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Isolation and Characterization of Anaephenes A–C, Alkylphenols from a Filamentous Cyanobacterium (*Hormoscilla* sp., Oscillatoriales)

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Abstract

Three related new alkylphenols, termed anaephenes A–C (**1–3**), containing different side chains, were isolated from an undescribed filamentous cyanobacterium (VPG 16–59) collected in Guam. Our 16S rDNA sequencing efforts indicated that VPG 16–59 is a member of the marine genus *Hormoscilla* (Oscillatoriales). The structures of anaephenes A–C (**1–3**) were elucidated by spectroscopic methods, and compounds assayed for growth inhibitory activity against prokaryotic and eukaryotic cell lines. Anaephene B (**2**), possessing a terminal alkyne, displayed moderate activity against *B. cereus* and *S. aureus* with MIC values of 6.1 µg/mL. While **1** and **3** showed no pronounced activity in these assays, their structural features highlight the unusual biosynthetic capacity of this cyanobacterium and warrant further study.

Graphical Abstract

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Supporting Information

The Supporting Information is available free of charge on the ACS Publication website. This document contains ¹H NMR, ¹³C NMR, COSY, HSQC, and HMBC spectra of anaephenes A–C (**1–3**) in CDCl₃. Additionally, the NOESY spectrum of anaephene C, and ¹³C NMR chemical shift comparisons of anaephenes A and B versus reported examples have been provided (PDF).

Cyanobacteria represent a largely untapped stock of structurally intriguing and biologically active natural products. The secondary metabolites associated with marine cyanobacteria can be categorized into nonribosomal peptides, ribosomal peptides, polyketides, or hybridizations of these classifications.^{1–2} These pathways give rise to natural product scaffolds that include linear or cyclic peptides, linear lipopeptides, depsipeptides, fatty acid derivatives, and various macrocycles.³ The pharmacological value of these compounds are exemplified by a broad range of biological activities.^{3–5} Here we detail the structural and initial biological characterization of a unique family of alkylphenols from an undescribed filamentous cyanobacterium from the genus *Hormoscilla*.⁶

The HRMS spectrum of compound **1** displayed a $[\text{M}-\text{H}]^-$ ion at m/z 217.1601 indicative of the molecular formula $\text{C}_{15}\text{H}_{22}\text{O}$, with five degrees of unsaturation. Following the interpretation of COSY, HSQC and HMBC experiments (Table 1), the ^1H and ^{13}C NMR signals were assignable to one terminal methyl, six methylenes, two olefinic methines, four aromatic methines and one hydroxy group. The COSY data and the coupling constants for the four aromatic protons H-2 (δ_{H} 6.65, br s) H-4 (δ_{H} 6.75, d, $J = 7.7$ Hz), H-5 (δ_{H} 7.13, t, $J = 7.7$ Hz) and H-6 (δ_{H} 6.63, m) (Table 1) indicated the presence of a 1,3-disubstituted aromatic ring. HMBC correlations from H-5 to the phenolic C-1 (δ_{C} 155.6) and C-3 (δ_{C}

145.0) carbons and H-1 (δ_{H} 4.79, br s, OH) to both C-2 (δ_{C} 115.4) and C-6 (δ_{C} 112.6) justified the assignment of the -OH group and the side chain to positions 1 and 3, respectively. A combination of COSY and HMBC correlation data (Figure 1) established the planar structure. The olefinic carbons were then identified via three-bond HMBC correlations from H-3' to C-5' (δ_{C} 130.3), and H-8' to C-6' (δ_{C} 130.6). The assignment of the olefin configuration in **1** was nontrivial due to signal overlap in the ^1H NMR spectrum. The *E* configuration of the C-5' and C-6' olefin was determined based on comparative chemical shift arguments. Previously reported examples of similarly alkylated resorcinol type structures have relied on the diagnostic allylic carbon chemical shifts (Figure S1, Supporting Information).^{7–10} In the case of *Z* configuration, these allylic carbons, analogous to C-4' (32.6 ppm) and C-7' (34.9 ppm) in **1**, resonate around ~ 26.9 and 27.2 ppm respectively.^{7–8} Additionally, our determined configuration was in agreement with published metabolites containing similar *E* olefins.^{9–10}

