CHAPTER 4

RESULTS AND DISCUSSION

A collection of *Lyngbya majuscula* was obtained from Ratchamonkol Beach, Trung Province, Thailand, in April 2002. The organic extract (CH_2Cl_2/MeOH) of the alga was fractionated using silica gel liquid chromatography. Results from the icthyotoxicity assay led us to further fractionate a moderately polar fraction, using a combination of size exclusion Sephadex LH-20, RP-Sep-Pak, and RP-HPLC, to afford colorless oil of compounds A (**26**, 31.6 mg), B (**27**, 4.6 mg) and C (**28**, 4.4 mg). Herein we describe the physical properties and structure elucidation of depsipeptides **26**, **27** and **28**.

4.1 Physical Properties of Isolated Compounds

4.1.1 Compound A

colorless oil; $[\alpha]^{28}_{D}$ –68.45° (c = 1.68x10^{-3}, MeOH); UV (MeOH) λ_{max} 205 nm (ε 23 000); IR (neat) 3353, 2970, 2939, 1734, 1654, 1527, 1451, 1249, 1197 cm^{-1}; HRFABMS m/z $[M+1]^+$ 723.4329 (calcd for $C_{40}H_{59}N_4O_8$, -0.4 mmu dev); ^{1}H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$) see Table 4.1.

4.1.2 Compound B

colorless oil; $[\alpha]^{27}_{D}$ –34.37° (c = 0.32x10^{-3}, MeOH); UV (MeOH) λ_{max} 204 nm (ε 17 000); IR (neat) 3354, 2960, 2929, 2858, 1736, 1654, 1521, 1454 cm^{-1}; HRFABMS m/z $[M+1]^+$ 725.4442 (calcd for $C_{40}H_{61}N_4O_8$, -4.8 mmu dev); ^{1}H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$) see Table 4.2.

4.1.3 Compound C

colorless oil; $[\alpha]^{27}_{D}$ –46.66° (c = 0.30x10^{-3}, MeOH); UV (MeOH) λ_{max} 204 nm (ε 13 000); IR (neat) 3344, 2960, 2929, 2852, 1741, 1659, 1526, 1454, 1250, 1193

cm^{-1}; HRFABMS m/z $[M+1]^+$ 727.4548 (calcd for $C_{40}H_{63}N_4O_8$, -9.8 mmu dev); ^1H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$) see Table 4.2.

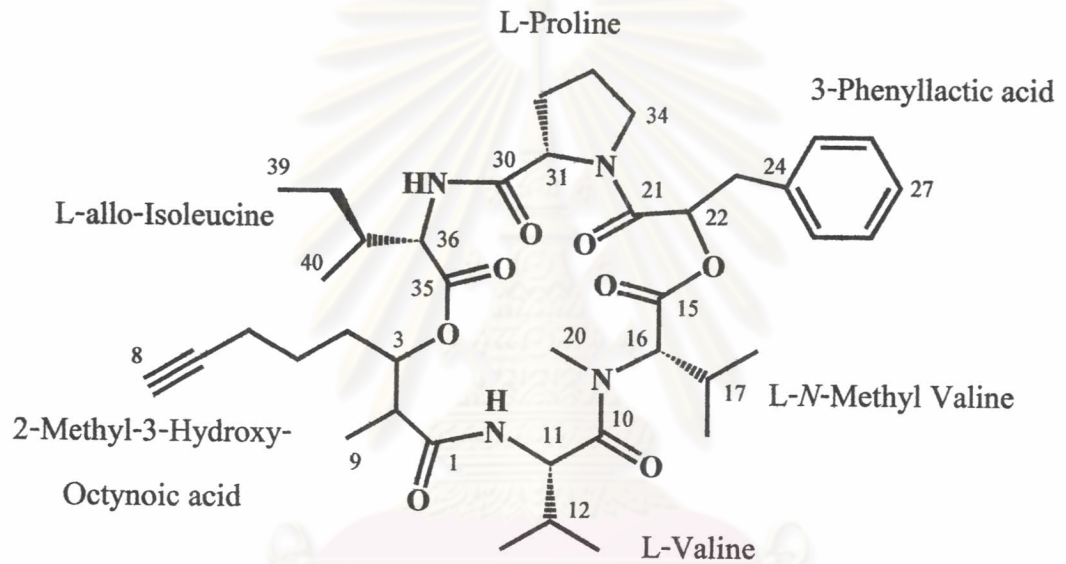
4.2 Structure Elucidation of Isolated Compounds

