-50. All other nitrogen atoms, except for the free amine of Trp-1, are bonded to other carbons.

Fragment b. Structure elucidation of fragment **b** was straightforward. A free amino group was evidenced by a broad peak at 8.52 ppm. When trifluoroacetic acid

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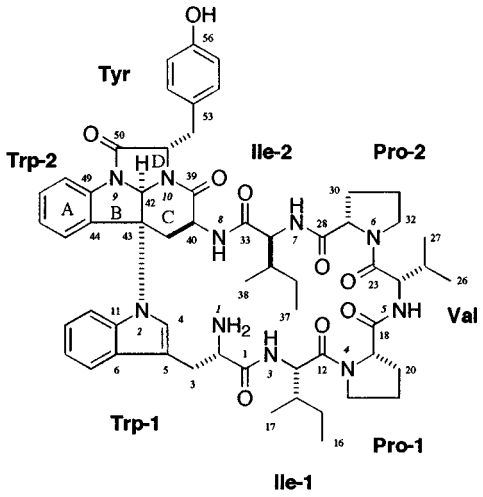
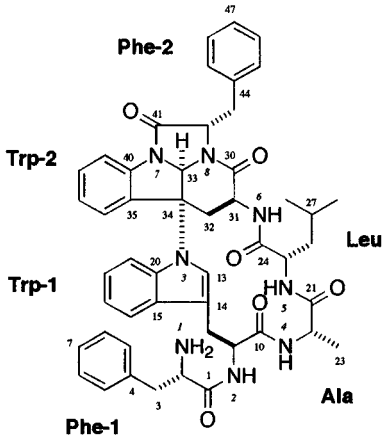
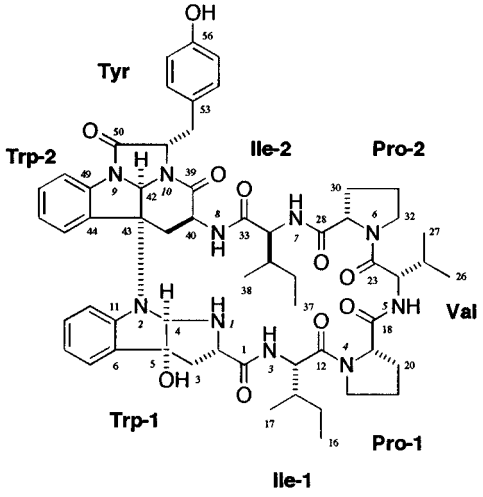
Chart 1



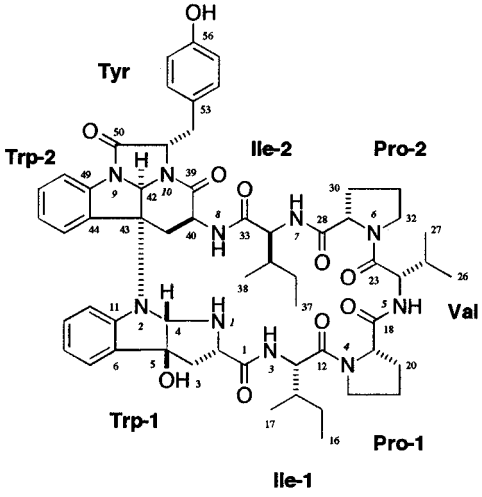
kapakahine A (1)



kapakahine B (2)



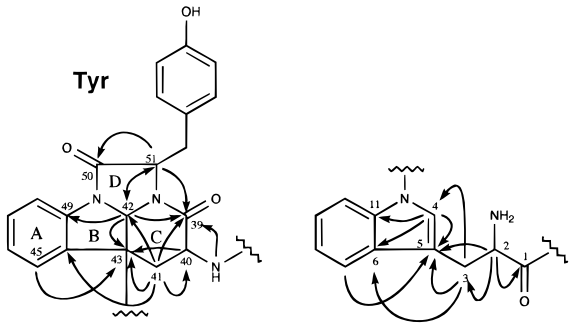
kapakahine C (3)



kapakahine D (4)

(TFA) was added to the solution, the peak became sharper and shifted to 7.26 ppm. The sharpening of the peak permitted observation of a COSY correlation with an α-proton at 4.85 ppm (H-2). A HMBC spectrum demonstrated that the free amine is part of Trp-1. Acetylation afforded a single isolable material, which showed a diacetylated ion peak at *m*/*z* 1137 in FAB-MS, indicating that kapakahine A has two acetylatable functional groups, the phenolic OH of Tyr and the free amino group of Trp-1. Unfortunately, this acetylated material gave NMR spectra that showed peak doubling, apparently caused by two conformers, which precluded structural analysis.