Based on the observed $[\text{M}-\text{H}]^-$ ion at m/z 241.1602 the molecular formula of compound **2** was determined to be $\text{C}_{17}\text{H}_{22}\text{O}$ with seven degrees of unsaturation. Analysis of the NMR data (Table 1) indicated that compound **2** contained four aromatic methines, seven methylenes, two additional sp^2 methines, and one sp methine. The presence of the terminal alkyne in **2** was proposed based on the diagnostic proton triplet at δ_{H} 1.95 and associated ^{13}C NMR signals at δ_{C} 68.4, and 87.6 (Table 1).^{11–12} Elucidation of the meta-substituted benzene of **2** and the C-1' bridge evoked similar COSY and HMBC arguments used in the assignment of **1** (Figure 1). These arguments were further supported by two new HMBC correlations from the phenolic proton at δ_{H} 4.66 to both the C-2 (δ_{C} 115.4) and C-6 (δ_{C} 112.7) carbons. The C-6' carbon was first inferred based on COSY correlations between H-6' (δ_{H} 5.36, dt, $J = 15.5, 6.7$ Hz) and H-7' (δ_{H} 2.09, m), and further confirmed via a weak HMBC correlation from H-6' to C-8' (δ_{C} 28.5). Informed by HSQC data, the observed COSY correlations between H-7' and H-8' (δ_{H} 1.58, m), and H-8' and H-9' (δ_{H} 2.18, m) assigned the C-7' (δ_{C} 31.6), C-8' (δ_{C} 28.5), and C-9' (δ_{C} 17.9) positions. HMBC signals from H-6' to C-8' and H-7' to C-9' further solidified these assignments. The aforementioned terminal alkyne was elucidated via long range COSY correlations between H-9' and H-11', and HMBC signals from H-9' to C-11' and H-8' to C-10'. The determined *E* configuration of the internal olefin was first justified via coupling constant values extracted from the H-5' (δ_{H} 5.43, dt, $J = 15.5, 6.7$ Hz) and H-6' (δ_{H} 5.36, dt, $J = 15.5, 6.7$ Hz) resonances. Contrary to **1**, in the case of compound **2**, both olefinic signals exhibited first order coupling but displayed a notable 'roof effect' because of their close chemical shift. The *E* configuration was additionally supported by allylic C-4' (δ_{C} 32.5) and C-7' (δ_{C} 31.6) carbon resonances when compared to literature values (Figure S1, Supporting Information).

The molecular formula of **3** was determined to be $\text{C}_{15}\text{H}_{20}\text{O}$ with six degrees of unsaturation and was consistent with the $[\text{M}-\text{H}]^-$ ion at m/z 215.1445. Analysis of the NMR spectra (Table 1) of **3** indicated the presence of four aromatic methines, four sp^2 methines, four methylenes and one methyl group. The *meta* substituted benzene ring system and methylene C-1' (δ_{C} 35.9) bridge in **3** were assigned based on arguments presented in **1** and **2** above (Figure 1). The C-2' (δ_{C} 34.4) and C-3' (δ_{C} 131.0) positions were established via sequential COSY correlations between H-1' (δ_{H} 2.65, t, $J = 7.6$ Hz) and H-2' (δ_{H} 2.36, m),

and H-2' and H-3' (δ_{H} 5.59, m). The assignment of the C-4' (δ_{C} 131.1) olefin was determined via a three-bond HMBC correlation from H-2'. The final degree of unsaturation was attributed to an additional olefin based on the unaccounted sp^2 methine carbons. In order to assign these positions, the alkyl chain was elucidated starting from the C-9' (δ_{H} 0.90) methyl. The C-7' (δ_{C} 34.8) C-8' (δ_{C} 26.6) carbons were identified based on an HMBC correlation from the terminal H-9' methyl to C-8' in addition to a COSY between H-8' (δ_{H} 1.40, m) and H-7' (δ_{H} 2.03, m). The remaining sp^2 carbons were then assigned via three-bond HMBC signals from H-8' (δ_{H} 1.40, m) to C-6' (δ_{C} 133.0), and H-7' (δ_{H} 2.03, m) to C-5' (δ_{C} 130.4). Determination of the diene configuration based on coupling constant arguments or correlations observed in the NOESY data was non-trivial due to spectral overlap of the H-4'/H-6', and H-3'/H-5' proton signals. Due to these challenges the diene configuration was justified based on similar arguments presented for **1** (Figure S1, Supporting Information). As the configurational assignment of internal olefins in long alkyl chains has historically been difficult, literature precedence supports the allylic C-2' (δ_{C} 30.9) and C-7' (δ_{C} 34.9) carbons as diagnostic handles. As demonstrated by the provided alkyl amide type structures (Figure S 1, Supporting Information), the *Z* configuration results in the shielding of the allylic carbon (δ_{C} ~26.7) as opposed to shifts observed in the *E* case (δ_{C} ~32.1).^{13–14} Furthermore, based on these examples, the configuration of the C-4'/5' olefin has minimal (i.e < 1.0 ppm) influence on the allylic C-7' carbon resonance.¹⁴ Based on these data the diene of **3** was assigned to the *E,E* configuration. The proposed s-trans conformation of the diene was first justified based on thermodynamic stability arguments as the s-cis conformer would be highly congested and subject to thermal isomerization.¹⁵ Additionally, the NOESY spectrum of **3** lacked correlations consistent with the s-cis conformer, as this conformation would bring the H-7', H-8' and H-9' protons proximal to H-3', H-4' and H-5'.

