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Venturicidin C, A New 20-Membered Macrolide Produced by *Streptomyces* sp. TS-2-2

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Abstract

Venturicidin C (1), a new 20-membered macrolide along with the known venturicidins A (2) and B (3) were isolated from the crude extract of the Appalachian bacterial strain *Streptomyces* sp. TS-2-2. Additionally, nine other known compounds namely nocardamine, dehydroxynocardamine, desmethylenlnocardamine, ferrioxamine E (FOE), adenosine, riboflavin, *cyclo*(D)-*trans*-4-OH-Pro-(D)-Phe, *cyclo*(D)-Pro-(D)-Phe, and *N*-(2-phenylethyl)-acetamide were also isolated and identified. The structure of the new macrolide 1 was elucidated by the cumulative analyses of NMR and HR-MS spectrometry data. Complete NMR assignments for the known venturicidins A (2) and B (3) are also provided, for the first time, in this report. Venturicidins A-C did not inhibit the proliferation of A549 lung cancer cell lines but all displayed potent antifungal activity.

Keywords

Streptomycetes; apoptolidin; microbial; secondary metabolite; natural products

INTRODUCTION

As a part of our ongoing natural product discovery initiative program, we are investigating soil actinomycetes collected near thermal vents emanating from a range of underground coal mine fire sites throughout Appalachia.[1, 2] Notable alteration of soil composition in and around the vents due to the fire-related emission of greenhouse gases and the dispersion of

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Supplementary Information including the full spectroscopic data (UV, NMR and mass) of venturicidins A-C (**1-3**) are available on the *Journal of Antibiotics* website (http>//www.nature.com/ja).

Conflict of Interest

The authors report competing interests. JST is a co-founder of Centrose (Madison, WI).

volatile organic and inorganic species through surface vents of the mine, in conjunction with the natural biodiversity of Applachian Mountain, supports the contention that such collection sites present unique ecological environments soil actinobacteria-based natural product discovery.[3-11] Located in the upper Elkhorn No. 3 coal bed of Appalachian Mountain of Eastern Kentucky, the Truman Shepherd coal bed was last mined in 1930s and the corresponding Truman Shepherd coal mine fire first observed in 2009.[8] More than 70 pure bacterial cultures isolated from the soil samples collected around the vents of the Truman Shepherd fire displayed actinobacteria-like morphology on solid agar media. Comparison of HPLC-High Resolution Mass Spectrometry (HPLC-HR-MS) molecular ions of the *Streptomyces* sp. TS-2-2 culture extracts to Antibase[12] revealed the potential presence of novel metabolites, with the corresponding MS fragmentation patterns suggestive of glycosylated natural products. In this report, we describe the isolation, structure elucidation and biological activity of a new 20-membered glycosylated macrolide venturicidin C (**1**), along with the two known counterparts venturicidin A (**2**) and B (**3**), from a mycelial extract of *Streptomyces* sp. TS-2-2 (Figure 1). Also reported herein for the first time are the full NMR spectral datasets for venturicidins A (**2**) and B (**3**) to complement the previously published isolation/characterization data.[13-20] Nine additional known compounds namely nocardamine,[21, 22] dehydroxynocardamine,[21] desmethylenlnocardamine,[21] ferrioxamine E (FOE),[23, 24] adenosine, riboflavin, *cyclo*(D)-*trans*-4-OH-Pro-(D)-Phe,[25] *cyclo*(D)-Pro-(D)-Phe,[25] and *N*-(2-phenylethyl)-acetamide[26] were also isolated during the culture extract work up.

RESULTS AND DISCUSSION

Streptomyces sp. TS-2-2 was fermented in liquid medium for both small scale (50 mL) screening and large scale (8L) production of metabolites. Screening of metabolites in low resolution LC-ESI-MS revealed three compounds which displayed similar retention time, UV profile and MS-fragmentation pattern. A difference of 231 *amu* between the base MS ion and the fragmentation MS ion for compounds **1** and **2**, and 170 *amu* for compound **3** suggested a parental glycoside. The extract did not reveal any major UV-visible spots (at 254 or 365 nm) on TLC and treatment with anisaldehyde/sulfuric acid followed by heating revealed a spot with an intensive blue coloration which turned dark-green within a few minutes. This suggested a predominance of non-aromatic compounds within the extract. Subsequent purification of compounds from a large scale fermentation extract using various chromatographic techniques led to the isolation of one new macrolide, venturicidin C (**1**, 3.2 mg l^{-1}), along with the two known antifungal macrolide antibiotics venturicidin A (**2**; 40.0 mg l^{-1}) and B (**3**; 10.8 mg l^{-1}) (see experimental and supporting information for details). The structure of these molecules were determined by a cumulative consideration of 1D and 2D NMR spectroscopy and high resolution mass spectrometry (ESI) data.

Structure elucidation

The physicochemical properties of compounds **1~3** are summarized in Table 1. Compound **1** was isolated as white powder (3.2 mg l^{-1}) from the mycelial extract using various chromatographic techniques (Figure S3). The molecular formula of **1** was deduced as $C_{42}H_{69}NO_{11}$ on the basis of HR-ESI-MS (*m/z* 786.4799 $[M + Na]^+$, Calcd. 786.4763 for

$C_{42}H_{69}NO_{11}Na$) and ^{1}H and ^{13}C NMR. The HRESI-MS spectrum of **1** also revealed a fragmentation ion (*m/z* 555.4067) consistent with the cleavage of a glycosidic bond and the elimination of two water molecules (Figure 2). The proton NMR spectrum of **1** in CD_3OD (Table 2) was rich in aliphatic proton signals with four additional olefinic signals. The triplet signal observed at δ 0.98, five doublets at δ 1.27, 0.98, 0.91, 0.88 and 0.82 along with two singlets at δ 1.43 and 1.48, indicated the presence of nine methyl groups in the molecule. When the solvent was changed to DMSO-d_6, an additional broad signal comprising two protons were observed at δ 6.48, suggesting the presence of an amino group (-NH_2).

The ^{13}C NMR/HSQC spectra of **1** (Table 3) displayed 42 signals which corresponded to nine methyl groups, ten methylene groups, six quaternary carbons, and seventeen methine groups of which eight were oxygenated. In addition, carbon signals pertaining an acetal (δ 99.8), a ketal (δ 95.3), six olefinic (δ 140.1~118.3) an amide (δ 159.7), a lactone/carboxylic acid/amide (δ 174.0), and a ketone (δ 218.0) functionality appeared in the downfield region of the spectrum. The presence of 3'-*O*-carbamoyl-*β*-D-olivose sugar moiety in compound **1** was confirmed through both the MS fragmentation pattern and the cumulative analyses of ^{1}H-^{1}H-COSY/HMBC spectra (Figure 3). The ^3J HMBC correlation observed between H-3' and the quaternary carbon at δ 159.7, confirmed the attachment of the C-3' sugar carbamoyl group. A substructure search of AntiBase 2012[12] using the 3'-*O*-carbamoyl-*β*-D-olivose moiety as a prompt revealed only four 20-membered macrolides - venturicidin A (**2**),[27-29] X-14952B (**4**),[30] 17-hydroxyventuricidin A (**5**),[14] and irumamycin (**6**).[31] Comparison of the ^{13}C NMR chemical shifts of **1** with that reported for X-14952B (**4**) revealed both compounds to share an identical glycosylated macrolactone core with structural divergence at C-17 (Table 3). Specifically, compared to **4, 1** lacks a C-17 hydroxyl as indicated by a notable C-17 ^{13}C NMR chemical shift (δ 43.8 in **1** compared to δ 78.2 in **4**). The ^3J HMBC cross peaks observed between H-13 and C-1' (δ 99.8), and between H-1' and C-13 (δ 84.2) are consistent with the attachment of 3'-*O*-carbamoyl-*β*-D-olivose sugar moiety at C-13 position of **1** as previously observed for **2** and **4-6**.[14, 30, 31] All of the remaining HMBC correlations (Figure 3) and NMR data (Table 2 and 3) are in full agreement with structure **1**. The relative configuration at the stereocenters were indirectly established through the analyses of NOESY correlations (Figure 4) and their comparison to those of the reported analogues **2-9**.[16, 32] Thus, thorough analyses of ^{1}H, ^{13}C, HSQC, ^{1}H-^{1}H-COSY, TOCSY, HMBC and NOESY spectra cumulatively established the structure of compound **1** as depicted in Figure 3 and was subsequently named as venturicidin C.

