Induced Production of Emericellamides A and B from the Marine-Derived Fungus *Emericella* sp. in Competing Co-culture

Dong-Chan Oh, Christopher A. Kauffman, Paul R. Jensen, and William Fenical*

Center for Marine Biotechnology and Biomedicine, Scripps Institution of Oceanography, University of California, San Diego, La Jolla, California, 92093-0204

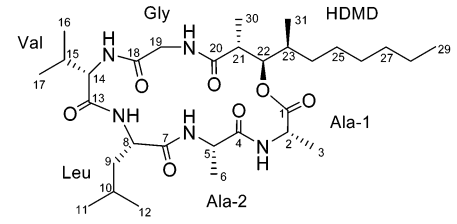
Received August 3, 2006

Induction of the production of emericellamides A and B (**1**, **2**), by the marine-derived fungus *Emericella* sp., was observed during co-culture with the marine actinomycete *Salinispora arenicola*. The planar structures of these new cyclic depsipeptides, which incorporate 3-hydroxy-2,4-dimethyldecanoic acid and 3-hydroxy-2,4,6-trimethyldodecanoic acid, were assigned by combined chemical and spectral methods. The absolute configurations of the amino acids, and those of the chiral centers on the side chain, were established by application of the Marfey's method, by *J*-based configuration analysis, and by application of the modified Mosher method. Emericellamides A and B show modest antibacterial activities against methicillin-resistant *Staphylococcus aureus* with MIC values of 3.8 and 6.0 μM, respectively.

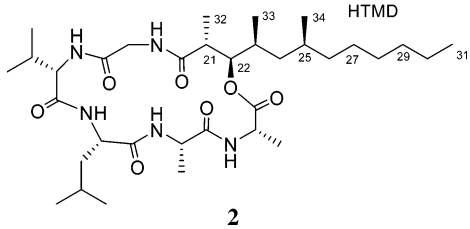
The rediscovery of known secondary metabolites is a chronic problem in natural products chemistry,[1] suggesting overall that new approaches must be adopted to enhance the discovery of novel chemotypes. Several culture-dependent approaches have been reported. For example, hydroponic plant cultures, with the application of various elicitors, have been reported.[2] This strategy, however, provided only limited evidence for the induction of bioactivity in the root extracts and did not result in the isolation and full characterization of new compounds. Cultivation of the cyanobacterium *Scytomena ocellatum,* with added elicitors, such as fungal wall polysaccharides, increased production of the previously known metabolite tolytoxin.[3] Most recently, the marine-derived fungus *Phomopsis asparagi,* when cultured in the presence of the sponge metabolite jasplakinolide, shifted its biosynthesis to produce new analogues of the well-known chaetoglobosins.[4]

Given that microorganisms interact with each other in the natural environment and these interactions are, arguably, the driving force to produce necessary secondary metabolites, simulating microbial habitats by culturing two different microbial strains in one culture vessel (i.e., co-culture) would seem to be an effective way to harvest new molecules. Following this hypothesis, several co-culturing experiments have been reported to result in an increase of biological activities;[5] however the chemical constituents responsible for the enhanced biological activities were not fully identified. Our co-culture experiments have, however, produced promising results. A previous study of a mixed cultivation of the marine-derived fungus *Pestalotia* sp. with a marine α-proteobacterium, closely related to *Thalassospira lucentensis,* yielded a new antimicrobial metabolite, pestalone.[6] In addition, a more recent co-culture experiment with the marine-derived fungus *Libertella* sp. and the same bacterial strain used in the previously mentioned pestalone case induced the production of new cytotoxic diterpenoids, which are not produced in either microorganism in single culture.[7]

In this paper, we report another example of induction during co-culture with a 100-fold increase in the production of new secondary metabolites. This co-culture, involving the marine-derived fungus *Emericella* sp. and the marine actinomycete *Salinispora arenicola,* led to isolation of two new antimicrobial cyclic depsipeptides, emericellamides A and B (**1** and **2**).



1



2

Results and Discussion

In the course of screening more than 50 co-cultures with marine-derived fungi and marine actinomycetes, the co-culture of an *Emericella* sp. (strain CNL-878) and a *Salinispora arenicola* (strain CNH-665) displayed a significantly different metabolite profile compared to the single culture (Figure 1). A distinct major peak in the co-culture was identified to have molecular weight 609 ($[M + H]^+$ *m/z* at 610, $[M + Na]^+$ at 632) by LC/MS analysis. This new peak was subsequently isolated and characterized as a new cyclic lipopeptide, emericellamide A (**1**). Having the new metabolite in hand, we found that this compound was produced in very minor amounts, undetectable by normal LC-MS analysis, by the fungal strain *Emericella* sp. (strain CNL-878). The relative enhancement in the production of **1** was approximately 100 times based on the ion counts of **1** in the co-culture and the single culture. The minor compound isolated, emericellamide B (**2**), was also significantly increased (∼100 times) by co-culture.

