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Bacillusin A, an Antibacterial Macrodiolide from *Bacillus amyloliquefaciens* AP183

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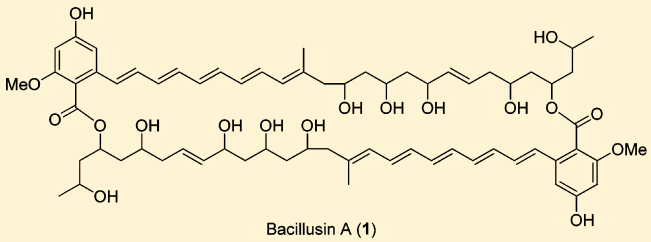
Supporting Information



Bacillusin A (**1**)

Antibacterial Activities (MIC, μg/mL)

	MRSA ATCC 33591	*E. faecium* ATCC 700221
Bacillusin A (**1**)	1.2	0.6
Vancomycin	1.6	>100
Ciprofloxacin	0.4	>100
Methicillin	>100	>100

ABSTRACT: Bioassay-guided fractionation of the organic extracts of a *Bacillus amyloliquefaciens* strain (AP183) led to the discovery of a new macrocyclic polyene antibiotic, bacillusin A (**1**). Its structure was assigned by interpretation of NMR and MS spectroscopic data as a novel macrodiolide composed of dimeric 4-hydroxy-2-methoxy-6-alkenylbenzoic acid lactones with conjugated pentaene-hexahydroxy polyketide chains. Compound **1** showed potent antibacterial activities against methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant *Enterococcus faecium* with minimum inhibitory concentrations in a range of 0.6 to 1.2 μg/mL. The biosynthetic significance of this unique class of antibiotic compounds is briefly discussed.

Bacillus, a genus of Gram-positive bacteria, have been screened for antimicrobial activities over the past few decades.[1−4] Many *Bacillus* spp. have been shown to produce antibiotic polyketides such as macrolactins, difficidins, and oxidifficidins[1] as well as lipopeptides such as surfactins, iturins, and fengycins.[2,3] Some *Bacillus* spp. have also been used as biocontrol agents against plant and animal pathogens in agriculture and aquaculture.[5−7] In our search for new antibiotics against drug-resistant bacteria, the *B. amyloliquefaciens* subsp. plantarum strain AP183 was shown to produce potent activity against methicillin-resistant *Staphylococcus aureus* (MRSA) (IC_{50} of <10 μg/mL). Initial LC-MS analysis indicated that surfactins, iturins, and fengycins were major metabolites of this strain. However, preliminary fractionation of the crude extracts afforded activity-enriched fractions that contained an apparently unknown active compound with strong UV absorptions. Thus, a scale-up fermentation of this *Bacillus* strain was conducted, leading to the isolation and identification of a new antibacterial macrocyclic polyene antibiotic compound (**1**), designated bacillusin A.

Compound **1** was obtained as a yellow powder from organic extracts of cell pellets and the culture supernatant of *B. amyloliquefaciens* AP183 (see the Experimental Section) and exhibited a molecular ion peak at m/z 1219.6172 for $[M + Na]^+$ in the high-resolution ESIMS spectrum. The ^{13}C NMR, DEPT, and HSQC spectra in DMSO-d_6 (Table 1) showed 34 carbon resonances including two methyl groups, one methoxy group, six methylene groups, six oxymethine groups, 13 olefinic or aromatic tertiary carbons, five olefinic or aromatic quaternary carbons, and one carboxylic ester carbon. Note that the resonance at δ_C 131.5 correlating with two olefinic protons at δ_H 6.80 and 6.32 in the HSQC spectrum represented two carbons. Thus, compound **1** should be a symmetric dimer with a molecular formula of $C_{68}H_{92}O_{18}$ possessing aromatic, polyene, and polyol structural moieties (Figure 1).

