Kulolide: A Cytotoxic Depsipeptide from a Cephalaspidean Mollusk, *Philinopsis speciosa*[1]

Michael T. Reese, Nanda K. Gulavita, Yoichi Nakao, Mark T. Hamann, Wesley Y. Yoshida, Stephen J. Coval, and Paul J. Scheuer*

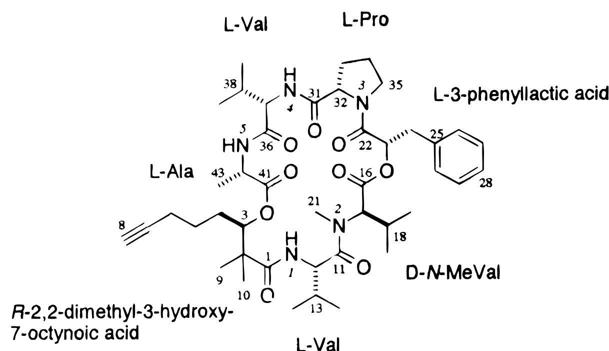
Contribution from the Department of Chemistry, University of Hawaii at Manoa, 2545 The Mall, Honolulu, Hawaii 96822-2275

Received June 17, 1996[⊗]

Abstract: Kulolide, a cyclic depsipeptide, was isolated from a cephalaspidean mollusk, *Philinopsis speciosa* Pease, 1860. Kulolide is made up of five amino acid residues, one each of L-Ala, L-Pro, and *N*-Me-D-Val and two of L-Val, and two carboxylic acids, L-3-phenyllactic acid and the unprecedented (*R*)-3-hydroxy-2,2-dimethyl-7-octynoic acid. The kulolide structure was elucidated by spectral techniques and hydrolytic reactions. Kulolide is active against L-1210 leukemia cells and P388 murine leukemia cells at IC_{50} values of 0.7 and 2.1 μg/mL, respectively. Kulolide caused morphological change against rat 3Y1 fibroblast cells at the concentration of 50 μM.

Small (<1000 Da) peptides, acyclic and cyclic, many of which display remarkable structural and biological diversity, have recently been isolated in impressive numbers from marine sponges[2] and ascidians[3] but only infrequently from mollusks. Notable exceptions are the dolastatins from an anaspidean (sea hare),[4] the kahalalides from a sacoglossan,[5,6] and the onchidins from a pulmonate (air-breathing) mollusk.[7] We now report the structure and bioactivity of kulolide,[8] a bidepsipentapeptide, which was isolated from a cephalaspidean (bubble shell), *Philinopsis speciosa*.

We first collected the animals some 15 years ago in the tidepools of Shark's Cove on the north shore of Oahu during incoming tides on midsummer nights, when the animals emerge from the sand to feed and mate. A hexane extract yielded as major constituents two unexceptional polypropionates, the niuhinones,[9,10] and an intriguing minor alkylpyridine, pulou-pone,[9,11] which is structurally related to navenone A,[12] one of the constituents of the alarm pheromone of *Navanax inermis*, a closely related cephalaspidean. When the freeze-dried animals were, instead of hexane, first extracted with methanol followed by methylene chloride and the combined extracts purified by chromatography, the isolate surprisingly was a peptide, kulolide (**1**), of composition $C_{43}H_{63}N_5O_9$.[13]



From 80 animals, weighing 150 g (dry) and collected at various times, we isolated 10 mg of kulolide (**1**) as a colorless amorphous solid after chromatography on an ODS column ($MeOH/H_2O$, 1:1; CH_2Cl_2/MeOH, 1:1) followed by Sephadex LH-20 (CH_2Cl_2/MeOH, 1:1) and eventually by HPLC on an ODS column (H_2O/MeCN, 35:65). The earliest ^1H NMR spectra measured at 300 MHz displayed doubling of the signals, which we attributed to an unsymmetrical dimer or to the existence of two conformers. This feature persisted in solvents ranging from DMSO-d_6 to toluene-d_8 and at temperatures ranging from −30 to 90 °C, with signal intensity varying in ratios between 1:1 and 2:1. This observation indicated that **1** was not an unsymmetrical dimer but was existing as a mixture of two conformers. Subsequent spectra at 500 MHz could be interpreted less ambiguously. Complete NMR spectral data of both conformers are listed in Table 1.

