Published in final edited form as:
J Nat Prod. 2011 May 27; 74(5): 928–936. doi:10.1021/np200077f.

Cytotoxic Veraguamides, Alkynyl Bromide-containing Cyclic Depsipeptides from the Marine Cyanobacterium cf. *Oscillatoria margaritifera*

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Abstract

A family of cancer cell cytotoxic cyclodepsipeptides, veraguamides A-C (**1**-**3**) and H-L (**4**-**8**), were isolated from a collection of cf. *Oscillatoria margaritifera* obtained from the Coiba National Park, Panama as part of the Panama International Cooperation Biodiversity Group (ICBG) program. The planar structure of veraguamide A (**1**) was deduced by 2D NMR spectroscopy and mass spectrometry whereas the structures of **2-8** were mainly determined by a combination of ^{1}H NMR and MS^{2}/MS^{3} techniques. These new compounds are analogous to the mollusk-derived kulomo'opunalide natural products, with two of the veraguamides (C and H) containing the same terminal alkyne moiety. However, four veraguamides, A, B, K and L, also feature an alkynyl bromide, a functionality that has only been previously observed in one other marine natural product, jamaicamide A. Veraguamide A showed potent cytotoxicity to the H-460 human lung cancer cell line (LD_{50} = 141 nM).

Marine cyanobacteria are exceptionally prolific producers of structurally diverse secondary metabolites, of which many have intriguing biological properties.[1] An emerging biosynthetic theme in cyanobacterial natural products is the frequent combination of polyketide synthase (PKS) and non-ribosomal peptide synthetase (NRPS) derived portions, and this results in a highly diverse suite of nitrogen-rich structural frameworks, most of which are lipid soluble.[2] A number of these cyanobacterial metabolites possess terminal

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Supporting Information Available: ^{1}H NMR, ^{13}C NMR, COSY, TOCSY, NOESY, HSQC and HMBC spectra in $CDCl_3$ for veraguamide A (**1**). ^{1}H NMR spectra in $CDCl_3$ for veraguamides B (**2**) and K (**3**). ^{1}H NMR, TOCSY, HSQC and HMBC spectra in $CDCl_3$ for veraguamide L (**8**). MS^{2} chromatograms for veraguamides A-C and H-L (**1**-**8**). MS^{2}/MS^{3} algorithm results for veraguamides BC and H-I. Biological assay results for veragumide A. Morphological description of the veraguamide producer. This material is available free of charge via the Internet at http://pubs.acs.org.

alkyne functionalites in the PKS-derived sections, including carmabin A,[3] georgamide,[4] pitipeptolide A,[5] yanucamides,[6] antanapeptin,[7] trungapeptin A,[8] hantupeptin,[9] wewakpeptins,[10] dragonamide,[11] and viridamide A.[12] Similar metabolites have also been obtained from several species of mollusks, namely, *Onchidium* sp. and *Dolabella auricularia*, yielding onchidins A[13a] and B[13b] and a family of kulolides,[14] respectively. Due to the strong and distinctive similarity between these secondary metabolites isolated from mollusks and those of cyanobacterial origin, it is highly likely that the mollusks obtain these compounds from their diet of cyanobacteria.

Since 1998, the International Cooperative Biodiversity Group (ICBG) in Panama, a program of the Fogarty International Center of the National Institutes of Health, has enabled unique opportunities to conduct integrated natural products investigations, biodiversity inventories and conservation, infrastructure development, and educational training.[15] Moreover, the country of Panama permits the study of marine cyanobacteria from two very different tropical environments, the Caribbean Sea in the Western Atlantic, and the Eastern Pacific. Some of these sites are quite pristine and of exceptional biodiversity, such as the Coiba National Park (CNP), some 15 kilometers off the Pacific coast of Panama. The CNP was formed in 2003 as a result of a developing recognition of its high number of indigenous and endemic plant, animal and microbial species, and in 2005 it was named a World Heritage Site by UNESCO.[16]

Several filamentous tuft-forming species of marine cyanobacteria were collected from the CNP in 2010, and their extracts evaluated in a number of biological assays. Two reduced complexity fractions from one extract, subsequently tentatively identified as *Oscillatoria margaritifera*, were found to be highly cytotoxic to H-460 human lung cancer cells in vitro (2% survival at 3 μg/mL), and these were chosen for further investigation. As a result of a bioassay-guided fractionation process, one major and several minor new cytotoxic lipopeptides were isolated and structurally defined. The major compound, named veraguamide A (**1**) (the CNP lies within the Panamanian state of Veraguas),[17] was highly cytotoxic to H-460 cells (LD_{50} = 141 nM); the minor compounds were all of lesser potency to this cancer cell line. As described below, the structure of **1** was fully characterized, including the absolute configuration at all chiral centers, whereas the planar structures of the minor compounds were largely determined by integrated ^{1}H NMR and MS^2/MS^3 analysis. Additionally, a new iteration of a recently developed computer algorithm was applied to the MS^2/MS^3 data and allowed deduction of the structures of the minor metabolites.[18]

During the final stages of this project, a parallel effort in the Luesch and Paul laboratories in Florida found several of the same compounds (veraguamides A, B, and C) (**1**-**3**) as well as several new derivatives from an Atlantic collection, and these form the substance of a parallel report.[19] It is interesting and potentially insightful to the origin and evolution of the genetic pathways responsible for veraguamide biosynthesis that these same distinctive metabolites have been isolated from cyanobacteria collected from these two well-separated oceans.

Results and Discussion

The tentatively identified cyanobacterium *O. margaritifera* was collected by hand from shallow waters (1-5 m deep) in the CNP, Panama, in February 2010. The ethanol-preserved collection was repetitively extracted (CH_2Cl_2-MeOH, 2:1) and fractionated using normal-phase vacuum liquid chromatography (VLC). Two fractions that eluted with 100% EtOAc and 75% EtOAc/MeOH were cytotoxic to H-460 human lung cancer cells (both exhibiting 2% survival at 3 μg/mL). Further fractionation with reversed-phase solid-phase extraction

(SPE) yielded 2.3 mg of veraguamide A (**1**), a pure amorphous solid, and between 0.1 to 0.5 mg of several analogues, veraguamides B, C, and H-L (**2**-**8**).

HRESIMS of **1** gave a $[M+H]^+$ at *m/z* 767.3594 as well as peaks for the $[M+Na]^+$ and $[M+K]^+$ adducts at *m/z* 789.3405 and 805.3148, respectively, indicating a molecular formula of $C_{37}H_{59}N_4O_8Br$ and requiring 10 degrees of unsaturation. IR spectroscopy suggested a peptide with a strong absorption band at 1763 cm^{-1}, and this was supported by observation of six ester or amide type carbonyls by ^{13}C NMR analysis (δ_C 173.5, 172.2, 170.9, 170.7, 169.7, and 166.0). The ^1H NMR spectrum also suggested a peptide with one amide (NH) proton resonating at δ_H 6.28 and two *N*-methyl groups at δ_H 3.01 and 2.95. The ^{13}C NMR spectrum also revealed the presence of an unusually polarized alkyne functionality (δ_C 79.4 and 38.4), accounting for a further 2 degrees of unsaturation. Thus, 8 of the 10 degrees of unsaturation were explained, and indicated that veraguamide A must possess two rings.