The aromatic system in **1** was established first as follows: the two characteristic singlets at δ_H 6.34 (H4) and 6.63 (H6) correlated with two carbons at δ_C 98.7 (C4) and 102.5 (C6), respectively, in the HSQC spectrum. In the HMBC spectrum, the singlet at δ_H 6.34 (H4) correlated with five carbons resonating at δ_C 102.5 (CH, C6), 115.0 (C, C2), 157.3 (C, C3), 159.3 (C, C5), and 167.3 (C, C1), while the singlet at δ_H 6.63 (H6) showed cross-peaks with five carbons resonating at δ_C 98.7 (CH, C4), 115.0 (C, C2), 127.9 (CH, C8), 159.3 (C, C5), and 167.3 (C, C1) (Figure 2, Table 1). The carbon at δ_C 157.3 (C, C3) was confirmed to be attached to a methoxy group by its HMBC correlation with the methoxy protons

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Table 1. NMR Data of Bacillusin A (1) in DMSO-d_6

C/H	δ_C	DEPT	δ_H mult (J, Hz)	HMBC	ROESY
1 (1′)	167.3	C			
2 (2′)	115.0	C			
3 (3′)	157.3	C			
4 (4′)	98.7	CH	6.34 s	C-1 (1′), 2 (2′), 3 (3′), 5 (5′), 6 (6′)	H-34 (34′)
5 (5′)	159.3	C			
6 (6′)	102.5	CH	6.63 s	C-1 (1′), 2 (2′), 4 (4′), 5 (5′), 8 (8′)	H-9 (9′)
7 (7′)	136.1	C			
8 (8′)	127.9	CH	6.38 d (15)	C-2 (2′), 6 (6′), 9 (9′), 7 (7′)	
9 (9′)	131.5	CH	6.80 dd (15.2, 10.7)	C-7 (7′), 11 (11′)	H-6 (6′), 11 (11′)
10 (10′)	135.7	CH	6.48 dd (15, 10)	C-9 (9′), 11 (11′)	
11 (11′)	132.1	CH	6.35 dd (15, 10)	C-10 (10′)	
12 (12′)	131.5	CH	6.32 dd (15.2, 10.7)	C-10 (10′), 11 (11′), 14 (14′)	
13 (13′)	135.1	CH	6.36 dd (15, 10)	C-11 (11′), 12 (12′)	
14 (14′)	130.5	CH	6.16 dd (15, 11)	C-16 (16′), 15 (15′),	H-16 (16′)
15 (15′)	130.7	CH	6.46 dd (15, 10)	C-14 (14′), 17 (17′)	H-33 (33′)
16 (16′)	126.9	CH	5.82 d (11)	C-15 (15′), 18 (18′), 33 (33′)	H-14 (14′), 18 (18′)
17 (17′)	138.6	C			
18 (18′)	48.1	CH_2	2.00 m, 2.08 m	C-16 (16′), 17 (17′) 19 (19′), 20 (20′), 33 (33′)	H-16 (16′)
19 (19′)	67.7	CH	3.74 m		
20 (20′)	44.6	CH_2	1.37 m	C-18 (18′), 21 (21′), 22 (22′)	
21 (21′)	67.6	CH	3.67 m		
22 (22′)	45.1	CH_2	1.43 m, 1.50 m	C-20 (20′), 21 (21′), 23 (23′), 24 (24′)	
23 (23′)	69.7	CH	4.03 q (10, 5)	C-21 (21′), 22 (22′), 24 (24′), 25 (25′)	H-25 (25′)
24 (24′)	135.7	CH	5.37 dd (15, 6.7)	C-23 (23′), 25 (25′), 26 (26′)	H-26 (26′)
25 (25′)	126.3	CH	5.51 dt (15, 6.7)	C-23 (23′), 24 (24′), 26 (26′), 27 (27′)	H-23 (23′), 27 (27′)
26 (26′)	41.1	CH_2	1.98 m, 2.04 m	C-24 (24′), 25 (25′), 27 (27′), 28 (28′)	
27 (27′)	66.3	CH	3.56 m		H-25 (25′)
28 (28′)	42.2	CH_2	1.63 m	C-29 (29′)	
29 (29′)	70.2	CH	5.37 m	C-1′ (1)	
30 (30′)	44.7	CH_2	1.58 m	C-29 (29′)	
31 (31′)	62.5	CH	3.74 m		
32 (32′)	24.3	CH_3	1.05 d (5)	C-30 (30′), 31 (31′)	
33 (33′)	17.2	CH_3	1.69 s	C-15 (15′), 16 (16′), 17 (17′), 18 (18′)	
34 (34′)	55.5	CH_3	3.67 s	C-3 (3′)	H-4 (4′)

(Me34) at δ_H 3.67. These observations are consistent with the structural moiety of a 4-hydroxy-2-methoxybenzoic acid ester.