A molecular formula of $C_{43}H_{63}N_5O_9$ was determined by HR-FABMS. Hydrolysis of 1 mg of kulolide with 6 N HCl in a sealed tube followed by amino acid analysis revealed four amino acids—two units of valine, one alanine, and one proline. Analysis of 2D NMR spectra including COSY, HMQC,[14] and HMBC[15] allowed for the complete spectral assignment of these

⊗ Abstract published in *Advance ACS Abstracts,* October 15, 1996.

(1) In part from (a) the M.S. Thesis of M. T. Reese, University of Hawaii at Manoa, Honolulu, HI, 1991, and (b) the Ph.D. Dissertation of N. K. Gulavita, University of Hawaii at Manoa, Honolulu, HI, 1987.

(2) Fusetani, N.; Matsunaga, S. *Chem. Rev.* **1993**, *93*, 1793−1806.

(3) Davidson, B. S. *Chem. Rev.* **1993**, *93*, 1771−1791.

(4) Pettit, G. R.; Kamano, Y.; Herald, C. L.; Dufresne, C.; Bates, R. B.; Cerney, R. L.; Schmidt, G. M.; Haruhisa, K. *J. Org. Chem.* **1990**, *55*, 2989−2990 and earlier papers cited.

(5) Hamann, M. T. Ph.D. Dissertation, University of Hawaii at Manoa, Honolulu, HI, 1992.

(6) Hamann, M. T.; Scheuer, P. J. *J. Am. Chem. Soc.* **1993**, *115*, 5825−5826.

(7) (a) Rodriguez, J.; Fernandez, R.; Quinoa, E.; Riguera, R.; Debitus, C.; Borchet, P. *Tetrahedron Lett.* **1994**, *35*, 9239−9242. (b) Fernandez, R.; Rodriguez, J.; Quinoa, E.; Riguera, R.; Debitus, C. Symposium Proceedings. Eighth International Symposium On Marine Natural Products, Tenerife, Canary Islands, Spain, 1995.

(8) Kulo (Hawaiian) meaning "long waiting", in recognition of the time lapse between our first collection of *P. speciosa* and this report.

(9) Coval, S. J. Ph.D. Dissertation, University of Hawaii at Manoa, Honolulu, HI, 1985.

(10) Coval, S. J.; Schulte, G. R.; Matsumoto, G. K.; Roll, D. M.; Scheuer, P. J. *Tetrahedron Lett.* **1985**, *26*, 5359−5362.

(11) Coval, S. J.; Scheuer, P. *J. Org. Chem.* **1985**, *50*, 3025−3026.

(12) Sleeper, H. L.; Fenical, W. *J. Am. Chem. Soc.* **1977**, *99*, 2367−2368.

(13) Presence of a peptide was first observed by S. J. Coval; see footnote 82 in ref 1b.

(14) Summers, M. F.; Marzilli, L. G.; Bax, A. *J. Am. Chem. Soc.* **1986**, *108*, 4285−4294.

(15) Bax, A.; Azolos, A.; Dinya, Z.; Sudo, K. *J. Am. Chem. Soc.* **1986**, *108*, 8056−8063.

S0002-7863(96)02030-6 CCC: $12.00 © 1996 American Chemical Society

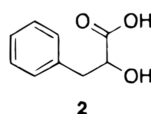
Table 1. Correlated ^{1}H and ^{13}C NMR Data for Both Conformers of Kulolide (**1**) in CD_2Cl_2