Sequencing the Amino Acids. Connectivity between neighboring amino acids could be demonstrated by a combination of NOE experiments and an HMBC spectrum. Sequential HMBC correlations from the NH proton to a neighboring carbonyl were seen between Trp-2/Ile-2 (NH-8/C-33). The correlations from the α-proton to carbonyl carbon of the neighboring amino acid were also observed between Val/Pro-1 (H-24/C-18), Pro-2/Val (H-29/C-23), and Ile-2/Pro-2 (H-34/C-28). Between Ile-1/Trp-1, an HMBC correlation was seen from NH-3 to C-1 in the experiment using a different sample. Though



a b

there was no HMBC correlation between Ile-1 and Pro-1, an NOE was observed between the α-protons of both amino acids (H-13/H-19), suggesting a *cis*-amide bond.

As for connectivity of Trp-1 and -2 through the C-43/N-*2* bond, NOE values could be seen between H-4/H-40 and 41a, which had also been observed in kapakahine B (between H-13/H-31 and 32a), but only a weak HMBC correlation was observed between H-4/C-43 (not at all in kapakahine B, H-13/C-34). To confirm this connectivity,

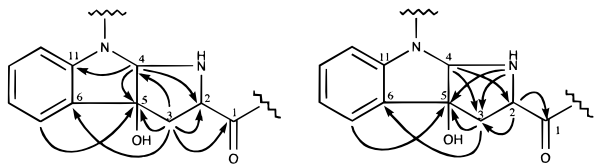
a selective INEPT experiment[9] was carried out with 14.4 mg of kapakahine A in CD_3OD. In this experiment, a clear correlation between H-4 and C-43 was observed and enabled us to connect Trp-1 and -2 through the unusual C−N bond.

Kapakahines C and D. The molecular formulas of kapakahines C (**3**) and D (**4**) were identical: $C_{58}H_{72}N_{10}O_{10}$ [**3**, $(M + H)^+$ *m/z* 1069.5549 (Δ +3.8 mmu); **4**, $(M + H)^+$ *m/z* 1069.5439 (Δ −7.2 mmu)] on the basis of HR-FABMS, differing from kapakahine A (**1**) by one additional oxygen atom. Amino acid analysis after hydrolysis of **3** showed 1 mol each of Val and Tyr and 2 mol each of Ile and Pro for both **3** and **4**.

Kapakahine C. The NMR spectra of **3** indicated a roughly 4:1 mixture of two conformers, whose corresponding proton signals showed negative crosspeaks in the NOESY spectrum.[10] The NMR spectra were analyzed for the major conformer, and Pro, Val, Ile, and Tyr residues were assigned together with fragment **a**.

Sequential HMBC crosspeaks observed between Ile-1/Trp-1 (NH-3/C-1), Val/Pro-1 (NH-5/C-18), Ile-2/Pro-2 (NH-7/C-28), and Trp-2/Ile-2 (NH-8/C-33) and NOESY correlations between H-13/H-19 and H-24/H-32a and 32b suggested the same amino acid sequence as in **1**. Diagnostic NOESY correlations between H-4/H-40 and 41a were also seen for the unconventional C−N bond between Trp-1/Trp-2.

The difference between kapakahines A (**1**) and C (**3**) resides in the Trp-1 moiety. In kapakahine C, the amino nitrogen atom of Trp-1 (N-1) is joined to C-4 to produce fragment **c** which was secured by HMBC correlation between H-4/C-2 and comparison of the chemical shifts of C-4 and -5 with those of the flustraminols.[11]



c **d**

Kapakahine D. The NMR spectra of kapakahine D (**4**) were also those of a 3:1 mixture of two conformers. NMR analysis of the major conformer showed fragments **a** and **d** together with Pro, Val, Ile, and Tyr residues.

Sequential HMBC crosspeaks were seen between NH-3 and H-13/C-1, NH-5 and H-24/C-18, NH-7/C-28, and NH-8/C-33, and sequential ROESY[12] crosspeaks were present between α-protons of Ile-1/Pro-1 and Val/Pro-2. An HMBC correlation between H-4/C-43, together with a ROESY crosspeak between H-4/H-41a, connected Trp-1 and Trp-2, thereby indicating the same planar structure as in kapakahine C (**3**).