The assignment of a conjugated system of five double bonds attached to the aromatic ring was facilitated by the 2D NMR COSY, HSQC, and HMBC spectra. The COSY spectrum showed that the nine protons arising from this system in the range of δ_H 5.7−7.0 had no correlations with aliphatic protons in the upfield region. Only the doublet proton at δ_H 5.82 (1H, J = 11 Hz, H16) showed a long-range correlation with the vinyl methyl group (Me33) at δ_H 1.69, indicating that the conjugated system was terminated by this methyl group. There were two well-resolved doublets of doublets in this region at δ_H 6.32 (1H, dd, J = 15.2, 10.7 Hz, H12) and 6.80 (1H, dd, J = 15.2, 10.7 Hz, H9). The remaining six protons resonating at δ_H 6.1−6.6 were overlapping in the 1D ^{1}H NMR spectrum. However, the HSQC spectrum showed that the five protons had doublet of doublets coupling patterns with similar coupling constants of approximately 15 and 11 Hz, and one proton exhibited a doublet with a coupling constant of 15 Hz, which was assigned H8 as attached to C8, which showed an HMBC correlation with the aromatic proton (H6) at δ_H 6.63. The defined coupling constants suggested that the five double bonds were all *trans* configured in a conjugated manner. The presence of this aromatic-polyene system also explains the UV maximum absorptions at 378 and 401 nm for the molecule.

The remaining polyketide chain comprised six methylenes, six oxymethines, one double bond, and one terminal aliphatic methyl group. Using HSQC, each protonated carbon was assigned (Table 1). The connectivity of these molecular fragments was then determined through ^2J and ^3J H−C correlations (Figure 2) by HMBC experiments. The ^3J HMBC correlation between the vinyl methyl protons (Me33) at δ_H 1.69 and the methylene carbon at δ_C 48.1 confirmed the assignment of C18 as well as H18 at δ_H 2.08 and 2.00. H18 showed a ^2J HMBC correlation with the C19 oxymethine at δ_C 67.7 and a ^3J HMBC correlation with the C20 methylene at δ_C 44.6. In the same manner, the connectivity from H20 to C21 and C22 and from H22 to C23 and C24 was established, indicating that the sixth double bond in the molecule resonating at δ_H 5.37 (1H, dd, J = 15.0, 6.7 Hz, H24) and 5.51 (1H, dt, J = 15.0, 6.7 Hz, H25) was located between C24 and C25. Although H24 was overlapping with another proton (H29), its coupling pattern was again resolved by the HSQC spectrum, confirming a *trans* configuration for this double bond. In addition, an HMBC correlation between H23 (δ_H 4.03) and C22 was observed. Extension of the polyketide chain beyond the isolated double bond was further proven from the HMBC correlations of H25 to C26 and C27, H26 to C27 and C28, and H28 to C29. As H29 was resonating at δ_H 5.37 in the downfield region, lactonization was expected to occur at C29,

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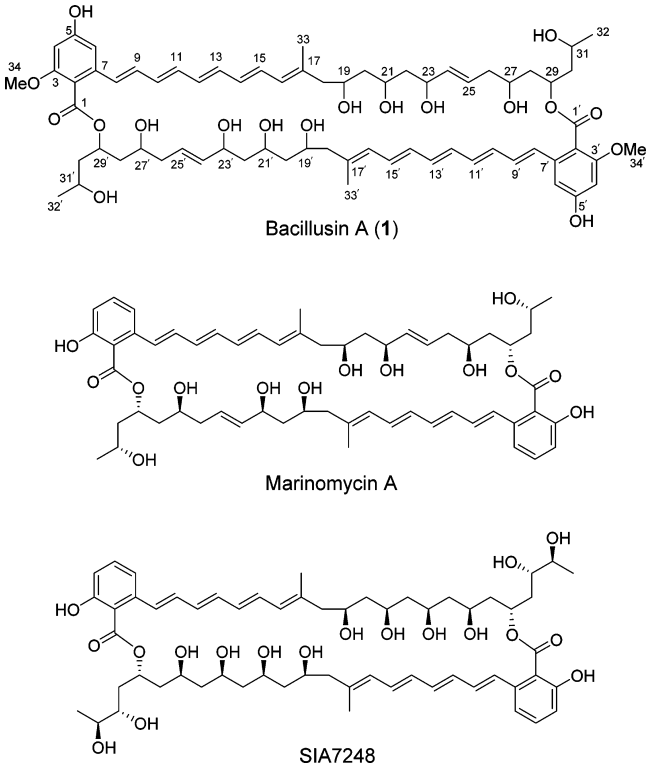