Emericellamide A (**1**) was isolated as a white powder, which analyzed for the molecular formula $C_{31}H_{55}N_5O_7$ (7 degrees of unsaturation), by ESI high-resolution mass spectrometry (obsd $[M + H]^+$ at *m/z* 610.4173, calcd $[M + H]^+$ 610.4174). This molecular formula was also supported by ^1H and ^{13}C NMR spectral data (Table 1). The ^1H NMR spectrum displayed characteristics of a typical peptide, illustrating five amide NH signals [δ_H: 8.08, 8.01, 7.93, 7.50, 7.39], six α-amino protons [δ_H: 4.30, 4.07, 4.05, 4.01, 3.97,

* To whom correspondence should be addressed. Tel: (858) 534-2133. Fax: (858) 558-3702. E-mail: wfenical@ucsd.edu.

10.1021/np060381f CCC: $37.00
Published on Web 02/27/2007

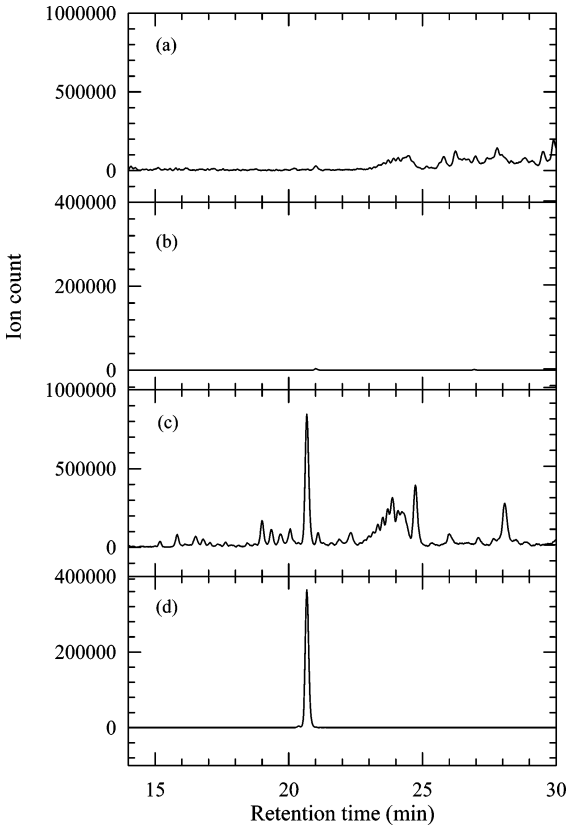


Figure 1. LC/MS traces (ion counts) of (a) the pure culture of CNL-878, (b) ion extraction for the ion *m*/*z* 610 in the pure culture of CNL-878, (c) the co-culture of CNL-878 and CNH-665, and (d) ion extraction for the *m*/*z* 610 ion in the co-culture. The LC/MS analyses were achieved with 10−100% aqueous CH_3CN over 30 min. The crude extracts were prepared by extraction of 25 mL of cultures, and 5 μL of each sample was analyzed by LC/MS.

3.61], and one ester carbinol proton [δ_H: 4.92]. In the ^{13}C NMR spectrum, six amide or ester resonances [δ_C: 172.6, 171.1, 171.0, 170.9, 170.5, 168.4] and one oxygenated sp^3 carbon [δ_C: 76.3] were observed. IR absorption bands observed at 1631 and 1755 cm^{-1} illustrated the presence of amide and ester functionalities. Since six carbonyl carbons accounted for six of the seven unsaturations, emericellamide A must be a monocyclic depsipeptide.

Interpretation of HSQC, DEPT, $^1H-^1H$ COSY, and $^1H-^1H$ TOCSY NMR data led to the construction of six partial structures: glycine (Gly), two alanine residues (Ala-1 and Ala-2), valine (Val), leucine (Leu), and 3-hydroxy-2,4-dimethyldecanoic acid (HDMD). The amino acid constituents of **1**, and HDMD, were then connected on the basis of HMBC heteronuclear couplings and correlations observed in a ROESY NMR experiment. The α-amino proton of Ala-1 [H-2, δ_H: 4.01] and the analogous α-proton of Ala-2 [H-5, δ_H: 4.07] both were observed to correlate with the carbonyl carbon of Ala-2 [C-4, δ_C: 171.1] in the HMBC spectrum. A ROESY NMR cross-peak between the NH amide proton of Ala-1 [δ_H: 8.01] and α-amino proton of Ala-2 (H-5) also supported the sequence of Ala-1−Ala-2. The linkage of Ala-2 to Leu was established by HMBC correlations from the NH of Ala-2 [δ_H: 7.39] and the α-amino proton of Leu [H-8, δ_H: 4.05] to the Leu amide carbonyl [C-7, δ_C: 170.5]. This linkage was also confirmed by the observed dipolar coupling between the NH of Ala-2 and the α-amino proton of Leu (H-8). The connection between Leu and Val was achieved through interpretation of HMBC data, which demonstrated correlations from the α-amino proton of Leu [H-8, δ_H: 4.05] and the α-amino proton of Val [H-14, δ_H: 3.97] to C-13 [δ_C: 170.9]. The strong ROESY correlation between the amide NH proton of Leu [δ_H: 8.08] and the α-amino proton (H-14) of Val also confirmed this connectivity. The subsequent connection of Val to Gly was achieved on the basis of HMBC correlations from the α-amino proton of Val [H-14, δ_H: 3.97] and the α-amino protons of Gly [H-19a, b, δ_H: 4.30, 3.61] to the Gly carbonyl carbon [C-18, δ_C: 168.4]. This connectivity was also supported by the presence of a ROESY NMR correlation between the amide NH proton of Val [δ_H: 7.93] and one of the α-amino protons of Gly [H-19b, δ_H: 3.61]. Further, HMBC correlations from H-21 [δ_H: 2.85] and the amide NH proton of Gly [δ_H: 7.50] to C-20 [δ_C: 172.6] led to the assignment of the Gly to HDMD linkage. A ROESY correlation was also observed between these two protons, in full support of this assignment. Finally, the location of the ester linkage was established by the observation of a long-range HMBC coupling from H-22 [δ_H: 4.92] to C-1 [δ_C: 171.0], completing the full planar structure of emericellamide A (**1**).