A distinction between kapakahines C (**3**) and D (**4**) appears to lie in the geometry of the amide bonds between Val and Pro-2. The carbon chemical shift difference between β- and γ-carbons of Pro-2 (3.2 ppm in **3**, 9.1 ppm in **4**),[13] together with the NOE values (δH Pro-2/αH Val in **3**, αH Pro-2/αH Val in **4**), indicated *cis* and *trans* amide bonds, respectively. Furthermore, an HMBC correlation was observed between H-4 and C-43 in **4**,

Table 1. NMR Data for Kapakahine A (in CD_3CN)

		^{13}C	^{1}H mult	*J* (Hz)	HMBC (C no.)	NOE (H; *NH* no.)
Trp-1	1	169.1				
	2	55.9	4.68 bd	12.2	1, 3, 5	3ab, 4; *3*
	3a	27.8	3.58 dd	15.9, 12.2	2, 4, 5, 6	2, 3b, 7; *3*
	3b		3.38 bd	15.9	4, 5	2, 3a
	4	124.5	8.09 s		5, 6, 11, 43	2, 40, 41a
	5	109.7				
	6	131.0				
	7	120.3	7.62 d	7.6	5, 6, 9, 11	3a, 8, 9
	8	121.0	7.04 t	7.6	6, 10	7, 9
	9	123.5	6.91 dd	6.82	7, 11	7, 8, 10
	10	111.8	6.35 d	8.2	6, 8	9, 42
	11	135.8				
	N-1		8.52 bs			
Ile-1	12	170.4				
	13	57.1	4.17 bdd	6.4, 5.1		14, 17, 19
	14	39.6	1.90 m			13, 17
	15a	25.6	1.63 m		17	15b
	15b		1.16 m		14, 17	15a, 16
	16	11.6	0.88 t	7.6	14, 15	15b
	17	14.9	0.91 d	6.7	13, 14, 15	13, 14, 19, 22b
	N-3		7.91 bd	5.1		2, 3a
Pro-1	18	172.5				
	19	62.5	4.54 d	7.6	18, 20, 21, 22	13, 17, 20a
	20a	33.0	2.19 m		22	19
	20b		2.15 m		18, 19, 21	
	21a	23.1	1.94 m			21b
	21b		1.75 m			21a, 22a, 27
	22a	47.4	3.65 m			21b, 22a; *5*
	22b		3.51 m			17, 22a
Val	23	174.8				
	24	58.5	4.35 dd	10.1, 7.8	18, 23, 25, 26, 27	26, 27, 32ab; *5*
	25	30.8	2.18 m		24, 26, 27	26
	26	19.4	0.95 d	6.7	24, 25, 27	24, 32b
	27	19.5	0.99 d	6.7	24, 25, 26	21b, 24
	N-5		8.08 d	7.8		22a, 24
Pro-2	28	171.4				
	29	62.2	4.75 d	7.6	23, 28, 30, 31, 32	30ab; *7*
	30a	27.0	2.52 bdd	11.6, 5.5	28, 31, 32	29, 30b, 31b
	30b		1.73 m		28, 29	29, 30a
	31a	25.7	2.03 m		29, 30	31b, 32a
	31b		1.85 m			30a, 31a, 32b
	32a	49.2	4.08 dt	10.4, 7.0	29, 31	24, 31a, 32b
	32b		3.70 bt	10.4	30	24, 26, 31b, 32a
Ile-2	33	173.5				
	34	58.4	4.45 dd	7.6, 5.5	28, 33, 35, 36, 38	35, 38; *7*, *8*
	35	39.9	1.69 m			34, 38, 36a; *8*
	36a	25.5	1.37 m		35, 37	35, 36b, 37
	36b		1.06 m		37, 38	36a, 37
	37	11.9	0.83 t	7.3	35, 36	36ab
	38	15.9	0.87 d	7.0	34, 35, 36	34, 35
	N-7		8.17 d	7.6		29, 34
Trp-2	39	168.3				
	40	48.2	5.03 ddd	12.8, 8.0, 1.5	39, 41, 43	4, 41a, 42; *8*
	41a	38.3	3.15 dd	14.3, 1.5	39, 40, 42, 43	4, 40, 41b, 45
	41b		2.44 dd	14.3, 12.8	39, 40, 43, 44	41a
	42	82.5	5.68 s		39, 41, 43, 49, 51	10, 40, 54, 58
	43	68.4				
	44	135.3				
	45	124.6	6.76 d	7.3	43, 47, 49	41a, 46, 47
	46	126.8	6.99 dd	7.3 7.5	44, 48	45, 47
	47	131.3	7.34 dd	7.5 7.9	45, 49	45, 46, 48
	48	115.6	7.61 d	7.9	44, 46, 49	47
	49	140.3				
	N-8		7.47 d	8.0	33, 39	34, 35, 40
Tyr	50	172.8				
	51	65.9	4.93 dd	4.6, 5.2	39, 42, 50, 52, 53	52ab
	52a	36.5	3.18 dd	14.2, 4.6	50, 51, 53, 54, 58	51, 52b, 54, 58
	52b		3.06 dd	14.2, 5.2	50, 51, 53, 54, 58	51, 52a, 54, 58
	53	127.5				
	54	131.3	7.07 d	8.4	52, 56, 58	42, 52ab, 55
	55	116.2	6.56 d	8.4	53, 56, 57	54
	56	157.3				
	57	116.2	6.56 d	8.4	53, 55, 56	58
	58	131.3	7.07 d	8.4	52, 54, 56	42, 52ab, 57