Figure 1. Structures of bacillusin A (**1**) from *B. amyloliquefaciens* AP183, marinomycin A from *Marinispora* sp., and SIA7248 from *Streptomyces* sp. A7248.

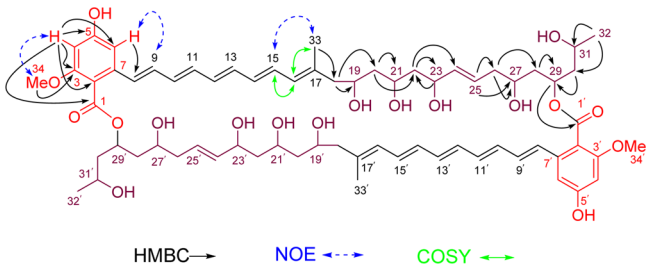

Figure 2. Key 2D NMR correlations establishing the C−C connectivity of bacillusin A (**1**).

which was supported by a ^3J HMBC correlation between H29 and the carbonyl carbon at δ_C 167.3 (C1′). Finally, the key HMBC correlations of the terminal methyl group Me32 (δ_H 1.05) to C31 and C30 and of H30 (δ_H 1.58) to C29 furnished the C−C connectivity of the molecule. The molecular formula indicated compound **1** to be a symmetric macrodiolide. The ROESY spectrum of compound **1** was also acquired and showed key NOE correlations (Table 1), e.g., H4 and Me34, H6 and H9, and H15 and Me33, supporting the assigned structure.

Compound **1** showed strong *in vitro* antibacterial activities against multiple drug-sensitive and -resistant *Staph. aureus* and *Enterococcus* spp. with minimum inhibitory concentrations (MICs) in the range 0.6−1.2 μg/mL, and its potency was compared against vancomycin, ciprofloxacin, and methicillin (Table 2). It was noted that compound **1** exhibited an MIC of 0.6 μg/mL against *E. faecium* ATCC 700221 that was resistant to the three aforementioned antibiotics tested at 100 μg/mL. Compound **1** did not show any antifungal activity against *Candida albicans* ATCC 90028 at 20 μg/mL.

Compound **1** is structurally analogous to marinomycins A−D isolated from a marine actinomycete of the recently discovered genus *Marinispora*[8] and SIA7248 isolated from the marine isolate *Streptomyces* sp. A7248,[9] but possesses a larger macrocyclic ring with four additional C2 extender units, making it more difficult to determine the absolute or even relative configuration for the stereogenic centers. In addition, compound **1** is chemically unstable in organic solvents due to the conjugated aromatic-pentaene system as indicated by LC-MS (Supporting Information). This phenomenon appears to be similar to what has been observed for marinomycin A, which photoisomerized to give marinomycins B and C with reduced antibacterial activities due to olefin isomerization.[8] The photochemical instability of this compound further increased the challenges for determination of its absolute configuration. It was indeed observed that the antibacterial activity of compound **1** decreased when it was stored in DMSO for more than 1 week (its biological activity data in Table 2 were obtained from a fresh sample in DMSO).