Analysis of 1D and 2D NMR spectra (COSY, TOCSY, ROESY, HSQC and HMBC) led to the identifications of four amino acids [one valine (Val), two *N*-methyl-valines (*N*-MeVal) and one proline (Pro)], one hydroxy acid [2-hydroxy-3-methylpentanoic acid (Hmpa)] and one extended chain polyketide. The proton chemical shifts of the Hmpa residue were very similar to those reported for isoleucine, however, the carbon chemical shift for the α-carbon was significantly downfield (δ_C 76.1), consistent with a hydroxy acid. The identity of the extended polyketide residue was deduced from a combination of COSY and HMBC correlations. A $CHCH_3$ constellation formed one spin system, and a deshielded methine adjacent to three sequential methylene residues formed a second spin system. By HMBC, the two methine centers were found to be adjacent, and thus a nearly 90° angle must exist between their proton substituents. HMBC between the H-30 methine, as well as its attached secondary methyl group (H_3-37), and an amide-type carbonyl at δ 170.9 completed one terminus of this residue. At the other end, HMBC cross peaks were observed between the methylene protons H-34a/H-34b and both carbons C-35 and C-36, whereas methylene protons H-33a and H-33b showed only correlations with C-36. The chemical shift of the distal carbon of the alkyne was quite unusual (δ_C 38.4), but matched quite well with that reported for the alkynyl bromide present in jamaicamide A, the only other marine natural product reported with the this functionality.[20] Thus, this last residue in veraguamide A (**1**) was identified as a derivative of 8-bromo-3-hydroxy-2-methyloct-7-ynoic acid (Br-Hmoya).

As the proline residue accounted for one additional degree of unsaturation, the tenth and final degree of unsaturation must arise from veraguamide A (**1**) having an overall cyclic constitution; this was apparent from the residue connectivities observed by HMBC and ROESY (Table 1). HMBC correlations from the two *N*-Me groups and the NH to their respective adjacent carbonyls and α-carbons were used to connect three of the residues in veraguamide A. A correlation from the α-hydroxy proton of the Br-Hmoya residue (H-31) to the carbonyl of *N*-MeVal-1 (C-1) served to connect these two residues. Similarly, the Hmpa and *N*-MeVal-2 residues were connected by a HMBC cross peak from the α-hydroxy proton of the Hmpa residue (H-13) to the C-18 carbonyl of the *N*-MeVal-2 residue. Finally, a ROESY correlation was used to make the concluding connection between the Pro and Hmpa residues. Thus, veraguamide A was deduced to have a cyclo-[*N*-MeVal – Pro – Hmpa - *N*-MeVal – Val – Br-Hmoya] structure.

The absolute configuration of the four α-amino acids in veraguamide A (**1**) were determined by LC-MS analysis of the acid hydrolysate appropriately derivatized with Marfey's reagent (DFDAA). The six standards, L-Pro, D-Pro, L-Val, D-Val, L-*N*-MeVal, and D,L-*N*-MeVal were also reacted with D-FDAA and compared to the derivatized hydrolysate by LC-MS. From the retention times and co-injections it was clear that all four of the amino acids, Pro, Val and

two *N*-MeVal residues, were of the L configuration (see Experimental Section and Supporting Information).

The absolute configuration of the Hmpa residue was determined by comparing the GC-MS retention time of the methylated residue liberated by acid hydrolysis with authentic standards. The four standards, L-*allo*-Hmpa, L-Hmpa, D-*allo*-Hmpa, and D-Hmpa, were synthesized from L-*allo*-Ile, L-Ile, D-*allo*-Ile, and D-Ile, respectively, following literature procedures.[21] The four standards each possessed distinctly different retention times by GC-MS [44.86 (L-*allo*-Hmpa), 45.06 (D-*allo*-Hmpa), 45.26 (D-Hmpa), and 45.63 min (L-Hmpa)]. The methylated residue from the acid hydrolysate gave a single peak at 45.63 min, thus indicating its configuration as LHmpa.

To determine the absolute configuration of the Br-Hmoya residue, compound **1** was hydrogenated with 10% Pd/C to remove simultaneously the bromine atom and fully reduce the terminal alkyne functionality. This hydrogenation product was then hydrolyzed with 6 N HCl in a microwave reactor to yield the free residues. An aliquot of the methylated hydrolysate was treated with the *S*-Mosher's acid chloride [*S*-(+)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride, *S*-(+)-MTPA-Cl) and compared to four synthetic standards, as described below. Two core standards, 2*S*,3*S*-Hmoaa and 2*S*,3*R*-Hmoaa, were synthesized using a published procedure.[13b] To create the four chromatographic standards, 2*S*,3*S*-Hmoaa and 2*S*,3*R*-Hmoaa were each separately treated with *S*-MTPA-Cl and *R*-MTPA-Cl, yielding four diastereomeric compounds. These four standards were then compared to the *S*-MTPA-Cl derivatized hydrolysate of veraguamide A (**1**). Two of the standards (2*S*,3*S*-Hmoaa and 2*S*,3*R*-Hmoaa reacted with *S*-MTPA-Cl) are each identical to a possible configuration of the natural residue, whereas the other two standards (2*S*,3*S*-Hmoaa and 2*S*,3*R*-Hmoaa reacted with *R*-MTPA-Cl) are enantiomeric to the other two possible configurations of the natural residue (2*R*,3*R*-Hmoaa and 2*R*,3*S*-Hmoaa, respectively). A GC-MS instrument equipped with a DB5-MS column was then used to compare the retention times of the four diastereomeric standards with the derivatized hydrolysate. The retention time of the hydrolysate product (47.13 min) matched 2*S*,3*R*-Hmoaa that was reacted with *S*-MTPA-Cl, identifying that the absolute configuration of the Hmoya residue in **1** is 30*S*,31*R*. In summary, the above experiments established that veraguamide A (**1**) has 2*S*, 8*S*, 13*S*, 14*S*, 19*S*, 25S, 30*S* and 31*R* absolute configuration.

Several analogues of compound **1** were isolated from the more polar chromatographic fraction (eluted with 75% EtOAc/MeOH) of the crude extract. Because these analogues were obtained in quite small yield (0.1-0.5 mg), we were motivated to examine their structures using a newly reported computer analysis of MS^2/MS^3 data obtained for cyclic peptides.[18] Additionally, because ^1H NMR analysis of several of these analogues showed them to be similar in overall structure to veraguamide A (**1**), the position of structural modifications could be determined based on mass shifts in characteristic fragments. With the structure of **1** rigorously determined by a full spectrum of spectroscopic and chemical techniques, it was possible to use this parent structure to determine the characteristic fragmentation pattern for this family of metabolites. Thus, by both a manual comparison of MS^2 fragmentation pattern for each of the analogues to that of **1**, and by application of this newly developed computer algorithm for cyclic peptides, the location and nature of the structural modifications to the veraguamide A (**1**) parent structure were determined readily. In most cases, confirmatory ^1H NMR data were also obtained.

Compound **2** was isolated as a slightly more polar secondary metabolite in approximately 0.3 mg yield, and by HRESIMS indicated a molecular formula of $C_{36}H_{57}N_4O_8Br$. This mass is 14 Da less than that of veraguamide A (**1**), and thus, veraguamide B (**2**) possesses one fewer fully saturated carbon atom. Consistent with this observation, ^1H NMR analysis

showed a nearly identical spectrum as obtained for veraguamide A with only small differences observed in the high field methyl and methylene regions. To localize this mass offset, the MS ion dataset tree for **2**, containing both MS^2 and a series of MS^3 spectra, were subjected to the comparative dereplication algorithm.[18] This algorithm compares the MS dataset to the Norine database plus any user inputted sequences (such as veraguamide A); as expected, **1** was the top hit with the 14 Da difference located to the Hmpa residue.[22] To verify this assignment, the MS^2 spectra for compounds **1** and **2** were compared manually, and this also indicated that the 14 Da structural difference was present in the Hmpa residue (Figure 2). Thus, the Hmpa residue in veraguamide A (**1**) was replaced by a 2-hydroxy-3-methyl-butanoic acid (Hmba) residue in veraguamide B (**2**). Due to the small amount of compound obtained, and the desire to explore the biological properties of these veraguamide A analogues (discussed below), the absolute configuration was not established for compound **2**, but we speculate that it is likely identical to that of veraguamide A (**1**).