The relative configurations of the chiral centers in HDMD were determined by *J*-based configurational analysis using $^3J_{HH}$, $^3J_{CH}$, and NOE correlations.[8] Three-bond C−H coupling constants were readily defined in the gHSQMBC experiment[9] in a $CDCl_3-CD_3OD$ (550 μL:150 μL) solvent mixture. Since $^3J_{HH}$ between H-21 and H-22 was measured as 9.5 Hz (in the $CDCl_3-CD_3OD$ mixture solvent), only A and F are feasible configurations among the six possible rotamers shown. On the basis of the strong NOE correlations observed between H-23 and H-30 (1D NOE experiment), only rotamer A can define the relative configuration of the C-21 and C-22 stereogenic centers (Figure 2a). The small coupling constant (2.0 Hz) between H-22 and H-23, which was not definitive, suggested that C−H coupling constant analysis could be useful for configuration analysis. The $^3J_{CH}$ between C-31 and H-22 was large enough (5.5 Hz) to assign the *anti*-conformation between these two nuclei by comparison with model β-hydroxy-carbonyl compounds.[10] The *anti*-relationship between C-31 and H-22 ruled out the rotamers B′, C′, D′, and E′. The strong NOE between H-21 and H-23 allowed the relative configuration to be assigned as in A′ (Figure 2b). The selected rotamer combination assigned, A−A′ (21*R**, 22*R**, and 23*S**), was also supported by strong NOE correlations between H-21 and H-31 and between H-23 and H-30.

Hydrolysis of emericellamide A (**1**) using 6 N HCl yielded the free amino acid units. The hydrolysis products were derivatized using the Marfey reagent and analyzed by LC/MS.[11] Comparison with the retention times of authentic Marfey standards of L- and D-Ala, Val, and Leu showed that these amino acids possess L configurations.

The absolute stereochemistry of the HDMD unit in **1** was determined by Mosher derivatization of the methanolysis product of **1** (Figure 3).[12] Methanolysis of **1** generated the methyl ester alcohol, which was acylated with *R*-(−)- and *S*-(+)-α-methoxy-α-(trifluoromethyl)phenyl acetyl chloride (MTPA-Cl) to furnish the corresponding *S*- and *R*-MTPA esters, respectively. Analysis of ^1H NMR, COSY, and TOCSY spectra allowed the assignment of the proton chemical shifts for both esters in proximity of the esterified carbon. Calculation of $\Delta\delta_{S-R}$ values clearly established the absolute configuration of C-22 as *R*. This allowed the assignment of C-21 and C-23 as *R* and *S*, respectively.

Emericellamide B (**2**) was obtained as a white powder, which analyzed for the molecular formula $C_{34}H_{61}N_5O_7$ by HRESI mass spectrometry (obsd $[M + Na]^+$ at *m*/*z* 674.4468, calcd $[M + Na]^+$ 674.4463). Using the same approach as in the assignment of **1**, the overall structure of compound **2** was determined by interpretation of 1D and 2D NMR spectroscopic data (Table 2). The molecular composition of **2** indicated the addition of C_2H_6 to the formula of **1**. The ^1H NMR spectral data of emericellamide B showed almost identical features to those found for **1**, displaying five amide protons [δ_H: 8.53, 8.51, 8.07, 7.47, 7.35], six α-amino protons [δ_H: 4.25, 4.05, 4.03, 3.97, 3.96, 3.60], and one ester carbinol proton [δ_H: 4.92]. The ^{13}C NMR spectrum of **2**, which illustrated three additional aliphatic resonances, showed that the difference was in the 3-hydroxy acid component.

