Published in final edited form as:
J Nat Prod. 2019 July 26; 82(7): 1930–1934. doi:10.1021/acs.jnatprod.9b00312.

Bulbiferates A and B: Antibacterial Acetamidohydroxybenzoates from a Marine Proteobacterium, *Microbulbifer* sp.

Dinith R. Jayanetti[†], **Doug R. Braun**[†], **Kenneth J. Barns**[†], **Scott Raymond Rajski**[†], **Tim S. Bugni**[*,†]

[†]Pharmaceutical Sciences Division, School of Pharmacy, University of Wisconsin, Madison, Wisconsin 53705, United States

Abstract

Here we report the discovery of two new 3-acetamido-4-hydroxybenzoate esters, bulbiferates A (**1**) and B (**2**), isolated from *Microbulbifer* sp. cultivated from the marine tunicate *Ecteinascidia turbinata*. The structures of **1** and **2** were determined by analysis of 2D NMR and MS data. Additionally, three synthetic analogs (**3**–**5**), differing in ester sizes/lengths were prepared for the purposes of evaluating potential structure:activity relationships; no clear correlations tying ester lengths to activity were evident. Bulbiferates A (**1**) and B (**2**) demonstrated antibacterial activity against both *Escherichia coli* (*E. coli*) and methicillin-sensitive *Staphylococcus aureus* (MSSA), whereas the synthetic analogs **3** and **4** displayed activity only against MSSA.

Graphical Abstract

The phylum proteobacteria is the most predominent phylum in marine ecosystems and comprises a wide array of gram-negative bacteria including *Microbulbifer* spp.[1–6] The α- and γ-groupings within the proteobacterial family are often found in high abundance in marine environments, whereas β-proteobacteria are most commonly found in lakes and rivers.[7–11] Despite being the most widely encountered phylum in the marine environment, very few bioactive compounds have been described from proteobacteria contrasting sharply

[*]**Corresponding Author** Tel: (608)-263-2519. Fax: (608)-262-5345., tim.bugni@wisc.edu.

The authors declare no competing financial interest.

ASSOCIATED CONTENT

Supporting Information

1D and 2D NMR spectra for compounds **1** and **2** and 1D NMR data for compounds **3**–**5**. This material is available free of charge via the Internet at http://pubs.acs.org.

with actinobacteria from which vast numbers of bioactive natural products are derived.[2, 12–16] Some of the rare examples of novel bioactive molecules isolated from marine proteobacteria include thalassospiramides A and B isolated from *Thalassospira* sp.,[17] tropodithietic acid discovered from *Phaeobacter* spp.,[18] the antibacterial thiomarinol obtained from *Pseudoalteromonas* sp.,[19] and didemnin B, an anti-cancer agent isolated from *Tistrella mobilis*.[20]

Although not extensively studied, the proven track record of marine proteobacteria as a source of novel bioactive metabolites inspires continued investigation of these microorganisms as sources of novel drug leads.[17–20] The genus *Microbulbifer* in particular, has been largely neglected as a producer of novel bioactive molecules. This genus was first described by Gonzales *et al.* in 1997 and is well known for its capacity to degrade a wide variety of polysaccharides including cellulose, agar, chitin, alginate, and xylan.[21–25] Indeed, these degradative abilities have bolstered interest in these organisms and their enzymatic machineries since lignocellulose breakdown represents a current bottleneck in converting plant biomass to exploitable biofuels;[26, 27] a process now deemed essential to meeting long-term global energy needs. Notably, *para*-hydroxybenzoic acids (parabens) are the only class of natural products reported thus far from *Microbulbifer* spp.[28, 29] Thus, beyond serving as potential repositories of enzymes with value in the biofuels sector, *Microbulbifer* spp. represent an interesting and underexplored source for the discovery of new, and potentially novel, bioactive substances.[26, 27]

In our efforts to explore the potential of *Microbulbifer* spp. as a source of biologically active metabolites with new or novel skeletons, we investigated 16 *Microbulbifer* strains isolated from the marine tunicate *Ecteinascidia turbinata.* Of these organisms, strain WMMC-695 displayed a small zone of inhibition against *E. coli*. Hence, this strain was selected for further investigation with the objective of isolating the compound/s responsible for Gram-negative antimicrobial activity