In a similar fashion, the structures for compounds **3**, **4**, **5**, and **6** were also determined, with each possessing only a single modified residue in comparison with either veraguamide A (**1**) or veraguamide B (**2**). Veraguamides C (**3**) and H (**4**) were found to be analogues of compounds **1** and **2**, respectively; however, they lacked the alkynyl bromine atom but retained the alkyne functionality. Veraguamides I (**5**) and J (**6**) also proved to be analogues of compounds **1** and **2**, respectively; in this case they lack both the bromine atom as well as the alkynyl functionality in the polyketide section of the molecule. Again, due to the low yields of compounds **3-6**, their absolute configurations were not determined experimentally; it may be that they are the same as veraguamide A (**1**).

Two additional veraguamides, K (**7**) and L (**8**), were isolated from the more polar and biologically active VLC fraction; however, their structures could not be determined by the MS^2/MS^3 method because the algorithm is currently designed specifically for the analysis of cyclic peptides. Additional development of the algorithm is underway to expand its ability to distinguish between linear and cyclic peptides using mass spectrometry data, as this is a longstanding problem in the proteomics and peptidomics fields. Nevertheless, using 600 MHz cryoprobe NMR it was possible to obtain a nearly complete 2D NMR data set for **8** (HSQC, HMBC, and TOCSY). Additionally, HRESIMS of **8** gave **a** $[M+Na]^+$ peak at *m/z* 821.3673, indicating a molecular formula of $C_{38}H_{63}N_4O_9Br$ (9 degrees of unsaturation), differing from veraguamide B (**2**) by C_2H_6O and one less degree of unsaturation. ^{13}C NMR shifts were deduced by a combination of HMBC and HSQC data, and revealed the presence of six ester- or amide-type carbonyls (δ_C 176.0, 172.5, 172.5, 171.0, 170.0, 166.7) and an alkynyl bromide (δ_C 79.7 and 38.0), accounting for 8 degrees of unsaturation. As detailed below, a proline in **8** accounted for the ninth and final degree of unsaturation in veraguamide L, signifying that **8** is a linear depsipeptide.

The NMR spectra of veraguamide L (**8**) possessed similar ^1H and ^{13}C NMR shifts to most of the resonances present for veraguamide A (**1**). Analysis of the 1D and 2D NMR spectra led to the assignments of four amino acids [valine (Val), two *N*-methyl-valines (*N*-MeVal) and proline (Pro)] as well as one hydroxy acid [2-hydroxy-3-methylbutyric acid (Hmba)] and 8-bromo-3-hydroxy-2-methyloct-7-ynoic acid (Br-Hmoya). In addition, HMBC correlations were observed from a deshielded methylene (δ_H 4.15) to both a methyl carbon (δ_C 14.3) and a carbonyl (δ_C 171.0), features not observed for compound **1**. By TOCSY, this same deshielded methylene was directly adjacent to the new methyl group, thus defining an ethyl ester at the carboxylic acid terminus of veraguamide L (**8**). Subsequently, comparison of the MS^2 data for compounds **7** and **8** revealed that the only difference between these two compounds is in the hydroxy acid residue. In **7**, this residue is Hmpa (comparable to **1**) while in **8** it is Hmba (comparable to **2**). At this point, we are uncertain if veraguamide K (**7**)

and L (**8**) are artifacts of the preservation of the original sample in ethanol, or if they represent true natural products of the cyanobacterium.

Only compounds **1**, **2**, **3**, **7**, and **8** were available in sufficient quantity for evaluation in the H-460 cytotoxicity assay. Compound **1** showed potent activity (LD_{50} = 141 nM), while compounds **2**, **3**, **7**, and **8** all exhibited activity in the low micromolar range, but due to insufficient quantities, no further evaluation of these analogues was possible. However, two structural analogues of veraguamide A, kulomo'opunalide-1 and -2, have similar or identical NRPS portions of the molecule but lack the alkynyl bromide in the PKS portion. These two compounds were previously tested against P388 cells, but were reported to exhibit only moderate cytotoxicity,[14] suggesting that the alkynyl bromide may be an essential structural feature for the potent cytotoxic activity observed for veraguamide A (**1**).

Taxonomy of the Veraguamide-producing Strain

A taxonomic investigation of the veraguamide-producing cyanobacterium (PAC-17-FEB-10-2) showed that the morphology agreed relatively well with the current definition of *Oscillatoria margaritifera* (for morphological description, see Supporting Information).[23] *O. margaritifera* was described initially from brackish and marine environments of northern Europe,[23] which makes it geographically and environmentally unlikely that tropical marine PAC-17-FEB-10-2 would belong to the same taxon.[24] Moreover, specimens of *Oscillatoria* have overlapping morphological characters with the genus *Lyngbya*,[25] and phylogenetic analysis is therefore essential to delineate these morphologically similar but evolutionarily unrelated genera.[26] Phylogenetic inferences of the SSU (16S) rRNA gene of PAC-17-FEB-10-2 revealed that this strain nested within the *Oscillatoria* lineage with *O. sancta* PCC 7515 as the closest related reference strain.[24] However, the *Oscillatoria* lineage forms two distinct sister clades, one temperate *sensu stricto* (including PCC 7515) and one tropical marine (including PAC-17-FEB-10-2). The DNA bar-coding gap between the two clades was 4.2 (mean p-distance: inter-clade = 2.3 %; intra-clade = 0.6 %), which may support the separation of temperate and tropical marine *Oscillatoria* into two distinct genera. However, because such a revision in the taxonomy of tropical marine *Oscillatoria* has not yet occurred, at the present time the best taxonomic definition of the veraguamide-producing strain PAC-17-FEB-10-2 is cf. *Oscillatoria margaritifera*.

Experimental Section

General Experimental Procedures

Optical rotations were measured on a JASCO P-2000 polarimeter, UV spectra on a Beckman Coulter DU-800 spectrophotometer, and IR spectra were obtained using a Nicolet IR-100 FT-IR spectrophotometer using KBr plates. NMR spectra were recorded with chloroform as internal standard (δ_C 77.0, δ_H 7.26) on a Varian Unity 500 MHz spectrometer (500 and 125 MHz for ^1H and ^{13}C NMR, respectively), on a Varian VNMRS (Varian NMR System) 500 MHz spectrometer equipped with a Cold Probe (500 and 125 MHz for ^1H and ^{13}C NMR, respectively). Also used were a Bruker 600 MHz spectrometer equipped with a 1.7 mm MicroCryoProbe (600 and 150 MHz for ^1H and ^{13}C NMR, respectively) and a JOEL 500 MHz spectrometer (500 and 125 MHz for ^1H and ^{13}C NMR, respectively). LR- and HRESIMS were obtained on a ThermoFinnigan LCQ Advantage Max mass detector and Thermo Scientific LTQ Orbitrap-XL mass spectrometer, respectively. MS^2/MS^3 spectra were obtained on Biversa Nanomate with nanoelectrospray ionization on a ThermoFinnigan LTQ-MS which utilized Tune Plus software version 1.0. HPLC was carried out using a Waters 515 pump system with a Waters 996 PDA detector. All solvents were either distilled or of HPLC quality. Acid hydrolysis was performed using a Biotage (Initiator) microwave reactor equipped with high pressure vessels.