The physicochemical properties of compound **2** (40.0 mg l^{-1}) were similar to those of **1**. Low resolution ESI MS revealed a 749-Dalton-base peak suggesting its molecular weight as 14 *amu* less than that of **1**. The molecular formula of compound **2** was deduced as $C_{41}H_{67}NO_{11}$ based on the molecular ion peak observed at *m/z* 772.4676 $[M + Na]^+$ in HR-ESI-MS spectrum. The presence of an additional fragment peak at *m/z* 541.3908 was consistent with generation of an aglycone fragment via the elimination of the sugar moiety and two water molecules (Figure 2). Like **1**, the proton NMR spectrum of **2** indicated nine methyl groups. The methyl triplet at δ 0.98 in **1** (24-$CH_2\underline{CH_3}$) was missing in case of **2** and instead, a methyl doublet was displayed at δ 0.96 in **2** (24-$\underline{CH_3}$). The ^{13}C NMR spectrum revealed 41 signals. The chemical shift in the sugar residue and the lactone part of the aglycone moiety in **2** were nearly identical to those of **1** in both CD_3OD and DMSO-d_6 solvents (Tables 2 and 3). Thorough analyses of NMR spectra indicated **2** and **1** to share an identical 20-membered lactone core with the sole structural difference C-24 ($-CH_2CH_3$ in **1** versus $-CH_3$ in **2**). A substructure search in AntiBase 2012 revealed the identity of **2** as venturicidin A, also known as aabomycin A1 (**2**). Although this molecule was isolated previously from several *Streptomyces* species (including *Streptomyces* sp. No. 325-17, *Streptomyces griseolus*, *Streptomyces xanthophaeus* and *Streptomyces aureofaciens*) and its structure subsequently determined through chemical degradation and through partial NMR analyses, we report here for the first time the complete physicochemical and NMR spectroscopic data (Figures 5-6 and Tables 1-3).[27-29]

Likewise, compound **3** was isolated as white powder (10.8 mg l^{-1}) and displayed similar physicochemical properties to **1** and **2**. ESI-MS spectrum of **3** revealed a molecular weight of 706, 43 *amu* less than that of **2**. The molecular formula of **3** was deduced as $C_{40}H_{66}O_{10}$ (*m/z* 729.4594 $[M + Na]^+$ with an identical fragment peak at *m/z* 541.3915 as **2**, indicating the same aglycon in both compounds (Figure 2). This implicated structural divergence of the appended sugar wherein the 43 *amu* molecular weight difference between **3** and **2** could correlate to the sugar C-3' amide group ($-CONH_2$). Consistent with this, the carbamoyl carbon signal observed at δ 159.6 in the ^{13}C NMR spectrum of compound **2** was absent in the spectrum for **3.** Thus, through the cumulative analysis of NMR data and relevant precedent in the literature, the structure of compound **3** was confirmed as depicted in Figures 7-8 – the known molecule venturicidin B (aabomycin A2). While venturicidin B was previously reported as a metabolite of *Streptomyces aureofaceins*,[13] we report here for the first time the complete physicochemical and NMR spectroscopic data.

Including ventruricidin C reported herein, the venturicidins make up 9 of the 17 naturally-occurring 20-membered macrolide glycosides. The closely related congeners venturicidins A,[13] B,[13] X,[16] X-14952B,[30] 17-hydroxyventuricidin A,[14] irumamycin,[31] 3′-decarbamoylirumamycin[14] and irumanolide II[32] are summarized in Figure 1. Their remarkable potency against different fungal strains[14, 15] and their anti-trypanosomal activities[18] are notable. Consistent with this precedent, the antifungal activities of venturicidins A, B, and C against *Cladosporium cucumerium* ATCC 26212 were comparable in disc diffusion assays (supporting information, figure S48). Mechanistically, venturicidins inhibit F_0F_1-ATPase and are also known to inhibit ATP synthesis in both fungi and in bacteria.[17,19,33] However, unlike their structurally/mechanistically similar cytotoxic counterparts (ossamycin, cytovaricin and apoptolidin),[34] venturicidins A, B or C did not exhibit significant cytoxoxicity against non-small cell carcinoma cell line A549 (supporting information, figure S47). This distinction is noteworthy in the context of considering the further development of non-toxic anti-infective members of this unique family. None of these molecules exhibited antibacterial activities against bacterial test strains *S. aureus* ATCC6538 and *S enterica* ATCC10708 up to 124 μM concentrations.

EXPERIMENTAL SECTION

General Experimental Procedures

UV spectra were recorded on an Ultrospec 8000 spectrometer (GE, Pittsburgh, USA). NMR spectra were measured on Varian VnmrJ 500 (1H, 500 MHz; 13C, 125.7 MHz) and VnmrJ 400 (1H, 399.8 MHz; 13C, 100.5 MHz) spectrometers; the δ-values were referenced to the respective solvent signals. ESI mass spectra were recorded on a Finnigan LCQ ion trap mass spectrometer. HRESI mass spectra were recorded on AB SCIEX Triple TOF® 5600 System. HPLC-MS analyses were carried out in Waters 2695 LC module (Waters corp. Milford, MA, USA) using a Symmetry Anal C18 5 μm column (4.6 × 250 mm, (Waters corporation, Milford, MA 01757) and a gradient elution profile (solvent A: H2O, solvent B: acetonitrile; flow rate: 0.5 mL min-1; 0-4 min 90% A and 10% B, 4-22 min, 90-0% A, 22-27 min 0% A and 100% B, 27-29 min 0-90% A, 29-35 min 90% A and 10 % B). For preparative scale separation, Phenomenex (Torrance, CA 90501-1430) C18 column (10 × 250 mm, 5 μm) was used on a Varian (Varian, Palo Alto, CA, USA) ProStar Model 210 equipped with a photodiode diode array detector and a gradient elution profile (solvent A: H2O, solvent B: acetonitrile; flow rate: 5.0 mL min-1; 0-2 min 75% A and 25% B, 2-15 min, 75-0% A, 15-17 min 0% A and 100 % B, 17-18 min 0-75% A, 18-19 min 75% A and 25% B). All solvents used were of ACS grade and purchased from the Pharmco-AAPER (Brookfield, CT). Silica gel (230–400 mesh) for column chromatography was purchased from Silicycle (Quebec City, Canada). Rf values were measured on Polygram SIL G/UV254 (Macherey-Nagel & Co., Dueren, Germany). Amberlite XAD-16 was obtained from Sigma-Aldrich (St. Louis, MO, USA). Size exclusion chromatography was performed on Sephadex LH-20 (25 ~ 100 μm; GE Healthcare, Piscataway Township, NJ, USA).

Isolation of *Streptomyces* sp. TS-2-2 and its Taxonomy

A soil sample containing TS-2-2 was collected from the Truman Shepherd underground mine fire, Floyd County, KY (coordinates: N 37° 28.218' and W 83° 51.132'). *Streptomyces* sp. TS-2-2 was isolated following previously reported methods.[35] Genomic DNA was isolated from a fully grown colony using InstaGene Matrix (Biorad, Hercules, CA, USA). DNA was purified using QIAquick PCR purification kit (Qiagen, Valencia, CA, USA). The partial 16S rRNA gene fragment was amplified using universal primers (27F, 5′-AGAGTTTGATCMTGGCTCAG-3′; 1492R, 5′-GGTTACCTTGTTACGACTT-3′) and Advantage GC2 DNA polymerase (Clontech, Mountain View, CA, USA).[36] PCR conditions were as follows: initial denaturation for 95 °C for 3 min followed by 30 cycles at 94 °C for 30 sec, 48 °C for 30 sec, 68 °C for 1 min 30 sec, followed by a final extension temperature at 68 °C for 5 min. QIAquick gel extraction kit (Qiagen) was used to gel-purify the amplified product. The amplified fragment was sequenced (1,256 bp) and the sequence was used to search the NCBI 16S rRNA library for bacteria and archaea by Basic Local Alignment Search Tool (BLAST). This comparison revealed a 99% identity to the 16S rRNA gene sequence of *Streptomyces bingchenggensis* BCW-1. The sequence of 16S rRNA has been deposited in the NCBI nucleotide database under the accession number KC526988.

Cell Viability Assay

Conversion of resazurin (7-hydroxy-10-oxido-phenoxazin-10-ium-3-one) to its fluorescent product resorufin was monitored to assess viability of human lung non-small cell carcinoma cell line A549. DMEM/F-12 Kaighn's modification and MEM/EBSS media (Thermoscientific, Rockford, IL, USA) were used to grow A549 (ATCC, Manassas, VA, USA) with 10% heat-inactivated fetal bovine serum (FBS), 100 U ml^{-1} penicillin, 100 μg ml^{-1}, streptomycin, 2 mM L-glutamine. Cells were seeded at a density of 2×10^3 cells per well in 96-well clear bottom culture plates (Corning, NY, USA), incubated 24 hours at 37 °C in a humidified atmosphere containing 5% CO_2 and were subsequently exposed to known toxin (1.5 mM hydrogen peroxide, 10 μg ml^{-1} actinomycin D) and test compounds for two days. To assess cell viability, 150 μM of resazurin (Sigma, St. Louis, MO, USA) was added to each well, plates were shaken briefly for 10 seconds and incubated for another 3 hours (A549 cells) at 37 °C to allow viable cells to convert resazurin into resorufin. The fluorescence intensity for resorufin was detected on a scanning microplate spectrofluorometer FLUOstar Omega (BMG Labtech, Cary, NC, USA) using an excitation wavelength of 560 nm and an emission wavelength of 590 nm. The assay was repeated in three independent experiment replications. In each replication, the resorufin values of treated cells were normalized to, and expressed as percent of, the mean resorufin values of untreated, metabolically active cells (100%, all cells are viable). In addition, the CellTox Green Cytotoxicity Assay (Promega, Madison, WI) was applied for assessing the level of dead cells, or cytotoxicity, in the same A549 cell culture. Before assaying viability with resazurin, 20μl of CellTox™ Green Dye (a proprietary asymmetric cyanine dye) reagent was added to each well of plate with cells, plate was mixed on shaker for 1 minute, incubated for 15 minutes at room temperature and fluorescent intensity of dye intercalated with dead cells DNA was measured at an excitation wavelength of 485 nm and an emission wavelength of 520 nm on a scanning microplate spectrofluorometer FLUOstar Omega, then resazurin was added and viability was assayed as described above. Cytotoxicity values were converted to corresponding viability values and plotted on the same graph with resazurin assay data.