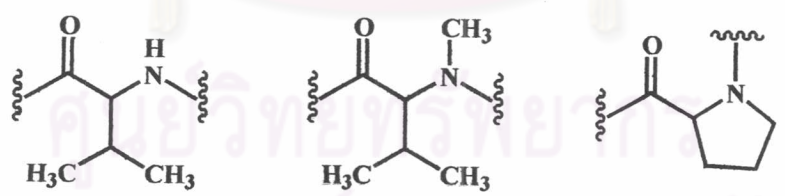
4.2.1 Compound A

High resolution FABMS of compound A (**26**) gave an $[M+1]^+$ peak of 723.4329 analyzed for molecular composition of $C_{40}H_{58}N_4O_8$ which required fourteen degrees of unsaturation. The IR spectrum of **26** displayed strong absorption bands at 1734 and 1654 cm^{-1}, indicating the presence of ester and amide functional groups, respectively. The ^{13}C NMR and DEPT spectra of **26** exhibited the presence of six ester/amide carbonyls and a monosubstituted phenyl ring (Table 4.1), accounting for ten of fourteen degrees of unsaturation.

Partial structures **26a-26d** (Figure 4.1) were deduced as amino acids valine (Val), N-methylvaline (N-Me-Val), proline (Pro), isoleucine (Ileu), based on 1D and 2D NMR spectral data. One of the last two partial structures (**26e**) revealed resonances at δ7.26-7.32 (H25-H29), 3.17 (H23a), 3.34 (H23b) and 5.07 (H22), which are very similar to those reported for phenylalanine. However, the HSQC spectrum of **26** indicated that H22 is attached to an oxymethine carbon (δ 74.79). Thus, residue **26e** was assigned to be 3-phenyllactic acid. For the final partial structure **26f**, the H2-H6/H9 spin system was constructed based on the TOCSY and COSY spectral data. The diagnostic signals in the ^{13}C spectrum at δ 69.17 (C8) for a methine carbon and at δ 83.66 (C7) for a quaternary carbon indicated the presence of a terminal alkyne functional group. The HMBC correlation showing from $H_3$9 (δ 1.17) to a carbonyl carbon at δ 172.68 (C1) and oxygenated methine carbon at δ 74.27 (C3), supportive to partial structure **26f** as 3-hydroxy-2-methyl-7-octynoic acid (Hmoya), and fulfilled two out of three remaining degrees of unsaturation. Thus, compound **26** is a monocyclic depsipeptide.

The partial structures of **26** were connected through interpretation of the HMBC spectral data. Correlation observed from the α-CH's to the neighboring carbonyl carbons were used to deduce the Hmoa/Val/N-MeVal/Pla and Pro/Ileu sequences. The correlation shown from H3 of Hmoa to the carbonyl carbon of Ileu (C35) linked the two sequences together. Three-bond coupling from H34b to the carbonyl carbon (C21) of Pla completed the planar structure of compound A (**26**). Acid hydrolysis and derivatization of **26** with Marfey's reagent[45] followed by HPLC analysis demonstrated the L- stereochemistry of alanine, N-Me-valine, proline, and allo-isoleucine residues.





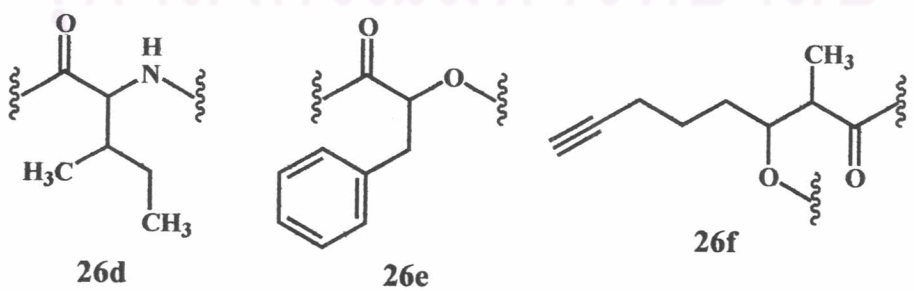


Figure 4.1 Partial Structures of Compound A (**26**)