Table 1. NMR Data for Emericellamide A (**1**) in DMSO-d_6

	position	δ_H[a]	mult (*J* in Hz)	δ_C[b]		key ROESY/NOE[c]	HMBC
L-Ala-1	1			171.0	C		
	2	4.01	m	47.9	CH	H-22, H-24	C-1, C-3, C-4
	3	1.24	d (7.5)	16.0	CH_3		C-1, C-2
	2-NH	8.01	d (3.5)			H-5	
L-Ala-2	4			171.1	C		
	5	4.07	m	47.0	CH	H-22, 2-NH, 19-NH	C-4, C-6
	6	1.21	d (7.5)	18.0	CH_3		C-4, C-5
	5-NH	7.39	d (7.5)			H-8, H-14, H-19a	C-5, C-7
L-Leu	7			170.5	C		
	8	4.05	m	51.5	CH	5-NH, 19-NH	C-7, C-9, C-13
	9	1.55	m	39.1	CH_2		C-7, C-8, C-10
	10	1.55	m	24.2	CH		C-9, C−11, C-12
	11	0.80	d (6.5)	20.4	CH_3		C-9, C-10
	12	0.89	d (6.5)	22.9	CH_3		C-9, C-10
	8-NH	8.08	d (8.0)			H-14	
L-Val	13			170.9	C		
	14	3.97	dd (8.5, 8.5)	59.8	CH	5-NH, 8-NH, 19-NH	C-14, C-15, C-18
	15	1.88	m	29.9	CH		C-13, C-14, C-16, C-17
	16	0.87	d (7.0)	18.5	CH_3		C-14, C-15
	17	0.88	d (7.0)	18.7	CH_3		C-14, C-15
	14-NH	7.93	d (8.5)			H-19	
Gly	18			168.4	C		
	19a	4.30	dd (17.5, 5.5)	42.1	CH_2	H-22, 5-NH, 14-NH	C-18, C-20
	19b	3.61	dd (17.5, 2.5)			14-NH	C-18,
	19-NH	7.50	dd (5.5, 2.5)			H-5, H-8, H-14, H-21	C-20
HDMD	20			172.6	C		
	21	2.85	dq (10.0, 7.0)	40.8	CH	19-NH, H-23, H-30, H-31	C-20, C-22, C-30
	22	4.92	dd (10.0, 2.0)	76.3	CH	H-2, H-5, H-19a, H-23, H-24, H-30	C-1, C-21, C-23, C-31
	23	1.66	m	32.9	CH	H-21, H-22, H-30, H-31	C-24, C-25, C-31
	24a	1.10	m	33.2	CH_2	H-2, H-22	C-22, C-23, C-25
	24b	1.02	m			H-2, H-22	C-23, C-25
	25	1.20	m	26.3	CH_2		
	26	1.20	m	28.6	CH_2		
	27	1.20	m	30.9	CH_2		
	28	1.22	m	21.8	CH_2		C-26, C-27, C-29
	29	0.84	t (7.0)	13.7	CH_3		C-27, C-28
	30	0.90	d (7.0)	14.0	CH_3	H-21, H-22, H-23	C-20, C-21, C-22
	31	0.82	d (7.0)	12.7	CH_3	H-21	C-22, C-23, C-24

[a] Assignment by gHSQC and DEPT NMR methods at 500 MHz. [b]Assignment by gHSQC and DEPT NMR methods at 125 MHz. [c]ROESY and 1D NOE correlations within amino acid units are excluded.

Overall NMR data, including analysis of information from HMQC, COSY, and TOCSY experiments, revealed the same amino acids and sequence as found in **1**. Analysis of ^{1}H NMR COSY and 2D HMBC data allowed the side chain to be assigned as 3-hydroxy-2,4,6-trimethyldodecanoic acid (HTMD). Three bond ^{1}H−^{1}H couplings of H-21 [δ_H: 2.85] with H-32 [δ_H: 0.90] and H-22 [δ_H: 4.92] established the connectivity from H-32 to H-22. A proton NMR COSY correlation between H-23 [δ_H: 1.81] and H-33 [δ_H: 0.82], as well as HMBC NMR correlations from H-33 to C-22 [δ_C: 75.7], C-23 [δ_C: 30.1], and C-24 [δ_C: 40.7], firmly established an identical 3-hydroxy-2,4-dimethyl constellation. In addition, a long-range HMBC coupling from H-34 [δ_H: 0.79] to C-24, C-25 [δ_C: 28.7] and C-26 [δ_C: 36.8] clearly showed that the additional methyl group was positioned at C-25. The remainder of the hydrocarbon chain was readily assigned by continued analysis of COSY, TOCSY, and HMBC data as 3-hydroxy-2,4,6-trimethyldodecanoic acid (HTMD).

The relative configurations of the chiral centers in HTMD at C-21, C-22, and C-23 were proposed as the same in HDMD (21*R**, 22*R**, and 23*S**) in **1**, on the basis of the high degree of similarity of their analogous ^{1}H coupling constants and 1D NOE correlations. Interpretation of 1D NOE experiments allowed the stereochemistry at C-25 to be related to that at C-23 (Figure 4). A key NOE correlation between H-22 and H-25 revealed that the aliphatic chain was folded at C-25. The relative configuration at C-25 was assigned as *R** on the basis of the NOE correlation observed between H-23 and H-34. The alternate configuration (25*S**) cannot be accommodated by this NOE result.

Hydrolysis of emericellamide B was accomplished as in **1**, and the absolute configurations of the amino acid units in **2** were similarly determined by Marfey derivatization and LC/MS analysis. The amino acid residues were two L-Ala's, one L-Val, and one L-Leu as observed in **1**. ROESY NMR experiments with **2** showed identical correlations to those observed in **1** (Figure 5). Specifically, consistent ROESY correlations between H-2 and H-22, and between 19-NH and H-22, were highly indicative of the same absolute configuration at C-22. On the basis of consistent ROESY correlations, the absolute stereochemistry of HTMD was assigned as 21*R*, 22*R*, 23*S*, and 25*S*. The similar optical rotation values observed ($[\alpha]_D = -34$ and -43 for **1** and **2**, respectively) also support this proposal.

