More Peptides and Other Diverse Constituents of the Marine Mollusk *Philinopsis speciosa*

Yoichi Nakao,[1] Wesley Y. Yoshida, Christina M. Szabo,[2] Bill J. Baker,[3] and Paul J. Scheuer*

Department of Chemistry, University of Hawai'i at Manoa, 2545 The Mall, Honolulu, Hawaii 96822-2275

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In addition to five new depsipeptides related to kulolide-1 (**1**), further examination of the mollusk *Philinopsis speciosa* has yielded a linear peptide, pupukeamide (**14**), and an unprecedented macrolide, tolytoxin-23-acetate (**7**). Chemical makeup suggested that the compounds originate from blue-green algae and are transmitted via herbivorous mollusks to *P. speciosa*. This could be verified by laboratory experiment. The structures were determined largely by spectroscopic techniques and the stereochemistry by hydrolysis of the peptides followed by chiral chromatography.

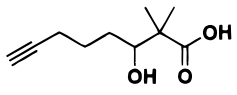
Introduction

The relatively large and slow-moving sea hares are herbivorous gastropod mollusks which may be found worldwide in shallow water. Not surprisingly, they are among the earliest marine invertebrates to attract the attention of natural product chemists.[4] A study of the metabolites of red algae of the genus *Laurencia*, which is an observed diet of some sea hares, led to a suggestion that secondary metabolites isolated from sea hares are in fact products of their algal diet.[5] We were able to draw a parallel conclusion for the defensive secretion of a carnivorous gastropod mollusk, the nudibranch *Phyllidia varicosa*, and its prey, a sponge *Ciocalypta* sp. (previously *Hymeniacidon* sp.).[6] In contrast to these uncomplicated examples of marine chemical ecology, the subject of this report, the constituents of the cephalaspidean mollusk *Philinopsis speciosa*, provides a far more complex and fascinating account of ecological relationships on a coral reef.

We first collected *P. speciosa* over 15 years ago at Shark's Cove on the north shore of O'ahu, HI, on midsummer nights, when the animals emerge from the sand to feed and mate. Our initial results, isolation of polypropionate[7] and alkylpyridine[8] constituents, conformed to the conventional wisdom of the time. A hint that a peptide might be present in the polar extract of *P. speciosa* led to its isolation and, in time, to the structural elucidation of the depsipeptide kulolide.[9] To secure the final stereochemical details of the structure, recollection became necessary. In our experience, the population density of *P. speciosa* would vary from virtual absence to rich abundance. Luckily, the 1994 and 1995 seasons provided a bountiful harvest, which not only allowed completion of the kulolide structure, but yielded a cornucopia of cyclic depsipeptides, a linear peptide (pupukeamide), and a derivative of the known macrolide tolytoxin. Moreover, we gained a fortuitous glimpse into complex ecological relationships.

The mollusk *Philinopsis speciosa* (9.0 kg, wet weight) was extracted with EtOH and then $CHCl_3$/MeOH (1:1). The combined extracts were evaporated and separated by solvent partition, ODS flash chromatography, gel filtration, and repetitive ODS HPLC, which yielded kulolide-1 (**1**, 128.0 mg), kulolide-2 (**2**, 5.0 mg), kulolide-3 (**3**, 2.4 mg), kulokainalide-1[10] (**4**, 8.0 mg), kulomo'opunalide-1[11] (**5**, 4.1 mg), kulomo'opunalide-2 (**6**, 4.2 mg), and tolytoxin 23-acetate (**7**, 2.5 mg) (Chart 1).

Kulolide-2 (2) and -3 (3). The molecular weights of kulolide-2 (**2**) and -3 (**3**) differed from that of kulolide-1 by 2 and 4 mass units, respectively, on the basis of FABMS analysis. The NMR spectral signals of kulolide-2 and -3 represented a mixture of two conformers in 1:1 ratio, which had also been the case for kulolide-1 (**1**). The ^{1}H NMR spectra revealed the difference in three new olefinic protons (δ 5.84, 5.03, and 4.97) in the spectrum of **2**, while no olefinic proton was seen in the spectrum of **3**. Neither spectrum displayed an acetylenic proton resonance. These observations and the mass difference among these three compounds implied that the terminal triple bond of Dhoya (**8**) in **1** must be reduced to a double or a single bond to 2,2-dimethyl-3-hydroxyoctenoic acid (Dhoea, **9**) in **2** and 2,2-dimethyl-3-hydroxyoctanoic acid (Dhoaa, **10**) in **3**.



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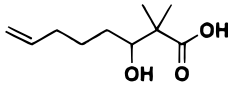

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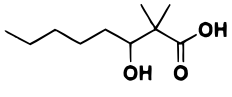

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Detailed analysis of 2D NMR spectra of **2** and **3**, including COSY, HMQC,[12] and HMBC,[13] showed that the

(1) Present address: Laboratory of Aquatic Natural Products, The University of Tokyo, Tokyo, Japan.
(2) NSF−REU participant, summer 1996.
(3) Department of Chemistry, Florida Institute of Technology, Melbourne, Fl 32901.
(4) Tanaka, T.; Toyama, Y. *J. Chem. Soc. Jpn., Pure Chem. Sec.* **1959**, *80*, 1329−1332.
(5) Irie, T.; Yasunari, Y.; Suzuki, T.; Imai, N.; Kurosawa, E.; Masamune, T. *Tetrahedron Lett.* **1965**, 3619−3624.
(6) Burreson, B. J.; Scheuer, P. J.; Finer, J.; Clardy, J. *J. Am. Chem. Soc.* **1975**, *97*, 4763−4764.
(7) Coval, S. J.; Scheuer, P. J. *J. Org. Chem.* **1985**, *50*, 3024−3025.
(8) Coval, S. J.; Schulte, G. R.; Matsumoto, G. K.; Roll, D. M.; Scheuer, P. J. *Tetrahedron Lett.* **1985**, *26*, 5359−5362.
(9) Reese, M. T.; Gulavita, N. K.; Nakao, Y.; Hamann, M. T.; Yoshida, W. Y.; Coval, S. J.; Scheuer, P. J. *J. Am. Chem. Soc.* **1996**, *118*, 11081−11084.
(10) Kaina means younger sibling in Hawaiian.
(11) Mo'opuna is grandchild in Hawaiian.

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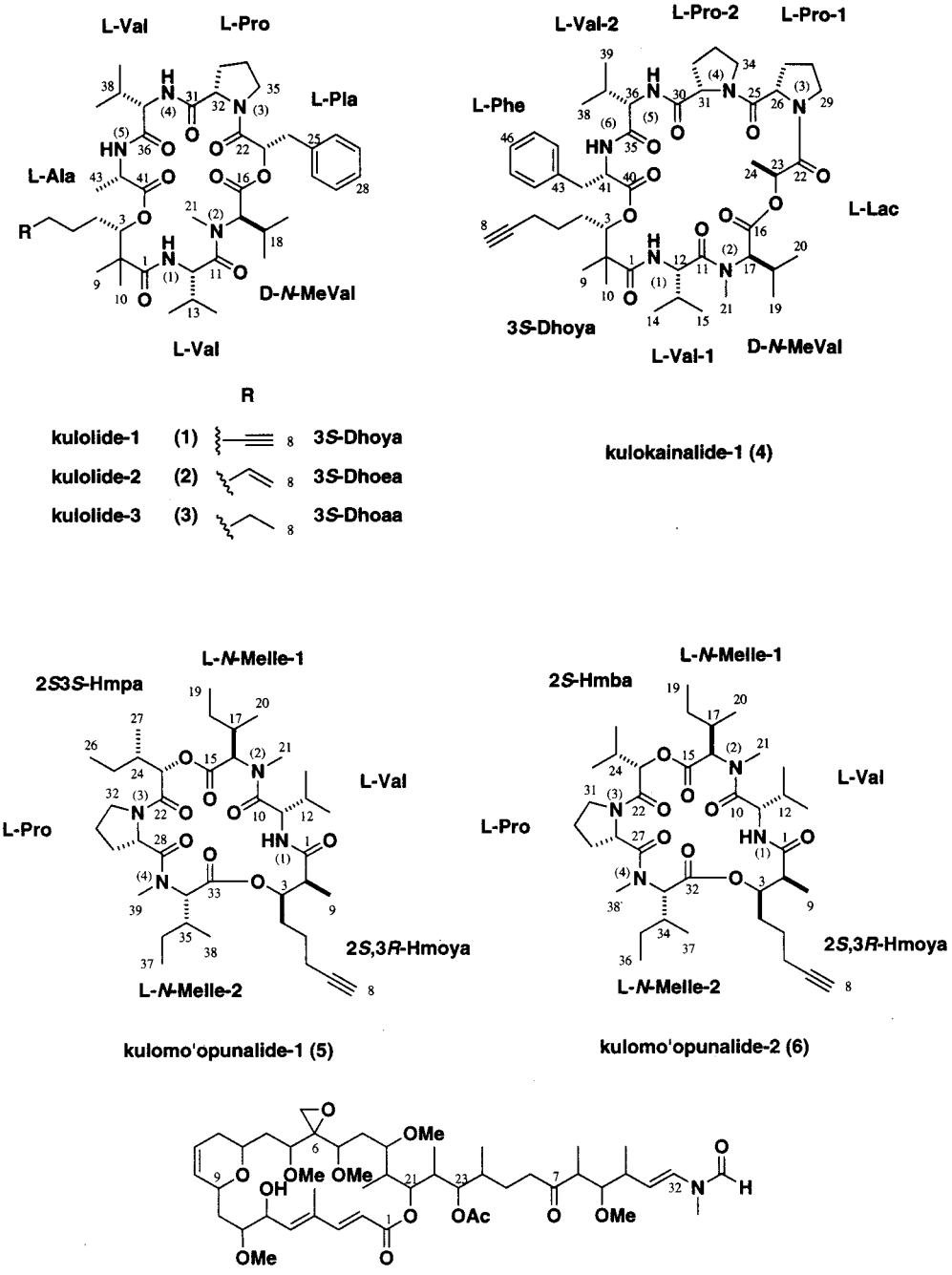
Chart 1



