Pergamon

Tetrahedron, Vol. 53, No. 5, pp. 1577-1582, 1997

0040-4020/97 $17.00 + 0.00

PII: S0040-4020(96)01136-2

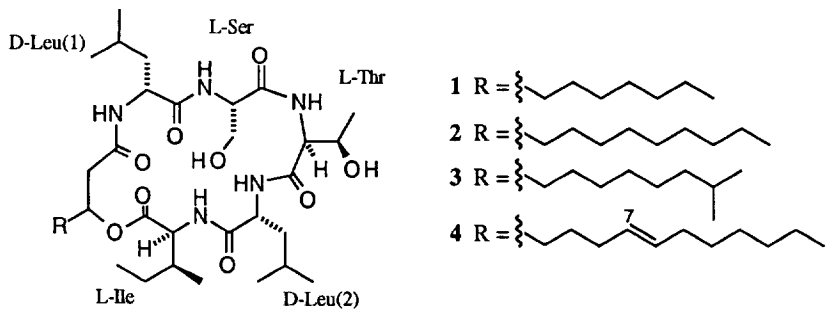
Kailuins A-D, New Cyclic Acyldepsipeptides from Cultures of a Marine-Derived Bacterium

George G. Harrigan, Beth L. Harrigan, and Bradley S. Davidson*

Department of Chemistry
University of Hawaii at Manoa, Honolulu, Hawaii 96822, USA.

Abstract: Four new cyclic acyldepsipeptides (**1-4**), assigned the trivial names kailuins A-D, have been isolated from liquid cultures of a Gram-negative bacterium (BH-107) obtained from driftwood collected at Kailua beach, Oahu. Structure elucidation employed 2-D NMR analyses, HRFABMS, and chemical derivatizations. Kailuins A-D exhibited mild cytotoxicity when tested against certain human tumor cell lines. © 1997, Elsevier Science Ltd. All rights reserved.

Secondary metabolites from marine microorganisms are represented in chemotypes of diverse biosynthetic origin and therefore constitute a source of new and potentially valuable bioactive compounds.[1,2] Indeed, marine microorganisms are now considered a promising biomedical resource[3] and have afforded such compounds as the cytotoxic and antiviral macrolactins,[4] the antibiotic bioxalomycins,[5] the cytotoxic octalactins,[6] and alteramide A, a toxic tetracyclic alkaloid.[7] We have recently reported the isolation of the cytotoxic caprolactins A and B,[8] γ-indomycinone,[9] and the antimicrobial wailupemycins[10] from marine-derived bacteria. Our continuing interest in marine microorganisms as producers of biologically active natural products has led us to investigate organisms cultured from a variety of souce materials. Although wood collected from the marine environment has been studied extensively as a source of fungi, it has received less attention as a source of bacteria. We would now like to report the discovery and structure determination of four new acyldepsipeptides, kailuins A-D (**1-4**), from a bacterial isolate cultured from a piece of driftwood obtained at Kailua beach, Oahu.



Chromatography of the EtOAc extract of a liquid culture of the Gram-negative bacterium BH-107 on Sephadex LH-20 followed by semi-preparative reversed phase HPLC afforded kailuins A-D (**1-4**) as clear, optically active oils. The IR spectra for all of the compounds were identical with prominent broad peaks at 1650 and 1539 cm^{-1}, consistent with the presence of amide carbonyl groups, and a smaller peak at 1733 cm^{-1}, suggesting that the compounds also each incorporate an ester carbonyl.

Table I. NMR Data for Kailuin A (1).