Emericellamides A and B appear to be derived from two distinct biosynthetic pathways. The amino acid components are clearly derived by nonribosomal peptide synthetase pathways (NRPS), while the 3-hydroxy acids (HDMD and HTMD) are of an apparent polyketide synthase (PKS) origin. The HDMD unit has previously been reported as a component of a fungal peptide. The relative configurations of the chiral centers in HDMD (emericellamide A) are identical to those in the HDMD component of lipodepsipeptide 15G256γ previously isolated from the marine-derived fungus *Hypoxylon oceanicum*, but the absolute configurations are opposite.[13] The HTMD component of emericellamide B has not been previously reported as a component of a lipopeptide. This chain-extended unit may be derived by the incorporation of an additional methylmalonyl building block (C-24, C-25, and C-34) in the PKS chain elongation process.

Emericellamide A (**1**) displayed moderate antimicrobial activity against methicillin-resistant *Staphylococcus aureus* (MIC: 3.8 μM), but weak cytotoxicity against the HCT-116 human colon carcinoma cell line (IC_{50}: 23 μM), and no significant inhibition of amphot-

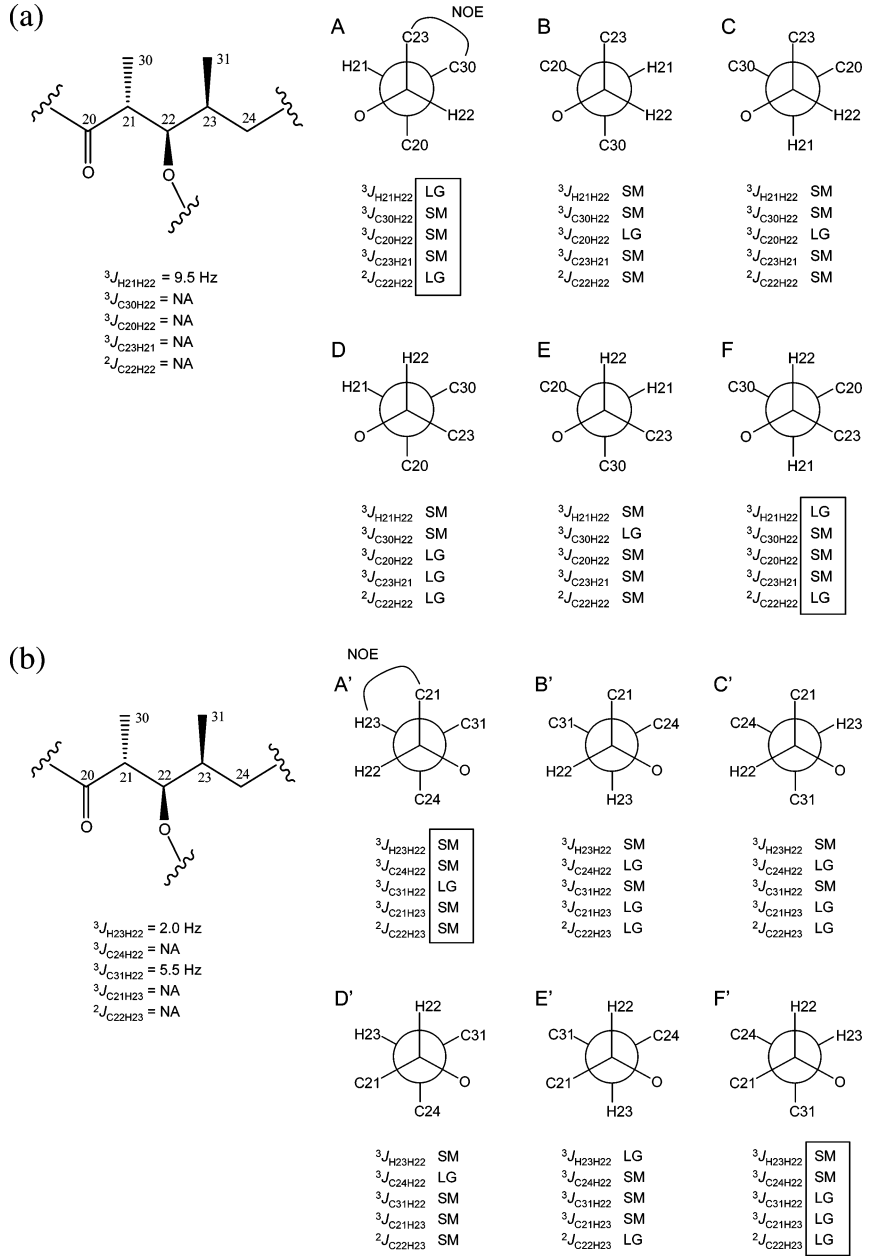


Figure 2. *J*-based configuration analysis for (a) C-21 and C-22 and (b) C-22 and C-23 chiral centers. All possible conformational rotamers are shown.

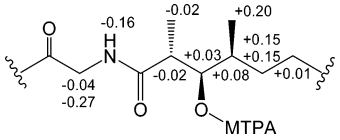


Figure 3. $\Delta\delta_{S-R}$ values in ppm for the C-22 *S*- and *R*-MTPA esters of the methanolysis product of **1** in DMSO-d_6.

ericin-resistant *Candida albicans*. The biological activities of emericellamide B (**2**) were slightly weaker than **1**, showing an MIC value of 6.0 μM against methicillin-resistant *S. aureus* and an IC_{50} against HCT-116 of 40 μM.