Cyanobacterial Collections and Morphological Identification

The veraguamide-producing cyanobacterium PAC-17-FEB-10-2 was collected by hand using snorkel gear in shallow water off Isla Canales de Afuera on the Pacific coast of Panama (7°41.617′N, 81°38.379′E). Morphological characterization was performed using an Olympus IX51 epifluorescent microscope (1000X) equipped with an Olympus U-CMAD3 camera. Morphological comparison and putative taxonomic identification of the cyanobacterial specimen was performed in accordance with modern classification systems.[25,27]

Extraction and Isolation

The cyanobacterial biomass (9.75 g, dry wt) was extracted with 2:1 CH_2Cl_2-CH_3OH to afford 1.8 g of dried extract. A portion of the extract was fractionated by silica gel VLC using a stepwise gradient solvent system of increasing polarity starting from 100% hexanes to 100% MeOH (nine fractions, A-I). The two fractions eluting with 100% EtOAc (fraction G) and 75% EtOAc in MeOH (fraction H) were separated further using RP SPE [500 mg SPE, stepwise gradient solvent system of decreasing polarity starting with 20% CH_3CN in H_2O to 100% CH_2Cl_2, to produce four fractions (1-4) each] to yield pure veraguamide A (**1**). Further fractionation by RP HPLC using a Phenomenex 4 μm Synergi Fusion analytical column, with a gradient from 50% CH_3CN/H_2O to 100% CH_3CN over 30 min, yielded pure veraguamides B, C and K-L (**2**-**8**).

Veraguamide A (1)

amorphous solid; $[\alpha]^{22}_D$ -14.7 (*c* 0.33, CH_2Cl_2); UV (MeCN) λ_{max} (log ε) 204 (4.00), 266 (2.83) nm; IR (neat) ν_{max} 3327, 2964, 2930, 1734, 1700, 1456, 1272, 1194, 1128 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (500 MHz, $CDCl_3$), see Table 1; ESIMS/MS *m/z* 741.26 ($C_{36}H_{61}N_4O_7{}^{80}Br$), 654.13 ($C_{31}H_{50}N_3O_7{}^{78}Br$), 574.13 ($C_{26}H_{44}N_3O_6{}^{80}Br$), 542.20 ($C_{25}H_{40}N_3O_5{}^{80}Br$), 463.20 ($C_{20}H_{35}N_2O_5{}^{80}Br$), 456.26 ($C_{23}H_{42}N_3O_6$), 438.24 ($C_{23}H_{40}N_3O_5$), 343.17 ($C_{17}H_{31}N_2O_5$), 325.12 ($C_{17}H_{29}N_2O_4$), 297.19 ($C_{16}H_{29}N_2O_3$), 228.12 ($C_{12}H_{22}NO_3$); HRESIMS $[M+H]^+$ *m/z* 767.3594 (calcd for $C_{37}H_{61}N_4O_8{}^{78}Br$ 767.3594).

Veraguamide B (2)

amorphous solid; $[\alpha]^{23}_D$ -13.1 (*c* 0.25, CH_2Cl_2); ^1H NMR (600 MHz, $CDCl_3$) δ 0.90 (3 H, d, *J* = 6.7 Hz), 0.94 (3H, d, *J* = 7.3 Hz), 0.95 (3H, d, *J* = 6.7 Hz), 0.96 (3H, d, *J* = 5.8 Hz), 1.00 (3H, d, *J* = 7.6 Hz), 1.01 (3H, d, *J* = 7.3 Hz), 1.04 (3H, d, *J* = 6.7 Hz), 1.12 (3H, d, *J* = 6.7 Hz), 1.27 (3H, d, *J* = 4.7 Hz), 1.27 (1H, m), 1.45 (1H, m) , 1.81 (2H, m), 1.97-2.12 (4H, m), 2.14-2.39 (5H, m), 2.96 (3H, s), 3.02 (3H, s), 3.14 (1H, m), 3.62 (1H, q, *J* = 7.4 Hz), 3.81 (1H, q, *J* = 7.4 Hz), 3.95 (1H, d, *J* = 10.3 Hz), 4.16 (1H, d, *J* = 9.3 Hz), 4.73 (1H t, *J* = 6.2 Hz), 4.86 (2H, d, *J* = 8.1 Hz), 4.96 (1H, t, *J* = 6.1 Hz), 6.27 (1H, d, *J* = 8.2 Hz); ESIMS/MS *m/z* 727.21 ($C_{35}H_{59}N_4O_7{}^{80}Br$), 642.12 ($C_{30}H_{48}N_3O_7{}^{80}Br$), 574.13 ($C_{26}H_{44}N_3O_6{}^{80}Br$), 542.20 ($C_{25}H_{40}N_3O_5{}^{80}Br$), 463.20 ($C_{20}H_{35}N_2O_5{}^{80}Br$), 442.21 ($C_{22}H_{40}N_3O_6$), 424.19 ($C_{22}H_{38}N_3O_5$), 329.13 ($C_{16}H_{29}N_2O_5$), 311.06 ($C_{16}H_{27}N_2O_4$), 283.15 ($C_{15}H_{27}N_2O_3$), 214.11 ($C_{11}H_{20}NO_3$); HRESIMS $[M+Na]^+$ *m/z* 775.3257 (calcd for $C_{36}H_{57}N_4O_8{}^{78}BrNa$ 775.3252).

Veraguamide C (3)

amorphous solid; $[\alpha]^{23}_D$ -13.0 (*c* 0.17, CH_2Cl_2); ^1H NMR (600 MHz, $CDCl_3$) δ 0.86-0.89 (6H, m), 0.94 (3H, d, *J* = 6.4 Hz), 0.96 (3H, d, *J* = 6.6 Hz), 1.00 (3H, d, *J* = 6.4 Hz), 1.01 (3H, d, *J* = 7.1 Hz), 1.03 (3H, d, *J* = 7.0 Hz), 1.12 (3H, d, *J* = 6.6 Hz), 1.26 (3H, s), 1.81 (1H, m), 1.93-2.12 (7H, m), 2.15-2.40 (4H, m), 2.95 (3H, s), 3.02 (3H, s), 3.12 (1H, m), 3.63 (1H, m), 3.86 (1H, m), 3.95 (1H, d, *J* = 10.7 Hz), 4.15 (1H, d, *J* = 10.8 Hz), 4.71 (1H, m),

4.88 (1H, m), 4.90 (1H, d, *J* = 9.2 Hz), 4.96 (1H, m), 6.27 (1H, m); ESIMS/MS *m/z* 661.35 ($C_{36}H_{61}N_4O_7$), 576.23 ($C_{31}H_{50}N_3O_7$), 496.25 ($C_{26}H_{46}N_3O_6$), 462.30 ($C_{25}H_{40}N_3O_5$), 456.25 ($C_{23}H_{42}N_3O_6$), 438.22 ($C_{23}H_{40}N_3O_5$), 383.26 ($C_{20}H_{35}N_2O_4$) 343.17 ($C_{17}H_{31}N_2O_5$), 325.12 ($C_{17}H_{29}N_2O_4$), 297.19 ($C_{16}H_{29}N_2O_3$); HRESIMS $[M+Na]^+$ *m/z* 711.4302 (calcd for $C_{37}H_{60}N_4O_8Na$ 711.4303).