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Table 2. NMR Data for the Major Conformers of Kapakahines C and D[a] (in CD_3OH)

		3					4				
		^{13}C	^1H mult	*J* (Hz)	HMBC (C no.)	NOE (H; *NH* no.)	^{13}C	^1H mult	*J* (Hz)	HMBC (C no.)	NOE (H; *NH* no.)
Trp-1	1	173.7					177.0				
	2	61.6	3.44 bq	8.0		3ab, 45; *3*	64.7	4.14 m		1, 3	4
	3a	46.9	2.47 d	8.0	1, 2, 4, 5, 6	2, 4, 7	47.1	2.68 dd	13.4, 7.6	5	
	3b		2.47 d	8.0	1, 2, 4, 5, 6	2, 4, 7		1.93 dd	13.4, 8.5	6	
	4	87.2	6.02 bs		2, 5, 11	3ab, 40, 41a	93.2	5.66 d	6.7	2, 3, 43	2, 41a, 45
	5	89.6					88.3				
	6	132.8					134.5				
	7	125.2	7.21 dd	1.4, 7.4	5, 9, 11	3ab	124.0	7.12 dd	7.5, 1.5	5, 9, 11	
	8	119.1	6.59 ddd	0.6, 7.4, 7.4	6, 10		121.1	6.67 bdd	7.2, 7.5	6, 10	
	9	130.2	6.69 ddd	1.4, 7.4, 8.2	7, 11		130.2	6.71 ddd	1.5, 7.2, 8.4	7, 11	
	10	108.5	5.37 d	8.2	6, 8		111.9	5.61 d	8.4	6, 8	
	11	148.8					147.6				
	N-1		4.61 m					4.46 dd	6.7, 6.4	3, 5	40, 41a
Ile-1	12	173.3					171.4				
	13	58.4	4.22 dd	8.6, 3.8	12, 14, 15	14, 15b, 19, 37; *5*	56.1	4.33 dd	8.2, 8.4	1, 12, 14, 15, 17	19
	14	37.6	1.71 m			13; *3*	40.2	1.73 m			
	15a	26.6	1.68 m				25.7	1.63 m			
	15b		1.28 m			13		1.29 m			
	16	11.1	0.91 t	7.2	14, 15		11.7	0.78 t	7.3	14, 15	
	17	15.1	0.89 d	7.1	13, 14, 15	19	15.2	0.85 d	6.7	13, 14, 15	
	N-3		8.37 d	3.8	1, 13	2, 14		7.57 d	8.4	1, 12	40
Pro-1	18	172.9					172.6				
	19	62.7	4.75 d	6.8	18, 21, 22	13, 17, 20ab; *5*	60.7	4.74 dd	2.1, 8.2	20, 21, 22	13; *5*
	20a	30.8	2.58 m		22	19, 20b, 21b	32.3	2.30 m			
	20b		1.86 m		18	19, 20a,		2.11 m			
	21a	22.5	1.97 m		19	22a	22.9	1.97 m			
	21b		1.74 m			20a, 22b		1.90 m			
	22a	46.7	3.69 ddd	12.0, 9.5, 8.6		21a, 22b	47.7	3.82 m			
	22b		3.38 ddd	1.8, 9.2, 12.0		21b, 22a		3.82 m			
Val	23	174.9					174.8				
	24	59.2	4.41 dd	8.3, 8.0	23, 25, 26	25, 26, 27, 32ab; *5*	59.0	4.18 m		18, 25	29
	25	31.3	2.61 m		24	24, 26, 27	31.8	2.06 m			
	26	19.6	1.18 d	6.8	24, 25, 27	24, 25; 32b; *7*	19.2	1.00 d	6.7	24, 25, 27	
	27	20.2	1.15 d	6.5	24, 25, 26	24, 25; *5*	19.8	1.17 d	6.7	24, 25, 26	
	N-5		9.54 d	8.0	18	13, 19, 24, 27		8.68 bd	5.6	18	19
Pro-2	28	173.1					173.8				
	29	62.6	4.52 dd	8.3, 2.7	28, 30, 31	30a; *7*	62.9	4.92 bd	6.7	28, 30, 31, 32	24