Residue/position		^{13}C[a]	^{1}H (J) in Hz[b]	COSY	HMBC[c]
D-LEU(1)	NH	-	7.42, d (8.4)	Hα	-
	Cα	51.3, d	4.55, m	NH, Hβ	Hβ
	Cβ	42.2, t	ca. 1.50-1.60	Hα	Hα, Hγ, Hδ
	Cγ	24.7,d	ca. 1.60		Hα, Hγ, Hδ
	Cδ	22.7, q	ca. 0.92		Hβ, Hδ
	Cδ	22.9, q	ca. 0.92		Hβ, Hδ
	CO	174.6, s	-	-	Hα, L-Ser NH
L-SER	NH	-	8.09, d (5.0)	Hα	-
	Cα	58.1, d	4.11, m	NH, Hβ	NH
	Cβ	61.3, t	3.85, dd (2.6, 11.9)	Hα	NH, Hα
			4.00, dd (3.9, 11.9)		
	CO	170.5, s	-	-	Hα, Hβ, L-Thr NH
L-THR	NH	-	7.21, d (7.9)	Hα	-
	Cα	58.2, d	4.28, br d (7.9)	NH, Hβ	NH, Hγ,
	Cβ	66.8, d	4.55, m	NH, Hα, Hγ	Hα, Hγ
	Cγ	19.4, q	1.16, d (6.4)	Hβ	Hα, Hβ
	CO	171.7, s	-		Hα, Hβ, D-Leu(2) NH
D-LEU(2)	NH	-	7.55, d (8.3)	Hα	-
	Cα	50.6, d	4.46, dt (8.3, 6.9)	NH, Hα, Hβ	NH, Hβ,
	Cβ	37.9, t	ca. 1.70		Hα, Hγ, Hδ
	Cγ	24.6, d	ca. 1.60		Hα
	Cδ	21.7, q	ca. 0.90		
	Cδ	22.1, q	ca. 0.88		
	CO	173.3, s	-	-	Hα, L-Ile NH
L-ILE	NH	-	7.45, d (7.7)	Hα	-
	Cα	57.9, d	4.09, dd (7.2, 7.7)	NH, Hα, Hβ,	NH, Hγ1, Hγ2,
	Cβ	35.7, d	ca. 1.70		NH, Hα, Hγ1,
	Cγ1	25.5, t	ca. 1.20-1.50		Hα
	Cγ2	15.3, q	ca. 0.86		Hα, Hγ1
	Cδ	11.1, q	ca. 0.89		Hγ1
	CO	173.6, s	-	-	Hα
β-ACYLOXY ACID	C-1	171.5, s	-	-	H-2, D-Leu(1) NH
	C-2	42.5, t	2.41, dd (2.4, 16.6)	H-3	
			2.49, dd (5.3, 16.6)		
	C-3	73.0, d	5.07, m	H-2, H-4	H-2
	C-4	35.3, t	ca. 1.50-1.70		H-2
	C-5	25.3, t	ca. 1.20		
	C-6	29.0,* t	ca. 1.20		
	C-7	29.1,* t	ca. 1.20		
	C-8	31.6, t	ca. 1.20		H-10
	C-9	22.5, t	ca. 1.20		
	C-10	14.0, q	0.83, t (6.9)		

[a]Multiplicity deduced from DEPT experiment; [b]Because of extensive overlap in the ^{1}H NMR spectrum many of the chemical shifts have been derived from 2-D NMR spectra, primarily HMQC. This is denoted by the ca. prefix; [c]Proton showing correlation to indicated carbon; *Chemical shifts with same superscript within a given column are interchangeable.

The NMR spectra of compounds **1-4** (Tables I-III) were also very similar. The presence of 6 signals for carbonyls in each of the ^{13}C NMR spectra, combined with the occurrence of only 5 signals in the ^{1}H NMR spectra assignable to amide NH protons, suggested that the ester functionality is incorporated into the macrocyclic ring. Therefore, the kailuins are depsipeptides each containing 5 amino acids and a hydroxyacid moiety. Furthermore, the occurrence of an ABX spin system, consisting of a pair of doublets of doublets centered at around δ 2.45 and a one-proton multiplet at δ 5.1, together with a prominent multiple proton signal at δ 1.2 in each of the ^{1}H NMR spectra suggested that compounds **1-4** were structurally related to halobacillin,[11] a cyclic depsipeptide incorporating a β-acyloxy fatty acid moiety. The spectral similarities of compounds **1-4** to each other suggested they all contain the same sequence of amino acid residues, but differ in the identity of the β-acyloxy fatty acid component.