Media, Fermentation and Isolation

A-Medium—Glucose (10.0 g), yeast extract (5.0 g), soluble starch (20.0 g), peptone (5.0 g), NaCl (4.0 g), K_2HPO_4 (0.5 g), $MgSO_4{\cdot}7H_2O$ (0.5), and calcium carbonate (2 g) were dissolved in 1 liter of demineralized water. The suspension (pH 7.0) was sterilized by autoclaving for 33 min at 121 °C.

M_2-agar plates—Glucose (4.0 g), malt extract (10.0 g), yeast extract (4.0 g), and agar (15.0 g) were dissolved in 1 liter of demineralized water. The suspension (pH 7.2) was sterilized by autoclaving for 33 min at 121 °C.

Fermentation, Extraction and Isolation—The terrestrial *Streptomyces* sp. TS2-2 was cultivated on M_2-agar plates at 28 °C for 3 days. To prepare the seed culture, chuncks of grown agar plate were used to inoculate three-250 ml baffled flasks, each containing 100 ml of A-medium, and the cultures grown at 28 °C with shaking (210 rpm) for 3 days. An aliquot of seed culture (3 ml) was subsequently used to inoculate 80 250 ml baffled flasks, each containing 100 ml of A-medium. Fermentation was continued at 28°C with shaking

(210 rpm) for five days. The obtained reddish-brown culture broth was centrifuged and filtered over celite. The supernatant was mixed with XAD-16 (4%) resin overnight, followed by filtration. The resin was washed with water (4 × 800 ml) and then extracted with methanol (3 × 600 ml). The methanol extract was subsequently evaporated *in vacuo* at 38 °C to afford 15.1 g of reddish-brown solid crude extract. The biomass (mycelium) was extracted with methanol (3 × 500 ml) followed by acetone (1 × 800 ml) and then evaporated *in vacuo* at 38 °C to yield 8.8 g of reddish-brown solid crude extract. Both extracts revealed different sets of metabolites based upon HPLC and TLC analyses wherein the targeted metabolites were found to be mostly present in the mycelium-extract. Thus, this extract was subjected to the following work up and isolation procedure.

The mycelial extract (8.8 g) was dissolved in MeOH/50% H_2O (40 ml) and was fractionated with a gradient of H_2O/0-100% CH_3CN using RP-18 column (column 3 × 30 cm, 75 g) chromatography. This resulted in the generation of following fractions: 0.25 ml 0% CH_3CN and 0.25 ml 10% CH_3CN → fraction FI (1.6 g); 0.25 L 20% CH_3CN → fraction FII (0.8 g); 0.25 L 30% CH_3CN → fraction FIII (0.6 g); 0.25 L 40% CH_3CN → fraction FIV (0.25 g); 0.25 L 60% CH_3CN, 0.25 ml 50% CH_3CN, 0.25 ml 25% CH_3CN (combined based on the HPLC and TLC similarity) → fractions FV-FVII (1.2 g); 0.5 L 100% CH_3CN → fraction FVIII (0.92 g). TLC analysis followed by anisaldehyde sulfuric acid development indicated that the target dark-green band was present mainly in fraction FVIII (0.92 g) (see supporting information, Figure S4). Further purification of fraction FVIII using sephadex LH-20 (CH_2Cl_2/40% MeOH, 2.5 × 50 cm) and then by HPLC (see supporting information Figure S6, for HPLC conditions) afforded venturicidins A (**2**; 40.0 mg ml^{-1}), B (3; 10.8 mg ml^{-1}) and C (1; 3.2 mg ml^{-1}) as white powders. Fractions FI, FII and FV-FVII were avoided based on the HPLC/MS and TLC analysis, as no related compounds were observed. Further purifications of fractions FIII and FIV using Sephadex LH-20 (MeOH, 2.5 × 40 cm) led to the isolation of nocardamine-Fe-complex (240.0 mg, red solid, molecular weight 654 daltons) and ferrioxamine E (45.8 mg, orange solid), respectively.

Similarly, the XAD-extract (15.1 g) was dissolved in MeOH/50% H_2O (60 ml) and then subjected to fractionation using RP-18 column (column 3 × 30 cm, 75 g) chromatography and gradients of H_2O/0-100% CH_3CN systems. This resulted in the generation of nine fractions as follows: 0.25 ml 0% CH_3CN and 0.25 ml 10% CH_3CN → fraction FI (5.5 g); 0.15 L 20% CH_3CN → fraction FII (1.3 g); 0.25 L 20% CH_3CN → fraction FIII (1.25 g); 0.25 L 30% CH_3CN → fraction FIV (0.65 g); 0.25 L 40% CH_3CN → fraction FV (2.5 g); 0.25 L 50% CH_3CN → fraction FVI (0.20 g); 0.25 L 60% CH_3CN → fraction FVII (0.15 g); 0.25 L 80% CH_3CN → fraction FVIII (0.55 g); 0.5 L 100% CH_3CN → fraction FIX (0.21 g). Fractions FI and FIX were discarded as no significant metabolites were observed by TLC or HPLC/MS. Fractions FII, FIII and FIV were combined based on their HPLC/MS and TLC similarities. Further purification of the combined fractions using Sephadex LH-20 and HPLC afforded adenosine (131.2 mg, white powder), riboflavin (120.8 mg, yellowish-green solid) and *cyclo*(D)-*trans*-4-OH-Pro-(D)-Phe (28.3 mg, colorless solid).[25] In a similar manner, fractions FVI and FVII were combined and purified to afford *cyclo*(D)-Pro-(D)-Phe[25] (29.8 mg, colorless solid) and *N*-(2-phenylethyl)-acetamide[26] (10.8 mg, colorless solid) in pure forms. HPLC purification of fraction FIII (0.55 g) led to the isolation of nocardamine[21, 22]

(28.3 mg), dehydroxynocardamine[21] (4.8 mg) and desmethylenlnocardamine[21] (3.7 mg) as white solids. Fine crystals were observed in fraction FV (2.5 g) when it was kept overnight in H_2O/40% CH_3CN. Filtration of fraction of crystals followed by washing with water afforded nocardamine (1.6 g, white needles). The filtrate was not further analyzed as it mainly contained an unknown nocardamine-Fe-complex[23] (1.31 g, red solid, molecular weight 654 Daltons), (Figure S3).

Antibacterial and Antifungal Activity Tests

The fungal strain *S. cerevisiae* (ATCC 204508), and the two bacterial strains *S. aureus* (ATCC 6538) and *S. enterica* (ATCC 10708) were used as model strains for susceptibility assays. *S. cerevisiae, S. enterica* and *S. aureus* were grown in liquid or on agar plates using YAPD (ATCC medium number 1069), nutrient broth (BD 234000) and in tryptic soy broth (BD211825) media, respectively. Individual strains were grown in 5 ml medium for 16 h at 37 °C with shaking (200 rpm). An aliquot of a fully grown culture (100 μL) was diluted to 30 ml using sterile liquid medium. Aliquots (160 μL) of each diluted culture were then transferred into 96 well plates supplied with 2 μL of venturicidins (final concentration of range 1-125 μm). Negative controls tested in parallel include vehicle alone (DMSO) while positive controls ampicillin and kanamycin sulfate (200 μg each for *S. enterica* and *S. aureus*) or cycloheximide (50 μg for *S. cerevisiae*) were employed. The culture plate was incubated at 37°C for 16 h with shaking (200 rpm) and the OD_{600} was subsequently measured using a scanning microplate spectrofluorometer FLUOstar Omega (BMG Labtech, Cary, NC, USA). The acquired OD_{600} values were normalized considering the negative control wells have 100% viability.

The fungal strain *Cladosporium cucumerinum* was used in disc diffusion assays. Solutions of amphotericin B and test compounds were made in DMSO. Each sterile paper disc was loaded with 20 μL solution and was allowed to dry in the biosafety cabinet for 4 hours. The dried discs were then placed on the V8 agar plate following the homogenous distribution of *Cladosporium cucumerinum* spores. DMSO was used as a negative control. The plates were then incubated at 24°C for three days under the dark condition. Inhibition zone were then measured.