This report provides another example of the production of new secondary metabolites during co-culture conditions. While these depsipeptides are, in fact, produced in *very* low yields by *Emericella* sp. (approximately 0.025 mg/L for **1**), these yields would not facilitate their isolation and structure elucidation. In co-culture the yields of these depsipeptides (2.5 mg/L for **1**) were enhanced by 100-fold. It is still unclear how the interaction between the fungus and the actinomycete induces the production of the emericellamides. However, this result underscores that co-culturing represents a potentially important strategy for discovery of new bioactive secondary metabolites.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a Rudolph Research Autopol III polarimeter with a 10 cm cell. IR spectra were recorded using a Perkin-Elmer 1600 FT-IR spectrometer. ^{1}H, ^{13}C, and 2D NMR spectral data were recorded on Varian Inova 500 MHz and Varian Inova 300 MHz NMR spectrometers. High-resolution mass spectroscopic data were acquired on an Agilent ESI-TOF mass spectrometer at The Scripps Research Institute, La Jolla. LC/MS data were obtained using a Hewlett-Packard series 1100 LC/MS system with a reversed-phase C_{18} column (Phenomenex Luna C_{18}(2), 4.6 mm × 100 mm, 5 μm) with CH_3CN-H_2O gradient solvent system.

The Marine-Derived Fungus *Emericella* (CNL-878) and the Marine Actinomycete *Salinispora arenicola* (CNH-665). The marine-derived fungus (strain CNL-878) was isolated from the surface of a green alga of the genus *Halimeda*, which was collected at Madang Bay

Table 2. NMR Data for Emericellamide B (**2**) in DMSO-d_6

	position	δ_H[a]	mult (*J* in Hz)	δ_C[b]		key ROESY/NOE[c]	HMBC
L-Ala-1	1			171.3	C		
	2	3.97	dd (8.5, 7.5)	48.2	CH	H-22, H-25, H-26	C-1, C-3, C-4
	3	1.24	d (7.5)	16.3	CH_3		C-1, C-2
	2-NH	8.07	d (3.5)			H-5	C-4
L-Ala-2	4			171.3	C		
	5	4.03	m	47.0	CH	H-22, 2-NH, 19-NH	C-4, C-6
	6	1.20	d (7.5)	18.4	CH_3		C-4, C-5
	5-NH	7.47	d (7.5)			H-8, H-14, H-19a	C-5, C-7
L-Leu	7			171.0	C		
	8	4.05	m	51.7	CH	5-NH, 19-NH	C-7, C-9, C-13
	9	1.55	m	39.6	CH_2		C-7, C-8, C-10
	10	1.55	m	24.4	CH		C-9, C-11, C-12
	11	0.82	d (6.5)	20.7	CH_3		C−9, C-10
	12	0.86	d (6.5)	23.2	CH_3		C-9, C-10
	8-NH	8.53	d (8.0)			H-14	
L-Val	13			171.2	C		
	14	3.96	m	60.3	CH	5-NH, 8-NH, 19-NH	C-14, C-15, C-18
	15	1.92	m	30.1	CH		C-13, C-14, C-16, C-17
	16	0.86	d (7.0)	18.8	CH_3		C-14, C-15
	17	0.86	d (7.0)	19.0	CH_3		C-14, C-15
	14-NH	8.51	d (8.5)			H-19	
Gly	18			168.6	C		
	19a	4.25	dd (17.5, 5.5)	41.1	CH_2	H-22, 5-NH, 14-NH	C-18, C-20
	19b	3.60	dd (17.5, 2.5)			14-NH	C-18
	19-NH	7.35	dd (5.5, 2.5)			H-5, H-8, H-14, H-21	
HTMD	20			172.7	C		
	21	2.85	dq (10.0, 7.0)	40.0	CH	19-NH, H-23, H-32, H-33	C-20, C-22, C-30
	22	4.92	dd (10.0, 2.0)	75.7	CH	H-2, 19-NH, H-23, H-24, H-25, H-32	C-1, C-21, C-23, C-33
	23	1.81	m	30.1	CH	H-21, H-22, H-32, H-33, H-34	C-24, C-33
	24a	1.10	m	40.7	CH_2	H-22	C-23, C-25
	24b	0.79	m			H-22	
	25	1.53	m	28.7	CH	H-2, H-22	
	26a	1.19	m	36.8	CH_2	H-2	
	26b	1.01	m			H-2	C-27
	27	1.22	m	29.0	CH_2		
	28	1.22	m	26.1	CH_2		
	29	1.22	m	31.3	CH_2		
	30	1.24	m	22.0	CH_2		
	31	0.85	t (7.0)	13.9	CH_3		C-29, C-30
	32	0.90	d (7.0)	14.2	CH_3	H-21, H-22, H-23	C-20, C-21, C-22
	33	0.82	d (7.0)	13.5	CH_3	H-21, H-23	C-22, C-23, C-24
	34	0.79	d (6.5)	19.4	CH_3	H-23, H-25	C-24, C-25, C-26

[a] Assignment by gHMQC NMR method at 500 MHz. [b]Assignment by gHMQC NMR method at 75 MHz. [c]ROESY and 1D NOE correlations within amino acid units are excluded.