Compounds **1–3** were assayed for antimicrobial activity against *Pseudomonas aeruginosa*, *Bacillus cereus*, *Mycobacterium tuberculosis*, and *Staphylococcus aureus* (Table 2). Compound **2** displayed the most notable activities against *B. cereus* (MIC 6.1 $\mu\text{g/mL}$) and *S. aureus* (MIC 6.1 $\mu\text{g/mL}$). Additionally, **1** was found to show weak activity against *B. cereus* (MIC 11 $\mu\text{g/mL}$). The assessment of **1–3** in the antifungal assay showed no activity against *Saccharomyces cerevisiae*. The effects of compounds **1–3** on the growth of mammalian cells were tested in the MTT cell viability assay against human colon carcinoma cells (HCT116), breast adenocarcinoma (MDA-MB-231), and lung carcinoma (A549) cells. The three compounds showed insignificant activity against these cancer cell lines ($\text{IC}_{50} > 20 \mu\text{M}$) (Table 2).

Our continued study of the chemical diversity associated with Guamanian marine ecosystems has resulted in the discovery of three structurally intriguing cyanobacterial natural products. The similarity of **1–3** to those found in terrestrial plants raises questions about their biosynthetic origins. Terminal alkynes are not a rarity in cyanobacteria – with examples including pitipeptolide A,¹¹ jamaicamides A and B,¹² and carmabin B¹⁶ – and generally display potency against Gram-positive bacteria. These observations were consistent with the bioactivity profile of the terminal alkyne associated with **2** (Table 2). While only marginally active in these bioassays, these discoveries highlight the biosynthetic

capacity of cyanobacterial species and thus warrant further exploration of the underlying biochemical origins of these metabolites through an integrative approach relying on genomic and transcriptomic tools.

Phylogenetic reconstruction based on 16S rDNA identified VPG16–59 as an undescribed species of the genus *Hormoscilla* (Oscillatoriales)¹⁷ (Figure 2). BLASTn search identified numerous relatives published from environmental sequencing studies of sponges collected from various coral reef systems including Guam,^{18–21} with some of these sponges linked to the production of polybrominated diphenyl ethers, a group of compounds well known from tropical Dysideidae sponges that contain *H. spongelliae* as an abundant symbiont.²¹ These sequences sometimes lack valuable taxonomic information thus confounding the accurate description and study of new environmental isolates. In these situations, specimens are often reported as “Uncultured cyanobacterium” or are labelled as “*Oscillatoria* sp.” or “*Oscillatoria spongelliae*”, since redefined as *Hormoscilla spongelliae* (Gomont).⁶ More recently, several environmental clones were published as “Uncultured *Hormoscilla* sp.” reflecting this taxonomic change.²¹ In a BLASTn search, VPG 16–59 showed 97–99% sequence identity when compared with reported members of the *Hormoscilla* clade. Such values represent 3–16 bp mismatches along the sequence, thus supporting our hypothesis that this genus harbors multiple undescribed species. When specifically compared to sequence data from reported Guamanian *Hormoscilla* spp. isolates, VPG 16–59 was found to be distinct (i.e. a new species) (Figure 2). Overall, based on our analysis, the *Hormoscilla* spp. clade appears broadly distributed in the tropical Atlantic and Indo-Pacific oceans with both free living (benthic) and symbiotic representatives (endozoic).