V8-medium—6 gram of calcium carbonate was added into 360 mL V8 juice and stirred the mixture for 30 minutes. Supernatant liquid was collected following centrifugation at 4000 × g for 10 minutes. pH of the solution was adjusted to 7.0 using 1 M KOH solution. The solution was then diluted to four fold with water. Bacto agar was added to 1.5% (W/V) and then autoclaved to prepare V8 agar plates.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

1. Shaaban KA, Wang X, Elshahawi SI, Ponomareva LV, Sunkara M, Copley GC, Hower JC, Morris AJ, Kharel MK, Thorson JS. Herbimycins D-F, Ansamycin Analogues from *Streptomyces* sp. RM-7-15. Manuscript submitted.
2. Wang X, Shaaban KA, Elshahawi SI, Ponomareva LV, Sunkara M, Zhang Y, Copley GC, Hower JC, Morris AJ, Kharel MK, Thorson JS. Frenolicins C–G, Pyranonaphthoquinones from *Streptomyces* sp. RM-4-15. Manuscript submitted.
3. Petranka, JW. Salamanders of the United States and Canada. Smithonian Institute Press; Washington, DC: 1998.
4. Bernardo J, Spotila JR. Physiological constraints on organismal response to global warming: Mechanistic insights from clinally varying populations and implications for assessing endangerment. Biol Lett. 2006; 2(1):135–139. [PubMed: 17148347]
5. Neves, RJ.; Bogan, AE.; Williams, JD.; Ahlstedt, SA.; Hartfield, W. Aquatic Fauna in Peril: The Southeastern Perspective. Special Publication No. 1, Southeastern Research Institute, Lenz Design and Communications; Decatur, GA: 1997.
6. Stein, BA.; Kutner, LS.; Adams, JS. Precious Heritage: The Status of Biodiversity in the United States. Oxford University Press; Oxford, UK: 2000.
7. History of Coa l in Kentucky. Frankfort, KY: Dec 27. 2012 p. 1-101.
8. O'Keefe JMK, Henke KR, Hower JC, Engle MA, Stracher GB, Stucker JD, Drew JW, Staggs WD, Murray TM, Hammond ML, Adkins KD, Mullins BJ, Lemley EW. CO_2, CO, and Hg emissions from the Truman Shepherd and Ruth Mullins coal fires, eastern Kentucky, USA. Sci Total Environ. 2010; 408(7):1628–1633. [PubMed: 20071005]
9. Zhao Y, Zhang J, Chou C, Li Y, Wang Z, Ge Y. Trace element emissions from spontaneous combustion of gob piles in coal mines, Shanxi, China. Int J Coal Geol. 2008; 73:52–62.
10. Carras JN, Day SJ, Saghafi A, Williams DJ. Greenhouse gas emissions from lowtemperature oxidation and spontaneous combustion at open-cut coal mines in Australia. Int J Coal Geol. 2009; 78:161–168.
11. Hower JC, Henke K, O'Keefe JMK, Engle MA, Blake DR, Stracher GB. The Tiptop coalmine fire, Kentucky: Preliminary investigation of the measurement of mercury and other hazardous gases from coal-fire gas vents. Int J Coal Geol. 2009; 80(1):63–67.
12. Laatsch, H. AntiBase 2012: The natural compound identifier. Wiley-VCH; Weinheim: 2012. AntiBase 2012: The natural compound identifier.
13. Brufani M, Cellai L, Musu C, Keller-Schierlein W. Metabolic products of microorganisms. 102. The structure of venturicidin A and B. Helv Chim Acta. 1972; 55(7):2329–2346. [PubMed: 4637271]
14. Fourati-Ben Fguira L, Fotso S, Ben Ameur-Mehdi R, Mellouli L, Laatsch H. Purification and structure elucidation of antifungal and antibacterial activities of newly isolated *Streptomyces* sp. strain US80. Res Microbiol. 2005; 156(3):341–347. [PubMed: 15808937]
15. Fourati-Ben Fguira L, Smaoui S, Karray-Rebai I, Bejar S, Mellouli L. The antifungal activity of the terrestrial *Streptomyces* US80 strain is induced by heat-killed fungi. Biotechnol J. 2008; 3(8): 1058–1066. [PubMed: 18481262]
16. Laatsch H, Kellner M, Lee YS, Wolf G. Isolation of Venturicidin-X, the Aglycon of Venturicidin A and Venturicidin B from *Streptomyces* sp. Z Naturforsch (B). 1994; 49(7):977–980.
17. Morales-Rios E, de la Rosa-Morales F, Mendoza-Hernandez G, Rodriguez-Zavala JS, Celis H, Zarco-Zavala M, Garcia-Trejo JJ. A novel 11-kDa inhibitory subunit in the F1FO ATP synthase of *Paracoccus denitrificans* and related alpha-proteobacteria. FASEB J. 2010; 24(2):599–608. [PubMed: 19783785]
18. Otoguro K, Ishiyama A, Namatame M, Nishihara A, Furusawa T, Masuma R, Shiomi K, Takahashi Y, Yamada H, Omura S. Selective and potent in vitro antitrypanosomal activities of ten microbial metabolites. J Antibiot. 2008; 61(6):372–378. [PubMed: 18667785]

19. Perlin DS, Latchney LR, Senior AE. Inhibition of *Escherichia coli* H+-ATPase by venturicidin, oligomycin and ossamycin. Biochim Biophys Acta. 1985; 807(3):238–44. [PubMed: 2859888]
20. Walter P, Lardy HA, Johnson D. Antibiotics as Tools for Metabolic Studies. X. Inhibition of Phosphoryl Transfer Reactions in Mitochondria by Peliomycin Ossamycin and Venturicidin. J Biol Chem. 1967; 242(21):5014–5018. [PubMed: 4228672]
21. Lee HS, Shin HJ, Jang KH, Kim TS, Oh KB, Shin J. Cyclic peptides of the nocardamine class from a marine-derived bacterium of the genus *Streptomyces*. J Nat Prod. 2005; 68(4):623–625. [PubMed: 15844966]
22. Meyer JM, Abdallah MA. The Siderochromes of Non-Fluorescent Pseudomonads - Production of Nocardamine by *Pseudomonas stutzeri*. J Gen Microbiol. 1980; 118:125–129.
23. Essen SA, Johnsson A, Bylund D, Pedersen K, Lundstrom US. Siderophore production by Pseudomonas stutzeri under aerobic and anaerobic conditions. Appl Environ Microbiol. 2007; 73(18):5857–5864. [PubMed: 17675442]
24. Mawji E, Gledhill M, Milton JA, Tarran GA, Ussher S, Thompson A, Wolff GA, Worsfold PJ, Achterberg EP. Hydroxamate Siderophores: Occurrence and Importance in the Atlantic Ocean. Environ Sci Tech. 2008; 42(23):8675–8680.
25. Fdhila F, Vazquez V, Sanchez JL, Riguera R. dd-diketopiperazines: antibiotics active against *Vibrio anguillarum* isolated from marine bacteria associated with cultures of *Pecten maximus*. J Nat Prod. 2003; 66(10):1299–1301. [PubMed: 14575426]
26. Maskey RP, Asolkar RN, Kapaun E, Wagner-Dobler I, Laatsch H. Phytotoxic arylethylamides from limnic bacteria using a screening with microalgae. J Antibiot. 2002; 55(7):643–649. [PubMed: 12243454]
27. Aizawa S, Nakamura Y, Shirato S, Taguchi R, Yamaguchi I. Aabomycin A, a new antibiotic. I. Production, isolation and properties of aabomycin A. J Antibiot. 1969; 22(10):457–462. [PubMed: 5350501]
28. Seino A, Sugawara H, Shirato S, Misato T. Aabomycin A, a new antibiotic. 3. Taxonomic studies on the aabomycin producing strain, *Streptomyces hygroscopicus* subsp. aabomyceticus Seino subsp. nov. J Antibiot. 1970; 23(4):204–209. [PubMed: 5429509]
29. Yamaguchi I, Taguchi R, Huang KT, Misato T. Aabomycin A, a new antibiotic. II. Biological studies on aabomycin A. J Antibiot. 1969; 22(10):463–466. [PubMed: 5350502]
30. Omura S, Nakagawa A, Imamura N, Kushida K, Liu CM, Sello LH, Westley JW. Structure of a new macrolide antibiotic, X-14952B. J Antibiot. 1985; 38(5):674–676. [PubMed: 3839500]
31. Omura S, Tanaka Y, Nakagawa A, Iwai Y, Inoue M, Tanaka H. Irumamycin, a new antibiotic active against phytopathogenic fungi. J Antibiot. 1982; 35(2):256–257. [PubMed: 7076572]
32. Sadakane N, Tanaka Y, Omura S. New 20-membered lactones, irumanolides I and II, produced by a mutant of *Streptomyces*. J Antibiot. 1983; 36(7):931–933. [PubMed: 6885645]
33. Griffiths DE, Houghton RL. Studies on energy-linked reactions: modified mitochondrial ATPase of oligomycin-resistant mutants of *Saccharomyces cerevisiae*. Eur J Biochem. 1974; 46(1):157–167. [PubMed: 4277672]
34. Salomon AR, Voehringer DW, Herzenberg LA, Khosla C. Apoptolidin, a selective cytotoxic agent, is an inhibitor of F0F1-ATPase. Chem Biol. 2001; 8(1):71–80. [PubMed: 11182320]
35. Abdelfattah MS, Kharel MK, Hitron JA, Baig I, Rohr J. Moromycins A and B, isolation and structure elucidation of C-glycosylangucycline-type antibiotics from *Streptomyces* sp. KY002. J Nat Prod. 2008; 71(9):1569–1573. [PubMed: 18666798]
36. Lane, DJ. 16S/23S rRNA sequencing. John Wiley and Sons Ltd.; New York, N.Y.: 1991. p. 115-147.