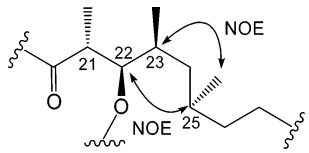


Figure 4. Key NOE correlations used to assign the relative configuration of C-25 of **2** and demonstrate the back-folded conformation of the side chain.

in Papua New Guinea, in 1997. Sequence analysis of the D2 expansion region of the large subunit rRNA gene (Accugenix) placed CNL-878 within the genus *Emericella* on the basis of the 98.9% sequence identity with *Emericella parvathecia*. The marine actinomycete (strain CNH-665) was isolated from a sediment sample collected from the Bahamas in 1999. It was identified as *S. arenicola* on the basis of the 100% 16S rDNA sequence identity with the type strain of this species (NCBI accession number AY040619).

Fermentation and Extraction. Five liters of the fungal strain CNL-878 was cultivated in YPM medium (4 g of mannitol, 2 g of yeast extract, 2 g of peptone in 1 L of seawater, in a 2.8 L Fernbach flask) at 27 °C with shaking at 215 rpm. The actinomycete (1 L) was cultured in the same manner as the fungus but in TCG medium (3 g of tryptone, 5 g of casitone, 4 g of glucose in 1 L of seawater). On day 3, 10 mL of actinomycete culture was inoculated into each 1 L of the fungal cultures to initiate the co-culture experiment. The co-culture and the single cultures of the fungus and the actinomycete were analyzed by LC/MS to identify changes of secondary metabolite production. For LC/MS monitoring, 25 mL of each culture was extracted with EtOAc.

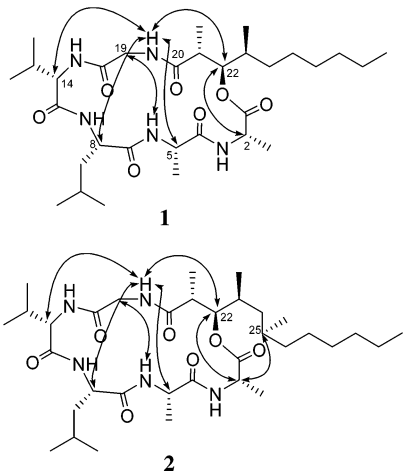


Figure 5. Transannular ROESY correlations observed in **1** and **2**.

After incubation for two additional days, the co-culture was extracted using a solid-phase resin extraction with Amberlite XAD-7 resin (20 g/L). The culture and resin were shaken at 215 rpm for 2 h, and the resin was filtered and extracted with acetone to yield 1.9 g of solid material after removal of solvents *in vacuo*.

Isolation of Emericellamides A and B (1, 2). The crude extract was fractionated by Si gel vacuum column chromatography (4.5 cm

diameter, 8 cm height, 200−425 mesh), eluting with EtOAc−MeOH mixtures. Emericellamides A and B were observed in the fractions eluted with 10:1 and 5:1 EtOAc−MeOH mixtures. These fractions were further purified by RP C_{18} HPLC with 65% aqueous CH_3CN (Alltech Altima C_{18} semipreparative column, 5 μm, 10 mm × 250 mm, 2 mL/min, refractive index detection). Emericellamides A (12.5 mg) and B (3.8 mg) were eluted at 17 and 39 min, respectively.

Emericellamide A (1): white powder; $[\alpha]_D$ −43 (*c* 0.23, MeOH); IR (neat, $CHCl_3$) ν_{max} 3307, 2931, 1755, 1631, 1549 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) and ^{13}C NMR (125 MHz, DMSO-d_6), see Table 1; HRESI $[M + Na]^+$ *m*/*z* 632.4173.

Emericellamide B (2): white powder; $[\alpha]_D$ −34 (*c* 0.076, MeOH); IR (neat, $CHCl_3$) ν_{max} 3310, 2934, 1754, 1630, 1550 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) and ^{13}C NMR (75 MHz, DMSO-d_6), see Table 2; HRESI $[M + Na]^+$ *m*/*z* 674.4468.

Stereochemical Determination of Amino Acid Residues. Hydrolysis of emericellamides A and B (0.8 mg each) was achieved, in separate experiments, by the addition of 1 mL of 6 N HCl at 115 °C for 18 h. The solution was concentrated under a stream of N_2. The residue was resuspended in 1 mL of H_2O and dried under a stream of N_2 twice to remove traces of HCl. The resulting hydrolysate was dissolved in 1 N $NaHCO_3$ (100 μL) and treated with 1-fluoro-2,4-dinitrophenyl-5-L-alanine amide (L-Marfey reagent) in acetone (10 mg/mL, 50 μL). The reaction was incubated at 80 °C for 3 min, then neutralized by adding 2 N HCl (50 μL), and diluted with 50% aqueous CH_3CN (300 μL). The Marfey derivative mixture was analyzed by LC/MS (Hewlett-Packard Series 1100, Phenomenex Luna C_{18}(2), 4.6 mm × 100 mm, 5 μm) with a linear gradient from 10% to 50% aqueous CH_3CN (0.1% TFA) over 45 min. The retention times of the derivatives were compared with those of authentic derivatized standards (L-Ala: 29.9 min, D-Ala: 32.6 min, L-Val: 36.1 min, D-Val: 40.2 min, L-Leu: 40.9 min, and D-Leu: 44.7 min), which showed that the two alanine, the valine, and the leucine amino acids of emericellamides A and B possessed L configurations.