Veraguamide H (4)

amorphous solid; ESIMS/MS *m/z* 647.33 ($C_{35}H_{59}N_4O_7$), 562.21 ($C_{30}H_{48}N_3O_7$), 496.25 ($C_{26}H_{46}N_3O_6$), 462.30 ($C_{25}H_{40}N_3O_5$), 442.23 ($C_{22}H_{40}N_3O_6$), 424.20 ($C_{22}H_{38}N_3O_5$), 365.24 ($C_{19}H_{33}N_2O_4$) 329.14 ($C_{16}H_{29}N_2O_5$), 311.07 ($C_{16}H_{27}N_2O_4$), 283.18 ($C_{15}H_{27}N_2O_3$); HRESIMS $[M+Na]^+$ *m/z* 697.4141 (calcd for $C_{36}H_{58}N_4O_8Na$ 697.4147).

Veraguamide I (5)

amorphous solid; ESIMS/MS *m/z* 665.37 ($C_{36}H_{65}N_4O_7$), 580.28 ($C_{31}H_{54}N_3O_7$), 500.28 ($C_{26}H_{50}N_3O_6$), 466.34 ($C_{25}H_{44}N_3O_5$), 456.25 ($C_{23}H_{42}N_3O_6$), 438.22 ($C_{23}H_{40}N_3O_5$), 383.26 ($C_{20}H_{35}N_2O_4$) 343.17 ($C_{17}H_{31}N_2O_5$), 325.12 ($C_{17}H_{29}N_2O_4$), 297.19 ($C_{16}H_{29}N_2O_3$); HRESIMS $[M+Na]^+$ *m/z* 715.4619 (calcd for $C_{37}H_{64}N_4O_8Na$ 715.4616).

Veraguamide J (6)

amorphous solid; ESIMS/MS *m/z* 651.35 ($C_{35}H_{63}N_4O_7$), 566.20 ($C_{30}H_{52}N_3O_7$), 500.25 ($C_{26}H_{50}N_3O_6$), 467.05 ($C_{25}H_{45}N_3O_5$), 442.23 ($C_{22}H_{40}N_3O_6$), 424.20 ($C_{22}H_{38}N_3O_5$), 365.24 ($C_{19}H_{33}N_2O_4$) 329.14 ($C_{16}H_{29}N_2O_5$), 311.07 ($C_{16}H_{27}N_2O_4$), 283.18 ($C_{15}H_{27}N_2O_3$); HRESIMS $[M+Na]^+$ *m/z* 699.4298 (calcd for $C_{36}H_{62}N_4O_8Na$ 699.4303).

Veraguamide K (7)

amorphous solid; $[\alpha]^{23}_D$ -21.4 (*c* 0.33, CH_2Cl_2); ^1H NMR (600 MHz, $CDCl_3$) δ 0.85 (3H, d, *J* = 6.9 Hz), 0.88 (3H, d, *J* = 7.7 Hz), 0.90 (3H, t, *J* = 6.5 Hz), 0.91 (3H, d, *J* = 6.5 Hz), 0.99 (3H, d, *J* = 6.5 Hz), 1.00 (6H, d, *J* = 6.9 Hz), 1.04 (3H, d, *J* = 6.5 Hz), 1.16 (1H, m), 1.20 (3H, d, *J* = 6.9 Hz), 1.25 (3H, t, *J* = 7.0 Hz), 1.47 (1H, m), 1.47 (2H, m), 1.74 (1H, m), 1.89 (1H, m), 2.13-2.30 (7H, m), 2.41 (1H, m), 2.93 (1H, d, *J* = 5.5 Hz), 3.10 (3H, s), 3.13 (3H, s), 3.68 (1H, m), 3.79 (1H, t, *J* = 6.5 Hz), 3.89 (1H, m), 4.15 (1H, m), 4.17 (1H, m) 4.81 (1H, m), 4.86 (1H, t, *J* = 7.0 Hz), 4.88 (1H, d, *J* = 10.8 Hz), 4.90 (1H, m), 6.36 (1H, d, *J* = 8.6 Hz); ESIMS/MS *m/z* 769.24 ($C_{37}H_{60}N_4O_8{}^{80}Br$), 656.21 ($C_{31}H_{49}N_3O_7{}^{80}Br$), 559.14 ($C_{26}H_{42}N_2O_6{}^{80}Br$), 484.22 ($C_{25}H_{46}N_3O_6$), 443.10 ($C_{20}H_{33}N_2O_4{}^{78}Br$), 438.22 ($C_{23}H_{40}N_3O_5$), 371.06 ($C_{19}H_{35}N_2O_5$), 325.11 ($C_{17}H_{29}N_2O_4$), 297.20 ($C_{16}H_{29}N_2O_3$);HRESIMS $[M+Na]^+$ *m/z* 835.3831 (calcd for $C_{38}H_{65}N_4O_9{}^{78}BrNa$ 835.3827).

Veraguamide L (8)

amorphous solid; $[\alpha]^{22}_D$ -27.9 (*c* 0.50, CH_2Cl_2); ^1H NMR (600 MHz, $CDCl_3$) δ 0.85 (3H, d, *J* = 6.7 Hz), 0.88 (3H, d, *J* = 6.7 Hz), 0.89 (3H, d, *J* = 6.6 Hz), 0.95 (3H, d, *J* = 6.6 Hz), 0.97 (3H, d, *J* = 6.5 Hz), 0.98 (3H, d, *J* = 6.2 Hz), 1.00 (3H, d, *J* = 6.9 Hz), 1.04 (3H, d, *J* = 1.04 Hz), 1.17 (3H, d, *J* = 7.1 Hz), 1.23 (3H, t, *J* = 7.l Hz), 1.46 (2H, dt, *J* = 7.1, 6.9 Hz), 1.51 (1H, m), 1.72 (1H, m), 1.87 (1H, m), 2.00 (1H, m), 2.06 (1H, m), 2.15 (1H, m), 2.19 (1H, m), 2.20 (1H, m), 2.22 (1H, m), 2.24 (1H, m), 2.26 (1H, m), 2.39 (1H, m), 2.93 (1H, d, *J* = 3.81 Hz), 3.09 (3H, s), 3.13 (3H, s), 3.67 (1H, dt, *J* = 7.7, 7.5 Hz), 3.78 (1H, t, *J* = 6.6 Hz), 3.85 (1H, dt, *J* = 7.7, 7.5 Hz), 4.14 (1H, m), 4.17 (1H, m), 4.81 (1H, dt, *J* = 6.2, 7.8 Hz), 4.82 (1H, d, *J* = 8.6 Hz), 4.85 (1H, d, *J* = 10.5 Hz), 4.87 (1H, d, *J* = 10.3 Hz), 4.90 (1H, dd, *J* = 8.5, 6.3 Hz), 6.37 (1H, d, *J* = 8.8 Hz); ESIMS/MS *m/z* 755.24 ($C_{36}H_{58}N_4O_8{}^{80}Br$), 642.21 ($C_{30}H_{47}N_3O_7{}^{80}Br$), 545.14 ($C_{25}H_{40}N_2O_6{}^{80}Br$), 470.21 ($C_{24}H_{44}N_3O_6$), 445.11 ($C_{20}H_{33}N_2O_4{}^{80}Br$), 424.21 ($C_{22}H_{38}N_3O_5$), 357.06 ($C_{18}H_{33}N_2O_5$), 311.10 ($C_{16}H_{27}N_2O_4$),

283.20 ($C_{15}H_{27}N_2O_3$); HRESIMS $[M+Na]^+$ *m/z* 821.3673 (calcd for $C_{38}H_{63}N_4O_9{}^{78}BrNa$ 821.3671).