This is the first isolation of a macrodiolide from a *Bacillus* sp. *Bacillus* is a member of the phylum Firmicutes, while both *Marinispora* and *Streptomyces* from which marinomycins and SIA7248 were isolated, respectively, belong to the different phylum Actinobacteria.[8,9] A recent study shows that two oral Actinobacteria (*Propionibacterium propionicum* F0230a and *Actinomyces timonensis* DSM 23838) identified from human microbiomes contain biosynthetic gene clusters similar in their domain architecture to a gene cluster from *Streptomyces* sp. A7248 that encodes the production of SIA7248.[10]

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured on an Autopol IV polarimeter. UV spectra were obtained in MeOH using a Varian Cary 50 Bio UV visible spectrophotometer. The 1D and 2D NMR (DQF-COSY, HMQC, HMBC, and ROESY) spectra using standard pulse programs were recorded at room temperature on a Bruker Avance DRX 500 FT spectrometer operating

Table 2. *In Vitro* Antibacterial Activities of Bacillusin A (1) (IC_{50}/MIC/MBC, μg/mL)[a]

	S. aureus ATCC 29213	MRSA ATCC 33591[b]	MRSA EAMC30[c]	*E. faecalis* ATCC 51299	*E. faecalis* ATCC 29212	*E. faecium* ATCC 700221[d]
1	0.04/1.2/2.5	0.04/1.2/1.2	<0.02/0.6/0.6	0.2/0.6/1.2	0.2/0.6/2.5	0.1/0.6/–[e]
vancomycin	0.7/1.6/50	0.8/1.6/1.6	0.2/0.4/0.4	3.4/6.2/–[f]	1.0/1.6/50	–/–/–[f]
ciprofloxacin	<0.1/0.4/0.4	<0.1/0.4/0.8	29.4/100/–[f]	0.2/0.4/6.2	0.2/0.8/6.2	–/–/–[f]
methicillin	0.4/3.1/12.5	–/–/–[f]	10.1/50/–[f]	14.2/50/50	15.3/25/50	–/–/–[f]

[a]IC_{50}: 50% growth inhibition. MIC: minimum inhibitory concentration. MBC: minimum bactericidal concentration. The highest test concentration for compound **1** was 20 μg/mL; the highest test concentrations for vancomycin, ciprofloxacin, and methicillin were 100 μg/mL. [b]Methicillin-resistant *Staphylococcus aureus* strain. [c]Methicillin-resistant *Staphylococcus aureus* clinical isolate. [d]Vancomycin-resistant *Enterococcus faecium* strain. [e]Not active at 20 μg/mL. [f]Not active at 100 μg/mL.

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at 500 (^{1}H) and 125 (^{13}C) MHz. The chemical shift values were calibrated using the NMR solvent residual signals. HRESIMS data were obtained on an Agilent Series 1100 SL mass spectrometer. Column chromatography was performed using normal-phase silica gel (230 × 400 mesh, J. T. Baker, USA) and reversed-phase silica gel (RP-18, 40 μm, J. T. Baker, USA). TLC was carried out on silica gel sheets (Alugram Sil G/UV254, Macherey-Nagel, Germany) and reversed-phase plates (RP-18 F254S, Merck, Germany) and visualized by UV 254 nm and spraying 10% H_2SO_4 followed by heating. LC-MS was carried out on an Agilent 1290 Infinity series chromatograph with a dual pump, autosampler, thermostated column compartment, and a diode array detector. The chromatograph was coupled with an Agilent 6120 single quadruple mass spectrometer with a dual APCI/ESI source operated in both the positive and negative modes. The system was controlled by ChemStation software. A Waters Acquity UPLC BEH C_{18} column (2.1 × 150 mm, 1.7 μm) was used. The experiments were carried out at a gradient elution from 10% to 100% CH_3CN in H_2O. The quadrupole mass analyzer was operated in the scan mode with the mass range from 100 to 1500. The drying gas flow was 10 L/min at 300 °C, the nebulizer pressure was 35 psi, and the vaporizer temperature was 200 °C. The capillary voltage used was 3 kV, the corona current was 10 μA, and the charging voltage was 4 kV. The fragmentor was set to 120 V.