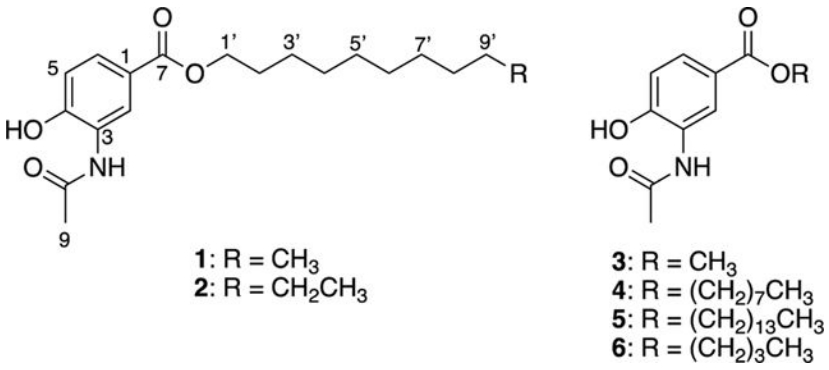
Bioassay-guided fractionation of strain WMMC-695 (10 L) led to the isolation of two new acetamidobenzoate esters, exhibiting weak activity against *Escherichia coli* (*E. coli*) and methicillin-sensitive *Staphylococcus aureus* (MSSA), which we named bulbiferates A (**1**) and B (**2**). Both **1** and **2** differ from the commonly isolated parabens from this genus by the presence of an acetamide group *ortho* to the phenolic OH functionality. This is the first report of compounds with this substitution pattern from a *Microbulbifer* sp. We are aware of only one other related report, where butyl 3-acetamido-4-hydroxybenzoate (**6**) and octyl 3-acetamido-4-hydroxybenzoate (**5**) esters, produced by a *Pelagiobacter sp.,* were used as fat-soluble UV absorbers in cosmetics.[30] Three analogs (**3**–**5**) of bulbiferates A (**1**) and B (**2**) with differing ester chain lengths were also synthesized in order to assess how lipophilicity impacts the biological activity of these agents.

Bulbiferate A (**1**) was obtained as a yellow powder and the molecular formula was determined to be $C_{19}H_{29}NO_4$ with six degrees of unsaturation, based on the HR-ESI-MSsignal at *m/z* 358.1994 $[M + Na]^+$. The IR spectrum showed absorptions typical of phenolic-OH (2980 cm^{-1}), amide-NH (3659 cm^{-1}), ester carbonyl (1738 cm^{-1}) and amide carbonyl (1693 cm^{-1}) bonds.

The ^{1}H NMR spectrum of **1** (Table 1) revealed two methyl groups [δ_H 0.88 (3H, t, J = 7.20 Hz), 2.30 (3H, s)], three aromatic protons [(δ_H 7.03 (1H, d, J = 8.52 Hz), 7.67 (1H, d, J = 2.0 Hz), 7.80 (1H, dd, J = 2.0, 8.52 Hz)], along with three resolved [(δ_H 4.27 (2H, t, J = 6.60 Hz), 1.74 (2H, br p, J = 13.52, 6.60 Hz), 1.42 (2H, m)] and six overlapped (1.30–1.37 ppm) methylenes as deduced on the basis of relative integrations. The ^{13}C NMR and HSQC spectra of compound **1** (summarized in Table 1) revealed the presence of one ester and one amide carbonyl (δ_C 171.5 and 166.7, respectively), five aromatic carbons (δ_C 119.9, 122.4, 124.5, 125.9, 129.0), one oxygenated aromatic carbon (δ_C 153.6), nine sp^3 methylenes (δ_C 23.8, 26.2, 28.9, 29.4, 29.5, 29.7, 29.8, 32.1 and 65.5), and two methyls (δ_C 14.3, 22.9) in the structure. The NMR spectroscopic features (Table 1) were characteristic of a 1,2,4-trisubstituted benzene ring with one of the substituents containing a long alkyl chain. COSY and HSQC analyses revealed two isolated spin systems: (**a**) C(5)H–C(6)H, and (**b**) C(1′)H_2–C(10′)H_3. It is important to note here that the determination of the length of the carbon chain from C1′-C10 was supported by MS data, in addition to the NMR data discussed above. The HMBC correlations from H-2 to C-4/C-6/C-7, H-5 to C-1/C-3, H-6 to C-2/C-4/C-7, further confirmed the presence of the 1, 2, 4-trisubstituted benzene ring and suggested linkage of the ester carbonyl group (C-7) to C-1. Further HMBC correlations seen from H_2-1′ to C-7, along with the spin system b, made clear that the side chain spanning C-1′–C-10′ is linked to the ester carbonyl group C-7 via an oxygen.