Table 4.1 Correlated ^{1}H and ^{13}C NMR Data for Compound A (**26**) in $CDCl_3$

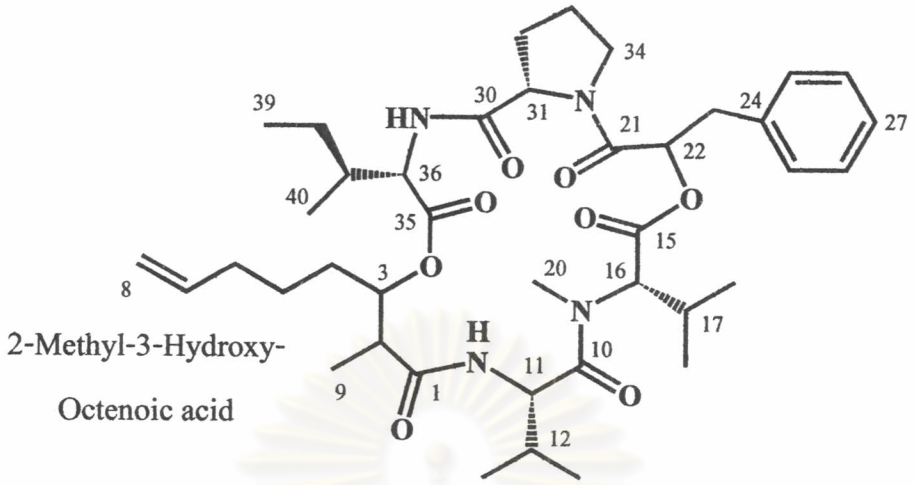
unit	C/H no.	δ_H (J in Hz)	δ_C	HMBC
Hmoa	1		172.68 (s)	
	2	2.45 m	43.19 (d)	C: 1, 3, 9
	3	4.98 dt (10.1, 3.5)	74.29 (d)	C: 1, 2, 5, 35
	4a	1.85 m	28.26 (t)	
	4b	1.99 m		
	5a	1.41 m	24.19 (t)	C: 7
	5b	1.52 m		
	6a	2.22 m	17.73 (t)	C: 4, 5, 7, 8
	6b			
	7		83.66 (s)	
	8	1.99 t (2.7)	69.17 (d)	C: 5 ,6
	9	1.17 d (6.9)	12.40 (q)	C: 1, 2, 3
Val	10		173.54 (s)	
	11	4.61 t (9.7)	53.60 (d)	C: 1 ,10, 12, 13
	12	2.05 m	31.49 (d)	C: 10, 11
	13	0.95 d (6.7)	18.53 (q)	C: 11, 12, 14
	14	0.92 d (6.6)	19.64 (q)	C: 11, 12, 13
	NH-1	5.97 d (9.7)		C: 1 ,9
N-Me Val	15		171.56 (s)	
	16	4.42 d (7.7)	65.35 (d)	C: 10, 15, 18, 19, 20
	17	2.41 m	29.29 (d)	C: 15
	18	1.06 d (6.8)	19.94 (q)	C: 16, 17
	19	1.37 d (6.9)	21.11 (q)	C: 16, 17
	20	3.00 s	30.37 (q)	C: 10, 16

Table 4.1 (Continued)