Overall, our continued work with Guamanian reef systems and their associated cyanobacteria resulted in the discovery of three new natural products, anaephenes A-C (**1–3**), with mixed/uncharacterized biosynthetic origins, and the identification of a new environmental clone *Hormoscilla* sp. (VPG 15–59). While previous studies have produced structures containing long alkyl chains with varying degrees of unsaturation,^{22–23} few examples contain the meta-phenolic group. The closest relatives to **1–3**, the hierridins, were previously reported in species of pico- and filamentous cyanobacteria.^{24–25} Similarly, the hierridin scaffold contains a polyphenolic head group and a long aliphatic tail. Additional examples of similar natural products are reported from terrestrial plant species including: *Spondias tuberosa*,^{26–27} *Ozoroa insignis*,²⁸ and *Piper villiramulum*.^{29–30}

In light of their structural novelty with respect to reported cyanobacterial metabolites, the anaephenes hint at the existence of more interesting/undiscovered chemistry. Though our initial characterization of the therapeutic/biomedical applications of anaephenes A-C (**1–3**) showed only modest antibacterial activities (Table 2), further examinations of their endogenous ecological functions and biosynthetic origins are warranted.

EXPERIMENTAL SECTION

General Experimental Procedures.

All UV/Vis data were collected on a SpectraMax M5 (Molecular Devices) spectrometer. The ¹H, ¹³C and 2D NMR spectra were recorded on a Bruker AVANCE II 600 MHz

spectrometer. The chemical shifts of the ^1H NMR spectra were referenced using the residual solvent signal of CDCl_3 at 7.26 ppm and at the center of the CDCl_3 triplet at 77.0 ppm in the ^{13}C NMR spectra. All reported HRMS data were collected on an Agilent 6220 ESI-TOF.

Biological Material.

The sample was collected as an epilithic grey filamentous marine cyanobacterium from Anae Island, Guam in December 2016. Nucleic acids were extracted with a plant genomic kit (Epoch Life Science Inc, Texas), subjected to PCR amplification of the 16S ribosomal gene (16S rDNA) with cyanobacterial primers, and sequenced according to standard protocols.³¹ The sequence obtained for VPG 16–59 was then used on NCBI's GenBank to retrieve its 1000 closest homologues via BLASTn. An exploratory phylogenetic tree was built (not shown) to place VPG 16–59 in a broad phylogenetic context. Relevant sequences were then selected with TREE2FASTA³² to reduce the data set to the genus *Hormoscilla* and a few outgroup taxa. Final phylogenetic reconstruction was then performed with RAXML with the GTR model with 1000 restarts and 1000 bootstrap.³³ A voucher specimen (VPG 16–59) is stored at the Smithsonian Marine Station, Fort Pierce, FL and its 16S sequence was deposited in GenBank as MH578561.

Extraction and Isolation.

The freeze-dried material (10.81 g) was first extracted with EtOAc–MeOH (1:1) followed by H_2O –EtOH (1:9). Concentration of these extracts furnished 1.95 g (18.0%) of an EtOAc–MeOH soluble less-polar fraction and 0.857 g (7.9%) of a H_2O –EtOH soluble polar fraction. The less-polar fraction (1.95 g) was chromatographed on a column of SiO_2 (20 g) using a step gradient of hexanes–30% EtOAc, EtOAc, EtOAc–20% MeOH and MeOH to give four sub-fractions. The 30% EtOAc in hexanes fraction was subjected to another round of SiO_2 gel chromatography using hexanes with increasing concentrations of EtOAc. The fraction that eluted at 10% EtOAc in hexanes was further purified by semi-preparative reversed-phase HPLC (Phenomenex Luna C18, 5 μm , flow rate, 2.0 mL/min) using a linear gradient of MeCN– H_2O (70–100% for 15 min and then 100% MeCN for 10 min) to give compounds **1** (t_{R} 19.0 min, 8.3 mg), **2** (t_{R} 16.2 min, 0.5 mg), and **3** (t_{R} 17.2 min, 0.4 mg).

Anaephene A (1): colorless solid; UV (MeOH) λ_{max} (log ϵ) 224 (2.42), 270 (2.39) nm; ^1H and ^{13}C NMR data, Table 1; HRESIMS m/z 217.1601 $[\text{M}-\text{H}]^-$ (calcd for $\text{C}_{15}\text{H}_{21}\text{O}$, 217.1592).

Anaephene B (2): colorless solid; UV (MeOH) λ_{max} (log ϵ) 220 (3.02), 272 (2.74) nm; ^1H and ^{13}C NMR data, Table 1; HRESIMS m/z 241.1602 $[\text{M}-\text{H}]^-$ (calcd for $\text{C}_{17}\text{H}_{21}\text{O}$, 241.1592).