The HMBC correlations from H_3−9 to C-8 established the connectivity of H_3-9 to the carbonyl group C-8, which, based on the ^{13}C chemical shift (166.7 ppm) and the requirement of one N by the molecular formula, was assigned as an amide carbonyl. Finally, the molecular formula required the presence of one OH group in the structure. HMBC correlations from the broad singlet at 8.17 ppm, characteristic of a phenolic-OH, to C-4 (153.6 ppm) placed this OH moiety directly on C-4, thereby enabling unambiguous assignment of the structure for **1**.

The molecular formula of compound **2** was found to be $C_{20}H_{31}NO_4$, as established by HR-ESI-MS, with six degrees of unsaturation. Comparison of NMR (Table 1) and HR-ESI-MS data with that of compound **1** suggested that the only difference between **1** and **2** is the presence of an additional methylene group in **2**. Combined COSY, HMBC and MS data suggested that the additional CH_2 group in **2** resides within the aliphatic side chain making **2** an undecyl ester variant of **1**. The structures of **1** and **2** were confirmed by comparison of spectral data with compounds produced by chemical synthesis (*vide infra*).



In addition to bulbiferates A (**1**) and B (**2**), three purine-based natural products were found in the crude extract of WMMC-695; these species were identified as previously known 4′, 5′-didehydro-5′-deoxyinosine, 2′-O-methyladenosine, and 5′-methylthioinosine based on comparisons of experimental ^{1}H NMR and MS data (Supporting Information) with previously reported data.[31–33] None of these compounds has thus far been noted to display any biological activities.[31–33]

Bulbiferates A (**1**) and B (**2**) were tested for antibacterial activity against *E.coli*, methicillin-resistant *Staphylococcus aureus* (MRSA; ATCC 33591), and MSSA in agar diffusion assays. Although both agents showed weak activity against *E.coli* and moderate activity against MSSA, neither compound displayed activity against MRSA (ATCC 33591). Parabens, the only other class of compounds reported from *Microbulbifer* spp., are also described as showing antibacterial activity against *Staphylococcus aureus* and their activity has been found to increase significantly with increasing chain length of the ester group.[28, 29] By the same token, we hypothesized that ester lengths might influence biological activities for the bulbiferates as well, especially if they were simply affecting membranes due to their lipophilicity. To assess this possibility, we synthesized compounds **3**–**5** using procedures described in the literature starting with the commercially available 3-amino-4-hydroxybenzoic acid.[34, 35] Specifically, N-acetylation of 3-amino-4-hydroxybenzoic acid was followed by esterifications of the resulting 3-acetamido-4-hydroxybenzoic acid using alcohols of desired chain lengths to attain **3**–**5**. Due to the relatively straightforward nature of this synthesis and the low titers of **1** (0.4 mg) and **2** (0.2 mg) from WMMC-695, we also made **1** and **2**. The synthetic approach easily generated more than enough material to carry out further biological studies. Compounds **3** and **4** consisted of shorter side chains (methyl and octyl respectively) and compound **5** possessed a longer side chain (tetradecyl) compared to bulbiferates A (**1**) and B (**2**). We envisioned that any impact upon bioactivities by the ester moieties would be easily revealed given the variance in ester side chain length from 1 14 methylene groups across compounds **1**–**5**. As summarized in Table 2, compounds **1**–**5** appeared to express varying extents of bioactivity against *E. coli* and MSSA in agar diffusion assays although no clear trend linking ester length to bioactivity is evident. Although differentiated from each other by their ester moieties, we found that antibacterial activities of **1**–**5** do not track with increasing or decreasing ester side chain lengths. This finding takes into consideration that **5** presented issues with solubility and therefore, its bioactivity could not be assessed with certainty.

To follow up the results of Table 2, and to gain more accurate bioactivity data for compounds **1**–**5** we determined minimum inhibitory concentrations (MIC) values for these agents against *E. coli* and MSSA. Assays using both organisms revealed that all five esters were characterized by MIC values > 200 μg/mL. Again, these assays suggested that no direct correlation exists between microbial activities and the side chain length of **1**–**5**. From these data we conclude that ester lipophilicities for these compounds do not appear to play a role in dictating antimicrobial activities against the organisms tested.

In conclusion, two acetamidohydroxybenzoates, **1** and **2**, were isolated from a marine-derived *Microbulbifer* sp. Both **1** and **2** exhibited weak activity against *E.coli* and MSSA; construction of three analogs made clear that no correlation exists between the length of the ester side chain present in these molecules and their antimicrobial activities. Notably, this is only the second report of natural products generated from this bacterial genus; the first being the production of parabens from *Microbulbifer* spp. A4B-17. The discovery of **1** and **2** represents the unveiling of the second class of molecules from *Microbulbifer* spp.

EXPERIMENTAL SECTION

General Experimental Procedures.