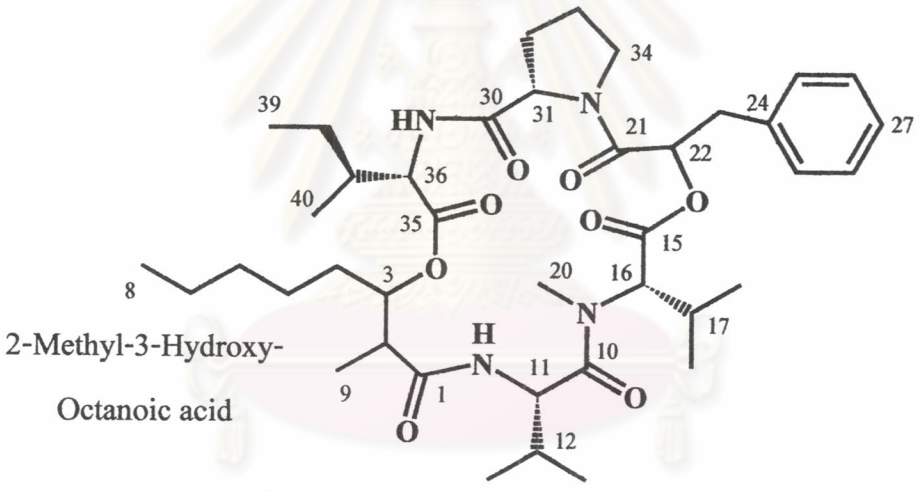
unit	C/H no.	δ_H (J in Hz)	δ_C	HMBC
Pla	21		168.96 (s)	
	22	5.07 dd (10.5, 5.6)	74.79 (d)	C: 15, 23
	23a	3.17 dd (12.9, 10.5)	38.05 (t)	C: 21, 22, 24, 25/29
	23b	3.34 dd (12.9, 5.6)		C: 22, 24, 25/29
	24		134.03 (s)	
	25/29	7.26 m	129.69 (d)	C: 23, 27
	26/28	7.32 m	129.08 (d)	C: 24, 25/29
	27	7.30 m	127.77 (d)	
Pro	30		170.01 (s)	
	31	3.31 d (7.6)	60.88 (d)	C: 34
	32a	0.95 m	30.53 (t)	C: 30
	32b	2.13 br dd (12.6, 6.4)		C: 30, 33, 34
	33a	1.45 m	21.70 (t)	
	33b	1.70 m		
	34a	3.38 m	46.38 (t)	C: 33
	34b	3.49 m		C: 21, 33
Ileu	35		170.15 (s)	
	36	4.17 t (8.4)	57.55 (d)	C: 30, 35, 38, 40
	37	1.85 m	34.50 (d)	
	38a	1.15 m	25.21 (t)	C: 37, 39, 40
	38b	1.37 m		C: 36, 37, 39
	39	0.81 t (7.6)	10.44 (q)	C: 36, 37, 38
	40	0.83 d (6.6)	15.66 (q)	C: 36, 37, 38
	NH-2	7.84 d (8.3)		C: 35

4.2.2 Compounds B and C

The HRFABMS of compounds B (**27**) and C (**28**) established their molecular formulas as $C_{40}H_{60}N_4O_8$ and $C_{40}H_{62}N_4O_8$, respectively. The nearly identical ^1H and ^{13}C NMR chemical shifts (Table 4.2) of compounds **27** and **28** to that of **26** indicated the structural similarities of the metabolites. The ^1H NMR spectra of **27** and **28** revealed the acetylenic proton signal at δ 1.99 for H-8 were replaced by the signals in the olefinic region at δ 5.79, 4.99 and 5.05 in **27** and by a high field methylene signals at δ 1.28 and a methyl signal at δ 0.87 in **28**. In addition, the ^{13}C NMR spectra of **27** and **28** displayed the lack of resonances at δ 69.17 and 83.66 for the acetylenic carbons with additional sp^2 carbon signals at δ 115.10 and 138.14 in **27** and high-field carbon resonances at δ 22.53 and 14.00 in **28**. These observations implied that their terminal triple bond of Hmoya in **26** had been reduced to a double bond in **27** and a single bond in **28**. Detailed analysis of 1D and 2D NMR data of compounds **27** and **28** confirmed that the only differences of the three compounds is the degree of unsaturation of the octanoic acid unit. Due to the similar spectroscopic properties of compounds **27** and **28** to those of **26**, therefore, it is likely that they are of the same enantiomeric series. Thus, the stereochemical analysis of **27** and **28** were not undertaken.