		conformer					
		1			2		
	no.	^{13}C NMR	^{1}H NMR	HMBC	^{13}C NMR	^{1}H NMR	HMBC
Dhoa	**1**	175.3			176.0		
	2	46.7			46.2		
	3	79.6	5.45 (d), 10.9	C: 1, 2, 5, 9, 10, 41	77.7	5.28 (dd), 2.5, 10.2	C: 1, 2, 5, 10, 41
	4a	27.4	1.83 (m)	C: 5	28.7	1.87 (m)	
	4b		1.62 (m)	C: 5		1.63 (m)	C: 3
	5a	25.0	1.55 (m)	C: 6	25.0	1.55 (m)	C: 6
	5b		1.46 (m)	C: 6		1.46 (m)	C: 6
	6	18.2[a]	2.28 (m)		18.1[a]	2.28 (m)	
	7	83.9[a]			83.8[a]		
	8	69.3	2.05 (t), 2.7	C: 7	69.3	2.04 (t), 2.7	C: 6, 7
	9	25.0	1.19 (s)	C: 1, 2, 3, 10	23.7	1.19 (s)	C: 1, 2, 3, 10
	10	16.9	1.28 (s)	C: 1, 2, 3, 9	18.6	1.33 (s)	C: 1, 2, 3, 9
Val-1	**11**	173.0			171.0		
	12	53.4	4.94 (dd), 6.5, 8.7	C: 1, 11, 13, 14, 15	54.6	4.67 (dd), 2.5, 7.9	C: 1, 11, 13, 14, 15
	13	32.7	1.97 (m)	C: 11, 12, 14, 15	29.7	2.13 (m)	C: 14, 15
	14	20.3	0.96 (d), 7.0	C: 12, 13, 15	20.6	1.04 (d), 6.5	C: 12, 13, 15
	15	17.5	0.89 (d), 6.7	C: 12, 13, 14	16.0	0.85 (d), 6.7	C: 12, 13, 14
	N-1		6.31 (d), 8.7	C: 1		5.89 (d), 7.9	C: 1
N-MeVal	**16**	173.5			172.5		
	17	65.0	4.28 (d), 10.4	C: 11, 16, 18, 19, 20, 21	70.0	3.19 (d), 8.9	C: 11, 16, 18, 19, 20, 21
	18	30.2	2.28 (m)	C: 16, 17, 19, 20	28.2	2.46 (m)	C: 16, 17, 19, 20
	19	20.6	1.01 (d), 6.5	C: 17, 18, 20	21.1	1.04 (d), 6.5	C: 17, 18, 20
	20	19.5	1.31 (d), 6.7	C: 17, 18, 19	19.5	0.86 (d), 6.9	C: 17, 18, 19
	21	29.6	2.95 (s)	C: 11, 17	40.3	3.31 (s)	C: 11, 17
Pla	**22**	169.4			170.0		
	23	75.3	5.48 (dd), 5.2, 10.7	C: 16, 24, 25	74.3	5.43 (dd), 5.2, 10.7	C: 16, 24, 25
	24a	38.3	3.62 (dd), 5.2, 12.9	C: 22, 23, 25, 26, 30	37.9	3.33 (dd), 5.2, 12.7	C: 22, 23, 25, 26, 30
	24b		3.18 (dd), 10.7, 12.9	C: 22, 23, 25, 26, 30		3.02 (dd), 10.7, 12.7	C: 22, 23, 25, 26, 30
	25	134.9			135.1		
	26	130.3	7.35 (m)	C: 24, 28, 30	130.5	7.32 (m)	C: 24, 28, 30
	27	129.3	7.37 (m)	C: 25, 29	129.1	7.35 (m)	C: 25, 29
	28	127.9	7.32 (m)	C: 26, 30	127.7	7.28 (m)	C: 26, 30
	29	129.3	7.37 (m)	C: 25, 27	129.1	7.35 (m)	C: 25, 27
	30	130.3	7.35 (m)	C: 24, 26, 28	130.5	7.32 (m)	C: 24, 26, 28
Pro	**31**	170.7			170.7		
	32	61.2	3.51 (d), 7.7	C: 22, 31, 33, 34, 35	61.1	3.43 (d), 7.4	C: 22, 31, 33, 34
	33a	30.4	2.03 (m)	C: 31, 34	30.9	1.97 (m)	C: 31, 35
	33b		0.85 (m)	C: 31, 34		0.94 (m)	C: 31, 32
	34a	21.9	1.69 (m)	C: 32	21.8	1.69 (m)	C: 32
	34b		1.47 (m)			1.47 (m)	
	35a	46.5	3.47 (m)	C: 22, 34	46.8	3.52 (m)	C: 22, 34
	35b		3.33 (m)	C: 22		3.35 (m)	C: 22
Val-2	**36**	170.5			171.8		
	37	62.0	3.81 (dd), 7.7, 7.5	C: 36, 38, 39, 40	63.6	3.64 (dd), 7.2, 10.2	C: 36, 39, 40
	38	29.4	2.10 (m)	C: 37, 39, 40	29.1	2.15 (m)	C: 36, 39, 40
	39	19.3	0.93 (d), 7.4	C: 37, 38, 40	19.9	0.95 (d), 7.0	C: 37, 38, 40
	40	19.4	0.92 (d), 7.0	C: 37, 38, 39	19.4	0.95 (d), 7.0	C: 37, 38, 39
	N-4		7.15 (d), 7.5	C: 31, 37, 38		8.01 (d), 7.2	C: 31, 37, 38
Ala	**41**	172.6			172.2		
	42	48.0	4.40 (dq), 7.0, 7.2	C: 41, 43	48.2	4.63 (dq), 8.2, 7.2	C: 41, 43
	43	18.5	1.24 (d), 7.2	C: 41, 42	17.3	1.54 (d), 7.2	C: 41, 42
	N-5		6.60 (d), 7.0	C: 36, 41, 42		6.40 (d), 8.2	C: 36, 42