Optical rotations were measured with a Perkin-Elmer 241 polarimeter. UV spectra were recorded with an Aminco/OLIS UV-Vis spectrophotometer. IR spectra were measured with a Bruker Equinox 55/S FT-IR spectrophotometer. 1D and 2D NMR data were recorded using a Bruker Avance 600 MHz spectrometer with a $^{1}H\{^{13}C/^{15}N\}$ cryoprobe and a 500 MHz spectrometer with a $^{13}C/^{15}N\{^{1}H\}$ cryoprobe, AVANCE-500, or DRX-400 spectrometers. Chemical shift values were referenced to the residual solvent peaks ($CDCl_3$: δ_H7.26, δ_C 77.18; methanol-d_4: δ_H 3.31, δ_C 49.15) HRMS data were acquired with a Bruker Maxis 4G QTOF mass spectrometer. Reverse phase (RP) HPLC was performed using a Shimadzu Prominence HPLC System and a Phenomenex Luna C-18 semi-prep column (250 × 10 mm, 5 μm), Phenomenex Luna phenyl-hexyl semi-prep column (250 × 10 mm, 5 μm) or a Phenomenex Luna phenyl-hexyl analytical column (250 × 4.6 mm, 5 μm).

Biological Material.

WMMC-695 was isolated from the tunicate *Ecteinascidia turbinata*, which was collected on August 13, 2014, in the Florida Keys (24° 39.393, 81° 26.268). A voucher specimen is housed at the University of Wisconsin-Madison. For cultivation, a sample of tunicate (1 cm^3) was rinsed with sterile seawater and macerated using a sterile pestle in a microcentrifuge tube, and dilutions were made in sterile seawater, with vortexing between steps to separate bacteria from heavier tissues. Dilutions were separately plated onto three media: i) ISP2 [4 g yeast extract, 4 g dextrose, 10 g malt extract in 1 L of deionized H_2O] supplemented with artificial sea water (ASW), ii) R2A [0.5 g yeast extract, 0.5 g peptone, 0.5 g casamino acids, 0.5 g dextrose, 0.5 g soluble starch, 0.3 g sodium pyruvate, 0.3 g dipotassium phosphate, 0.05 g magnesium phosphate, 15 g agar in 1 L deionized H_2O], and iii) M4 [0.1 g L-asparagine, 0.5 g dipotassium phosphate, 0.001 g iron(II) sulfate, 0.1 g $MgSO_4$, 2 g peptone, 4 g sodium propionate, 20 g NaCl, 15 g agar in 1 L deionized H_2O]. Plates were incubated at 28 °C for at least 28 d, and strain WMMC-695 was purified from an

R2A isolation plate containing nalidixic acid (25 μg/mL), cycloheximide (50 μg/mL), and nystatin (25 μg/mL).

Sequencing.

16S rRNA sequencing was performed on a single gene (851 bp) as previously described by our group.[36] WMMC-695 demonstrated a 99.7 % match with *M. taiwanensis* CC-LN1–12. The WMMC-695 partial sequence information corresponding to a single gene was deposited in GenBank and assigned the accession number MK636570.

Fermentation, Extraction, and Isolation.

Three 10 mL seed cultures (25 × 150 mm tubes) in medium DSC [5 g soluble starch, 10 g glucose, 5 g peptone, and 5 g yeast extract per liter of ASW] were inoculated with strain WMMC-695 and shaken (200 rpm, 28 °C) for 7 d. For making artificial sea water solutions I (415.2 g NaCl, 69.54 g Na_2SO_4, 11.74 g KCl, 3.40 g $NaHCO_3$, 1.7 g KBr, 0.45 g H_3BO_3, 0.054 g NaF) and II (187.9 g $MgCl_2{\cdot}6H_2O$, 22.72 g $CaCl_2{\cdot}2H_2O$, 0.428 g $SrCl_2{\cdot}6H_2O$) were made up separately and combined to give a total volume of 20 L. Baffled flasks (500 mL, 5×100 mL) containing artificial sea water-A (ASW-A) medium [20 g soluble starch, 10 g glucose, 5 g peptone, 5 g yeast extract, 5 g $CaCO_3$ per liter of ASW] were inoculated with 5 mL from the culture tube and shaken at 200 rpm at 28 °C for 7 d. Fernbach flasks (2.8 L, 10 ×1 L) containing medium ASW-A with Diaion HP20 (7 % by weight) were inoculated with 50 mL of the culture and shaken at 200 rpm at 28 °C for 7 d. Filtered HP20 was washed with distilled H_2O and extracted with acetone. The acetone extract (5.6 g) was subjected to a liquid-liquid partitioning using 10 % aqueous MeOH and hexane (1:1), and then increased to 30% aqueous methanol, and partitioned using $CHCl_3$ (1:1). The $CHCl_3$ soluble portion (676 mg) was subjected to a Sephadex LH-20 column with MeOH:$CHCl_3$; 1:1 to obtain 10 fractions. Fraction 7 (89 mg) was further subjected to RP HPLC (10−100% MeCN−H_2O with H_2O containing 0.1 % acetic acid over 35 min, 10.0 mg/mL) using a Phenomenex Luna semi-prep C18 column (250 × 10 mm, 5 μm) to obtain 12 fractions. Fraction 11 (1.0 mg, t_R 32.3 min) was purified by RP HPLC (70−100 % MeCN−H_2O over 35 min, 1 mg/mL) using a Phenomenex Luna analytical phenyl-hexyl column (250 × 4.6 mm, 5 μm), yielding **1** (0.4 mg, t_R 20.7 min) and **2** (0.2 mg, t_R 21.9 min).