Microorganisms and Growth Conditions. *Bacillus amyloliquefaciens* subsp. plantarum AP183, a plant growth-promoting rhizobacterium, was obtained from a collection of soil-derived *Bacillus* strains provided by the laboratory of Dr. Joseph Kloepper (Department of Entomology and Plant Pathology, Auburn University). It was identified by using a maximum likelihood analysis based on *gyrB* sequences from an available local database of *B. amyloliquefaciens* subsp. plantarum strains and strains available from the GenBank database including the subspecies plantarum type strain FZB42, using 1000 iterations for bootstrap support (data not shown). The *B. amyloliquefaciens* strain AP183 was grown on a tryptic soy agar plate and incubated at 30 °C for 1 day. A loop of the bacterium was inoculated into a 1 L Erlenmeyer flask containing 300 mL of tryptic soy broth and incubated on a rotatory shaker at 200 rpm, 30 °C, for 48 h. The whole culture was harvested by centrifugation for 15 min. The supernatant was extracted three times with EtOAc (300 mL each time for 1 L of supernatant), while the cell pellets were extracted with MeOH (100 mL for cell pellets from 1 L of culture) by sonication for 30 min followed by standing overnight. Both EtOAc and MeOH extracts were active against MRSA (ATCC 33591), with IC_{50} values of 8.0 and 2.5 μg/mL, respectively. For comparison, the commercially available iturin (a mixture of iturins A_2 and A_3) and surfactin (both from Sigma-Aldrich) gave IC_{50} values of 18.0 and >160 μg/mL, respectively. Surfactins, iturins, and fengycins were identified by LC-MS in both EtOAc and MeOH extracts (see Supporting Information).

Preliminary Fractionation. Extraction of 2 L culture prepared using the conditions described above afforded a MeOH extract (821 mg) and an EtOAc extract (463 mg). A portion (200 mg) of the MeOH extract was fractionated into 17 fractions (CFs) by normal-phase silica gel chromatography using $CHCl_3$–MeOH–H_2O (40:10:1). The fifth CF was the most active against MRSA (ATCC 33591), showing an IC_{50} of <0.85 μg/mL. The LC-MS data indicated that this CF contained a compound with a molecular weight (MW) of 1196 and strong UV absorptions in the range 300–450 nm (see the Supporting Information). A database search did not show any compound with the same MW and UV absorptions. In addition, a portion (440 mg) of the EtOAc extract was fractionated into 16 CFs by reversed-phase silica gel chromatography using a gradient solvent system of 50% to 100% MeOH. The ninth CF gave an IC_{50} of <1.1 μg/mL against MRSA (ATCC 33591), being the most active among all CFs. The same yellow compound that was present in the fifth CF from the MeOH extract was identified in this CF.

Extraction and Isolation. Extraction of the 30 L culture prepared using the conditions described above generated a MeOH extract (11.9 g) and an EtOAc extract (13.0 g). The MeOH extract was adsorbed on silica gel (35 g) and loaded on a normal-phase silica gel (450 g) column using a gradient solvent system (5–30% MeOH in $CHCl_3$) to afford 20 fractions (Fr.1–20). Fr.5 (110 mg), containing the active compound, was chromatographed on a reversed-phase silica gel column (C_{18} cartridge 10 g, Supelco Clean) eluting with 50–100% MeOH to afford a subfraction (13.2 mg), which was further purified by a reversed-phase silica gel flash column (C_{18}, 12 g) with 85% MeOH to give compound **1** (2.9 mg). Similarly, the EtOAc extract (13 g) was adsorbed on silica gel (40 g) and chromatographed on a normal-phase silica gel column (500 g) using 5–30% MeOH in $CHCl_3$ to afford 17 fractions (Fr.1–17). Fr.9 (1.12 g), containing the active compound, was chromatographed on reversed-phase silica gel (C_{18}, 45 g) using 85% MeOH to yield a subfraction (30 mg), which was further purified by a reversed-phase silica gel flash column (C_{18}, 15 g) with 85% MeOH to give compound **1** (4.5 mg). In addition, a small amount of macrolactin A (4.9 mg, IC_{50} > 20 μg/mL vs MRSA (ATCC 33591)) was isolated from Fr.4, and a large amount of surfactins as a mixture of surfactins A–D (>2 g) was isolated from Fr.6 and Fr.7. Iturins A_2 and A_3 were identified in Fr.11 by TLC and LC-MS.

Bacillusin A (1): yellow powder; $[\alpha]^{25}_D$ −227.9 (*c* 0.1, 20% MeOH in $CHCl_3$); UV (MeOH) λ_{max} log (ε) 363 (4.79, sh), 378 (4.85), 401 (4.72) nm; NMR (DMSO-d_6), see Table 1; HRESIMS *m*/*z* 1219.6172 (calcd for [M ($C_{68}H_{92}O_{18}$) + Na]$^+$) 1219.6176).