Hydrogenation, Acid Hydrolysis and Marfey's Analysis

Veraguamide A (**1**, 1 mg) was dissolved in 1 mL of EtOH and treated with a small amount of 10% Pd/C and then placed under an atmosphere of H_2 (g) for 5 h. The reaction product was treated with 1.5 mL of 6 N HCl in a microwave reactor at 160 °C for 5 min. An aliquot (~300 μg) of the hydrolysate was dissolved in 300 μL of 1 M sodium bicarbonate, and then 16 μL of 1% D-FDAA (1-fluoro-2,4-dinitrophenyl-5-D-alanine amide) was added in acetone. The solution was maintained at 40 °C for 90 min at which time the reaction was quenched by the addition of 50 μL of 6 N HCl. The reaction mixture was diluted with 200 μL of CH_3CN and 10 μL of the solution was analyzed by LC-ESIMS.

The Marfey's derivatives of the hydrolysate and standards were analyzed by RP HPLC using a Phenomenex Luna 5 μm C_{18} column (4.6 × 250 mm). The HPLC conditions began with 10% CH_3CN/90% H_2O acidified with 0.1% formic acid (FA) followed by a gradient profile to 50% CH_3CN/ 50% H_2O acidified with 0.1% FA over 85 min at a flow of 0.4 mL/min, monitoring from 200 to 600 nm. The retention times of authentic acid D-FDAA derivatives were D-Pro (66.49), L-Pro (69.30), D-Val (78.45), D-N-Me-Val (86.61), L-Val (88.00), and L-N-Me-Val (91.66); the hydrolysate product gave peaks with retention times of 69.49, 88.07, and 91.74 min, according to L-Pro, L-Val and L-N-Me-Val, respectively.

Preparation and GC-MS Analysis of 2-Hydroxy-3-methylpentanoic Acid (Hmpa)

Veraguamide A (**1**, 1 mg) was dissolved in 1 mL of ethanol and treated with a small amount of 10% Pd/C and H_2 (g). The reaction product was then treated with 1.5 mL of 6 N HCl at 110 °C for 16 h. The reaction product was dried under N_2 (g) then dissolved in 0.5 mL of MeOH and Et_2O and treated with diazomethane. L-Ile (20 mg) was dissolved in 5 mL of cold (0 °C) 0.2 N $HClO_4$, and then 2 mL of $NaNO_2$ (aq) were added with rapid stirring. The reaction mixture was stored at room temperature for 1 h. The solution was boiled for 3 min, cooled to room temperature, and then saturated with NaCl. The mixture was extracted three times with Et_2O, and the Et_2O layer was then dried under N_2 (g) to yield the oily 2*S*,3*S*-Hmpa. An aliquot was dissolved in 1.5 mL of MeOH and Et_2O and treated with diazomethane. The product was then dried under N_2 (g). Correspondingly, 2*R*,3*R*-Hmpa, 2*S*,3*R*-Hmpa, and 2*R*,3*S*-Hmpa were synthesized with the same procedure from D-Ile, L-*allo*-Ile, and D-*allo*-Ile, respectively.

Each authentic stereoisomer of Hmpa was dissolved in CH_2Cl_2 with retention times measured by GC using a Cyclosil B column (Agilent Technologies J&W Scientific, 30 m × 0.25 mm) under the following conditions: the initial oven temperature was 35 °C, and held for 15 min, followed by a ramp from 35 to 60 °C at a rate of 1 °C/min, and another ramp to 170 ° C at a rate of 10 °C/min, and held at 170 °C for 5 min. The retention time of the Hmpa residue in acid hydrolysate of **1** matched with 2*S*,3*S*-Hmpa (45.63 min; 2*S*,3*R*-Hmpa, 44.86 min; 2*R*,3*S*-Hmpa, 45.06; 2*R*,3*R*-Hmpa, 45.26).

Preparation and GC-MS Analysis of Methyl 3-Hydroxy-2-Methyloctanoate (Hmoaa)

2*S*,3*S*-Hmoaa and 2*S*,3*R*-Hmoaa were synthesized following literature conditions.[13b] A sample of 5 mg of each product was dissolved in 2 mL of dry CH_2Cl_2 and treated with 0.122 mmol of triethylamine and 16.4 mmol of DMAP, and each was separately treated with 0.126 mmol of both *R*-MTPA-Cl and *S*-MTPA-Cl for 17 h at room temperature. Each reaction was quenched with 2.5 mL of 1 N HCl and extracted with Et_2O to produce the four diastereomeric standards. An aliquot of the hydrolysate of veraguamide A (**1**, 0.3 mg) was

dissolved in 1 mL of CH_2Cl_2 and treated with 7.32 μmol of triethylamine, 0.964 mol of DMAP, and 7.56 μmol of *S*-MTPA for 18 h at room temperature.

The four stereoisomeric standards of Hmoaa as well as the derivatized hydrolysate product of compound **1** were dissolved in CH_2Cl_2 and analyzed by GCMS as described below. A DB-5MS GC column (Agilent Technologies J&W Scientific, 30 m × 0.25 mm) was used with the following conditions: initial oven temperature was 35 °C, held for 2 min, followed by a ramp from 35 to 140 °C at a rate of 25 °C/min, followed by another ramp to 165 °C at a rate of 1 °C/min and held for 15 min before it was finally ramped up to a temperature of 190 °C at 1 °C/min. The retention time of the Hmoaa residue from the derivatized hydrolysate mixture of **1** matched that of 2*S*,3*R*-Hmoaa which was reacted with *S*-MTPA-Cl (47.13 min; 2*S*,2*S*-Hmoaa reacted with *S*-MTPA-Cl, 48.17 min; 2*S*,3*R*-Hmoaa reacted with *R*-MTPA-Cl, 48.13 min; 2*S*,3*S*-Hmoaa reacted with *R*-MTPA-Cl, 47.63 min).

Tandem Mass Spectrometry Data Acquisition and Preprocessing

For the ion-trap data acquisition, each compound was prepared to a 1 μM solution using 50:50 MeOH-H_2O with 1% AcOH as solvent, and underwent nanoelectrospray ionization on a Biversa Nanomate (pressure: 0.3 p.s.i., spray voltage: 1.4–1.8 kV). Ion trap spectra were acquired on a Finnigan LTQ-MS (Thermo-Electron Corporation) running Tune Plus software version 1.0. Ion tree datasets were collected using automatic mode, in which, the $[M+H]^+$ of each compound was set as the parent ion. MS^n data were collected with the following parameters: maximum breadth, 50; maximum MS^n depth, 3. At $n = 2$, isolation width, 4; normalized energy, 50. At $n = 3$, isolation width, 4; normalized energy 30. The Thermo-Finnigan files (in RAW format) were then converted to an mzXML file format using the ReAdW (http://tools.proteomecenter.org/) and subject to analysis using algorithms as well as manual interpretation.[18]

Cytotoxicity Assay

H-460 cells were added to 96-well plates at 3.33×10^4 cells/mL of Roswell Park Memorial Institute (RPMI) 1640 medium with fetal bovine serum (FBS) and 1% penicillin/streptomycin. The cells, in a volume of 180 μL per well, were incubated overnight (37 °C, 5% CO_2) to allow recovery before treatment with test compounds. Compounds were dissolved in DMSO to a stock concentration of 10 mg/mL. Working solutions of the compounds were made in RPMI 1640 medium without FBS, with a volume of 20 μL added to each well to give a final compound concentration of either 30 μg/mL or 3 μg/mL. An equal volume of RPMI 1640 medium with FBS was added to wells designated as negative controls for each plate. Plates were incubated for approximately 48 h before staining with MTT. Using a ThermoElectron Multiskan Ascent plate reader, plates were read at 570 and 630 nm.