only difference of the three kulolides lay in the degree of unsaturation of the octanoic acid residues. This was subsequently confirmed by comparison of the ^{1}H NMR spectra after reducing the triple or double bond in **1** or **2** on Pd−C, leading to **3**. The ^{1}H NMR spectra of tetrahydrokulolide-1, dihydrokulolide-2, and kulolide-3 were superimposable. Thus the structures of **2** and **3**, including identical relative stereochemistry, were confirmed.

The same sign of optical rotation of tetrahydrokulolide-1, dihydrokulolide-2, and kulolide-3 indicated the same absolute stereochemistry of all residues in these compounds.

Kulokainalide-1 (4). The molecular formula of kulokainalide-1 (**4**) was determined as $C_{48}H_{70}N_6O_{10}$ by HR-FABMS. The NMR spectra of kulokainalide-1 (**4**) showed only one conformer, in contrast to those of the kulolides. Comparison of the ^{1}H and ^{13}C NMR spectra of **4** with those of **1** suggested that both compounds contain common residues of Val, *N*-methylvaline (MeVal), and Pro. A characteristic triplet signal of an acetylenic proton at 1.97 ppm, together with two singlet methyl signals at 1.14 and 1.23 ppm, recalled Dhoya (**8**) of kulolide, which was confirmed by COSY, HMQC, and HMBC experiments.

The most obvious difference between kulolide-1 (**1**) and kulokainalide-1 (**4**) is their molecular size. One additional Pro residue in kulokainalide-1 accounts for the difference of the molecular formulas (C_5H_7NO); the two

(12) Summers, M. F.; Marzilli, L. G.; Bax, A. *J. Am. Chem. Soc.* **1986**, *108*, 4285−4294.
(13) Bax, A.; Azolos, A.; Dinya, Z.; Sudo, K. *J. Am. Chem. Soc.* **1986**, *108*, 8056−8063.

Table 1. NMR Data for Kulokainalide (4) in CD_2Cl_2

		δ						δ			
	no.	C	H	HMBC	ROESY		no.	C	H	HMBC	ROESY
Dhoa	1	176.9				Pro-1	27b		1.62 m		
	2	46.6					28a	25.5	1.98 m		
	3	77.8	5.18 dd, 10.6, 1.2	C: 9, 10, 40			28b		1.79 m		
	4a	28.8	1.71 m				29a	47.9	3.58 dd, 7.1, 2.4		
	4b		1.42 dd, 10.3, 9.4	C: 3			29b		3.55 dd, 7.1, 8.5		
	5a	24.3	1.72 m	C: 7		Pro-2	30	169.6			
	5b		1.47 m	C: 7			31	61.5	3.89 d, 7.0	C: 30, 32, 33, 34	H: 26
	6	18.5	2.24 m	C: 5, 7, 8			32a	30.4	2.65 dd, 10.6, 5.3	C: 33	
	7	84.4					32b		1.83 m	C: 30	
	8	68.7	1.97 t, 2.5				33a	22.5	1.85 m		
	9	24.3	1.14 s	C: 1, 2, 3, 10			33b		1.63 m		
	10	17.2	1.23 s	C: 1, 2, 3, 9			34a	46.7	3.43 dd, 11.8, 10.9	C: 25	
Val-1	11	175.6					34b		3.39 dt, 7.0, 11.7	C: 25	
	12	55.7	4.44 t, 8.5	C: 11, 13, 14, 15		Val-2	35	169.8			
	13	31.0	2.04 m	C: 14, 15			36	55.8	4.57 dd, 8.5, 8.2	C: 35, 37, 38, 39	
	14	18.9	0.96 d, 6.8	C: 12, 13, 15			37	33.4	1.92 m	C: 35, 39	
	15	19.0	0.87 d, 7.0	C: 12, 13, 14			38	19.0	0.97 d, 6.7	C: 36, 37, 39	
	NH-1		6.38 d, 8.2	C: 1			39	18.2	0.82 d, 6.8	C: 36, 37, 38	
N-MeVal	16	171.5					NH-5		9.22 d, 6.8	C: 30	H: 23, 44, 48
	17	64.6	4.49 d, 11.4	C: 11, 16, 18, 19, 21		Phe	40	170.7			
	18	27.6	2.26 m				41	54.2	4.73 ddd, 5.6, 7.1, 12.3	C: 35, 40	
	19	20.3	1.00 d, 6.5	C: 17, 18, 20			42a	34.3	3.62 dd, 13.5, 12.3	C: 41, 43, 44, 48	
	20	19.7	0.98 d, 6.8	C: 17, 18, 19			42b		3.10 dd, 5.6, 13.8	C: 41, 43, 44, 48	
	21	30.8	3.23 s	C: 11, 17			43	139.3			
Lac	22	169.6					44	130.1	7.24 d, 8.2	C: 42, 45, 46, 48	
	23	69.8	5.51 q, 6.8	C: 16, 22, 24	NH: 5		45	128.0	7.08 t, 8.2	C: 43, 44, 47	
	24	17.2	1.55 d, 6.7	C: 22, 23			46	126.4	7.13 t, 8.5	C:	
Pro-1	25	170.3					47	128.0	7.08 t, 8.2	C: 43, 45, 48	
	26	58.9	2.92 t, 7.6	C: 27	H: 31 NH: 6		48	130.1	7.24 d, 8.2	C: 42, 44, 46, 47	
	27a	28.6	1.81 m				NH-6		7.22 d, 6.8	C: 35	

prolines could be assigned from 2D NMR data, as could two Val and one MeVal.

Further analysis of the 2D NMR spectra allowed us to assign Phe and Lac instead of Pla and Ala in **1**. On the basis of ^{1}H NMR spectra, we initially thought that Pla and Ala residues were retained in **4**. Interestingly, it turned out that the phenyl group of Pla in **1** was transferred to Phe in **4**.

Sequencing of the peptide was carried out by HMBC and ROESY[14] analyses. Sequential HMBC correlations were observed between NH1/C1; H17,H21/C11; H23/C16; $H_2$34/C25; NH5/C30; NH6,H41/C35; and H-3/C-40 connecting the sequence of Pro-1/Pro-2/Val-2/Phe/Dhoya/Val-1/*N*-MeVal/Lac. NOE's between H-26/H-31 and NH-5/NH-6 supported the sequence of Pro-1/Pro-2 and Val-2/Phe. A connection between Lac and Pro-1 was not observed in NMR spectra. However, it is the only remaining choice to connect these residues to satisfy the molecular formula. Table 1 summarizes all spectral data.