Table II. ^{1}H NMR Data for Kailuins B-D (2-4).

Residue/position		2 (*J*) in Hz[a]	3 (*J*) in Hz[a]	4 (*J*) in Hz[a]
D-LEU(1)	NH	7.54, d (8.1)	7.45, d (8.4)	7.45, d (8.3)
	Cα	4.55, m	4.54, m	4.55, m
	Cβ	ca. 1.45-1.60	ca. 1.40-1.65	ca. 1.52-1.58
	Cγ	ca. 1.58	ca. 1.60	ca. 1.60
	Cδ	ca. 0.90	ca. 0.92	ca. 0.92
	Cδ	ca. 0.90	ca. 0.92	ca. 0.92
L-SER	NH	8.15, d (5.2)	8.12, d (4.7)	8.21, d (4.9)
	Cα	4.11, m	4.12, m	4.11, m
	Cβ	3.82, dd (3.3, 11.8)	3.86, dd (3.2, 11.8)	3.88, dd (3.8, 11.9)
		3.96, dd (4.2, 11.8)	3.99, dd (3.9, 11.8)	4.01, dd (3.8, 11.9)
L-THR	NH	7.32, d (8.3)	7.22, d (8.4)	7.19, d (8.4)
	Cα	4.27, dd,(1.7, 8.3)	4.28, dd (1.5, 8.4)	4.29, dd (1.5, 8.4)
	Cβ	4.50, m	4.54, m	4.55, m
	Cg	1.15, d (6.5)	1.17, d (6.5)	1.17, d (6.4)
D-LEU(2)	NH	7.57, d (7.5)	7.58, d (8.1)	7.62, d (8.0)
	Cα	4.44, dt (8.3, 7.5)	4.44, dt (8.1, 7.5)	4.44, dt (8.0, 7.6)
	Cβ	ca. 1.60-1.75	ca. 1.60-1.80	ca. 1.70
	Cγ	ca. 1.58	ca. 1.50	ca. 1.50
	Cδ	ca. 0.89	ca. 0.90	ca. 0.90
	Cδ	ca. 0.86	ca. 0.88	ca. 0.88
L-ILE	NH	7.48, d,(8.1)	7.47, d (7.6)	7.47, d (7.8)
	Cα	4.11, m	4.10, m	4.10, dd (6.9, 7.8)
	Cβ	ca. 1.70	ca. 1.70	ca. 1.70
	Cγ1	ca. 1.15-1.45	ca. 1.15-1.40	ca. 1.20-1.40
	Cγ2	ca. 0.86	ca. 0.88	ca. 0.90
	Cδ	ca. 0.87	ca. 0.88	ca. 0.90
β-ACYLOXY ACID	C-2	2.40, dd (2.2, 16.6)	2.42, dd (2.2, 16.6)	2.42, dd (2.2, 16.8)
		2.47, dd (5.7, 16.6)	2.50, dd (5.7, 16.6)	2.52, dd (5.7, 16.8)
	C-3	5.10, m	5.09, m	5.11, m
	C-4	ca. 1.50-1.67	ca. 1.50-1.70	ca. 1.52-1.70
	C-5	ca. 1.20	ca. 1.20	ca. 1.30-1.50
	C-6	ca. 1.20	ca. 1.20	2.02, br q (7.2)
	C-7	ca. 1.20	ca. 1.20	5.36, br dt (7.2, 18.0)
	C-8	ca. 1.20	ca. 1.20	5.26, br dt (7.2, 18.0)
	C-9	ca. 1.20	ca. 1.20	1.97, br q (7.2)
	C-10	ca. 1.20	1.11, m	ca. 1.20-1.35
	C-11	ca. 1.20	0.83, d (6.7)	ca. 1.20-1.35
	C-12	0.83, t (7.0)	0.83, d (6.7)	ca. 1.25
	C-13	-	-	ca. 1.25
	C-14	-	-	0.86, t (7.2)

[a]Because of extensive overlap in the ^{1}H NMR spectrum many of the chemical shifts have been derived from 2-D NMR spectra, primarily HMQC. This is denoted by the ca. prefix.