***In Vitro* Antimicrobial Assay.** Microorganisms were obtained from the American Type Culture Collection (Manassas, VA, USA) and include the bacteria methicillin-susceptible *Staphylococcus aureus* ATCC 29213, methicillin-resistant *Staphylococcus aureus* ATCC 33591, *Enterococcus faecalis* ATCC 51299, *Enterococcus faecalis* ATCC 29212, and *Enterococcus faecium* ATCC 700221, as well as the yeast *Candida albicans* ATCC 90028. Methicillin-resistant *Staphylococcus aureus* isolate number EAMC30 is a clinical MRSA isolate obtained from East Alabama Medical Center, Opelika, AL, USA, provided by Dr. James Barbaree (Department of Biological Sciences, Auburn University). All organisms were tested using modified versions of the CLSI (formerly NCCLS) method,[11] which has been described previously.[12] Samples (dissolved in DMSO) were serially diluted in 20% DMSO–saline and transferred (10 μL) in duplicate to 96-well flat bottom microplates. Inocula were prepared by adjusting the OD_{630} of microbe suspensions in assay broth to afford the final desired CFU/mL. The positive control drugs included vancomycin, ciprofloxacin, and methicillin (all from ICN Biomedicals, OH, USA) for the antibacterial assay and amphotericin B (ICN Biomedicals) for the antifungal assay. The highest test concentrations of compound **1** and the control drugs are indicated in Table 2. IC_{50}'s (concentrations that afford 50% inhibition relative to controls) were calculated using XLfit software (IDBS, Alameda, CA, USA) with fit model 201. The MIC is defined as the lowest test concentration that allows no detectable growth. Minimum bactericidal concentrations (MBC) were determined by removing 5 μL from each clear well, transferring to fresh media, and incubating for 24–48 h. The MBC is defined as the lowest test concentration that allows no growth in fresh media.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

LC-MS data of crude extracts, antibacterial activity of column fractions, and NMR, UV, and high-resolution ESIMS spectra of bacillusin A (**1**). This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Hamdache, A.; Lamarti, A.; Aleu, J.; Collado, I. G. *J. Nat. Prod.* **2011**, *74*, 893−899.
(2) Stein, T. *Mol. Microbiol.* **2005**, *56*, 845−857.
(3) Mannanov, R. N.; Sattarova, R. K. *Chem. Nat. Compd.* **2001**, *37*, 117−123.
(4) Chen, X. H.; Koumoutsi, A.; Scholz, R.; Borriss, R. *J. Mol. Microbiol. Biotechnol.* **2009**, *16*, 14−24.
(5) Ongena, M.; Jacques, P. *Trends Microbiol.* **2008**, *16*, 115−125.
(6) Cook, R. J.; Thomashow, L. S.; Weller, D. M.; Fujimoto, D.; Mazzola, M.; Bangera, G.; Kim, D.-S. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 4197−4201.
(7) Ran, C.; Carrias, A.; Williams, M. A.; Capps, N.; Dan, B. C.; Newton, J. C.; Kloepper, J. W.; Ooi, E. L.; Browdy, C. L.; Terhune, J. S.; Liles, M. R. *PLoS One* **2012**, *7*, e45793.
(8) Kwon, H. C.; Kauffman, C. A.; Jensen, P. R.; Fenical, W. *J. Am. Chem. Soc.* **2006**, *128*, 1622−1632.
(9) Zou, Y.; Yin, H.; Kong, D.; Deng, Z.; Lin, S. *ChemBioChem* **2013**, *14*, 679−683.
(10) Donia, M. S.; Cimermancic, P.; Schulze, C. J.; Brown, L. C. W.; Martin, J.; Mitreva, M.; Clardy, J.; Linington, R. G.; Fischbach, M. A. *Cell* **2014**, *158*, 1402−1414.
(11) *Susceptibility Testing of Mycobacteria, Nocardia, and other Aerobic Actinomycetes; Tentative Standard, Approved Standard, M24-A*; National Committee on Clinical Laboratory Standards, 2003, *23*(18).
(12) Samoylenko, V.; Jacob, M. R.; Khan, S. I.; Zhao, J.; Tekwani, B. L.; Midiwo, J. O.; Walker, L. A.; Muhammad, I. *Nat. Prod. Commun.* **2009**, *4*, 791−796.

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