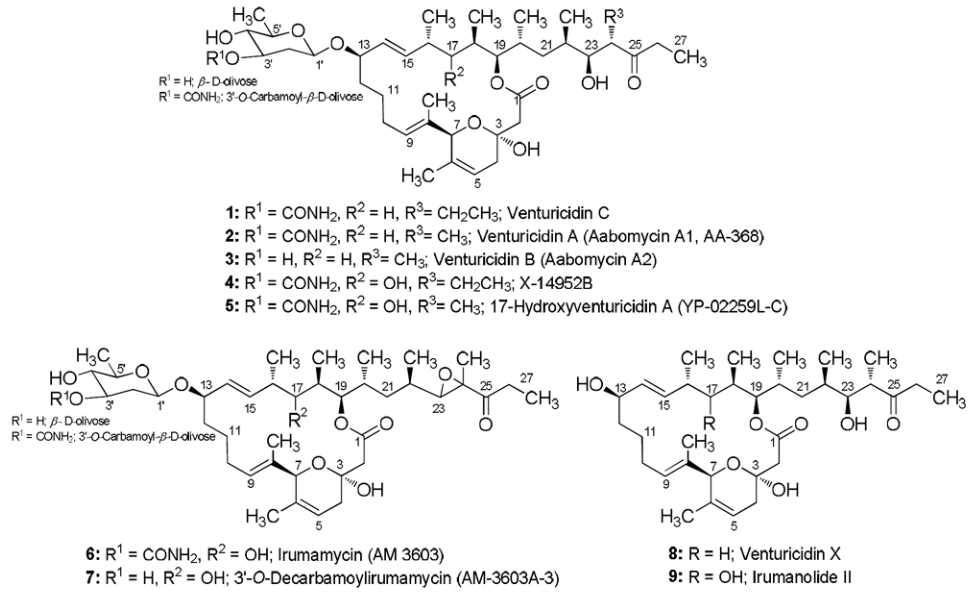

Figure 1.

Chemical structure of venturicidins C (**1**), A (**2**), and B (**3**), along with the related macrolides **4-8**

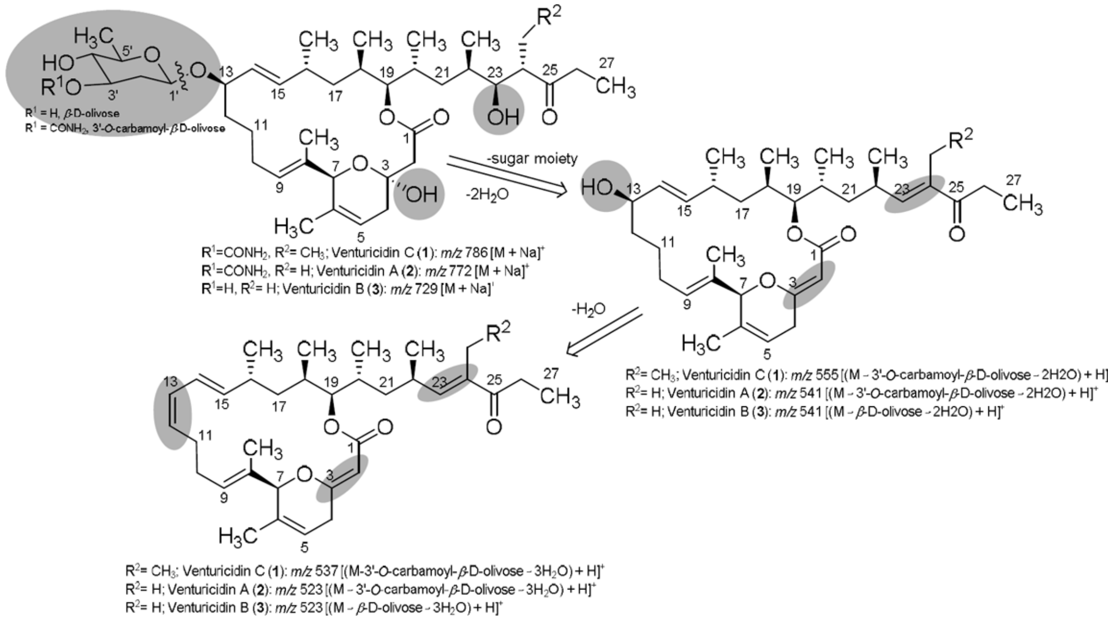

Figure 2.
ESI/MS fragmentation patterns of venturicidins C (**1**), A (**2**), and B (**3**)

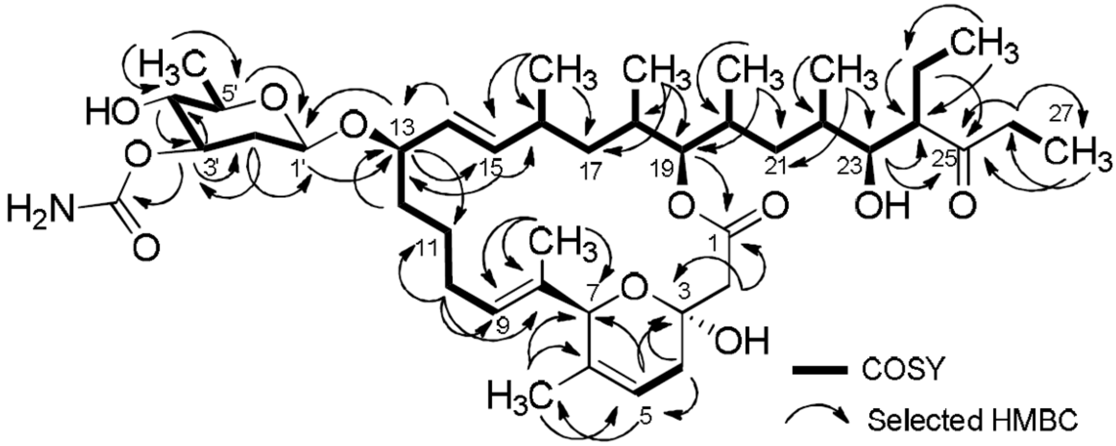

Figure 3.
Selected $^{1}H,^{1}H$-COSY (▬) and HMBC (→) correlations of venturicidin C (**1**)

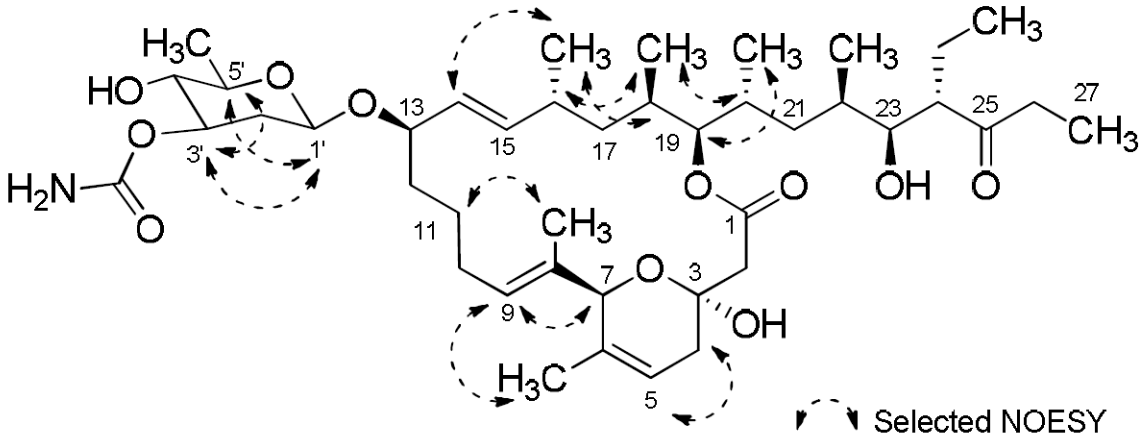

Figure 4.
Selected NOESY ( ) couplings of venturicidin C (**1**)

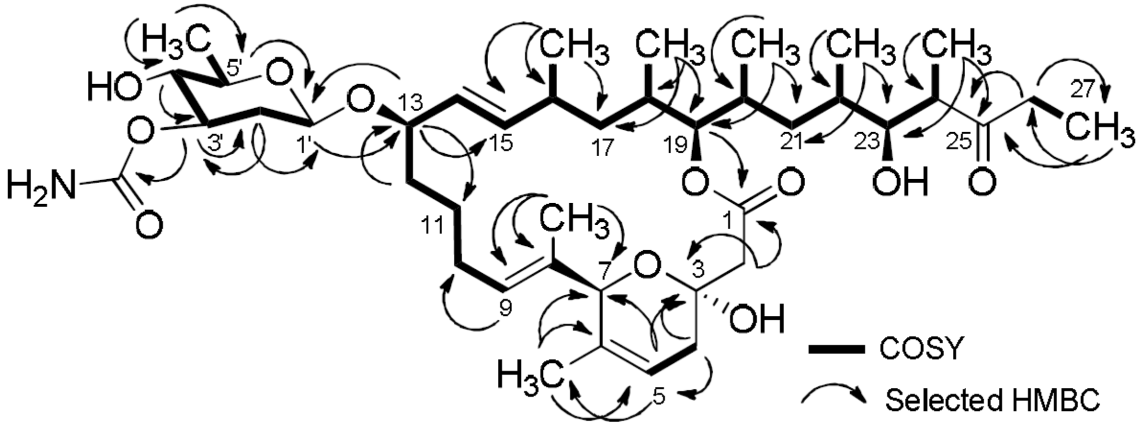

Figure 5.