Kailuin A (**1**) has a molecular formula of $C_{35}H_{63}N_5O_9$ as deduced from HRFABMS, indicating that one additional degree of unsaturation was required in addition to the 6 carbonyls. This confirmed that the molecule was indeed cyclic. The amino acid residues were identified as leucine (2 mol), isoleucine, serine, and threonine using 2-D NMR techniques including ^{1}H-^{1}H COSY, HMQC,[12] and HMBC[13] spectroscopy. Their absolute configurations were determined using Marfey's method.[14] HMBC correlations between each amide proton and the adjacent amino acid's α-carbon afforded the sequence D-Leu-L-Ser-L-Thr-D-Leu-L-Ile. The position of the β-acyloxy acid component was confirmed based on HMBC correlations between the β-acyloxy carbonyl (C-1) and the D-Leu(1) NH proton. The chirality of the β-carbinol position has not been determined. ^{13}C NMR chemical shifts and DEPT spectroscopy confirmed the presence of an *n*-alkyl chain establishing the identity of the β-acyloxy acid component as 3-hydroxydecanoic acid.

The similarity between the ^{1}H and ^{13}C NMR chemical shifts (see Tables I-III) of kailuin B (**2**) and those of compound **1** suggested that both compounds incorporate the same sequence of amino acids, an assignment that was confirmed based on HMBC data. Marfey's analysis also established that the chiralities of

the amino acids in compound **2** were identical to those in **1**. The molecular formula of kailuin B was assigned as $C_{37}H_{67}N_5O_9$ using HRFABMS, indicating that the β-acyloxy fatty acid moiety of compound **2** contains two more methylene units than are found in compound **1**. Analysis of the ^{13}C NMR chemical shifts and the DEPT data confirmed the presence of 3-hydroxydodecanoic acid.

HRFABMS of compound kailuin C (**3**) also indicated a molecular formula of $C_{37}H_{67}N_5O_9$, indicating that kailuins B and C are isomeric. However, the presence of a 6H doublet (J = 6.7 Hz) at δ 0.83 in the ^1H NMR spectrum (Table II), as opposed to a 3H triplet (J = 7.0 Hz) at δ 0.83 observed in the ^1H NMR spectra of compounds **1** and **2**, suggested that kailuin C contains an *iso*-alkyl chain rather than an *n*-alkyl chain. This proposal was supported by signals in the ^{13}C NMR and DEPT spectra at δ 22.5 (q, C-11, C-12), 27.9 (d, C-10) and 38.8 (t, C-9). The identity of the β-acyloxy component in compound kailuin C (**3**) was thus established as 3-hydroxyisododecanoic acid.

Table III. ^{13}C NMR Data for Kailuins B-D (2-4).