The stereochemistry of the Dhoya (**8**) unit in **4** was determined by comparing the optical rotation of Dhoaa (**10**), which could be obtained from the acid hydrolysates of tetrahydro-**1** and **4**. Dhoya (**8**) was not stable to acid hydrolysis (5 N HCl, 105 °C, 12 h) and we could not obtain **8** from the acid hydrolysates of intact peptides **1** and **4**. The hydrolysate of tetrahydro **4** was extracted with EtOAc, and both organic and aqueous layers were dried under N_2. The organic layer yielded Dhoaa (**10**) {$[\alpha]_D$ −30° (*c* 0.1, MeOH)} by ODS HPLC separation. The identical sense of rotation with **10** {$[\alpha]_D$ −38° (*c* 1.0, MeOH)} from kulolide-1 (**1**) indicated the same 3*S* stereochemistry of the acid.[15]

Stereochemistry of the amino acids in **4** was determined by Marfey analysis,[16] and the stereochemistry of Lac was determined by chiral HPLC analysis. The aqueous layer of the acid hydrolysate of **4** was divided in two portions and half was subjected to Marfey analysis to yield L-Pro, L-Val, L-Phe, and D-MeVal. The other half was subjected to chiral HPLC analysis which determined the stereochemistry of Lac as L.

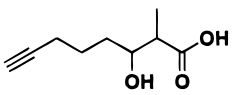
Kulomo'opunalide 1 (5). The molecular formula of kulomo'opunalide-1 (**5**) was determined by HR-FABMS analysis as $C_{39}H_{64}N_4O_8$. Analysis of COSY, HMQC, and HMBC spectra indicated that **5** contains four amino acids; one Pro, one Val, and two *N*-methylisoleucine (MeIle) residues. In the ^{1}H NMR spectrum of **5**, typical singlet methyl signals for Dhoya (**8**) were missing. Instead, there was a doublet methyl signal at 1.16 ppm (H9), from which we could follow the COSY correlation, via H2 (δ 2.80), up to H6 (δ 2.2). This signal, in turn, showed HMBC correlations with two carbons of a termi-

(14) Bothner-By, A. A.; Stephens, R. L.; Lee, J.; Warren, C. D.; Jeanloz, R. W. *J. Am. Chem. Soc.* **1984**, *106*, 811−813.

(15) This is a correction of the originally misassigned stereochemistry in ref 8.

(16) Marfey, P. *Carlsberg Res. Commun.* **1984**, *49*, 591−596.

nal acetylene, which completes the structure of 3-hydroxy-2-methyloctynoic acid (Hmoya, **11**). The other hydroxy acid in **5** displayed an Ile type spin system. From the multiplicity of the α-proton (doublet) and chemical shift of the α-carbon (77.6 ppm), it was assigned as 2-hydroxy-3-methylpentanoic acid (Hmpa, **12**).



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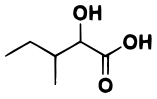

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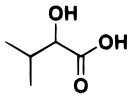
Sequencing of these residues was performed by HMBC and NOESY analyses. The sequence of Hmoya/Val/MeIle-1/Hmpa/Pro/MeIle-2 of **5** could be traced by the HMBC correlations between H11,NH1/C1; H16,H21/C10; H23/C15; and H34,H39/C28 and by the NOESY correlations between H23/$H_2$32. Although we could see neither HMBC nor NOESY correlation between Hmoya and MeIle-2, the only possibility to satisfy the molecular formula is to connect these two residues by an amide bond, which completes the planar structure of kulomo'opunalide-1 (**5**).[17]

Kulomo'opunalide-2 (6). The molecular formula of kulomo'opunalide-2 (**6**), which was determined as $C_{38}H_{62}N_4O_8$ by HR-FABMS, was smaller than that of **5** by a CH_2 unit. NMR spectra of **6** showed two conformers in 2:1 ratio. Analysis of COSY, HMQC, and HMBC spectra of the major conformer of **6** showed that all residues in **5** were retained in **6** except that Hmpa (**12**) in **5** was replaced by 2-hydroxy-3-methylbutanoic acid (Hmba, **13**) in **6**, which accounts for the difference of CH_2 between molecular formulas of **5** and **6**.



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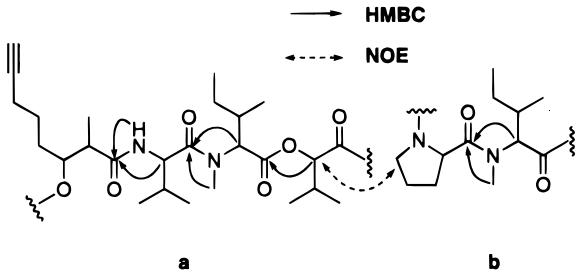
In the HMBC spectrum of **6**, correlations between H11,NH1/C1; H21/C10; H23/C15; and H33,H38/C27 allowed the sequence of Hmoa/Val/MeIle-1/Hmba (fragment **a**), and correlations between H33,H38/C27 connected Pro and MeIle-2 (fragment **b**).



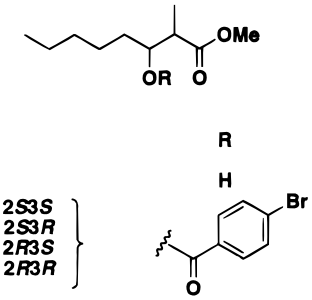
a **b**

Although we could not see any HMBC correlation between these two fragments, we could connect Hmba of **a** and Pro of **b** on the basis of NOESY correlation between H23/$H_2$31 (Table 2). Again the only choice to satisfy the molecular formula is to make the same 19-membered ring as **5**.[17]

Hmoya (**11**) units in kulomo'opunalides possess an additional stereocenter as compared to Dhoya (**8**) in **1**−**4**. Recently, Riguera and co-workers isolated onchidin B, which contains the same Hmoya (**11**) from the pulmonate mollusk *Onchidium* sp.[18] They determined the absolute stereochemistry of the Hmoya unit by comparing HPLC retention times with those of synthetic samples after derivatization with (−)-(*R*)-α-methoxy-α-(9-anthryl)acetic acid.

The stereochemistry of the Hmoya (**11**) units in **5** and **6** was determined by HPLC analysis after chemical derivatization. As we could not obtain Hmoya by acid hydrolysis of intact **5** and **6** (5 N HCl, 105 °C, 12 h), we hydrogenated kulomo'opunalides-1 and -2 (**5** and **6**; 0.3 mg each) on Pd−C prior to acid hydrolysis (5 N HCl, 105 °C, 12 h). Then we could see the signal of 3-hydroxy-2-methyloctanoic acid (Hmoaa; **14**) in the ^{1}H NMR spectrum as was the case of Dhoaa (**10**) in **1** and **4**. The EtOAc extract of the hydrolysate was dried under N_2 and treated with diazomethane to furnish the methyl ester of **14**. Each methyl ester derived from **5** and **6** was further reacted with *p*-bromobenzoyl chloride and separated by ODS HPLC to yield a *p*-bromobenzoate, which was analyzed by ^{1}H NMR and chiral HPLC.

Authentic *p*-bromobenzoyl Hmoaa methyl esters (**14a**−**d**) were prepared from the synthetic Hmoya methyl esters,[19] after hydrogenation on Pd−C. The diastereomers of 2*S**,3*S** (**14a**,**d**) and of 2*S**,3*R** (**14b**,**c**) could be distinguished by chemical shifts of α-methine (2*S**,3*S**, 2.89 ppm; 2*S**,3*R**, 2.79 ppm) and *β*-methine protons (2*S**,3*S**, 5.31 ppm; 2*S**,3*R**, 5.38 ppm) in the ^{1}H NMR spectra. The ^{1}H NMR spectra of both prepared *p*-bromobenzoates from **5** and **6** showed signals of α- and *β*-methine protons at 5.39 ppm (**5**; 5.38 ppm for **6**) and 2.80 ppm (**5**; 2.79 ppm for **6**), respectively, which indicated that the relative stereochemistry of **9** is 2*S**,3*R**in both **5** and **6**. In the chiral HPLC analysis, the authentic four samples gave peaks at different retention times [**14a** (2*S*,3*S*), 6.0 min; **14b** (2*S*,3*R*), 7.1 min; **14c** (2*R*,3*S*), 6.3 min; **14d** (2*R*,3*R*), 7.6 min]. Comparison of retention time and coinjection confirmed the absolute stereochemistry of the Hmoya units in **5** and **6** as 2*S*,3*R*.