Selected $^1H,^1H$-COSY (▬) and HMBC (→) correlations of venturicidin A (**2**)

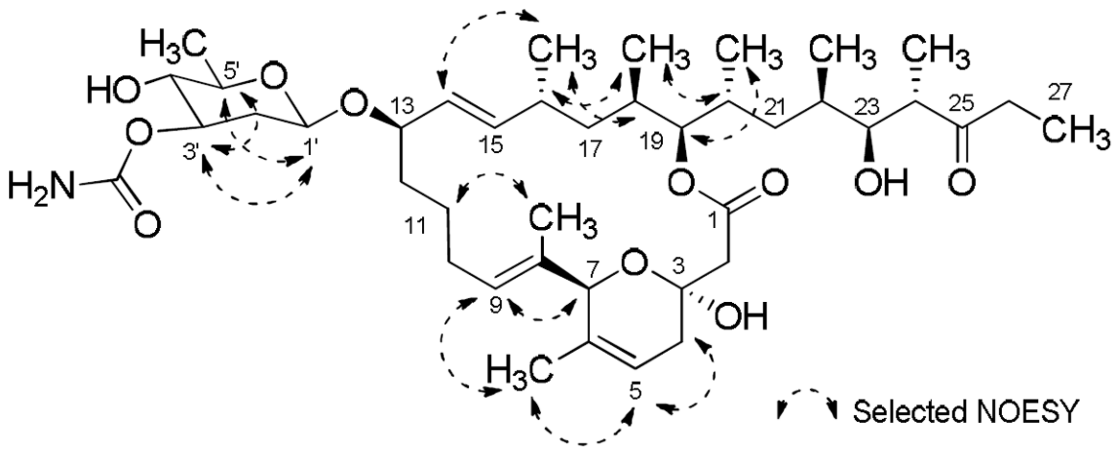

Figure 6.

Selected NOESY ( ) couplings of venturicidin A (**2**)

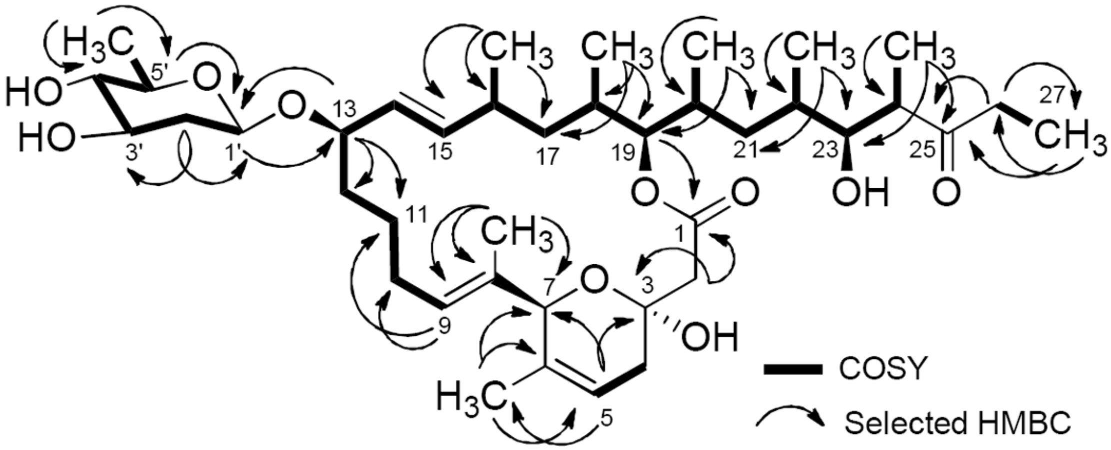

Figure 7.
Selected $^{1}H,^{1}H$-COSY (▬) and HMBC (→) correlations of venturicidin B (**3**)

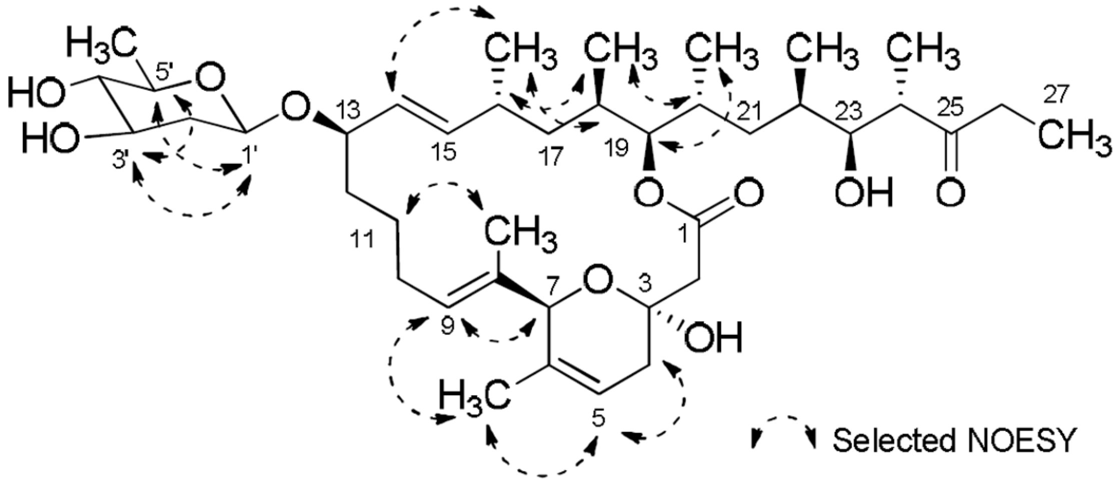

Figure 8.
Selected NOESY ( ) couplings of venturicidin B (**3**)

Table 1

Physico-chemical properties of macrolides **1-3**

	Venturicidin C (1)	**Venturicidin A (2)**	**Venturicidin B (3)**
Molecular Formula	$C_{42}H_{69}NO_{11}$	$C_{41}H_{67}NO_{11}$	$C_{40}H_{66}O_{10}$
Appearance	White powder, UV non-absorbing (254 nm)	White powder, UV non-absorbing (254 nm)	White powder, UV non-absorbing (254 nm)
R_f	0.24[a],0.34[b], 0.28[c]	0.23[a],0.33[b]	0.23[a],0.31[b]
HPLC-R_t[a)]	17.01 (min)	16.25 (min)	17.01 (min)
Anisaldehyde/H_2SO_4 reagent	Pink then dark-blue and few minutes later turned to dark-green	Pink then dark-blue and few minutes later turned to dark-green	Pink then dark-blue and few minutes later turned to dark-green
(+)-ESI-MS: *m/z*	786 $[M + Na]^+$, 555 $[(M - 3'$-*O*-carbamoyl-β-D-olivose – $2H_2O) + H]^+ - 2H_2O) + H]^+$,537 $[(M - 3'$-*O*-carbamoyl-β-D-olivose – $3H_2O) + H]^+$	772 $[M + Na]^+$, 541 $[(M - 3'$-*O*-carbamoyl-β-D-olivose – $2H_2O) + H]^+$, 523 $[(M - 3'$-*O*-carbamoyl-β-D-olivose – $3H_2O) + H]^+$	729 $[M + Na]^+$, 541 [(M – β-D-olivose $-2H_2O) + H]^+$, 523 [(M – β-D-olivose – $3H_2O) + H]^+$
(+)-HRESI-MS: *m/z*	786.4799 $[M + Na]^+$, 555.4067 $[(M - 3'$-*O*-carbamoyl-β-D-olivose – $2H_2O) + H]^+$	772.4676 $[M + Na]^+$, 559.4016 $[(M - 3'$-*O*-carbamoyl-β-D-olivose – $H_2O) + H]^+$, 541.3908 $[(M - 3'$-*O*-carbamoyl-β-D-olivose – $2H_2O) + H]^+$, 523.3795 $[(M - 3'$-*O*-carbamoyl-β-D-olivose – $3H_2O) + H]^+$	1435.9323 $[2M + Na]^+$, 729.4594 $[M+ Na]^+$, 559.4022 [(M – β-D-olivose – $H_2O) + H]^+$, 541.3915 [(M – β-D-olivose – $2H_2O) + H]^+$, 523.3806 [(M – β-D-olivose – $3H_2O) + H]^+$
Calcd.	786.4763 for $C_{42}H_{69}NO_{11}Na$ $[M + Na]^+$ and 555.4044 for $C_{35}H_{55}O_5$ $[(M - 3'$-*O*-carbamoyl-β-D-olivose – $2H_2O) + H]^+$	772.4606 for $C_{41}H_{67}NO_{11}Na$ $[M + Na]^+$, 559.3993 for $C_{34}H_{55}O_6$ $[(M - 3'$-*O*-carbamoyl-β-D-olivose – $H_2O) + H]^+$, 541.4044 for $C_{34}H_{53}O_5$ $[(M - 3'$-*O*-carbamoyl-β-D-olivose – $2H_2O) + H]^+$ and 523.3782 for $C_{34}H_{51}O_4$ $[(M - 3'$-*O*-carbamoyl-β-D-olivose – $3H_2O) + H]^+$	1435.9204 for $C_{80}H_{132}O_{20}Na$ $[2M+Na]^+$, 729.4548 for $C_{40}H_{66}O_{10}Na$ $[M + Na]^+$, 559.3993 for $C_{34}H_{55}O_6$ [(M – β-D-olivose – $2H_2O) + H]^+$, 541.4044 for $C_{34}H_{53}O_5$ [(M – β-D-olivose – $2H_2O) + H]^+$ and 523.3782 for $C_{34}H_{51}O_4$ [(M – β-D-olivose – $3H_2O) + H]^+$