Residue/position		2	3	4
D-LEU(1)	Cα	51.4, d	51.4, d	51.4, d
	Cβ	42.1, t	42.1,* t	42.2,* t
	Cγ	24.6,* d	24.7,^{x} d	24.7,^{x} d
	Cδ	22.7, q	22.7, q	22.7, q
	Cδ	22.8, q	22.9, q	22.9, q
	CO	174.7, s	174.5, s	174.6, s
L-SER	Cα	58.1, d	58.1, d	58.1, d
	Cβ	61.2, t	61.3, t	61.3, t
	CO	170.9, s	170.7, s	170.6, s
L-THR	Cα	58.4, d	58.2, d	58.2, d
	Cβ	66.8, d	66.7, d	66.9, d
	Cg	19.3, q	19.4, q	19.4, q
	CO	171.6, s	171.7, s	171.7, s
D-LEU(2)	Cα	50.7, d	50.7, d	50.7, d
	Cβ	37.4, t	37.2, t	37.0, t
	Cγ	24.5,* d	24.5,^{x} d	24.5,^{x} d
	Cδ	21.4, q	21.6, q	21.5, q
	Cδ	21.8, q	22.0, q	22.0, q
	CO	173.2, s	173.3, s	173.4, s
L-ILE	Cα	57.7, d	57.8, d	57.9, d
	Cβ	35.8, d	35.8, d	35.8, d
	Cγ1	25.3,^{x} t	25.3,^{xx} t	25.4,^{xx} t
	Cγ2	15.3, q	15.3, q	15.4, q
	Cδ	10.8, q	11.0, q	11.0, q
	CO	173.2, s	173.4, s	173.6, s
β-ACYLOXY ACID	C-1	171.5, s	171.5, s	171.5, s
	C-2	42.0, t	42.0,* t	42.1,* t
	C-3	72.7, d	72.8, d	72.8, d
	C-4	35.0, t	35.2, t	34.8, t
	C-5	25.3,^{x} t	25.4,^{xx} t	25.5,^{xx} t
	C-6	29.1,$^+$ t	29.1,$^+$ t	26.6, t
	C-7	29.1,$^+$ t	29.6,$^+$ t	130.9, d
	C-8	29.3,$^+$ t	27.15, t	128.5, d
	C-9	29.5,$^+$ t	38.9, t	27.2, t
	C-10	31.7, t	27.9, d	29.6, t
	C-11	22.5, t	22.5, q	28.9, t
	C-12	13.9, q	22.5, q	31.7, t
	C-13	-	-	22.6, t
	C-14	-	-	14.0, q

[a]Multiplicity deduced from DEPT experiment; *Chemical shifts with same superscript within a given column are interchangeable.

The molecular formula of kailuin D (**4**) was established as $C_{39}H_{69}N_5O_9$, indicating that, while compound **4** contains two more carbons in the alkyl side chain than compounds **2** and **3**, it must also possess an additional degree of unsaturation. The ^1H and ^{13}C NMR spectra confirmed the presence of a double bond, located at the C7-C8 position on the basis of HMBC correlations, specifically those between H-7 and C-6 and C-8 and between H-8 and C-7 and C-9. A coupling constant of 18.0 Hz between the two olefinic protons indicated that the double bond has a *trans* geometry. Thus, the β-acyloxy acid component of kailuin D was identified as a (*E*)-3-hydroxytetradec-7-enoic acid.

Kailuins A-D (**1-4**) exhibited mild cytotoxicity towards several human tumor cell lines (see Table 4). Superficially, they are structurally related to the marine-derived acyl depsipeptides halobacillin,[11] isohalobacillin,[15] and the baciricins;[16] however, the known compounds are produced exclusively by Gram-positive bacteria of the genus *Bacillus*, while the kailuins are produced by a Gram-negative organism. Furthermore, compounds **1-4** contain two fewer amino acids and lack the polar amino acids Glu and Asp, instead containing Ser and Thr. In fact, kailuins A-D represent a new class of lipopeptides, as they are the first

cyclic depsipeptides known to incorporate 5 amino acids and a β-acyloxy fatty acid moiety within a 19-membered ring.

Table IV. Tumor Cell Cytotoxicity of Kailuins A-D (1-4) (GI_{50} in μg/mL).

Compound	A-549 (lung)	MCF-7 (breast)	HT-29 (colon)
1	3	3	3
2	2	2	3
3	3	4	3
4	2	3	2
adriamycin	8×10^{-3}	3×10^{-1}	3×10^{-2}

EXPERIMENTAL SECTION

General. ^{1}H- and ^{13}C NMR spectra were recorded in 0.4 - 0.5 mL $CDCl_3$ containing 100 μL CD_3OD on a GE Omega 500 spectrometer at 500 MHz and 125 MHz, respectively, using residual $CHCl_3$ signals as internal reference. All 2-D NMR experiments were conducted on the same spectrometer. For HMQC experiments $^{1}J_{CH}$ = 150 Hz and for HMBC experiments $^{n}J_{CH}$ = 6 Hz.