	30a	29.0	2.28 m			29, 40	31.5	2.51 bdd	11.0, 5.2	32	
	30b		2.02 m					2.00 m		28	
	31a	25.8	2.05 m				22.4	2.02 m			
	31b		1.87 m		29	32b		1.72 m			
	32a	49.1	3.92 dt	6.5, 10.1		24, 32b	47.2	3.65 m			
	32b		3.76 ddd	3.0, 7.0, 10.1		24, 26, 31b, 32a		3.43 m			
Ile-2	33	172.3					173.8				
	34	58.7	4.34 dd	8.0, 8.5	28, 33, 35, 36, 38	38; *8*	61.4	4.16 m		28, 33, 35, 36, 38	
	35	39.1	1.73 m			36a; *7*	37.6	2.03 m			
	36a	26.0	1.52 m			35	25.4	1.87 m			
	36b		1.14 m					1.25 m			
	37	11.3	0.89 t	7.3	35, 36	13	11.7	0.93 t	7.3	35, 36	
	38	15.6	0.92 d	6.2	34, 35, 36	34	16.6	1.00 d	6.7	34, 35	
	N-7		7.14 d	8.5	28	26, 28, 35		7.60 d	8.2	28	
Trp-2	39	169.7					168.6				
	40	48.4	4.63 m			4, 30a	50.3	4.70 ddd	12.7, 7.0, 2.8		*1*, *3*
	41a	37.2	3.57 dd	14.4, 2.7	39, 42, 43	4, 45	38.1	2.96 dd	15.2, 2.8	39, 42	4; *1*
	41b		1.73 dd	14.4, 12.7	39, 44	–; *8*		2.72 dd	15.2, 12.7	39, 44	
	42	80.9	5.39 s		39, 41, 43, 49	54, 58	83.3	5.61 s		39, 43, 49	54, 58
	43	65.9					69.4				
	44	136.9					136.1				
	45	127.7	7.65 dd	0.6, 7.7	43, 47, 49	2, 41a	124.7	7.14 bd	7.6	43, 47, 49	4
	46	126.6	6.97 dt	0.6, 7.7	44, 48		126.8	6.93 ddd	1.2, 7.3, 7.6	44, 48	
	47	130.2	7.25 ddd	1.2, 7.7, 8.0	45, 49		130.2	7.18 ddd	1.2, 7.3, 8.0	45, 49	
	48	115.0	7.51 d	8.0	44, 46, 49		115.1	7.46 d	8.0	44, 46	
	49	140.2					141.7				
	N-8		8.13 d	6.5	33, 39	34, 41b		8.12 d	7.0	33	
Tyr	50	172.7					173.0				
	51	67.2	5.02 dd	4.6, 4.9	39, 42, 50, 52, 53	54, 58	66.4	4.97 dd	4.0, 4.6	39, 42, 50, 52, 53	
	52a	36.5	3.20 dd	14.2, 4.6	50, 51, 53, 54, 58	54, 58	36.6	3.29 dd	14.3, 4.6	50, 53, 54, 58	
	52b		3.06 dd	14.2, 4.9	50, 51, 53, 54, 58	54, 58		3.25 dd	14.3, 4.0	50, 53, 54, 58	
	53	127.2					127.5				
	54	131.4	7.05 d	8.6	52, 56, 58	51, 52ab	132.4	7.42 d	8.6	52, 55, 56, 58	42
	55	116.4	6.56 d	8.6	53, 56, 57		116.3	6.64 d	8.6	53, 56, 57	
	56	157.9					158.3				
	57	116.4	6.56 d	8.6	53, 55, 56		116.3	6.64 d	8.6	53, 55, 56	
	58	131.4	7.05 d	8.6	52, 54, 56	51, 52ab	132.4	7.42 d	8.6	52, 54, 56, 57	42

[a] Signals of minor comformer.

which was not seen in **3**. These observations implied that conformation of the 24-membered ring is different for **3** and **4**.