[a] Interchangeable.

four amino acids as well as for the assignment of signals for *N*-methylvaline (*N*-MeVal) and two non-amino acid moieties.

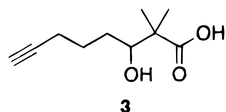
The first of the two non-amino acid units exhibited signals in the ^{1}H NMR spectrum reminiscent of those of phenylalanine. Resonances at 7.32−7.37 (H-26−H-30), 3.62 and 3.18 (H-24a and H-24b, respectively), and 5.48 (H-23) ppm are very similar to those reported for phenylalanine; however, the chemical shift of the α-carbon in this residue (C-23, 75.3 ppm) was that of an oxymethine, thus indicating that this residue is not phenylalanine but 3-phenyllactic acid (Pla, **2**).



2

The structure of the second non-amino acid fragment was deduced as follows. Geminal dimethyl protons (H_3-9 and H_3-10) showed HMBC correlations to a carbonyl carbon at 175.3 ppm (C-1), a quartenary carbon at 46.7 ppm (C-2), and an oxymethine carbon at 79.6 ppm (C-3). The COSY spectrum allowed for the connection of all protons from H-3 to H-6, the latter of which showed long range coupling to H-8 ($J = 2.7$ Hz). H-8 did not appear to show a crosspeak to a carbon in the HMQC spectrum, but instead, a one-bond CH satellite to C-8 with a large $^{1}J_{CH}$ coupling of 247 Hz was observed in the HMBC spectrum. Furthermore, a two-bond CH satellite from this proton to C-7 with $^{2}J_{CH} = 48.7$ Hz was also observed. These large CH couplings, together with carbon chemical shifts of C-7 (83.9 ppm) and C-8 (69.3 ppm), placed a triple bond between

these carbons and assigned the last fragment as 2,2-dimethyl-3-hydroxy-7-octynoic acid (Dhoa, **3**).



Sequencing of these residues was accomplished with the aid of an HMBC spectrum which displayed correlations from NH protons to neighboring carbonyl carbons between Val-1/Dhoa, Val-2/Pro, and Ala/Val-2. For residues lacking NH protons, correlations from α-protons to the carbonyl carbons of neighboring residues between *N*-MeVal/Val-1, Pla/*N*-MeVal, Pro/Pla, and Dhoa/Ala were observed, completing a 22-membered ring.

Stereochemistry of Kulolide

Acid hydrolysis and derivatization of **1** with Marfey's reagent[16] followed by HPLC analysis demonstrated L-stereochemistry of alanine and both valine residues and D-stereochemistry of *N*-MeVal. Proline and the carboxylic acids needed to be dealt with in a different manner. A GC−MS experiment of the Marfey adducts on a Chirasil-Val column confirmed the earlier results and also established the L-configuration for proline.

In order to determine the absolute stereochemistry of the two carboxylic acids, kulolide (**1**) was first hydrolyzed in acid. Phenyllactic acid was purified by HPLC on a ODS column and then analyzed on a Cu−ligand exchange resin establishing its L-stereochemistry. Determination of the absolute stereochemistry of the remaining chiral carbon (C-3) of the octynoic acid proved to be a lengthy process. While acid hydrolysis of kulolide (**1**) served well for the isolation of phenyllactic acid, the octynoic acid residue proved to be unstable during acid hydrolysis and also following its esterification with MTPA by the improved Mosher method.[17] Hydrogenation of kulolide prior to acid hydrolysis afforded chiral ($[\alpha]_D^{20}$ −38°) 3-hydroxy-2,2-dimethyloctanoic acid. However reaction with Mosher's reagent in pyridine once again yielded an unstable ester. The esterification was further attempted with (naphthylmethoxy)-acetic acid,[18] DCC, and DMAP in chloroform, resulting only in recovered starting material.