Collection and Isolation of Bacterium. The original culture of BH-107 was obtained from driftwood collected from an isolated area on the south of Kailua Beach, Oahu on October 25, 1995. It was isolated using Difco marine agar supplemented with polymyxin B (5 μg/mL) and penicillin (1 μg/mL). BH-107 is a Gram-negative rod that swarms on solid media. A fatty acid methyl ester (FAME) analysis of BH-107 performed by Microbial ID, Inc., failed to yield a conclusive result, providing only a weak match (similarity index = 0.285) to *Vibrio proteolyticus*. Consistent with, but not determinant to vibrios, growth of BH-107 is stimulated in the presence of sodium, it utilizes D-mannitol, and is incapable of denitrification. Further testing will be conducted in an effort to determine the bacterium's identity.

Extraction and Isolation. Eight 2 L Erlenmeyer flasks, each containing 500 mL of Marine Medium №. 1 (5 g/L starch, 7 g/L yeast extract and 30 g/L Instant Ocean), were inoculated from a stock culture maintained on Difco marine agar. Flask cultures were incubated at room temperature on an orbital shaker at 200 rpm for 5 days. The culture was then extracted with EtOAc and the organic phase was evaporated to give an oil (226.0 mg) which was subjected to chromatography over Sephadex LH-20 (Sigma), eluting initially with hexanes:CH_2Cl_2 (1:1) followed by CH_2Cl_2, CH_2Cl_2:$(CH_3)_2CO$ (1:1), $(CH_3)_2CO$, and finally MeOH. The hexanes:CH_2Cl_2 (1:1) and CH_2Cl_2 fractions were subjected to semi-preparative reversed-phase HPLC (Rainin C_{18} Microsorb-60Å, 1.0 X 25.0 cm; 2 mL/min; detection at 220 nm) using a CH_3CN-0.01% TFA linear gradient (20-100% over 20 min, and then 100% CH_3CN for 20 min). Compounds **1** (8.3 mg), **3** (2.1 mg), **2** (5.4 mg) and **4** (3.5 mg) were collected at Rt (min) 25.5, 28.7, 29.1, and 30.6, respectively.

Absolute Configuration of Amino Acids. Solutions of compounds **1** and **2** (1 mg) in 6N HCl were each heated at 108 °C for 18 h and then concentrated to dryness. The residues were each dissolved in H_2O (50 μL) and to the resulting mixtures were added a 1% solution of 1-fluoro-2,4-dinitrophenyl-5-L-alanine amide (Marfey's reagent) in acetone (100 mL) and 1M $NaHCO_3$ (20 μL). After heating at 37 °C for 1 h, the reaction was cooled, acidified with 2N HCl (10 mL), and evaporated to dryness. The resulting products were then resuspended in DMSO:H_2O (1:1) (2mL) and aliquots (10 μL) were subjected to reversed-phase HPLC analysis. (Rainin C_{18} Microsorb-MV, 0.4 cm X 25.0 cm, 2 mL/min; detection at 340 nm) using a CH_3CN-50 mM NH_4OAc linear gradient (10-50% over 60 min). The Rt values (min) were L-Ser (8.7), L-Thr (9.9), L-Ile (21.8), and D-Leu (30.9) respectively.

Kailuin A (1): clear oil, $[\alpha]_D$ + 8.6° (*c* 1.0, MeOH); IR (film) *v* 3301, 2958, 2926, 1733, 1650, 1539, 1468, 1202 cm^{-1}; HRFABMS *m/z* [MH]+ 698.4688 $C_{35}H_{64}N_5O_9$ requires 698.4704; ^1H NMR and ^{13}C NMR see Table I.

Kailuin B (2): clear oil, $[\alpha]_D$ + 9.3° (*c* 1.0, MeOH); IR (film) *v* 3301, 2958, 2926, 1733, 1650, 1539, 1468, 1202 cm^{-1}; HRFABMS *m/z* $[MH]^+$ 726.4998 $C_{37}H_{68}N_5O_9$ requires 726.5017; ^1H NMR see Table II; ^{13}C NMR see Table III.