Anaephene C (3): colorless solid; UV (MeOH) λ_{max} (log ϵ) 228 (3.07), 242 (3.06), 272 (2.94) nm; ^1H and ^{13}C NMR data, Table 1; HRESIMS m/z 215.1445 $[\text{M}-\text{H}]^-$ (calcd for $\text{C}_{15}\text{H}_{19}\text{O}$, 215.1436).

Antibacterial Assays.

S. aureus Seattle 1945 (ATCC 25923) and *P. aeruginosa* PAO1 (ATCC BAA-47) were inoculated in LB broth medium overnight at 37 °C and *B. cereus* NRS 248 (ATCC 10987) at

30 °C overnight. The culture was diluted 10⁶-fold in fresh medium. The diluted cultures were added in aliquots of 100 µL to each well in a 96-well plate starting from the second column. In the first column, 200 µL aliquots of the diluted culture were added to each well and were treated with 2-fold the final concentration of compounds **1–3**, negative control (DMSO or H₂O), and positive control (ciprofloxacin HCl, chloramphenicol or streptomycin). Each plate was serially diluted 2-fold (100 µL per well) starting from the first column. Each treatment was done in triplicate. The plates were incubated overnight. Concentrations starting at 100 µM were tested for compounds **1–3**, ciprofloxacin, chloramphenicol, and streptomycin started at 343 µM. The minimum inhibitory concentration (MIC) is reported as the lowest concentration in which no bacterial growth is observed as indicated by the presence of turbidity.

M. tuberculosis H37Ra (ATCC 25177) was inoculated in 10 mL of Middlebrook 7H9 medium and grown for two weeks. The culture was diluted in fresh medium to 0.01 at OD₆₀₀. The diluted culture was added in aliquots of 200 µL to each well in a 96-well plate starting from the second column. In the first column, 400 µL aliquots of the diluted culture were added to each well and were treated with 2-fold the final concentration of compounds **1–3**, negative control (DMSO or H₂O), and positive control (streptomycin). Each plate was serially diluted 2-fold (200 µL per well) starting from the first column. Each treatment was done in triplicate. The plates were incubated for a week at 37 °C.

Antifungal Assay.

S. cerevisiae wild-type (BY4741) cells were grown in YPD medium overnight at 30 °C. YPD medium aliquots of 50 µL was added to each well of a 96-well plate starting from the second column. Aliquots of 100 µL were added to the first column containing 2-fold the final concentrations of compounds **1–3**, negative control (DMSO) and positive control (nystatin). Each plate was serially diluted 2-fold (50 µL per well) starting from the first column. Each treatment was done in triplicate. The cell density was determined by measuring the absorbance at 660 nm using the SpectraMax M5 (Molecular Devices) spectrometer and aliquots of 50 µL containing 2-fold the final cell density (10 × 10⁵ cells/mL) were added to each well. Concentrations starting at 100 µM were tested for each compound, and 25 µM for nystatin. The plates were incubated overnight.

Cancer Cell Culture.

Human colon carcinoma cells (HCT116), breast adenocarcinoma (MDA-MB-231), and lung carcinoma (A549) were cultured in Dulbecco's modified Eagle's medium (DMEM, Invitrogen) supplemented with 10% fetal bovine serum (FBS, Sigma-Aldrich) and 1% antibiotic-antimycotic (Invitrogen) humidified at 37 °C with 5% CO₂.

Cell Viability Assay.

HCT116 and A549 cells were seeded in 96-well plates (10,000 cells/well) and MDA-MB-231 cells at slightly higher density (12,000 cells/well). After 24 h, cells were treated with varying concentrations of compounds **1–3** or solvent control (DMSO). After incubating for 48 h, cell viability was measured using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) according to the manufacturer's instructions

(Promega). Nonlinear regression analysis was carried out using GraphPad Prism 6 software for IC₅₀ calculations.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.
HMBC (black arrows); COSY (bold lines); long range COSY (dashed arrows) for 1–3

Figure 2.

16S rDNA maximum likelihood tree showing the phylogenetic position of the anaerobe producing VPG 16–59 (grey shaded regions) among other tropical and Guamanian (orange shaded regions) *Hormoscilla* spp. isolates. Published Genbank clone numbers (parenthesis) and geographic origins (brackets) are provided. Note that for clarity low bootstrap values (i.e. those below 70%) are not shown.