Stereochemistry of the Kapakahines. Kapakahines A and B. Absolute stereochemistry of the common amino acids of kapakahine A (Val, Pro, Ile, Trp-1, and Tyr) and kapakahine B (Ala, Leu, Phe, Trp-1) was determined by Marfey's method and shown to be L (Table 3).[14]

The relative stereochemistry of fragment **a** in **1** and **2** was determined as follows. The coupling constants

(14) Marfey, P. *Carlsberg Res. Commun.* **1984**, *49*, 591–596.

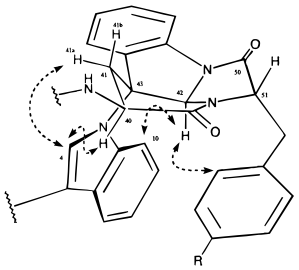
Table 3. Results of Marfey's Analyses of 1−4

	standard	1	2	3	4
L-ALa[a]	10.2		10.0		
D-ALa[a]	21.5				
L-Pro[a]	11.1	10.8		11.0	11.1
D-Pro[a]	20.5				
L-VaL[b]	12.6	12.7		12.6	12.9
D-VaL[b]	20.5				
L-Trp[b]	19.0	18.9	19.0		
D-Trp[b]	25.7				
L-ILe[b]	19.4	19.5		19.4	19.9
D-ILe[b]	35.1				
L-Leu[b]	22.1		22.1		
D-Leu[b]	38.6				
L-Phe[b]	22.0		22.1		
D-Phe[b]	34.4				
L-Tyr[a]	16.9, 30.7	16.6, 30.8		30.7, 39.7	30.6, 39.5
D-Tyr[a]	44.9, 32.0				
L-Tyr[c]	11.8	11.9		11.8	11.9
D-Tyr[c]	27.8				

[a] 20% MeCN + 50 mM NH_4OAc (solvent I). [b] 37.5% MeCN + 0.05% TFA (solvent II). [c] 38% MeCN + 50 mM NH_4OAc (solvent III).

between H-40 and H-41b (J = 12.8 Hz) of **1** indicated that both are axial [H-31 and H-32b (J = 12.8 Hz) in **2**]. A strong NOE between H-10/H-42 (H-19/H-33 in **2**) suggested that H-42 (H-33 in **2**) and Trp-1 are on the same side of the tetracycle, confirming *cis* linkage of B and C rings, which was further supported by NOE values between H-4/H-40 and H-41a (H-13/H-31 and H-32a in **2**); see fragments **e**.

The absolute stereochemistry of the C-42 chiral center was deduced from NOE values between H-42/H-54 and 58 (H-33/H-45 and 49 in **2**), which indicated that the benzene ring of Tyr (Phe in **2**) and H-42 (H-33 in **2**) are on the same side of ring D. Therefore, from the *S*-configuration at C-51 of L-Tyr, the absolute stereochemistry for C-40, 42, and 43 was deduced to be *S*, *R*, and *R*, respectively.



e

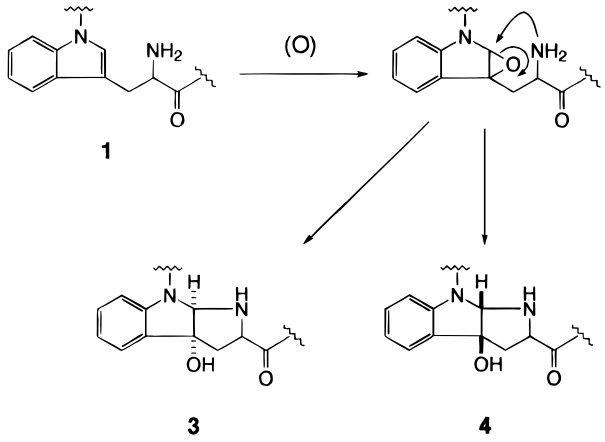
R= H 1
OH 2

Kapakahines C and D. The absolute configuration of Pro, Val, Ile, and Tyr residues of **3** and **4** was also determined as L by Marfey analysis (Table 3).