[a)] For HPLC purification, see figures S3-S6, S22-S23, S34-S35;

[a)] CH_2Cl_2/5% MeOH;

[b)] CH_2Cl_2/50% EtOAc;

[c)] CH_2Cl_2/7% MeOH

Table 2

^{1}H NMR spectroscopic data of venturicidins C (**1**), A (**2**), and B (**3**) (δ in ppm, mult., *J* in [Hz])

Position	**1** [a)]	**1** [b)]	**1** [c)]	**2** [a)]	**2** [b)]	**3** [a)]	**3** [b)]
	δ_H [d)]	δ_H [e)]	δ_H [d)]	δ_H [d)]	δ_H [e)]	δ_H [d)]	δ_H [e)]
2	2.75-2.60 (m)	2.95-2.50 (m)	2.70-2.45 (m)	2.70 (d, 16.0), 2.61 (d, 16.0)	2.90-2.40 (m)	2.70 (d, 16.0), 2.60 (d, 16.0)	2.78-2.46 (m)
3-OH	-	5.44 (brs)	-	-	5.43 (brs)		
4	2.10 (m), 1.45 (m)	2.08 (m), 1.55 (m)	2.10-2.25 (m)	2.10 (m), 1.50 (m)	2.08 (m), 1.50 (m)	2.10 (m), 1.50 (m)	2.08 (m), 1.50 (m)
5	5.49 (brm)	5.50-5.40 (brm)	5.47 (brm)	5.49 (brm)	5.48 (brm)	5.48 (m)	5.46 (m)
6-CH_3	1.48 (s)	1.41 (s)	1.46(s)	1.48 (s)	1.41 (s)	1.48 (s)	1.41 (s)
7	4.41 (brs)	4.32 (brs)	4.43 (brs)	4.42 (brs)	4.32 (brs)	4.42 (brs)	4.32 (brs)
8-CH_3	1.43 (s)	1.36 (s)	1.38 (s)	1.44 (s)	1.36 (s)	1.44 (s)	1.36 (s)
9	5.44 (m)	5.50-5.40 (brm)	5.38 (m)	5.44 (dd, 10.5, 5.5)	5.41 (m)	5.44 (m)	5.42 (m)
10	2.10 (m), 1.80 (m)	2.10 (m), 1.70 (m)	2.15 (m), 1.80 (m)	2.10 (m), 1.89 (m)	2.10 (m), 1.70 (m)	2.15 (m), 1.90 (m)	2.15 (m), 1.90 (m)
11	1.48-1.25 (m)	1.60-1.20 (m)	2.12 (m), 1.80 (m)	1.50-1.20 (m)	1.50-1.20 (m)	1.50-1.20 (m)	1.50-1.20 (m)
12	1.50-1.40 (m)	2.05 (m)	1.30-1.20 (m)	1.50-1.46 (m)	2.10 (m)	2.10 (m)	2.10 (m)
13	3.94 (m)	3.86 (m)	3.82 (m)	3.94 (m)	3.86 (m)	3.91 (m)	3.84 (m)
14	5.38 (dd, 15.0, 8.0)	5.38-5.20 (m)	5.38 (m)	5.39 (dd, 15.0, 7.5)	5.31 (dd, 15.2, 7.6)	5.38 (dd, 15.5, 8.5)	5.30 (dd, 15.2, 7.6)
15	5.33 (dd, 15.5, 8.0)	5.38-5.20 (m)	5.26 (m)	5.34 (dd, 15.0, 8.5)	5.22 (dd, 15.2, 8.4)	5.30 (dd, 15.0, 8.5)	5.21 (dd, 15.2, 8.4)
16	2.17 (m)	2.07 (m)	2.12 (m)	2.17 (m)	2.10 (m)	2.10 (m)	2.10 (m)
16-CH_3	0.98 (d, 7.0)	0.83 (d, 7.2)	1.07 (d, 7.0)	0.97 (d, 7.0)	0.83 (d, 6.8)	0.96 (d, 7.0)	0.85 (d, 7.2)
17	1.24 (m), 1.10 (m)	1.30 (m), 0.96 (m)	1.22 (m), 0.95 (m)	1.24 (m), 1.09 (m)	1.30 (m), 0.96 (m)	1.30 (m), 1.12 (m)	1.30 (m), 1.15 (m)
18	1.89 (m)	2.20-1.65 (m)	1.83 (m)	1.89 (m)	2.20-1.70 (m)	1.95-1.80 (m)	1.90-1.70 (m)
18-CH_3	0.88 (d, 7.0)	0.78 (d, 6.4)	0.91 (d, 6.5)	0.89 (d, 7.5)	0.78 (d, 6.0)	0.88 (d, 7.5)	0.78 (d, 6.4)
19	4.68 (m)	4.48 (m)	4.82 (m)	4.68 (dd, 8.0, 4.5)	4.49 (m)	4.69 (m)	4.57 (m)
20	1.88 (m)	1.80 (m)	1.70 (m)	1.89 (m)	1.80 (m)	1.85 (m)	1.80 (m)
20-CH_3	0.91 (d, 7.5)	0.81 (d, 6.4)	0.82 (d, 6.5)	0.91 (d, 7.0)	0.80 (d, 6.8)	0.89 (d, 7.0)	0.81 (d, 6.8)
21	1.40 (m), 1.0 (m)	1.55 (m), 0.95 (m)	1.20 (m), 0.95 (m)	1.41 (m), 1.0 (m)	1.55 (m), 0.95 (m)	1.50 (m), 1.0 (m)	1.55 (m), 0.95 (m)
22	1.69 (m)	1.80 (m)	1.55 (m)	1.68 (m)	1.70 (m)	1.70 (m)	1.72 (m)
22-CH_3	0.82 (brd, 5.0)	0.71 (brd, 6.0)	0.83 (d, 6.4)	0.82 (d, 6.5)	0.70 (d, 6.4)	0.82 (d, 7.0)	0.70 (d, 6.4)
23	3.56 (brdd, 9.5, 2.0)	3.18 (m)	3.51 (m)	3.56 (dd, 9.5, 2.0)	3.15 (m)	3.56 (dd, 10.0, 1.5)	3.38-3.20 (m)

Position	**1** [a)]	**1** [b)]	**1** [c)]	**2** [a)]	**2** [b)]	**3** [a)]	**3** [b)]
	δ_H [d)]	δ_H [e)]	δ_H [d)]	δ_H [d)]	δ_H [e)]	δ_H [d)]	δ_H [e)]
23-OH	-	5.03 (brd, 6.2)	-	-	5.03 (d, 6.8)	-	4.84 (brs)
24	2.70 (m)	2.90 (m)	2.73 (m)	2.78 (m)	3.15 (m)	2.78 (m)	3.03 (m)
24-CH3	-	-	-	0.96 (d, 7.0)	0.88 (d, 6.4)	0.97 (d, 7.0)	0.88 (d, 6.4)
24-$\underline{CH_2}CH_3$	1.42 (m), 1.30 (m)	1.35 (brm), 1.23 (brm)	1.70-1.50 (m)	-	-	-	-
24-$CH_2\underline{CH_3}$	0.98 (t, 7.5)	0.89 (t, 7.2)	0.84 (t, 6.5)	-	-	-	-
26	2.61 (m)	2.90-2.40 (m)	2.50 (m)	2.60 (m)	2.90-2.40 (m)	2.58 (m)	2.78-2.46 (m)
27	1.00 (t, 7.0)	0.89 (t, 8.0)	1.02 (t, 7.5)	1.00 (t, 7.0)	0.89 (t, 6.4)	1.01 (t, 7.0)	0.89 (t, 7.2)
1’	4.61 (dd, 10.0, 1.5)	4.59 (m)	4.53 (brd, 8.0)	4.61 (dd, 10.0, 1.5)	4.59 (brd, 6.4)	4.56 (brdd, 10.0, 1.0)	4.46 (dd, 9.2, 1.2)
2’	2.20 (m), 1.40 (m)	2.08 (m), 1.30 (m)	2.25 (m), 1.56 (m)	2.24 (m), 1.50 (m)	2.13 (m), 1.30 (m)	2.15 (m), 1.40 (m)	2.13 (m), 1.30 (m)
3’	4.56 (m)	4.47 (m)	4.63 (m)	4.56 (m)	4.43 (m)	3.48 (brdd, 7.5, 4.5)	3.38-3.20 (m)
3’-CO$\underline{NH_2}$	-	6.48 (brs)	-	-	6.46 (brs)	-	-
3’-OH	-	-	-	-	-	-	4.84 (brs)
4’	3.07 (dd, 9.5, 9.0)	3.36 (m)	3.22 (m)	3.10 (dd, 9.5, 9.0)	3.36 (brm)	2.88 (dd, 9.0, 9.0)	3.38-3.20 (m)
5’	3.26 (m)	3.20 (m)	3.22 (m)	3.26 (m)	3.36 (m)	3.17 (m)	3.38-3.20 (m)
5’-CH_3	1.27 (d, 6.0)	1.14 (d, 7.2)	1.30 (d, 6.0)	1.27 (d, 6.0)	1.13 (d, 6.4)	1.24 (d, 6.5)	1.11 (d, 6.0)

[a)] CD_3OD;

[b)] DMSO-d_6;

[c)] $CDCl_3$;

[d)] 500 MHz;

[e)] 400 MHz;

[f)] 100 MHz;

[g)] 100 MHz; See supporting information (Figures S10, S19, S31, S36, S43) for NMR spectra.