Trungapeptin B (**27**)



Trungapeptin C (**28**)

Table 4.2 ^{1}H and ^{13}C NMR Data for Compounds B (**27**) and C (**28**) in $CDCl_3$

Position	Compound B (**27**)		Compound C (**28**)	
	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)
1	172.77 (s)		172.86 (s)	
2	43.18 (d)	2.43 m	43.06 (d)	2.43 m
3	74.73 (d)	4.96 m	74.69 (d)	4.94 dt (9.6, 3.5)
4	29.63 (t)	2.39 m	29.67 (t)	2.39 m
		1.86 m		1.86 m
5	24.94 (t)	1.28 m	25.68 (t)	1.28 m
		1.41 m		1.41 m
6	33.16 (t)	2.06 m	31.38 (t)	2.06 m
7	138.14 (d)	5.79 m	22.53 (t)	1.28 m
8	115.10 (t)	4.99 br dd (10.1, 1.8)	14.00 (q)	0.87 t (7.2)
		5.05 br dd (15.2 ,1.8)		
9	12.11 (q)	1.14 d (6.9)	11.97 (q)	1.14 d (7.1)
10	173.60 (s)		173.64 (s)	
11	53.67 (d)	4.60 t (9.6)	53.72 (d)	4.60 t (9.5)
12	31.47 (d)	2.03 m	31.48 (d)	2.03 m
13	18.53 (q)	0.95 d (6.7)	18.55 (q)	0.95 d (6.6)
14	19.67 (q)	0.93 d (6.7)	19.68 (q)	0.92 d (6.4)
NH-1		5.96 d (9.6)		6.11 d (9.5)
15	171.53 (s)		171.45 (s)	
16	65.32 (d)	4.41 d (9.6)	65.32 (d)	4.42 d (9.6)
17	29.29 (d)	2.39 m	29.30 (d)	2.39 m
18	19.96 (q)	1.36 d (6.4)	19.97 (q)	1.36 d (6.7)
19	21.07 (q)	1.06 d (6.5)	21.06 (q)	1.06 d (6.7)
20	30.39 (q)	3.00 s	30.39 (q)	3.00 s

Table 4.2 (Continued)

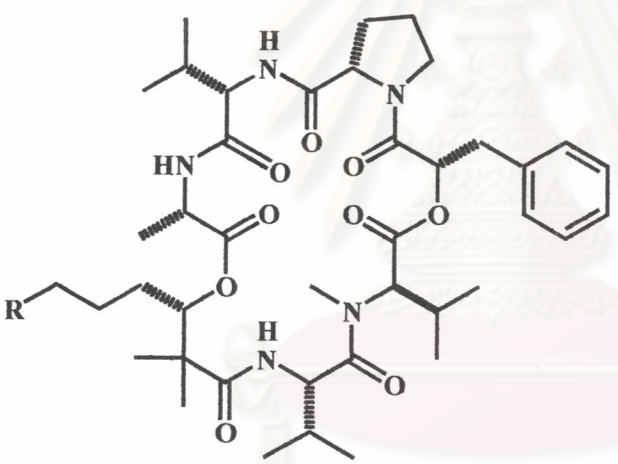
Position	Compound B (**27**)		Compound C (**28**)	
	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)
21	168.92 (s)		168.93 (s)	
22	74.86 (d)	5.10 dd (10.4, 5.6)	75.14 (d)	5.11 dd (10.5, 5.5)
23	38.07 (t)	3.16 dd (12.6, 10.4)	38.05 (t)	3.16 dd (12.8, 10.5)
		3.33 dd (12.6, 5.6)		3.33 dd (12.8, 5.5)
24	134.02 (s)		134.03 (s)	
25/29	129.74 (d)	7.24 m	129.73 (d)	7.25 m
26/28	129.05 (d)	7.31 m	129.00 (d)	7.31 m
27	127.73 (d)	7.26 m	127.72 (d)	7.29 m
30	169.90 (s)		169.92 (s)	
31	60.94 (d)	3.33 m	60.97 (d)	3.37 m
32	30.46 (t)	2.13 dd (12.4, 5.8)	30.46 (t)	2.15 dd (12.5, 6.1)
		0.90 dd (12.4, 6.7)		0.90 m
33	21.71 (t)	1.47 m	21.70 (t)	1.47 m
		1.68 m		1.68 m
34	46.35 (d)	3.36 m	46.29 (d)	3.36 m
		3.46 m		3.46 m
35	170.03 (s)		170.00 (s)	
36	57.39 (d)	4.21 t (8.4)	57.33 (d)	4.21 t (8.6)
37	34.59 (d)	1.86 m	34.58 (d)	1.84 dt (10.2, 3.5)
38	25.17 (t)	1.16 m	25.14 (t)	1.16 m
		1.37 m		1.37 m
39	10.50 (q)	0.81 t (7.4)	10.52 (q)	0.81 t (7.3)
40	15.68 (q)	0.84 d (6.7)	15.60 (q)	0.83 d (6.7)
NH-2		7.85 d (8.4)		7.85 d (9.0)