Recollections of *P. speciosa* during two summer seasons[19] eventually led to the conclusion of the research. All previous stereochemical results were confirmed, and the elusive chirality of C-3 was determined to be *R*. Treatment of kulolide (**1**) with NaOMe resulted in the isolation of fragment **4**. Subsequent treatment of **4** with Mosher's reagent showed unambiguously that C-3 has *R*-configuration (Figure 1).

Two related octynoic acids, 3-amino-2-methyl- and 3-hydroxy-2-methyl-7-octynoic acid, have been reported as constituents of the onchidins, cyclic peptides isolated from a pulmonate mollusk *Onchidium* sp.[7]

Kulolide (**1**) was found active against L-1210 leukemia cells and P388 murine leukemia cells at IC_{50}'s of 0.7 and 2.1 μg/mL, respectively; however, it did not show obvious toxicity against brine shrimp at a concentration of 1.0 ppm. Kulolide caused morphological change on rat 3Y1 fibroblast cells at a concentration of 50 μM and resulted in the formation of protuberances from the cell surface. This phenomenon is caused by actin polymerization inhibitors, such as bistheonellides,[20] swinholides,[21] mycalolides,[22] or latrunculins.[23] Further study on the actin-depolarizating activity of **1** is underway.

(16) Marfey, P. *Carlsberg Res. Commun.* **1984**, *49*, 591−596.
(17) Ohtani, I.; Kusumi, T.; Kashman, Y.; Kakisawa, H. *J. Am. Chem. Soc.* **1991**, *113*, 4092−4096.
(18) We are grateful to Professor T. Kusumi of Tokushima University for a generous gift of this new reagent.
(19) It has become evident over the years that the population of the mollusk varies greatly from year to year.

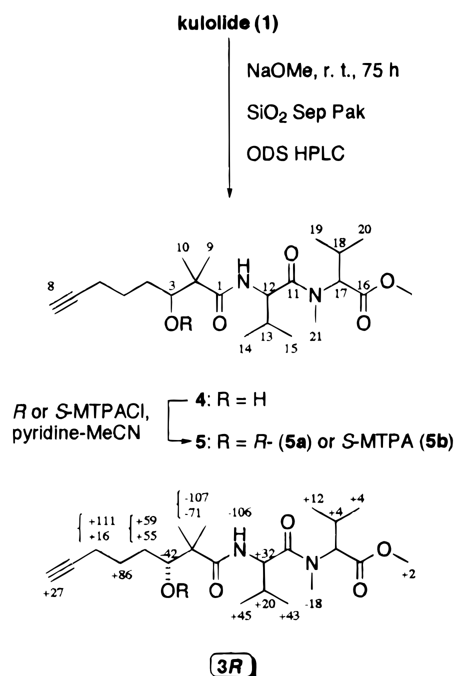


Figure 1. Preparation of **5a**,**b** and Δδ values (×10^{-3} ppm).

Experimental Section

Collection and Isolation. From 1981 on, *P. speciosa* Pease, 1860 (Aglajidae, Philinacea, Cephalaspidea, Opisthobranchia, Gastropoda) were collected on midsummer nights at Shark's Cove, Pupukea, Oahu. In 1984, 80 animals were collected, freeze-dried (150 g), and processed. Freeze-dried animals were extracted with MeOH; the residue was extracted with CH_2Cl_2, and the two extracts were combined and applied to an ODS column. Gradient elution [MeOH/H_2O (1:1), MeOH, MeOH/CH_2Cl_2 (1:1)] gave the peptide fraction in the MeOH/CH_2Cl_2 elute. Sephadex LH-20 (MeOH/CH_2Cl_2, 1:1) followed by ODS column chromatography (same solvent) and eventually ODS HPLC (H_2O/MeCN, 35:65) yielded the peptide (10 mg) as a colorless amorphous solid.