Table 1.

NMR Spectroscopic Data (¹H 600 MHz, ¹³C 150 MHz, CDCl₃) for 1–3

No	anaephene A (1)				anaephene B (2)				anaephene C (3)			
	δ_C , Type	δ_H (J in Hz)	HMBC	COSY	δ_C , Type	δ_H (J in Hz)	HMBC	COSY	δ_C , Type	δ_H (J in Hz)	HMBC	COSY
1	155.6, C				155.6, C				155.6, C			
2	115.4, CH	6.65, br s	1, 3, 4, 6, 1'		115.4, CH	6.65, br s	1, 3, 4, 6, 1'		115.5, CH	6.66, br s	1, 3, 4, 6, 1'	
3	145.0, C				144.9, C				144.0, C			
4	121.1, CH	6.75, d (7.7)	1, 2, 3, 5, 6, 1'	5	121.1, CH	6.75, d (7.7)	1, 2, 3, 5, 6, 1'	5	121.1, CH	6.76, d (7.7)	1, 2, 3, 5, 6, 1'	5
5	129.5, CH	7.13, t (7.7)	1, 3, 4, 6	4, 6	129.6, CH	7.14, t (7.7)	1, 3, 4, 6	4, 6	129.6, CH	7.14, t (7.7)	1, 3, 4, 6	4, 6
6	112.6, CH	6.63, m	1, 2, 4	5	112.7, CH	6.64, d (2.6)	1, 2, 4	5	112.9, CH	6.64, d (2.6)	1, 2, 4	5
1'	35.8, CH ₂	2.55, t (7.7)	2, 3, 4, 2'	2'	35.8, CH ₂	2.56, t (7.7)	2, 3, 4, 2'	2'	35.9, CH ₂	2.65, t (7.6)	2, 3, 4	2'
2'	30.9, CH ₂	1.60, m	3, 1', 3', 4'	1', 3'	30.8, CH ₂	1.60, m	3, 1', 3', 4'	1', 3'	34.4, CH ₂	2.36, m	1', 3', 4'	1', 3'
3'	29.4, CH ₂	1.38, m	2', 4', 5'	2', 4'	29.2, CH ₂	1.39, m	1', 5'	2', 4'	131.0, CH	5.59, m	1', 2'	4'
4'	32.6, CH ₂	2.01, m	3', 5', 6'	3'	32.5, CH ₂	2.01, m	3', 5'	3', 5'	131.1, CH	6.04, m	3', 5'	3'
5'	130.3, CH	5.38, m	4', 6'	6'	131.4, CH	5.43, dt (15.5, 6.7)		4', 6'	130.4, CH	5.99, m		6'
6'	130.6, CH	5.40, m	5', 7'	5', 7'	129.4, CH	5.36, dt (15.5, 6.7)	8'	5', 7'	133.0, CH	5.57, m		5'
7'	34.9, CH ₂	1.95, m	6', 8', 9'	6', 8'	31.6, CH ₂	2.09, m	6', 8', 9'	6', 8'	34.8, CH ₂	2.03, m	6', 9'	8'
8'	22.9, CH ₂	1.34, m	6', 9'	7', 9'	28.5, CH ₂	1.58, m	10'	7', 9'	26.6, CH ₂	1.40, m	6', 7', 9'	7', 9'
9'	13.8, CH ₃	0.88, t (7.4)	7', 8'	8'	17.9, CH ₂	2.18, m	11'	11'	13.9, CH ₃	0.90, t (7.4)	8'	8'
10'					87.6, C							
11'					68.4, CH	1.95, t (2.6)		9'				
1-OH		4.79, br s				4.66, s	1, 2, 6			4.66, s	1, 2, 6	

Table 2.

Biological Activities of Anaephene A (1), Anaephene B (2), and Anaephene C (3)

Compound	Antibacterial and Antifungal Activities (MIC in µg/mL)					Cytotoxicity (IC ₅₀ in µM)		
	<i>B. cereus</i>	<i>S. aureus</i>	<i>P. aeruginosa</i>	<i>M. tuberculosis</i>	<i>S. cerevisiae</i>	HCT116	MDA-MB-231	A549
1	11	22	>22	>22	>22	26	37	27
2	6.1	6.1	>24	>24	>24	71	28	33
3	22	22	>22	>22	>22	>100	49	42
Ciprofloxacin	0.03							
Chloramphenicol		4.0						
Streptomycin			6.2	3.1				
Nystatin					23			