Similar coupling constant values between H-40/H-41a and 41b [$J_{40,41a}$ (Hz), $J_{40,41b}$ (Hz), respectively: **1**, 1.5, 12.8; **3**, 2.7, 12.7; **4**, 2.8, 12.7] and NOE values between H-4/H-41a showed the same relative stereochemistry of rings C and D in **1**−**4**. NOE values between H-42/H-54 and 58 seen in **3** and **4** determined the same absolute stereochemistry (40*S*,42*R*,43*R*) of fragments **a** in **3** and **4** as in **1**.

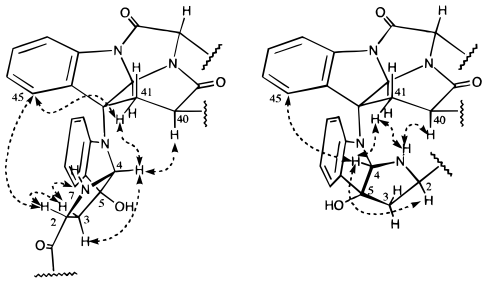
The difference between **3** and **4** lay in the stereochemistry of C-4 and C-5. In kapakahine C (**3**), NOE values were seen between H-2/H-3 and 45, H-3/H-4 and 7 and H-4/H-40 and 41a. On the other hand, in kapakahine D (**4**), NOE values were seen between H-2/H-4, H-4/H-45, and NH-1/H-40 and 41a, as well as between H-4/H-41a.

Scheme 1. Suggested Biogenetic Route of Kapakahines C (3) and D (4) from A (1)



1 **3** **4**

These NOE data can be explained by stereochemistry of 2*S*,4*R*,5*R* in **3** and 2*S*,4*S*,5*S* in **4** (fragments **f** and **g**); therefore kapakahines C and D are enantiomeric at C-4 and C-5.



f **g**

The difference between these structures appears reasonable if one considers a likely biogenetic route to **3** and **4** from **1** via oxidation of the imidazole of the Trp-1 residue (Scheme 1).

Biological Activities. Kapakahines A, B, and C showed moderate cytotoxicity against P388 murine leukemia cells at IC_{50} values of 5.4, 5.0, and 5.0 μg/mL, respectively. Kapakahine D did not show cytotoxicity at concentration of 10 μg/mL. Kapakahine A was tested for inhibitory activities against several enzymes [thrombin, trypsin, plasmin, elastase, papain, angiotensin converting enzyme (ACE), and protein phosphatase 2A (PP2A)], but showed only 15% inhibition against PP2A at a concentration of 30 μM (32 μg/mL). Against other enzymes, **1** did not show any activity at the concentration of 100 μg/mL.

Experimental Section

General Procedures. IR spectra were measured in $CHCl_3$ using KBr cells. Optical rotations were recorded on a digital spectropolarimeter. UV spectra were obtained using a diode array spectrophotometer. All NMR spectra were recorded at 500.115 MHz for ^1H and 125.766 MHz for ^{13}C. Glycerol was used as matrix for FAB-MS measurements.

Extraction and Isolation. The sponge *C. olemda* was collected at Pohnpei, Federated States of Micronesia, in April 1992, and recollected in August 1993.[15] The ethanolic extract of the frozen sponge (840 g wet weight) was partitioned

(15) From the 1992 collection we isolated a pregnane glycoside,[16] which was absent in the 1993 collection.

(16) Yeung, B. K. S.; Hamann, M. T.; Scheuer, P. J.; Kelly-Borges, M. *Tetrahedron* **1994**, *50*, 12593−12598.

(17) Singhal, A. K.; Chien, K.-Y.; Wu, W.-g.; Rule, G. S. *Biochemistry* **1993**, *32*, 8036−8044.

between ethyl acetate and water. The organic layer was subjected to the Kupchan separation scheme.[5] The methylene chloride fraction was chromatographed on Sephadex LH-20 (MeOH). The peptide-containing fractions (monitored by TLC) were separated by ODS HPLC (COSMOSIL $5C_{18}$-AR; H_2O/MeCN, 58:42, 0.05% TFA) and yielded three peptides, kapakahines A (**1**; 5.8 mg, 6.9×10^{-4}% yield based on wet weight), C (**3**; 2.6 mg, 3.1×10^{-4}% yield based on wet weight) and impure kapakahine B, which was further purified by HPLC on an amino column (MICROSORB NH_2 80-799-C_5; $CHCl_3$/MeOH, 98:2) furnishing 0.3 mg of pure compound (**2**; 3.6×10^{-5}% yield based on wet weight). Together with kapakahines obtained from a second sponge sample (4.0 kg), a total of 14.4 mg of kapakahine A (**1**) and 2.0 mg of kapakahine B (**2**) was obtained.