Kailuin C (3): clear oil, $[\alpha]_D$ + 10.0° (*c*. 1.0, MeOH); IR (film) *v* 3301, 2958, 2926, 1733, 1650, 1539, 1468, 1202 cm^{-1}; HRFABMS *m/z* ; $[MH]^+$ 726.5012 $C_{37}H_{68}N_5O_9$ requires 726.5017; ^1H NMR see Table II; ^{13}C NMR see Table III.

Kailuin D (4): clear oil, $[\alpha]_D$ + 9.5° (*c* 1.0, MeOH); IR (film) *v* 3301, 2958, 2926, 1733, 1650, 1539, 1468, 1202 cm^{-1}; HRFABMS *m/z* $[MH]^+$ 752.5152 $C_{39}H_{70}N_5O_9$ requires 752.5173; ^1H NMR see Table II; ^{13}C NMR see Table III.

ACKNOWLEDGMENTS

Funding was provided by the National Institute of Health (R29 CA57302) and the American Cancer Society for a Junior Faculty Research Award to B.S.D. Mass spectrometry was provided by the Washington University Mass Spectrometry Resource, an NIH Research Resource (Grant No. P41RR0954). We are indebted to Prof. C-j. Chang, Department of Medicinal Chemistry and Pharmacognosy, Purdue University, for providing cytotoxicity assays and to Wesley Yoshida for recording the NMR spectra.

REFERENCES

1. Fenical, W. *Chem. Rev.* **1993**, *93*, 1673.
2. Davidson, B.S. *Curr. Opinion Biotech.* **1995**, *6*, 284.
3. Jensen, P.R.; Fenical, W. *Annu. Rev. Microbiol.* **1994**, *48*, 559.
4. Gustafson, K.; Roman, M.; Fenical, W. *J. Am. Chem. Soc.* **1989**, *111*, 7519.
5. Zaccardi, J.; Alluri, M.; Ashcroft, J.; Bernan, V.; Korshalla, J.D.; Morton, G.O.; Siegle, M.; Tsao, R.; Williams, D.R.; Maiese, W.; Ellestad, G.A. *J. Org. Chem.* **1994**, *59*, 4045.
6. Tapiolas, D.M.; Roman, M.; Fenical, W.; Stout, T.J.; Clardy, J. *J. Am. Chem. Soc.* **1991**, *113*, 4682.
7. Shigemori, H.; Bae, M.A.; Yazawa, K.; Sasaki, T.; Kobayashi, J. *J. Org. Chem.* **1992**, *57*, 4317.
8. Davidson, B.S.; Schumacher, R.W. *Tetrahedron* **1993**, *49*, 6569.
9. Schumacher, R.W.; Davidson, B.S.; Montenegro, D.A.; Bernan, V.S. *J. Nat. Prod.* **1995**, *58*, 613.
10. Sitachitta, N.; Gadepalli, M.; Davidson, B.S. *Tetrahedron* **1996**, *52*, 8073.
11. Trischman, J.A.; Jensen, P.R.; Fenical, W. *Tetrahedron Lett.* **1994**, *35*, 5571.
12. Bax, A.; Summer, M.F. *J. Am. Chem. Soc.* **1986**, *108*, 2093.
13. Sklenar, V.; Bax, A. *J. Magn. Res.* **1987**, *71*, 379.
14. Marfey, P. *Carlsberg Res. Comm.* **1984**, *49*, 591.
15. Hasumi, K.; Takizawa, K.; Takahashi, F.; Park, J.K.; Endo, A. *J. Antibiotics* **1995**, *48*, 1419.
16. Kalinovskaya, N.I.; Kuznetsova, T.A.; Rashkes, Y.V.; MiVgrom, Y.V.; MiVgrom, E.G.; Willis, R.H.; Wood, A.I.; Kurtz, H.A.; Carabedian, C.; Murphy, P.; Elyakov, G.B. *Russ. Chem. Bull.* **1995**, *44*, 951.

(*Received in USA* 3 *October* 1996; *revised* 7 *November* 1996; *accepted* 3 *December* 1996)