In 1994, a total of 490 animals were collected at the same site. A portion of these animals (300 animals, 9 kg wet weight) was extracted with EtOH (3 × 3 L) 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 layer was evaporated to dryness and separated by the modified Kupchan procedure[21] 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 a solvent, second with aqueous MeCN) followed by gel filtration (Sephadex LH-20, MeOH) and amino short column chromatography [ϕ 1.5 × 3.5 cm, $CHCl_3$, $CHCl_3$/MeOH (9:1), $CHCl_3$/MeOH/H_2O (7:3:0.5), MeOH]. Each fraction was monitored by ^1H NMR and TLC, and the fractions

(20) (a) Saito, S.; Watabe, S.; Ozaki, H.; Fusetani, N.; Karaki, H. *J. Muscle Res. Cell Motil.* **1995**, *16*, 340. (b) Watabe, S.; Wada, S.; Saito, S.; Matsunaga, S.; Fusetani, N.; Ozaki, H.; Karaki, H. *Cell Struct. Funct.* **1996**, *21*, 199−212.
(21) Bubb, M. R.; Spector, I.; Bershadsky, A. D.; Korn, E. D. *J. Biol. Chem.* **1995**, *270*, 3463−3466.
(22) Saito, S.; Watabe, S.; Ozaki, H.; Fusetani, N.; Karaki, H. *J. Biol. Chem.* **1994**, *269*, 29710−29714.
(23) (a) Spector, I.; Shochet, N. R.; Kashman, Y.; Groweiss, A. *Science* **1983**, *219*, 493−495. (b) Coue, M.; Brenner, S. L.; Spector, I.; Korn, E. D. *FEBS Lett.* **1987**, *213*, 316−318.
(24) Kupchan, S. M.; Britton, R. W.; Ziegler, M. F.; Sigel, C. W. *J. Org. Chem.* **1973**, *38*, 178−179.

containing kulolide were combined and purified by ODS HPLC [COSMOSIL 5C_{18}-AR, MeCN/H_2O (7:3)] to yield 128 mg of kulolide (**1**): colorless amorphous solid; $[\alpha]_D^{20}$ −102° (*c* 1.00, MeOH); IR (KBr) 3405 (br), 3021, 2969, 1732, 1655 (br), 1508, 1221 cm^{-1}; UV (MeOH) 215 nm (ϵ 16 000), 254 (460), 260 (450), 266 (380); HR-FABMS (matrix, thioglycerol) $C_{43}H_{64}N_5O_9$ *m/z* 794.4692 (Δ −1.2 mmu); for ^1H and ^{13}C NMR data see Table 1.

Amino Acid Analysis. Kulolide (1 mg) was hydrolyzed in constant boiling 6 N HCl at 110 °C in vacuo for 24 h and analyzed on a Beckman 121 MB amino acid analyzer. Detection at 440 and 570 nm yielded proline, alanine, and valine in a ratio of 1:1:2.

Marfey Analysis of Amino Acids. Kulolide (0.1 mg) was dissolved in 5 N HCl (0.5 mL) 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 was added 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 furnish L-Ala (6.1 min; D-Ala 7.5 min), L-Pro (6.5 min; D-Pro 6.9 min), L-Val (9.4 min; D-Val 14.3 min), and *N*-Me-D-Val (16.5 min; *N*-Me-L-Val 13.0 min).

Stereochemistry of Pla (2). Kulolide (4 mg) was hydrolyzed (5 N HCl, 105 °C, 12 h) and extracted with EtOAc, and the organic layer was dried under a stream of N_2. The dried EtOAc extract was dissolved in MeOH and separated on an ODS column [YMC-ODS-AQ-323-5, MeCN/H_2O/TFA (50:50:0.05)] to yield phenyllactic acid (**2**; 0.6 mg). Phenyllactic acid was analyzed on a copper−ligand exchange resin [Nucleosil Chiral-1 column, MeCN/H_2O (1:9) with 2 mM $CuSO_4$] proving L-phenyllactic acid (12.3 min; D-Pla 16.2 min).

Hydrogenation and Acid Hydrolysis of Kulolide (1). Acid hydrolysis of kulolide did not yield a sufficient amount of 2,2-dimethyl-3-hydroxyoctynoic acid. Therefore, hydrogenation of kulolide (**1**; 10 mg) over 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 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 (2.2 mg, $[\alpha]_D^{20}$ −38°) along with 3-phenyllactic acid (2.0 mg).