Table 3

[13]C NMR spectroscopic data of venturicidins C (**1**), A (**2**), and B (**3**) in comparison with the literature data of X-14952B (**4**)[30], and 17-hydroxyventuricidin A (**5**), (δ in ppm)

Position	1 *a)*	1 *b)*	1 *c)*	2 *a)*	2 *b)*	3 *a)*	3 *b)*	4 *c)*	5 *c)*
	δ_C *d)*	δ_C *d)*	δ_C *d)*	δ_C *e)*	δ_C *d)*	δ_C *e)*	δ_C *d)*	δ_C *f)*	δ_C
1	174.0, C	171.9, C	173.2, C	174.3, C	171.9, C	174.8, C	171.9, C	173.7, C	173.8, C
2	44.8, CH_2	43.8, CH_2	43.8, CH_2	44.8, CH_2	43.6, CH_2	44.8, CH_2	43.6, CH_2	43.6, CH_2	43.2, CH_2
3	95.3, C	93.4, C	94.0, C	95.3, C	93.4, C	95.3, C	93.4, C	94.3, C	93.9, C
4	36.2, CH_2	34.6, CH_2	35.3, CH_2	36.2, CH_2	34.6, CH_2	36.2, CH_2	31.1, CH_2	35.3, CH_2	35.0, CH_2
5	118.3, CH	117.5, CH	117.0, CH	118.3, CH	117.5, CH	118.3, CH	117.5, CH	117.0, CH	116.7, CH
6	134.1, C	131.8, C	133.3, C	134.1, C	131.8, C	134.1, C	131.8, C	133.7, C	132.7, C
6-CH_3	19.5, CH_3	18.9, CH_3	19.5, CH_3	19.5, CH_3	18.9, CH_3	19.5, CH_3	19.5, CH_3	19.1, CH_3	19.1, CH_3
7	81.8, CH	79.2, CH	80.3, CH	81.7, CH	79.2, CH	81.8, CH	79.2, CH	80.3, CH	79.9, CH
8	135.8, C	134.1, C	136.2, C	135.8, C	134.1, C	135.8, C	134.1, C	135.4, C	134.7, C
8-CH_3	11.2, CH_3	10.9, CH_3	11.0, CH_3	11.2, CH_3	10.7, CH_3	11.2, CH_3	10.8, CH_3	11.0, CH_3	10.6, CH_3
9	131.1, CH	129.9, CH	129.7, CH	131.0, CH	129.9, CH	131.3, CH	130.0, CH	129.7, CH	129.3, CH
10	28.4, CH_2	26.9, CH_2	27.3, CH_2	28.4, CH_2	26.9, CH_2	28.4, CH_2	26.9, CH_2	27.3, CH_2	27.0, CH_2
11	27.3, CH_2	25.5, CH_2	26.1, CH_2	27.3, CH_2	25.5, CH_2	27.4, CH_2	25.5, CH_2	26.2, CH_2	25.9, CH_2
12	36.1, CH_2	34.3, CH_2	35.5, CH_2	36.1, CH_2	34.3, CH_2	36.8, CH_2	34.3, CH_2	35.5, CH_2	34.5, CH_2
13	84.2, CH	82.3, CH	83.3, CH	84.1, CH	82.3, CH	84.1, CH	82.3, CH	82.6, CH	82.2, CH
14	131.2, CH	129.1, CH	134.8, CH	131.2, CH	129.1, CH	131.1, CH	129.2, CH	134.8, CH	134.3, CH
15	140.1, CH	137.4, CH	134.6, CH	140.0, CH	137.4, CH	139.9, CH	137.2, CH	134.6, CH	133.9, CH
16	36.8, CH	34.8, CH	42.0, CH	36.8, CH	34.8, CH	36.8, CH	34.7, CH	42.3, CH	42.0, CH
16-CH_3	20.2, CH_3	19.4, CH_3	18.0, CH_3	20.2, CH_3	19.4, CH_3	20.2, CH_3	18.9, CH_3	17.4, CH_3	17.1, CH_3
17	43.2, CH_2	41.3, CH_2	43.8, CH_2	43.1, CH_2	41.3, CH_2	43.2, CH_2	41.3, CH_2	78.2, CH	77.7, CH
18	33.2, CH	31.2, CH	35.0, CH	33.2, CH	31.2, CH	33.2, CH	34.6, CH	34.9, CH	34.5, CH
18-CH_3	14.2, CH_3	13.4, CH_3	14.6, CH_3	14.2, CH_3	12.8, CH_3	14.2, CH_3	12.8, CH_3	5.7, CH_3	5.5, CH_3
19	84.8, CH	80.8, CH	82.9, CH	84.7, CH	80.8, CH	84.8, CH	80.6, CH	82.2, CH	81.8, CH
20	33.5, CH	31.5, CH	33.6, CH	33.4, CH	31.5, CH	33.5, CH	31.2, CH	33.5, CH	33.5, CH

Position	1 [a)]	1 [b)]	1 [c)]	2 [a)]	2 [b)]	3 [a)]	3 [b)]	4 [c)]	5 [c)]
	δ_C [d)]	δ_C [d)]	δ_C [d)]	δ_C [e)]	δ_C [d)]	δ_C [e)]	δ_C [d)]	δ_C [f)]	δ_C
20-CH_3	16.6, CH_3	15.8, CH_3	16.2, CH_3	16.6, CH_3	15.8, CH_3	16.6, CH_3	15.8, CH_3	16.0, CH_3	15.8, CH_3
21	38.6, CH_2	36.2, CH_2	37.0, CH_2	38.1, CH_2	36.2, CH_2	38.2, CH_2	37.5, CH_2	37.2, CH_2	35.9, CH_2
22	33.1, CH	31.1, CH	32.7, CH	33.0, CH	31.1, CH	33.1, CH	31.5, CH	32.8, CH	32.4, CH
22-CH_3	11.5, CH_3	11.7, CH_3	13.0, CH_3	11.5, CH_3	10.9, CH_3	11.5, CH_3	10.9, CH_3	12.9, CH_3	14.0, CH_3
23	78.8, CH	76.3, CH	78.0, CH	78.7, CH	76.3, CH	78.8, CH	76.6, CH	77.0, CH	77.6, CH
24	58.5, CH	56.6, CH	55.3, CH	50.8, CH	48.9, CH	50.9, CH	49.0, CH	55.4, CH	48.3, CH
24-CH_3	-	-	-	13.9, CH_3	13.4, CH_3	13.9, CH_3	13.4, CH_3	-	14.1, CH_3
24-$\underline{CH_2}CH_3$	23.2, CH_2	21.4, CH_2	23.0, CH_2	-	-	-	-	22.9, CH_2	-
24-$CH_2\underline{CH_3}$	12.2, CH_3	12.8, CH_3	12.0, CH_3	-	-	-	-	12.0, CH_3	-
25	218.0, C	214.5, C	217.1, C	217.9, C	214.5, C	218.0, C	214.5, C	217.5, C	216.8, C
26	37.2, CH_2	35.4, CH_2	37.0, CH_2	37.2, CH_2	35.4, CH_2	37.2, CH_2	35.4, CH_2	37.4, CH_2	35.9, CH_2
27	7.9, CH_3	7.4, CH_3	7.8, CH_3	7.9, CH_3	7.4, CH_3	7.9, CH_3	7.4, CH_3	7.6, CH_3	7.4, CH_3
1’	99.8, CH	97.6, CH	98.5, CH	99.8, CH	97.6, CH	100.3, CH	98.1, CH	98.4, CH	98.1, CH
2’	38.9, CH_2	37.5, CH_2	38.2, CH_2	38.6, CH_2	37.5, CH_2	41.0, CH_2	36.2, CH_2	37.0, CH_2	36.8, CH_2
3’	75.4, CH	72.7, CH	75.4, CH	75.3, CH	72.7, CH	72.5, CH	70.5, CH	75.2, CH	74.8, CH
3’-$\underline{CO}NH_2$	159.7, C	156.4, C	157.6, C	159.6, C	156.5, C	-	-	157.7, C	157.5, C
4’	75.6, CH	73.5, CH	75.8, CH	75.6, CH	73.5, CH	78.6, CH	76.3, CH	74.7, CH	75.0, CH
5’	73.4, CH	71.7, CH	72.2, CH	73.4, CH	71.7, CH	73.4, CH	71.5, CH	72.2, CH	71.9, CH
5’-CH_3	18.5, CH_3	18.0, CH_3	18.0, CH_3	18.5, CH_3	18.0, CH_3	18.5, CH_3	18.1, CH_3	17.7, CH_3	17.7, CH_3

[a)] CD_3OD;

[b)] DMSO-d_6;

[c)] $CDCl_3$;

[d)] 100 MHz;

[e)] 125 MHz;

[f)] 75 MHz; See supporting information (Figures S11-S21, S25-S33, S37-S45) for the NMR Spectra.