14
14a: 2*S*3*S*
14b: 2*S*3*R*
14c: 2*R*3*S*
14d: 2*R*3*R*

The absolute stereochemistry of Hmpa (**12**) in **5** and Hmba (**13**) in **6** was determined as 2*S*,3*S* for **12** and 2*S* for **13** by chiral HPLC analysis.

(17) The structures of **5** and **6** were supported by the FAB MS/MS analyses. In the FAB MS/MS of **5**, we could follow three major sequential fragments at *m*/*z* 617 (−Val), 466 (−Hmoya), 339 (−MeIle), and 242 (−Pro); 590 (−MeIle), 476 (−Hmpa), and 379 (−Pro); and 603 (−Hmpa), 476 (−MeIle), 377 (−Val), and 255 (−Hmoya). In case of **6**, the sequential fragments were also observed at *m*/*z* 603 (−Val), 452 (−Hmoya), 325 (−MeIle), and 228 (−Pro); 576 (−MeIle) and 476 (−Hmba); and 603 (−Hmba) and 476 (−MeIle).

(18) Fernández, R.; Rodríguez, J.; Quiñoá, E.; Riguera, R.; Muñoz, L.; Fernández-Suárez, M.; Debitus, C. *J. Am. Chem. Soc.* **1996**, *118*, 11635−11643.

(19) We are very grateful to Professor Ricardo Riguera of Universidad de Santiago deCompostela for generously providing us the synthetic samples.

Table 2. NMR Data for Kulomo′opunalide 1 (5) and the Major Conformer of 2 (6) in CD_3CN

		δ					δ	
	no.	C	H	HMBC	no.	C	H	HMBC
Hmoa	1	172.6			1	172.5		
	2	42.2	2.80 m	C: 1, 3, 4, 9	2	42.2	2.81 m	C: 1, 3, 4, 9
	3	77.7	4.79 m	C: 33	3	77.9	4.77 m	C: 1, 4, 5
	4a	30.3	2.07 m		4a	30.2	2.12 m	C: 3, 6
	4b		1.66 m		4b		1.64 m	C: 2, 3, 6
	5a	26.1	1.64 m	C: 7	5a	26.2	1.65 m	C: 4, 6, 7
	5b		1.50 m	C: 7	5b		1.47 m	C: 4, 6, 7
	6	18.7	2.2 m	C: 4, 5, 7, 8	6	18.8	2.22 m	C: 4, 5, 7, 8
	7	85.0			7	85.0		
	8	70.1	1.95 m		8	70.1	1.95 m	
	9	11.9	1.16 d, 7.2	C:1, 2, 3	9	12.4	1.17 d, 7.2	C:1, 2, 3
Val	10	173.6			10	173.6		
	11	53.9	4.68 dd, 3.4, 8.6	C: 1, 10, 12, 13, 14	11	53.8	4.71 dd, 3.3, 8.7	C: 1, 10, 12, 13, 14
	12	32.3	1.96 m		12	32.4	1.96 m	
	13	16.5	0.76 d, 6.9	C: 11, 12, 14	13	16.5	0.76 d, 6.8	C: 11, 12, 14
	14	20.6	0.88 d, 6.7	C: 11, 12, 13	14	20.6	0.87 d, 6.6	C: 11, 12, 13
	NH-1		6.42 d, 8.6	C: 1	NH-1		6.44 d, 8.7	C: 1
Me-Ile-1	15	170.7			15	170.7		
	16	66.6	3.93 d, 10.5	C: 10, 15, 17, 18, 20, 21	16	66.7	3.93 d, 10.5	C: 10, 15, 17, 18, 20, 21
	17	35.1	2.00 m		17	35.1	2.03 m	
	18a	26.5	1.50 m	C: 19	18a	26.6	1.51 m	C: 17, 19
	18b		1.02 m	C: 19	18b		1.02 m	C: 19
	19	11.6	0.94 t, 6.9	C: 17, 18	19	11.6	0.94 t, 6.6	C: 17, 18
	20	15.8	0.96 d, 6.3	C: 16, 17, 18	20	15.8	0.96 d, 6.8	C: 16, 17, 18
	21	29.7	2.93 s	C: 10, 16	21	29.8	2.95 s	C: 10, 16
Hmpa	22	167.1			22	166.9		
	23	77.6	4.85 d, 6.9	C: 15, 24, 25, 27	23	78.2	4.84 d, 6.2	C: 15, 24, 25, 26
	24	36.7	1.90 m		24	30.1	2.12 m	C: 22, 23, 25, 26
	25a	25.1	1.65 m		25	18.8	1.02 d, 6.8	C: 23, 24, 26
	25b		1.19 m		26	18.0	0.94 d, 6.8	C: 23, 24, 25
	26	11.4	0.87 t, 7.5	C: 24, 25	Pro	27	173.2	
	27	14.8	1.01 d, 6.3	C: 23, 24, 25		28	58.1	4.54 m	C: 27, 29, 30, 31
Pro	28	173.1				29a	30.1	2.21 m	C: 27, 30, 31
	29	58.2	4.54 dd, 6.1, 8.2	C: 28, 30, 31, 32		29b		1.67 m	C: 27, 28, 30, 31
	30a	30.1	2.20 m	C: 28, 31, 32		30a	26.1	1.98 m	C: 28, 29, 31
	30b		1.69 m	C: 28, 29, 31, 32		30b		1.91 m	C: 28, 29, 31
	31a	26.0	1.96 m	C: 29, 30, 32		31a	48.0	3.76 m	C: 28, 29, 30
	31b		1.89 m	C: 29, 30, 32		31b		3.49 m	C: 28, 29, 30
	32a	48.1	3.78 ddd, 9.5, 7.2, 5.5	C: 29, 30, 31	Me-Ile-2	32	172.1		
	32b		3.50 dt, 9.5, 7.0	C: 30, 31		33	64.4	4.02 d, 9.9	C: 27, 32, 34, 35, 37, 38
Me-Ile-2	33	172.2				34	36.6	1.97 m	
	34	64.4	4.02 d, 9.7	C: 28, 33, 35, 36, 38, 39		35a	27.1	1.42 m	C: 36
	35	36.6	1.98 m			35b		1.03 m	C: 36
	36a	27.2	1.40 m	C: 35, 37, 38		36	11.9	0.92 t, 7.2	C: 34, 35
	36b		1.03 m	C: 37, 38		37	16.2	0.96 d, 6.8	C: 33, 34, 35
	37	11.6	0.92 t, 6.9	C: 35, 36		38	29.6	2.89 s	C: 27, 33
	38	16.3	0.97 d, 6.3	C: 34, 35, 36					
	39	29.6	2.89 s	C: 28, 34					

Table 3. Constituents of the *Philinopsis* Peptides

	fatty acids	amino acids
kulolide-1 (**1**)	phenyllactic acid 2,2-dimethyl-3-hydroxyoctynoic acid (Dhoya)	Ala, Pro, 2 Val,*N*-MeVal
kulolide-2 (**2**)	phenyllactic acid (Plac) 2,2-dimethyl-3-hydroxyoctenoic acid (Dhoea)	Ala, Pro, 2 Val, *N*-MeVal
kulolide-3 (**3**)	phenyllactic acid (Plac) 2,2-dimethyl-3-hydroxyoctanoic acid (Dhoaa)	Ala, Pro, 2 Val, *N*-MeVal
kulokainalide-1 (**4**)	lactic acid (Lac) 2,2-dimethyl-3-hydroxyoctynoic acid (Dhoya)	2 Pro, 2Val, *N*-MeVal, Phe
kulomo'opunalide-1 (**5**)	2-hydroxy-3- methylpentanoic acid (Hmpa) 3-hydroxy-2- methyloctynoic acid (Hmoya)	Pro, Val, 2 *N*-MeIle
kulomo'opunalide-2 (**6**)	2-hydroxy-3- methylbutanoic acid (Hmba) 3-hydroxy-2- methyloctynoic acid (Hmoya)	Pro, Val, 2 *N*-MeIle
pupukeamide (**15**)	butanoic acid	Val, *N*-MeVal, *N*-MeIle, *N*,*O*-dimethyl-Tyr

Aqueous layers obtained from **5** and **6** were freeze-dried and derivatized with Marfey's reagent. HPLC analyses of both samples indicated L-Val, L-Pro, and L-MeIle stereochemistry. The composition of all *Philinopsis* peptides is summarized in Table 3.