Esterification of 2,2-Dimethyl-3-hydroxyoctanoic Acid. 2,2-Dimethyl-3-hydroxyoctanoic acid (1 mg each) was reacted with both (*R*)- and (*S*)-MTPACl (15 μL each) in pyridine (50 μL). The ^1H NMR spectrum of the (*S*)-MTPA ester showed that of a single major component, but the spectrum of the (*R*)-MTPA ester was that of a mixture, either of conformers or of decomposed products. Attempts to purify this mixture by SiO_2 Sep Pak were not successful. It was evident from ^1H NMR spectra, that all fractions were decomposed fragments of octanoic acid. Presumably the (*R*)-MTPA ester of octanoic acid was not stable enough on silica gel. We next attempted esterification with (naphthylmethoxy)acetic acid (NMA)[18] after protection of the carboxyl group. An excess of diazomethane in ether was added to octanoic acid and dried under N_2, but the ^1H NMR spectrum showed no trace of a methyl ester. Therefore, NMA esterification of unprotected octanoic acid (1.0 mg) was added to the NMR tube [1.0 mg each of (*R*)- and (*S*)-NMA, DCC (2.0 mg), DMAP (0.3 mg) in $CDCl_3$ (0.5 mL)] which resulted only in recovered starting material.

Methanolysis of Kulolide. Since neither octynoic nor octanoic acid was suitable for chemical derivatization, a more stable fragment was required. Fortunately, when kulolide (10 mg) was treated with 0.5 N NaOMe for 75 h at room temperature, followed by SiO_2 Sep Pak [$CHCl_3$, $CHCl_3$/MeOH (9:1), $CHCl_3$/MeOH/H_2O (7:3:0.5)] and ODS HPLC [COSMOSIL 5C_{18}-AR, MeCN/H_2O (6:4)] purification, a stable fragment (**4**; 3.7 mg) resulted, which contained Dhoa.

MTPA Esters of 4. Half of **4** was reacted with (*R*)-MTPACl and half with (*S*)-MTPACl (23 mg each) in pyridine/MeCN (1:10, 550 mL) with DMAP (10 mg) followed by purification on an ODS column (COSMOSIL 5C_{18}-AR, 80% MeCN) to yield the corresponding esters **5a**,**b**.

5a: ^1H NMR (CD_3OH) δ 7.432 (NH), 5.487 (dd, 9, 2.5, H-3), 4.544 (dd, 8.5, 8.0, H-12), 3.669 (s, MeO), 3.101 (s, H-21), 2.193 (m, H-8), 2.190 (m, H-6a), 2.130 (m, H-18), 2.095 (m, H-6b), 2.062 (m, H-13), 1.649 (m, H-4a), 1.600 (m, H-4b), 1.406 (m, H_2-5), 1.154 (s, H-9), 1.127 (s, H-10), 0.976 (d, 6.5, H-19), 0.908 (d, 7.0, H-14), 0.885 (d, 6.5, H-15), 0.755 (d, 7.0, H-20).

5b: ^1H NMR (CD_3OH) δ 7.326 (d, 8.0, NH), 5.445 (dd, 9.5, 2.5, H-3), 4.576 (t, 8.5, H-12), 3.671 (s, MeO), 3.083 (s, H-21), 2.222 (m, H-8), 2.206 (m, H_2-6), 2.134 (m, H-18), 2.082 (m, H-13), 1.708 (m, H-4a), 1.655 (m, H-4b), 1.492 (m, H_2-5), 1.083 (s, H-9), 1.020 (s, H-10), 0.980 (d, 6.5, H-19), 0.951 (d, 6.5, H-14), 0.930 (d, 6.5, H-15), 0.767 (d, 6.5, H-20).

Acknowledgment. We thank Dr. W. P. Niemczura and M. W. Burger for spectral analyses and E. G. Juagdan for reisolation of kulolide, Professor E. A. Kay for taxonomic identification, and Professor K. T. Yasunobu for amino acid analysis. Members and friends of the research group who assisted with collections over many years include Rhone Akee, Bill and Jill Baker, Jay Corgiat, Elen Juagdan, Jaroslaw Jurek, Helen and Peter Karuso, Robin Kinnel, Gayle Matsumoto, Tony Poiner, A. D. "Ito" Rodriguez, Gary Schulte, Bryan Yeung, and Toshio Ichiba. Without their help, there would not have been a *Philinopsis* project. Financial support from the National Science Foundation, the Sea Grant College Program, Pharma Mar S.A., and the Japan Society for the Promotion of Science for a fellowship to Y.N. is gratefully acknowledged.

Supporting Information Available: ^1H and ^{13}C NMR, COSY, HMQC, and HMBC spectra of **1** in CD_2Cl_2 and ^1H NMR spectra of **5a**,**b** in CD_3OH (7 pages). See any current masthead page for ordering and Internet access instructions.

JA9620301