Methanolysis and Mosher Derivatization of 1. Emericellamide A (**1**) (2.0 mg) was dissolved in 0.5 N NaOMe solution (2 mL of MeOH) and stirred at room temperature (RT) for 5 h. The mixture was neutralized by adding 1 N HCl. The volume of the solution was reduced *in vacuo*. The mixture was partitioned with H_2O and EtOAc, the EtOAc fraction was dried *in vacuo*, and the methanolysis product obtained was confirmed by LC/MS ($[M + H]^+$ *m*/*z* at 642, $[M + Na]^+$ at 664, molecular formula $C_{32}H_{59}N_5O_8$). The methanolysis product of **1** (0.5 mg, 0.8 μmol) was dissolved in 1.5 mL of freshly distilled, dry CH_2Cl_2, 2 drops of distilled triethylamine were added to the solution, and it was stirred for 5 min. A dry crystal of dimethylaminopyridine was added to the reaction vial, the solution was stirred at RT for 30 min, and then 20 μL of *R*-methoxy(trifluoromethyl)phenylacetyl chloride solution (5.36 μmol/μL) was added. Freshly distilled pyridine (500 μL) was added 1 h later, and the mixture was stirred at RT overnight. After removal of reactants *in vacuo*, the Mosher ester obtained was purified by RP HPLC (Waters Prep 4000, Alltech, Altima C_{18}, 10.0 mm × 250 mm, 5 μm, 2.0 mL/min, UV detection at 210 nm) using a gradient solvent system (0−10 min: 20% aqueous CH_3CN, 10−50 min: 20−100% aqueous CH_3CN, 50−70 min: 100% CH_3CN). The *S*-MTPA ester eluted at 61 min. The identical procedure was carried out to obtain the *R*-MTPA ester with *S*-methoxy(trifluoromethyl)phenylacetyl chloride. The unit masses of *R*- and *S*-MTPA esters ($[M + H]^+$ *m*/*z* = 858, $[M + Na]^+$ *m*/*z* = 880, molecular formula $C_{42}H_{66}F_3N_5O_{10}$) were identified by LC/MS analysis (ESIMS).

Acknowledgment. This research is a result of financial support from the National Cancer Institute under grant CA44848. D.-C.O. thanks Dr. T. S. Bugni for his generous assistance with the gHSQMBC NMR experiment.

Supporting Information Available: The ^1H, ^{13}C, DEPT, gCOSY, TOCSY, ROESY, gHSQC, and gHMBC spectra of **1** and ^1H, ^{13}C, gCOSY, TOCSY, ROESY, gHMQC, ad gHMBC spectra of **2**, ^1H chemical shift assignments for the MTPA derivatives of the methanolysis product of **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Butler, M. S. *J. Nat. Prod.* **2004**, *67*, 2141−2153.
(2) Poulev, A.; O'Neal, J. M.; Logendra, S.; Pouleva, R. B.; Timeva, V.; Garvey, A. S.; Doloressa, G.; Jenkins, I. S.; Halpern, B. T.; Kneer, R.; Cragg, G. M.; Raskin, I. *J. Med. Chem.* **2003**, *46*, 2542−2547.
(3) Patterson, G. M. L.; Bolis, C. M. *J. Phycol.* **1997**, *33*, 54−60.
(4) Christian, O. E.; Compton, J.; Christian, K. R.; Mooberry, S. L.; Valeriote, F. A.; Crews, P. *J. Nat. Prod.* **2005**, *68*, 1592−1597.
(5) (a) Mearns-Spragg, A.; Bregu, M.; Boyd, K. G.; Burgess, J. G. *Lett. Appl. Microbiol.* **1998**, *27*, 142−146. (b) Burgess, J. G.; Jordan, E. M.; Bregu, M.; Mearns-Spragg, A.; Boyd, K. G. *J. Biotechnol.* **1999**, *70*, 27−32. (c) Ueda, K.; Kwai, S.; Ogawa, H.-O.; Kiyama, A.; Kubota, T.; Kwanobe, H.; Beppu, T. *J. Antibiot.* **2000**, *53*, 979−982.
(6) Cueto, M.; Jensen, P. R.; Kauffman, C.; Fenical, W.; Lobkovsky, E.; Clardy, J. *J. Nat. Prod.* **2001**, *64*, 1444−1446.
(7) Oh, D.-C.; Jensen, P. R.; Kauffman, C. A.; Fenical, W. *Bioorg. Med. Chem.* **2005**, *13*, 5627−5673.
(8) Matsumori, N.; Kaneno, D.; Murata, M.; Nakamura, H.; Tachibana, K. *J. Org. Chem.* **1999**, *64*, 866−876.
(9) Williamson, R. T.; Marquez, B. L.; Gerwick, W. H.; Kover, K. E. *Magn. Reson. Chem.* **2000**, *38*, 265−273.
(10) (a) Furihata, K.; Seto, H. *Tetrahedron Lett.* **1999**, *40*, 6271−6275. (b) Williamson, R. T.; Marquez, B. L.; Sosa, A. C. B.; Koehn, F. E. *Magn. Reson. Chem.* **2003**, *41*, 379−385.
(11) Marfey, P. *Carlsberg Res. Commun.* **1984**, *49*, 591−596.
(12) Séco, J. M.; Quiñoa, E.; Riguera, R. *Tetrahedron: Asymmetry* **2001**, *12*, 2915−2925.
(13) Schlingmann, G.; Milne, L.; Williams, D. R.; Carter, G. T. *J. Antibiot.* **1998**, *51*, 303−322.

NP060381F