DNA Extraction, Amplification, and Sequencing

Algal biomass (~50 mg) was partly cleaned under an Olympus VMZ dissecting microscope. The biomass was pretreated using TE (10 mM Tris; 0.1M EDTA; 0.5 % SDS; 20 μg/mL^{-1}RNase)/lysozyme (1 mg/mL) at 37 °C for 30 min followed by incubation with proteinase K (0.5 mg/mL^1) at 50 °C for 1 h. Genomic DNA was extracted using the Wizard® Genomic DNA Purification Kit (Promega) following the manufacturer's specifications. DNA concentration and purity was measured on a DU® 800 spectrophotometer (Beckman Coulter). The 16S rRNA genes were PCR-amplified from isolated DNA using the modified lineage-specific primers, OT106F 5′-GGACGGGTGAGTAACGCGTGA-3′ and OT1445R 5′-AGTAATGACTTCGGGCGTG-3′. The PCR reaction volumes were 25 μL containing 0.5 μL (~50 ng) of DNA, 2.5 μL of 10 × PfuUltra IV reaction buffer, 0.5 μL (25 mM) of dNTP

mix, 0.5 μL of each primer (10 μM), 0.5 μL of PfuUltra IV fusion HS DNA polymerase and 20.5 μL dH_2O. The PCR reactions were performed in an Eppendorf® Mastercycler® gradient as follows: initial denaturation for 2 min at 95 °C, 25 cycles of amplification, followed by 20 sec at 95 °C, 20 sec at 55 °C and 1.5 min at 72 °C, and final elongation for 3 min at 72 °C. PCR products were purified using a MinElute® PCR Purification Kit (Qiagen) before subcloning using the Zero Blunt® TOPO® PCR Cloning Kit (Invitrogen) following the manufacturer's specifications. Plasmid DNA was isolated using the QIAprep® Spin Miniprep Kit (Qiagen) and sequenced with M13 primers. The 16S rRNA gene sequences are available in the DDBJ/EMBL/GenBank databases under acc. No. HQ900689.

Phylogenetic Inference

The 16S rRNA gene sequence of PAC-17-FEB-10-2 was aligned with evolutionary informative cyanobacteria using the L-INS-I algorithm in MAFFT 6.717[28] and refined using the SSU secondary structures model for *Escherichia coli* J01695[29] without data exclusion. The best-fitting nucleotide substitution model optimized by maximum likelihood was selected using corrected Akaike/Bayesian Information Criterion (AIC_C/BIC) in jModeltest 0.1.1.[30] The evolutionary histories of the cyanobacterial genes were inferred using Maximum likelihood (ML) and Bayesian inference algorithms. The ML inference was performed using GARLI 1.0[31] for the GTR+I+G model assuming a heterogeneous substitution rates and gamma substitution of variable sites (proportion of invariable sites (pINV) = 0.494, shape parameter (α) = 0.485, number of rate categories = 4) with 1,000 bootstrap-replicates. Bayesian inference was conducted using MrBayes 3.1[32] with four Metropolis-coupled MCMC chains (one cold and three heated) ran for 3,000,000 generations. The first 25% were discarded as burn-in and the following data set were sampled with a frequency of every 100 generations. The MCMC convergence was detected by AWTY.[33]

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank the government of Panama for permission to make the cyanobacterial collections, K. Tidgewell, H. Choi, P. Boudreau, M. Balunas and J. Nunnery for helpful discussions, and the UCSD mass spectrometry facilities for their analytical services. The 500 MHz NMR ^{13}C Cold Probe was supported by NSF CHE-0741968. We are grateful for data analysis using the NSF-funded CIPRES cluster at the San Diego Supercomputer Center. Financial support was provided by the Fogarty International Center's International Cooperative Biodiversity Groups (ICBG) program in Panama (ICBG TW006634). Funding of the Dorrestein Laboratory was provided by the Beckman Foundation and NIGMS grant NIHGM 086283 (to P.C.D. and P.A.P.). W-T.L. was supported, in part, by a study aboard grant SAS-98116-2-US-108 from Taiwan.

References and Notes

1. (a) Nunnery JK, Mevers E, Gerwick WH. Curr. Opin. Biotechnol. 2010; 21:787–793. [PubMed: 21030245] (b) Tan LT. J. Appl. Phycol. 2010; 22:659–676.(c) Gerwick WH, Coates RC, Engene N, Gerwick L, Grindberg RV, Jones AC, Sorrels CM. Microbe. 2008; 3:277–284.(d) Tidgewell, K.; Clark, BR.; Gerwick, WH. Comprehensive Natural Products Chemistry II. Moore, B.; Crews, P., editors. Vol. 8. Elsevier; Oxford, UK: 2010. p. 141-188.
2. (a) Gerwick WH, Tan LT, Sitachitta N. Alkaloids Chem. Biol. 2001; 57:75–184. [PubMed: 11705123] (b) Tan LT. Phytochemistry. 2007; 68:954–979. [PubMed: 17336349]
3. Hooper GJ, Orjala J, Schatzman RC, Gerwick WH. J. Nat. Prod. 1998; 61:529–533. [PubMed: 9584405]
4. Wan F, Erickson KL. J. Nat. Prod. 2001; 64:143–146. [PubMed: 11429989]

5. Luesch H, Pangilinan R, Yoshida WY, Moore R, Paul VJ. J. Nat. Prod. 2001; 64:304–307. [PubMed: 11277744]
6. Sitachitta N, Williamson RT, Gerwick WH. J. Nat. Prod. 2000; 63:197–200. [PubMed: 10691708]
7. Nogle LM, Gerwick WH. J. Nat. Prod. 2002; 65:21–24. [PubMed: 11809058]
8. Bunyajetpong S, Yoshida WY, Sitachitta N, Kaya K. J. Nat. Prod. 2006; 69:1539–1542. [PubMed: 17125217]
9. Tripathi A, Puddick J, Prinsep MR, Lee PPL, Tan LK. J. Nat. Prod. 2009; 72:29–32. [PubMed: 19093843]
10. Han B, Goeger D, Maier CS, Gerwick WH. J. Nat. Prod. 2005; 70:3133–3139.
11. Balunas MJ, Linington RG, Tidgewell K, Fenner AM, Ureña LD, Togna GD, Kyle DE, Gerwick WH. J. Nat. Prod. 2010; 73:60–66. [PubMed: 20030365]
12. Simmons TL, Engene N, Ureña DL, Romero LI, Ortega-Barría E, Gerwick L, Gerwick WH. J. Nat. Prod. 2008; 71:1544–1550. [PubMed: 18715036]
13. (a) Rodriguez J, Fernández R, Quiñoá E, Riguera R, Debitus C, Bouchet P. Tetrahedron Lett. 1994; 35:9239–9242.(b) Fernández R, Rodríquez J, Quiñoá E, Riguera R, Muñoz, Fernández-Suárez M, Debitus C. J. Am. Chem. Soc. 1996; 118:11635–11642.
14. Nakao Y, Yoshida WY, Szabo CM, Baker BJ, Scheuer PJ. J. Org. Chem. 1998; 63:3272–3280.
15. http://www.icbg.org/
16. http://whc.unesco.org/en/list/1138
17. Mevers, E.; Tidgewell, K.; Byrum, T.; Spadafora, C.; Gerwick, WH. Proceedings of the Joint Annual Meeting of the American Society of Pharmacognosy and the Phytochemical Society of North America; St. Petersburg Beach, FL. July 10-14, 2010; Abstract P-52
18. (a) Liu WT, Ng J, Meluzzi D, Bandeira N, Gutierrez M, Simmons TL, Schultz AW, Linington RG, Moore BS, Gerwick WH, Pevzner PA, Dorrestein PC. Anal. Chem. 2009; 81:4200–4209. [PubMed: 19413302] (b) Mohimani H, Yang YL, Liu WT, Doerrestein PC, Pevzner PA. J. Proteome. 2010 submitted for publication. (c) Ng J, Bandeira N, Liu WT, Ghassemian M, Simmons TL, Gerwick WH, Linnington RG, Dorrestein PC, Pevzner PA. Nat. Methods. 2009; 6:596–599. [PubMed: 19597502]
19. Salvador LA, Biggs JS, Paul VJ, Luesch H. J. Nat. Prod. 2011 ASAP.
20. Edwards DJ, Marquez BL, Nogle LM, McPhail K, Geoger DE, Roberts MA, Gerwick WH. Chem. Biol. 2004; 11:817–833. [PubMed: 15217615]
21. Mamer OA. Methods Enzymol. 2000; 324:3–10. [PubMed: 10989412]
22. Caboche S, Pupin M, Leclre V, Fontaine A, Jacques P, Kucherov G. Nucleic Acids Res. 2008; 36:326–331.
24. Johansen JR, Casamatta DA. Algol. Stud. 2005; 117:71–93.
25. Castenholz, RW.; Rippka, R.; Herdman, M. Bergey's Manual of Systematic Bacteriology. Boone, DR.; Castenholz, RW., editors. Vol. 1. Springer; New York: 2001. p. 473-599.
26. Engene N, Coates RC, Gerwick WH. J. Phycol. 2010; 46:591–601.
27. Komárek, J.; Anagnostidis, K. Süßwasserflora von Mitteleuropa. Büdel, B.; Gärtner, G.; Krienitz, L.; Schagerl, M., editors. Vol. 19/2. Gustav Fischer; Jena: 2005. p. 576-606.
28. Katoh K, Toh H. Brief Bioinform. 2008; 9:286–298. [PubMed: 18372315]
29. Cannone JJ, Subramanin S, Schnare MN, Collett JR, D'Souza LM, Du Y, Feng B, Lin N, Madabusi LV, Muller KM, Pnde N, Schang Z, Yu N, Gutell RR. BMC Bioinformatics. 2002; 3:1471–2105.
30. Posada D. Mol. Biol. Evol. 2008; 25:1253–1256. [PubMed: 18397919]
31. Zwickl, DJ. Genetic Algorithm Approaches for the Phylogenetic Analysis of Large Biological Sequence Datasets under the Maximum Likelihood Criterion. The University of Texas at Austin; 2006. Ph.D. Thesis
32. Ronquist F, Huelsenbeck JP. Bioinformatics (Oxf). 2003; 12:1572–1574.
33. Nylander JAA, Wilgenbusch JC, Warren DL, Swofford DL. Bioinformatics (Oxf.). 2008; 15:581–583.