4.3 Biological Activities of Compound A

Compound A (**26**) showed brine shrimp toxicity LD_{100} at 10 ppm and strong ichthyotoxicity at 6.25 ppm (20 min). However, neither compound exhibited cytotoxicity against KB and LoVo cells. Due to limited supply of compounds **27** and **28**, their biological activities were not evaluated.

4.4 Related Compounds of Trungapeptins A, B and C

The whole structures of these compounds are nearly identical to that of the kulolides (**29-31**), metabolites isolated from a cephalaspidean mollusk, *Philinopsis speciosa*.[46-47]



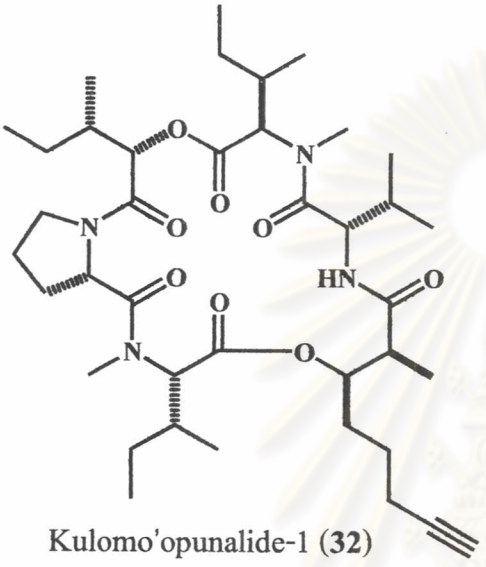
29 Kulolide-1 **R** =

30 Kulolide-2 **R** =

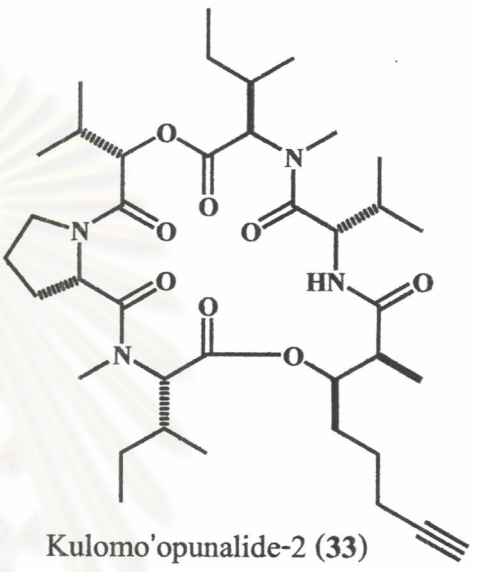
31 Kulolide-3 **R** =

However, the distinctive features of trangapeptins A (**26**), B (**27**) and C (**28**) include unique 3-hydroxy-2-methyl-7-octynoic acid (Hmoya), 3-hydroxy-2-methyl-7-octenoic acid (Hmoea), and 3-hydroxy-2-methyl-7-octanoic acid (Hmoaa) residues, respectively. The residue Hmoya in compound A (**26**) was previously reported as part of kulomo'opunalides (**32-33**),[47] onchidin B (**34**),[48-49] and antanapeptins A, D (**35, 38**)[50] which were isolated from the mollusk *Philinopsis speciosa*, the mollusk *Onchidium* sp. and the cyanobacterium *L. majuscula*, respectively. Both of these mollusk prey upon *stylocheilus longicaudus*, smaller marine mollusk which are known to feed on alga