Discovery of the remarkable macrolide constituent tolytoxin 23-acetate (**7**) resulted from bioassay experiments. Kulolide-1 is cytotoxic and also causes morphological change in rat fibroblast cells at a concentration of 50 μM.[9] Similar activity was observed with other kulolides; the strongest activity was seen in kulokainalide-1 (**4**) at a concentration of 5 μM.[20] The mode of action was reminiscent of tolytoxin, which was known from a blue-green alga.[21] Tolytoxin is a potent actin-

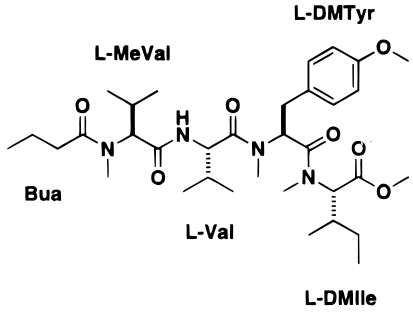
depolarizing agent and can cause morphological change at a concentration of 2−16 nM. This raised the question that kulokainalide-1 (**4**) might be contaminated with tolytoxin. It is potent enough to cause the same activity seen in kulokainalide-1 with only 0.3% contamination of tolytoxin. Repurification seemed advisable. Kulokainalide-1 was subjected to repetitive ODS HPLC analysis and submitted for bioassay. Indeed, the activity dropped and most certainly had been caused by about 0.3% contamination with tolytoxin. Kulolide-1 (**1**) caused the morphological changes in rat fibroblast cells at a concentration of 50 μM. Less than 0.1% contamination with tolytoxin might account for this activity. [Peptides **1**−**6** showed only moderate cytotoxicity against P388 cells, which made us suspect the possibility of small amounts of contaminating tolytoxins accounting for the cytotoxicity.]

Tolytoxin 23-acetate (**7**) was isolated from the fraction closest to that containing kulolide-1 and kulokainalide-1. FABMS analysis (matrix; NBA + NaCl) showed a peak at *m*/*z* 914 $(M + Na)^+$, which corresponds to that of monoacetyltolytoxin. Detailed analysis of 2D NMR data and comparison of ^{1}H and ^{13}C NMR data with those of tolytoxin indicated that the 23-hydroxy group in tolytoxin was acetylated in **7** [1.6 ppm downfield shift of 23-methine proton in **7** and an HMBC correlation from this proton to the acetyl carbonyl carbon (171.5 ppm)].

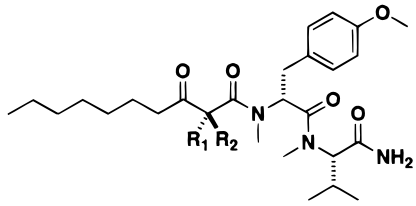
We tested the enzyme−inhibitory activity of these peptides in three inhibition assays (thrombin, trypsin, and Leu aminopeptidase), in which tolytoxin 23-acetate is inactive, but we found no activity.

The unexpected diversity of *Philinopsis* metabolites, polypropionates, an alkylpyridine, cyclodepsipeptides, a linear peptide, and a macrolide, prompted an examination of the animal's prey. Cimino et al. reported isolation of polypropionate constituents from a Mediterranean mollusk, *Aglaja depicta*, belonging to the Aglajidae family (as does *Philinopsis speciosa*) and from its prey, *Bulla striata*, an herbivorous mollusk, reported to feed on green algae.[22] Cimino and co-workers subsequently discovered an alkylpyridine in the related predator−prey pair of mollusks *Navanax inermis* and *Bulla gouldiana*.[23] We have isolated an alkylpyridine[8] and polypropionates[7] from *P. speciosa*, and during our 1994/1995 collection, we did encounter hundreds of *Bulla* sp. in the tidepool, although the animals are supposed to be abundant between January and April,[24] when Pupukea is inaccessible because of high surf. However, the chemical constituents of *Bulla* sp. remain to be investigated.

Searching our collection site for possible prey animals, we also encountered the herbivorous sea hares *Stylocheilus longicaudus* and *Dolabella auricularia*. We initially ignored these animals, since both had been resarched extensively. From *Stylocheilus* we had isolated the aplysiatoxins[25] and Pettit and co-workers have been isolating a host of dolastatins from *D. auricularia*.[26] It was only after we had isolated pupukeamide (**15**) from *P. speciosa*[27] and were struck by its similarity to majusculamide A (**16**) and B (**17**) that details of the intricate food chain revealed themselves: blue-green algae were the missing link! The majusculamides (**16**, **17**) are produced by *Lyngbya majuscula*,[28] which also is the source of the aplysiatoxins.[29] Even the macrolide tolytoxin 23-acetate (**7**) is a blue-green algal metabolite.[21] While no kulolides have so far been isolated from blue-green algae, the presence of tell-tale *N*-Me amino acids provide strong inference that blue-green algae provide the biosynthetic link.



pupukeamide (15)



	R1	R2
majusculamide A (16)	Me	H
majusculamide B (17)	H	Me

To support the suspected predator-prey relationship between *P. speciosa* and the sea hares *S. longicaudus* and *D. auricularia*, we carried out feeding experiments in the laboratory[30] and saw that *P. speciosa* readily accepted *S. longicaudus* and small *D. auricularia* as well as *Bulla* and *Dolabrifera* spp. Interestingly, we could collect small *D. auricularia* only outside the tidepool habitat of *P. speciosa*. We then reinvestigated *S. longicaudus* and succeeded in isolating kulolide-1 from this animal. The lower yield of kulolide-1 from *S. longicaudus* (4.8×10^{-4} % yield based on wet weight) than that from *P. speciosa* (1.4×10^{-3} % yield based on wet weight) suggests that *P. speciosa* derives kulolide-1 from its prey and accumulates it.

Experimental Section

General Procedures. IR spectra were measured in $CHCl_3$ using NaCl cells. UV spectra were measured with a diode

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array spectrophotometer. Optical rotations were measured on a digital spectropolarimeter. NMR spectra were recorded at 500.115 MHz for ^{1}H and 125.766 MHz for ^{13}C. ^{1}H NMR data are reported in the following format: δ value [multiplicity, J value in hertz (when applicable)]. Glycerol and thioglycerol were used as matrix for FAB-MS measurements.

Isolation. *P. speciosa* (300 animals, 9.0 kg wet weight) collected on midsummer nights in 1994 at Shark's Cove, Pupukea, O'ahu, HI, were extracted with EtOH (3 × 3L) and $CHCl_3$/MeOH (1:1, 3 L). The combined extracts were concentrated and extracted with $CHCl_3$. The aqueous layer was further extracted with *n*-BuOH, and the *n*-BuOH extract was combined with the $CHCl_3$ layer. The combined organic layers were evaporated to dryness and separated by the modified Kupchan procedure[31] to yield hexane, CH_2Cl_2, and aqueous MeOH extracts. The CH_2Cl_2 extract was evaporated to dryness and submitted to two-step ODS flash chromatography (first with aqueous MeOH as solvent, second with aqueous MeCN), followed by gel filtration (Sephadex LH-20, MeOH) and amino short column chromatography [i.d. 1.5 × 3.5 cm, $CHCl_3$, $CHCl_3$/MeOH (9:1), $CHCl_3$/MeOH/H_2O (7:3:0.5), and MeOH]. The $CHCl_3$/MeOH (9:1) fraction was separated by ODS HPLC [COSMOSIL 5C_{18}-AR, MeCN/H_2O (7:3)] giving nine fractions (fractions 1−9). Further separation of the fraction 3 by ODS HPLC [COSMOSIL 5C_{18}-AR, MeCN/H_2O (6:4)] yielded 8.0 mg of kulokainalide-1 (**4**, 8.9×10^{-5} % yield based on wet weight) and 128.0 mg of kulolide-1 (**1**, 1.4×10^{-3} % yield based on wet weight). Fraction 4 was separated by two-step ODS HPLC [COSMOSIL 5C_{18}-AR, 2-PrOH/H_2O (1:1), MeCN/H_2O (65:35)] yielding kulomo'opunalide-2 (**6**, 4.2 mg, 4.7×10^{-5} % yield based on wet weight). Fraction 6 was also separated by the same scheme [COSMOSIL 5C_{18}-AR, 2-PrOH/H_2O (1:1), MeCN/H_2O (65:35)] yielding kulomo'opunalide-1 (**5**; 4.1 mg; 4.6×10^{-5} % yield based on wet weight) and kulolide-2 (**2**; 5.0 mg; 5.6×10^{-5} % yield based on wet weight). Kulolide-3 (**3**, 2.4 mg, 2.7×10^{-5} % yield based on wet weight) was isolated from fraction 8 via three-step ODS HPLC [COSMOSIL 5C_{18}-AR, 2-PrOH/H_2O (1:1), MeCN/H_2O (7:3) and COSMOSIL 5C_{18}-MS, MeCN/H_2O (7:3)]