The aqueous layer from the first solvent partition was basified to pH 9 with $NaHCO_3$ and extracted with *n*-BuOH. The extract was neutralized with HCl before concentration. The concentrated extract was desalted on an ODS column and was combined with the CCl_4 fraction from the Kupchan separation scheme of the organic layer. The combined fractions were subjected to ODS flash chromatography using a stepwise gradient solvent system (25, 42% MeCN, and 100% MeOH). The fraction eluting with 42% MeCN was gel-filtered on a Sephadex LH-20 column with MeOH and monitored by TLC and ^{1}H NMR spectra. The fractions containing peptides were collected and separated by ODS HPLC (COSMOSIL $5C_{18}$-AR; H_2O/MeCN 6:4, 0.05% TFA), followed by HPLC on an amino column (MICROSORB NH_2 80-799-C_5; $CHCl_3$/MeOH, 98:2) yielding 1.8 mg of kapakahine D (**4**; 2.1×10^{-4} % yield based on wet weight)

Kapakahine A (1): colorless amorphous solid; $[\alpha]^{20}_D$ −131° (*c* 1.00, MeOH); UV (MeOH) 206 nm (ϵ 39 000), 232 (19 000), 274 (9500); IR ($CHCl_3$) 3360, 3040, 1660 cm^{-1}; ^{1}H and ^{13}C NMR (CD_3CN) see Table 1.

Kapakahine C (3): colorless amorphous solid; $[\alpha]^{20}_D$ −120° (*c* 0.50, MeOH); UV (MeOH) 208 nm (ϵ 44 000), 253 (ϵ 12 000), 279 (ϵ 4100), 305 (ϵ 1200); IR ($CHCl_3$) 3390, 3280, 1715, 1650 cm^{-1}; ^{1}H and ^{13}C NMR (CD_3OH) see Table 2.

Kapakahine D (4): colorless amorphous solid; $[\alpha]^{20}_D$ −30.7° (*c* 0.933, MeOH); UV (MeOH) 206 nm (ϵ 35 000), 247 (ϵ 9000), 279 (ϵ 2800), 304 (ϵ 690); IR ($CHCl_3$) 3400, 3340, 1670 cm^{-1}; ^{1}H and ^{13}C NMR (CD_3OH) see Table 2.

Marfey Analysis of 1−4. Kapakahines A−D (**1**−**4**), 100 μg each, were dissolved in 5 N HCl (500 μL) and freeze-dried for 2 min and then hydrolyzed at 105 °C for 12 h. The acid hydrolysate was dried under N_2, and to it were added 50 μL of a 0.1% FDAA solution in acetone and 100 μL of an 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 reverse phase HPLC with three isocratic solvent systems (COSMOSIL $5C_{18}$-MS; solvent I, 37.5% MeCN + 0.05% TFA; solvent II, 20% MeCN + 50 mM NH_4OAc; solvent III, 38% MeCN + 50 mM NH_4OAc).

The residues, Val, Ile, Leu, Trp, and Phe, were analyzed with solvent I to show all L configuration. Ala and Pro were analyzed with solvent II, indicating both to be L, which was comfirmed by coinjection with authentic standards.

Reaction of Tyr with Marfey's reagent can produce three derivatives: monosubstituted derivatives of phenolic OH or NH_2 groups and a disubstituted derivative. These derivatives were well analyzed on an ODS HPLC (COSMOSIL $5C_{18}$-MS; solvents II and III) column. The less polar O-substituted derivatives were analyzed with solvent III [L-Tyr (11.8 min) and D-Tyr (27.8 min)]. N-Substituted and N,O-disubstituted derivatives were analyzed with solvent II [N-substituted L-Tyr (16.9 min) and D-Tyr (44.9 min), N,O-disubstituted L-Tyr (30.7 min) and D-Tyr (32.0 min)]. All hydrolysates of **1**, **3**, and **4** with Marfey's reagent produced peaks corresponding to L-Tyr, whose intensity was increased by coinjection with a standard L-Tyr derivative (Table 3).

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Supporting Information Available: Selective INEPT of **1**, ^{1}H and ^{13}C NMR, COSY, NOESY, HMQC, and HMBC spectra for **1**, **3**, and **4** (25 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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