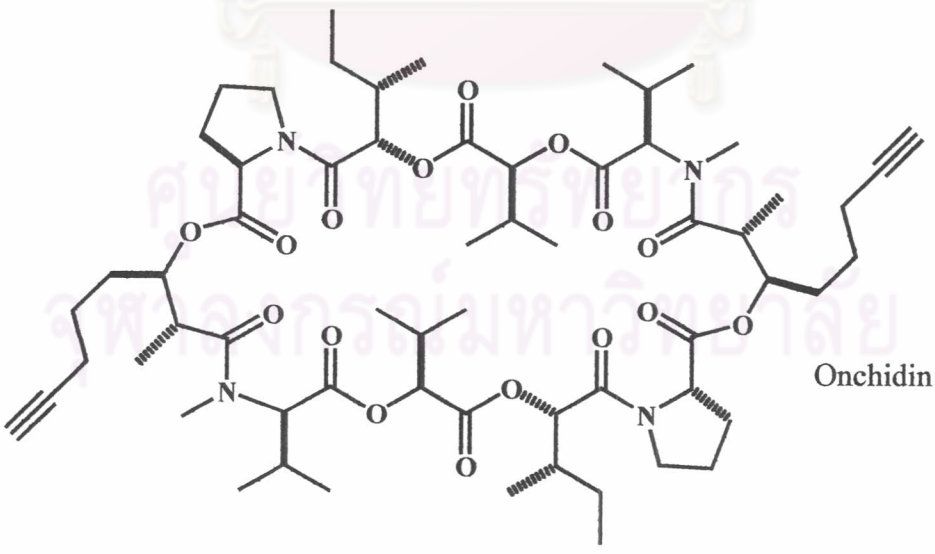
including cyanobacteria. Therefore, the kulomo'opunalides and onchidin B are likely to be produced by marine cyanobacteria and were transferred to *P. speciosa* and *Onchidium* sp. via food chain. Thus, compounds with the unique Hmoya unit are proposed to be biosynthesized marine cyanobacteria.



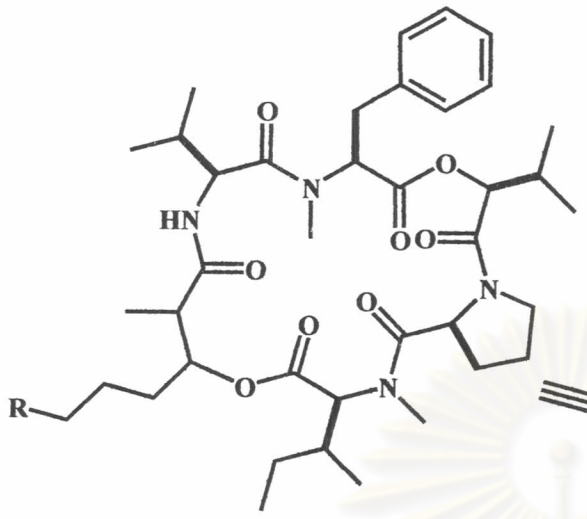
Kulomo'opunalide-1 (**32**)



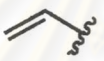
Kulomo'opunalide-2 (**33**)



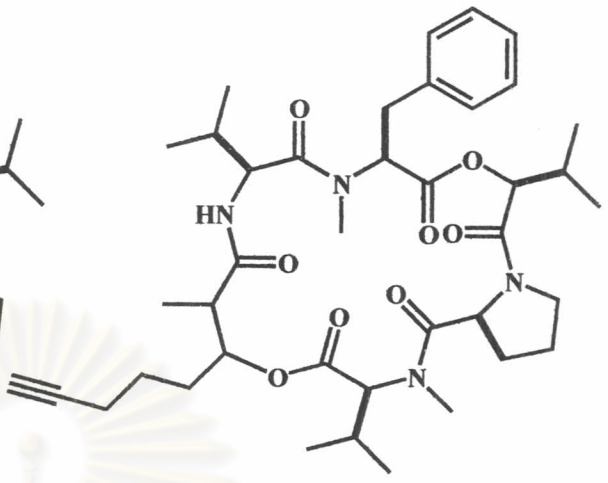
Onchidin B (**34**)



35 Antanapeptin A **R** = 

36 Antanapeptin B **R** = 

37 Antanapeptin C **R** = 



38 Antanapeptin D