Kulolide-2 (2): colorless amorphous solid; $[\alpha]^{31}_D$ −59° (*c* 1.0, MeOH); IR 1720, 1640 cm^{-1}; UV (MeOH) 206 nm (4300), 270 (210); FABMS *m*/*z* 796 $(M + H)^+$; ^{1}H NMR (CD_3CN) of conformer 1**,** 5.46 (dd, 10.3, 2.1, H3), 1.65 (m, H4a), 1.51 (m, H4b), 1.33 (m, H5), 2.13 (m, H6a), 2.05 (m, H6b), 5.84 (m, H7), 5.03 (m, 17.1, H8a), 4.97 (m, 10.3, H8b), 1.12 (s, H9), 1.25 (s, H10), 4.86 (dd, 6.9, 8.5; H12), 2.02 (m, H13), 0.94 (d, 6.7, H14), 0.87 (d, 6.7, H15), 6.38 (d, 8.5, NH1), 4.39 (d, 10.3, H17), 2.27 (m, H18), 1.26 (d, 6.9, H19), 0.98 (d, 6.7, H20), 2.91 (s, H21), 5.46 (dd, 10.0, 5.1, H23), 3.55 (dd, 5.1, 13.0, H24a), 3.13 (dd, 10.0, 13.0, H24b), 7.38 (m, H26, 30), 7.31 (m, H27, 29), 7.29 (m, H28), 3.46 (m, H32), 1.86 (dd, 5.9, 12.1, H33a), 1.11 (m, H33b), 1.70 (m, H34a), 1.44 (m, H34b), 3.50 (m, H35a), 3.35 (ddd, 11.8, 9.5, 2.3, H35b), 3.70 (dd, 7.4, 7.4, H37), 2.10 (m, H38), 0.90 (d, 6.9, H39), 0.91 (d, 6.9, H40), 7.08 (d, 7.4, NH4), 4.38 (qd, 7.2, 7.4, H42), 1.16 (d, 7.2, H43), 6.66 (d, 7.4, NH5); ^{1}H NMR (CD_3CN) of conformer 2, 5.31 (dd, 10.8, 2.6, H3), 1.69 (m, H4a), 1.54 (m, H4b), 1.34 (m, H5), 2.15 (m, H6a), 2.07 (m, H6b), 5.84 (m, H7), 5.03 (m, 17.1, H8a), 4.97 (m, 10.3, H8b), 1.12 (s, H9), 1.31 (s, H10), 4.57 (dd, 2.8, 7.7, H12), 2.16 (m, H13), 1.03 (d, 6.9, H14), 0.84 (d, 6.9, H15), 5.88 (d, 7.7, NH1), 3.25 (d, 9.0, H17), 2.40 (m, H18), 0.85 (d, 6.9, H19), 1.02 (d, 6.7, H20), 3.28 (s, H21), 5.38 (dd, 4.9, 10.3, H23), 3.31 (dd, 4.9, 12.6, H24a), 2.99 (dd, 10.3, 12.6, H24b), 7.34 (m, H26, 30), 7.35 (m, H27, 29), 7.27 (m, H28), 3.45 (m, H32), 1.93 (m, H33a), 0.96 (m, H33b), 1.70 (m, H34a), 1.44 (m, H34b), 3.45 (m, H35a), 3.28 (m, H35b), 3.53 (dd, 7.0, 10.3, H37), 2.18 (m, H38), 0.91 (d, 6.9, H39), 0.94 (d, 6.7, H40), 7.98 (d, 7.0, NH4), 4.54 (qd, 7.1, 8.2, H42), 1.50 (d, 7.1, H43), 6.35 (d, 8.2, NH5); ^{13}C NMR of (CD_3CN) conformer 1, 176.2 (C1), 47.3 (C2), 80.4 (C3), 28.4 (C4), 26.3 (C5), 34.0 (C6), 139.6 (C7), 115.6 (C8), 25.2 (C9), 17.1 (C10), 173.6 (C11), 54.3 (C12), 32.9 (C13), 20.2 (C14), 17.9 (C15), 174.3 (C16), 65.5 (C17), 30.8 (C18), 19.6 (C19), 20.7 (C20), 30.2 (C21), 170.0 (C22), 75.7 (C23), 38.6 (C24), 136.0 (C25), 131.0 (C26, 30), 129.7 (C27, 29), 128.5 (C28), 171.4 (C31), 61.7 (C32), 31.6 (C33), 22.3 (C34), 47.5 (C35), 62.8 (C37), 29.9 (C38), 19.6 (C39), 19.6 (C40), 173.2 (C41), 48.3 (C42), 18.8 (C43); ^{13}C NMR of (CD_3CN) conformer 2, 176.7 (C1), 46.8 (C2), 79.0 (C3), 29.5 (C4), 26.4 (C5), 34.1 (C6), 139.6 (C7), 115.6 (C8), 24.2 (C9), 18.6 (C10), 172.5 (C11), 55.6 (C12), 29.6 (C13), 20.6 (C14), 16.2 (C15), 173.7 (C16), 70.2 (C17), 28.9 (C18), 19.3 (C19), 21.1 (C20), 40.5 (C21), 170.5 (C22), 74.8 (C23), 38.3 (C24), 135.9 (C25), 131.2 (C26, 30), 129.9 (C27, 29), 128.4 (C28), 171.5 (C31), 61.8 (C32), 31.0 (C33), 22.3 (C34), 47.0 (C35), 64.4 (C37), 29.7 (C38), 19.6 (C39), 20.2 (C40), 173.0 (C41), 48.7 (C42), 17.7 (C43).