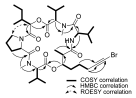

Figure 1.
Selected 2D NMR data for veraguamide A (1).

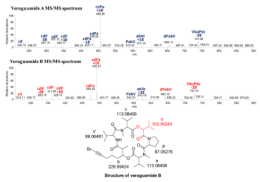

Figure 2.

Sequencing by ESI MS/MS fragmentations. To localize the residue reduced by 14 Da relative to veraguamide A (**1**), fragments that bear the 14 Da shift are labeled in red, with the non-shifting fragments labeled in blue. By comparing the shifted and non-shifted ions, the offset mass was located on residue “d”. All of the resulting fragments agreed with this new mass annotation.

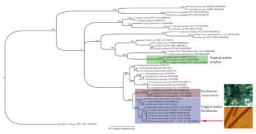

Figure 3.
Evolutionary tree of the veraguamide-producing strain PAC-17-FEB-10-2 (highlighted with a red arrow). Note that PAC-17-FEB-10-2 nests within the tropical marine *Oscillatoria* clade (blue box) and that the evolutionarily distance from the *Oscillatoria sensu stricto* (red box) suggests that tropical marine *Oscillatoria* should be revised as a new taxa. Tropical marine *Lyngbya*, which are often confused with the genus *Oscillatoria*, are highlighted with a green box. The cladogram is based on SSU (16S) rRNA gene sequences using the maximum-likelihood (GARLI) method and the support values are indicated as boot-strap at the nodes. The specimens are indicated as species, strain, and access number in brackets. Specimens designated with (^{T}) represent type-strains obtained from *Bergey's Manual*. The scale bar is indicated at 0.04 expected nucleotide substitutions per site.

Table 1

^{1}H and ^{13}C NMR Assignments for Veraguamide A (**1**) in $CDCl_3$.

Residue	position	δ_C [b]	δ_H (*J* in Hz) [a]	HMBC [a]	ROESY [a]
N-MeVal-1	1	170.7			
	2	65.0	3.94, d (10.7)	1, 3, 4, 7	8, 9b, 4, 5
	3	28.3	2.28, m	4	4, 6
	4	19.6	0.98, d (6.8)	2, 3, 5	3
	5	19.3	0.92, d (6.6)	2, 4	2, 3, 6
	6	29.5	3.01, s	2, 7	2, 3, 5
Pro	7	172.2			
	8	57.3	4.95, dd (8.5, 6.3)	9, 10, 11	2, 9a, 9b, 10a, 10b
	9a	28.7	2.28, m	8, 10, 11	6, 8, 9b
	9b		1.79, m	7, 8, 10, 11	8, 9a
	10a	25.0	2.03, m	8, 9, 11	8, 11b, 31
	10b		1.99, m	8, 9, 11	11b
	11a	47.3	3.85, dt (9.3, 7.1)	8, 9, 10	10a, 11b, 13
	11b		3.61, dt (9.3, 7.1)	9, 10	10b, 11a, 13
Hmpa	12	166.0			
	13	76.1	4.90, d (9.3)	12, 14, 15, 18	11a, 11b, 14, 16, 31
	14	35.7	1.98, m	17	13
	15a	24.9	1.54, m	14	15b, 16
	15b		1.13, m	14	15a
	16	20.3	1.00, d (6.8)	14, 15	14
	17	10.6	0.87, t (7.3)	13, 14, 15	15a, 16
N-MeVal-2	18	169.7			
	19	66.1	4.15, d (9.8)	18, 20, 22, 23, 24	20, 21, 22, 25
	20	28.5	2.25, m	19	19, 23
	21	20.4	1.11, d (6.3)	19, 20, 22	19
	22	20.2	0.99, d (6.8)	19, 20, 21	19, 23
	23	30.1	2.95, s	19, 24	20, 22, 28
Val	24	173.5			

J Nat Prod. Author manuscript; available in PMC 2012 May 27.

Residue	position	δ_C[b]	δ_H (*J* in Hz)[a]	HMBC[a]	ROESY[a]
	25	52.8	4.71, dt (6.3, 8.5)	24, 26, 27, 28, 29	19, 26, 27, NH-1
	26	32.1	1.90, m	25, 27	25
	27	20.2	0.94, d (6.8)	25, 26, 28	25
	28	17.5	0.88, d (6.8)	25, 26, 27	23
	*N*H-1		6.28, d (8.5)	29	25, 32, 37
Br-HMOYA	29	170.9			
	30	42.4	3.12, m	29, 31, 37	31, 32
	31	76.4	4.85, d (10.5)	1	10a, 30, 32, 33a, 33b, 34a, 34b, 37
	32	29.7	1.26, m	30, 31	*N*H-1
	33a	24.8	1.59, m	32, 34, 35	31
	33b		1.42, m	34, 35	31
	34a	19.5	2.20, m	33, 35, 36	31
	34b		1.97, m	33, 35, 36	31
	35	79.4			
	36	38.4			
	37	13.9	1.25, m	29, 30, 31	31, NH-1

[a] 500 MHz for ^{1}H NMR, HMBC, and ROESY

[b] 125 MHz for ^{13}C NMR