Kulolide-3 (3): colorless amorphous solid; $[\alpha]^{31}_D$ −95.2° (*c* 1.11, MeOH); IR 1720, 1640 cm^{-1}; UV (MeOH) 208 nm (ϵ 20 400), 213 (17 300), 287 (700); FABMS *m*/*z* 798 $(M + H)^+$; ^{1}H NMR (CD_3CN) of conformer 1, 5.41 (dd, 2.4, 10.9, H3), 1.59 (m, H4a), 1.52 (m, H4b), 1.34 (m, H5a), 1.32 (m, H5b), 1.32 (m, H6), 1.35 (m, H7), 0.91 (t, 7.0, H8), 1.16 (s, H9), 1.26 (s, H10), 4.95 (dd, 6.3, 8.7, H12), 1.96 (m, H13), 0.96 (d, 6.8, H14), 0.89 (d, 6.8, H15), 6.31 (d, 8.7, NH1), 4.26 (d, 10.4, H17), 2.29 (m, H18), 1.00 (d, 6.5, H19), 1.30 (d, 6.5, H20), 2.95 (s, H21), 5.49 (dd, 4.8, 10.8, H23), 3.63 (dd, 12.5, 4.8, H24a), 3.18 (dd, 12.5, 10.8, H24b), 7.34 (m, H26, 30), 7.32 (m, H27, 29), 7.33 (m, H28), 3.39 (d, 7.7, H32), 1.96 (m, H33a), 0.93 (m, H33b), 1.72 (m, H34a), 1.47 (m, H34b), 3.53 (m, H35a), 3.34 (m, H35b), 3.81 (dd, 7.7, 7.7, H37), 2.11 (m, H38), 0.93 (d, 6.8, H39), 0.92 (d, 6.8, H40), 7.20 (d, 7.7, NH4), 4.37 (qd, 7.2, 7.0, H42), 1.25 (d, 7.2, H43), 6.58 (d, 7.0, NH5); ^{1}H NMR (CD_3CN) of conformer 2, 5.26 (dd, 10.2, 2.9, H3), 1.64 (m, H4a), 1.52 (m, H4b), 1.32 (m, H5a), 1.26 (m, H5b), 1.34 (m, H6), 1.35 (m, H7), 0.91 (t 7.0, H8), 1.16 (s, H9), 1.32 (s, H10), 4.68 (dd, 8.1, 2.4, H12), 2.13 (m, H13), 1.04 (d, 6.8, H14), 0.85 (d, 6.8, H15), 5.90 (d, 8.1, NH1), 3.19 (d, 9.2, H17), 2.46 (m, H18), 1.04 (d, 6.5, H19), 0.86 (d, 6.8, H20), 3.31 (s, H21), 5.46 (dd, 10.8, 5.3, H23), 3.33 (m, H24a), 3.02 (dd, 12.6, 10.8, H24b), 7.32 (m, H26, 30), 7.28 (m, H27, 29), 7.27 (m, H28), 3.49 (d, 7.5, H32), 2.02 (m, H33a), 0.85 (m, H33b), 1.66 (m, H34a), 1.47 (m, H34b), 3.48 (m, H35a), 3.31 (m, H35b), 3.64 (dd, 9.7, 7.2, H37), 2.17 (m, H38), 0.95 (d, 5.8, H39), 0.95 (d, 6.5, H40), 8.04 (d, 7.2, NH4), 4.61 (dq 8.1, 7.0, H42), 1.54 (d, 7.0, H43), 6.39 (d, 8.1, NH5); ^{13}C NMR (CD_3CN) of conformer 1, 175.5 (C1), 46.7 (C2), 80.5 (C3), 28.6 (C4), 26.1 (C5), 32.0 (C6), 22.8 (C7), 14.2 (C8), 25.1(C9), 17.0 (C10), 173.0 (C11), 53.3 (C12), 32.8 (C13), 20.3 (C14), 17.5 (C15), 173.6 (C16), 65.0 (C17), 30.2 (C18), 20.5 (C19), 19.5 (C20), 29.7 (C21), 169.5 (C22), 75.4 (C23), 38.3 (C24), 135.0 (C25), 130.4 (C26, 30), 129.4 (C27, 29), 128.0 (C28), 170.7 (C31), 61.2 (C32), 31.9 (C33), 21.9 (C34), 46.9 (C35), 62.1 (C37), 29.5 (C38), 19.4 (C39), 19.5 (C40), 172.7 (C41), 48.1 (C42), 18.5 (C43); ^{13}C NMR (CD_3CN) of conformer 2, 176.2 (C1), 46.2 (C2), 78.3 (C3), 29.9 (C4), 26.1 (C5), 31.9 (C6), 22.8 (C7), 14.2 (C8), 23.8 (C9), 18.8 (C10), 171.7 (C11), 54.6 (C12), 29.6 (C13), 20.6 (C14), 16.0 (C15), 172.6 (C16), 70.1 (C17), 28.3 (C18), 21.2 (C19), 19.2 (C20), 40.3 (C21), 170.1 (C22), 74.4 (C23), 38.0 (C24), 135.2 (C25), 130.6 (C26, 30), 129.1 (C27, 29), 127.8 (C28), 170.7 (C31), 61.3 (C32), 30.4 (C33), 21.9 (C34), 46.5 (C35), 63.7 (C37), 29.1 (C38), 19.5 (C39), 20.0 (C40), 172.2 (C41), 48.3 (C42), 17.4 (C43).

Hydrogenation of Kulolide-1 (1) and −2 (2). Kulolide-1 (**1**, 1.6 mg) and -2 (**2**, 1.4 mg) were hydrogenated on Pd−C in MeOH at room temperature overnight. The reaction mixture was filtered [washed with MeOH and $CHCl_3$/MeOH/H_2O (6:4:1)], evaporated, and separated by ODS HPLC [COSMOSIL 5C_{18}-AR, 2-PrOH/H_2O (1:1)] to give tetrahydrokulolide-1 (0.8 mg) and dihydrokulolide-2 (0.9 mg), respectively.

Tetrahydrokulolide-1: colorless amorphous solid; $[\alpha]^{31}_D$ −82° (*c* 0.411, MeOH).

Dihydrokulolide-2: colorless amorphous solid; $[\alpha]^{31}_D$ −120° (*c* 0.473, MeOH).

Kulokainalide-1 (4): colorless amorphous solid; $[\alpha]^{20}_D$ −56° (*c* 1.0, MeOH); IR cm^{-1}; UV (MeOH) 260 nm (sh); HR-FABMS $C_{48}H_{71}N_6O_{10}$ *m*/*z* 891.5177 (Δ −5.5 mmu); for ^{1}H and ^{13}C NMR data, see Table 1.

Hydrogenation and Acid Hydrolysis of Kulokainalide-1 (4). Hydrogenation of kulokainalide-1 (**4**, 1 mg) over

(31) Kupchan, S. M.; Britton, R. W.; Ziegler, M. F.; Sigel, C. W. *J. Org. Chem.* **1973**, *38*, 178−179.

Pd−C at room temperature was carried out prior to acid hydrolysis (5 N HCl, 105 °C, 12 h). The hydrolysate was extracted with EtOAc and the extract was dried under N_2. The dried organic extract was dissolved in MeOH and separated by ODS HPLC [YMC-ODS-AQ-323−5, MeCN/H_2O/TFA (50:50:0.05)] to yield 2,2-dimethyl-3-hydroxyoctanoic acid (0.2 mg).

(3*S*)-2,2-Dimethyl-3-hydroxyoctanoic acid: oil; $[\alpha]^{20}_D$ −30° (*c* 0.1, MeOH).

The aqueous layer of above hydrolysate was dried under N_2; part of it was subjected to Marfey analysis and HPLC analysis on a chiral column.

Stereochemistry of Lactic Acid. Part of the aqueous layer of the acid hydrolysate was submitted to analysis on a copper ligand exchange resin (Nucleosil Chiral-1 column, 1 mM $CuSO_4$) to prove the sterochemisty as L-lactic acid (6.3 min; D-Lac, 6.9 min).

Marfey Analysis of Amino Acids from 4. Part of the aqueous layer of the acid hydrolysate was added to 50 μL of 0.1% FDAA solution in acetone and 100 μL of 0.1 N $NaHCO_3$, followed by heating at 80 °C for 3 min. After cooling to room temperature the reaction mixture was neutralized with 50 μL of 0.2 N HCl and diluted with 100 μL of MeCN/H_2O/TFA (50:50:0.05). This solution was analyzed by ODS HPLC [COSMOSIL 5C_{18}-MS, MeCN/H_2O/TFA (40:60:0.05)] to prove the following stereochemistries: L-Val (9.3 min; D-Val, 13.8 min), *N*-Me-D-Val (16.5 min; *N*-Me-L-Val, 12.5), and L-Phe (14.7 min; D-Phe, 21.0 min). L-Pro (10.6 min; D-Pro, 19.7 min) was analyzed with the solvent 50 mM NH_4OAc in MeCN/H_2O (2:8).

Kulomo'opunalide-1 (5): colorless amorphous solid; $[\alpha]^{31}_D$ −63° (*c* 0.67, MeOH); IR 3290, 1720, 1630 cm^{-1}; UV (MeOH) 206 nm (14 700), 276 (300); HR-FABMS $C_{39}H_{65}N_4O_8$ *m/z* 717.4716 (Δ −8.6 mmu); for ^{1}H and ^{13}C NMR data, see Table. 1.

Kulomo'opunalide-2 (6): colorless amorphous solid; $[\alpha]^{31}_D$ −45° (*c* 1.36, MeOH); IR 3290, 1720, 1640 cm^{-1}; UV (MeOH) 208 nm (ϵ 14 100), 212 (12 900), 269 (150), 284 (130); HR-FABMS $C_{38}H_{63}N_4O_8$ *m/z* 703.4636 (Δ −1.0 mmu); for ^{1}H and ^{13}C NMR data, see Table. 1.

Acid Hydrolysis and Marfey Analysis of Kulomo'opunalide-1 (5) and -2 (6). Kulomo'opunalide-1 (**5**, 0.3 mg) and -2 (**6**, 0.3 mg) were hydrogenated on Pd−C and hydrolyzed (5 N HCl, 105 °C, 12 h). Each acid hydrolysate was extracted with EtOAc, and the aqueous layer was submitted to Marfey analysis. In Marfey analysis, both **5** and **6** gave peaks of l-Pro (6.2 min; D-Pro, 6.7 min), L-Val (8.6 min; D-Val, 12.5 min), and L-MeIle (18.5 min; D-MeIle, 26.8 min). The possibility of *N*-Me-*allo*-Ile was excluded by ODS HPLC analysis of acid hydrolysates [COSMOSIL 5C_{18}-MS, MeCN/H_2O/TFA (5:95:0.05)], in which **5** and **6** gave peaks of MeIle with retention time of 7.0 min (*allo*-Ile 6.5 min).

Stereochemistry of Hmoya (9) in 5 and 6. The dried organic layer of the acid hydrolysate was treated with diazomethane and evaporated, and to the residue was added 100 μL each of 0.1 M *p*-bromobenzoyl chloride and 0.1 M DMAP in CH_2Cl_2. After stirring overnight, the reaction mixture was partitioned between 1 M $NaHCO_3$ and CH_2Cl_2, and the organic layer was backwashed with 1 N HCl. The organic layer was evaporated and separated by ODS HPLC [COSMOSIL 5C_{18}-AR-II, MeCN/H_2O (8:2)] to give methyl 3-*p*-bromobenzoyloxy-2-methyloctanoate (**14**), which was analyzed by chiral HPLC [CHIRALPAK AD, hexane/EtOH (9:1)]. Standard samples (**14a**−**d**) were prepared from the synthesized Hmoya methyl esters. Each methyl ester was hydrogenated and derivatized with *p*-bromobenzoyl chloride following the same procedure as above. In the HPLC analysis, both samples prepared from **5** and **6** gave a peak at the retention time of **14b** (2*S*,3*R*, 7.1 min) [**14a** (2*S*,3*S*, 6.0 min), **14c** (2*R*,3*S*, 6.3 min), **14d** (2*R*,3*R*, 7.6 min)].

Stereochemistry of Hmpa (12) and Hmba (13). The relative stereochemistry of Hmpa (**12**) of **5** was determined to be 2*S**,3*S** by ODS HPLC analysis [COSMOSIL 5C_{18}-MS, MeCN/H_2O/TFA (10:90:0.05)], in which the organic layer of acid hydrolysate of **5** gave a peak at 18.4 min (2*S**,3*R**, 19.4 min). The absolute stereochemistry of **12** was determined as 2*S*,3*S* on the basis of chiral HPLC analysis (Nucleosil Chiral-1, 1 mM $CuSO_4$) in which the organic layer of acid hydrolysate of **5** gave a peak at 5.4 min (2*R*,3*S*, 8.9 min).

Hmba (**13**) was isolated from the aqueous layer of acid hydrolysate of **6** by ODS HPLC [COSMOSIL 5C_{18}-MS, MeCN/H_2O/TFA (5:95:0.05)]. The acid (**13**) was analyzed on a chiral column (Nucleosil Chiral-1, 1 mM $CuSO_4$) to prove its absolute stereochemistry as 2*S*.

Fraction 2 was separated by two-step ODS HPLC [COSMOSIL 5C_{18}-AR, MeCN/H_2O (1:1) and 2-PrOH/H_2O (1:1)] to furnish 2.5 mg of tolytoxin 23-acetate (**7**, 2.8 × 10^{-5} % yield based on wet weight).

Tolytoxin 23-acetate (7): colorless amorphous solid; $[\alpha]^{31}_D$ −45° (*c* 1.36, MeOH); UV (MeOH) 208 nm (ϵ 14 100), 212 (12 900), 269 (150), 284 (130); HR-FABMS $C_{38}H_{63}N_4O_8$ *m/z* 703.4636 (Δ −1.0 mmu); ^{1}H NMR (CD_3CN) of conformer 1, 5.80 ppm (d, 15.9, H2), 7.40 (d, 15.9, H3), 5.78 (H5), 4.29 (ddd, 2.5, 9.2, 9.0, H6), 3.34 (H7), 1.54 (H8a), 1.32 (ddd, 14.2, 10.4, 2.7, H8b), 4.34 (H9), 5.63 (H10), 5.78 (H11), 1.89 (H12), 3.35 (H13), 1.51 (H14), 3.70 (t, 5.2, H15), 3.55 (dd, 4.2, 10.2, H17), 1.80 (ddd, 14.4, 10.2, 4.2, H18a), 1.40 (ddd, 13.9, 9.2, 4.5, H18b), 3.11 (dd, 4.5, 9.9, H19), 1.92 (H20), 5.12 (dd, 10.2, 1.2, H21), 2.17 (qd, 7.0, 7.5, H22), 4.63 (dd, 5.2, 7.7, H23), 1.97 (H24), 1.67 (H25a), 1.23 (H25b), 2.52 (H26a), 2.43 (H26b), 2.72 (H28), 3.24 (H29), 2.42 (H30), 5.05 (dd, 14.2, 9.2, H31), 6.59 (d, 14.2, H32), 1.83 (s, Me-4), 3.51 (s, MeO-7), 3.35 (s, MeO-15), 2.64 (d, 4.7, epoxy methylene-16), 2.63 (d, 4.7, epoxy methylene-16), 3.18 (s, MeO-17), 3.12 (s, MeO-19), 0.80 (d, 7.2, Me-20), 0.97 (d, 7.0, Me-22), 1.93 (s, Ac-23), 0.85 (d, 7.0, Me-24), 0.88 (d, 7.0, Me-28), 3.26 (s, MeO-29), 1.10 (d, 7.0, Me-30), 2.94 (s, N-Me), 8.24 (s, formyl); in comformer 2 spectral differences were seen for resonances at 2.44 ppm (H30), 5.13 (dd, 9.2, 14.9, H31), 7.05 (d, 14.7, H32), 0.88 (d, 7.0, Me-30), 3.12 (s, N-Me); ^{13}C NMR (CD_3CN) of comformer 1, 166.8 ppm (C1), 119.1 (C2), 150.2 (C3), 136.5 (C4), 140.4 (C5), 72.0 (C6), 83.1 (C7), 36.6 (C8), 71.3 (C9), 131.0 (C10), 125.6 (C11), 31.8 (C12), 67.3 (C13), 36.8 (C14), 79.4 (C15), 61.7 (C16), 77.1 (C17), 29.8 (C18), 77.3 (C19), 38.9 (C20), 73.7 (C21), 35.7 (C22), 79.5 (C23), 34.5 (C24), 25.2 (C25), 41.7 (C26), 214.8 (C27), 49.8 (C28), 88.6 (C29), 38.1 (C30), 111.8 (C31), 130.3 (C32), 12.9 (Me-4), 60.3 (MeO-7), 57.9 (MeO-15), 47.3 (epoxy methylene-16), 55.2 (MeO-17), 57.5 (MeO-19), 9.7 (Me-20), 10.7 (Me-22), 21.3, 171.5 (Ac-23), 17.0 (Me-24), 13.8 (Me-28), 61.3 (MeO-29), 19.4 (Me-30), 27.5 (N-Me), 163.3 (formyl); for comformer 2 differences were seen for 49.7 (C28), 88.5 (C29), 38.3 (C30), 114.0 (C31), 125.4 (C32), 17.0 (Me-30), 33.5 (N-Me), 162.1 (formyl).

Isolation of Kulolide-1 from *Stylocheilus longicaudus*. The frozen specimens of *S. longicaudus* (221 animals, 503 g wet weight) was extracted with EtOH (500 mL × 2) and EtOH/CH_2Cl_2 (1:1, 500 mL × 2). The combined extract was evaporated and partitioned between H_2O and $CHCl_3$. The aqueous layer was further extracted with *n*-BuOH, and both organic layers were combined and evaporated. The combined organic layer was subjected to the modified Kupchan separation procedure and the resulting $CHCl_3$ layer was dried and separated by two-step ODS flash chromatography (first with aqueous MeOH and second with aqueous MeCN). The fraction eluting with MeCN/H_2O (8:2) from the column was gel filtered on Sephadex LH-20 with MeOH. The fraction containing peptides was passed through ODS and amino short column prior to the final purification by ODS HPLC [COSMOSIL 5C_{18}-AR, 60% MeCN] to yield 2.4 mg of kulolide-1 (4.8 × 10^{-4} % yield based on wet weight) which was identified by ^{1}H NMR and FABMS analysis.

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Supporting Information Available: 1D and 2D NMR spectra of **2**−**7** in CD_2Cl_2 or CD_3CN, FAB MS/MS of **5** and **6**, and schematics for stereochemistry determination and NMR spectral proof of the structures of three kulolides (37 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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