*Bulbiferate A (**1**):* yellow powder; UV (MeOH) λ_{max} (log ε) 236 (3.93), 254 (3.83), 264 (3.82) nm; IR (ATR) ν_{max} 3659, 2980, 2888, 2350, 1738, 1693, 1590, 1380, 1231 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$) data, Table 1; HRESIMS *m*/*z* 358.1994 $[M + Na]^+$ (calcd for $C_{19}H_{29}NO_4Na$, 358.1985).

*Bulbiferate B (**2**):* yellow powder; UV (MeOH) λ_{max} (log ε) 236 (3.81), 254 (3.61), 264 (3.60) nm; IR (ATR) ν_{max} 3659, 2981, 2888, 2350, 1738, 1692, 1587, 1377, 1230 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$) data, Table 1; HRESIMS *m*/*z* 372.2153 $[M + Na]^+$ (calcd for $C_{20}H_{31}NO_4Na$, 372.2141).

Antibacterial Assays.

Compounds **1**–**5** were tested for antibacterial activity against E.coli (ATCC #25922) and MSSA (ATCC #25913), and MICs were determined using a dilution antimicrobial

susceptibility test for aerobic bacteria.[37] Compounds **1**–**5** were dissolved in DMSO, serially diluted to 10 concentrations (0.5–256 μg/mL), and tested in a 96-well plate. Geneticin was used as the positive control against *E. coli*, which showed an MIC of 0.25 μg/mL whereas vancomycin was used as a the positive control against MSSA, which exhibited an MIC of 1 μg/mL. Compounds **1**–**5** and the positive controls were tested in duplicate. Eight untreated media controls were included on each plate. The plates were incubated at 33 °C for 18 h. The MIC was determined as the lowest concentration that inhibited visible growth of bacteria.

3-acetamido-4-hydroxybenzoic acid.—To a solution of 3-amino-4-hydroxybenzoic acid (-, 1.08g, 7 mmol) in acetic acid (CH_3COOH, 7 mL) was added acetic anhydride [$(CH_3CO)_2O$, 1.05 ml (10.5 mmol)] in a dropwise manner over 10 min at 60 °C and the reaction was stirred for 2h. The reaction solution was then poured into water (10 mL) and stirred, the resulting solid was filtered, washed with water (4 mL × 3) and dried in vacuo to obtain 3-acetamido-4-hydroxybenzoic acid as a white solid (1.20 g, 88%), which was then used for the susbsequent syntheses of compounds **1**–**5**.[12] UV (MeOH) λ_{max} (log ε) 254 (2.37), 276 (2.71) nm; IR (ATR) ν_{max} 3426, 2988, 2361, 1667, 1596, 1542, 1409, 1376 cm^{-1}; ^1H NMR data for 3-acetamido-4-hyroxybenzoic acid (methanol-d_4, 400 MHz) δ_H 8.40 (1H, d, J = 2.0 Hz), 7.70 (1H, dd, J = 8.4, 2.0 Hz), 6.90 (1H, d, J = 8.4 Hz), 2.19 (3H, s); HRESIMS m/z 218.0425 $[M + Na]^+$ (calcd for $C_9H_9NO_4Na$, 218.0425).

3-acetamido-4-hydroxymethylbenzoate (3).—To a solution of 3-acetamido-4-hydroxybenzoic acid () (195 mg, 1 mmol) dissolved in dimethylformamide (DMF,2 mL) was added methanol (162 μL, 4 mmol) and 4-dimethylaminopyridine (DMAP) (12.2 mg, 0.1 mmol) and cooled to 0 °C in an ice bath while stirring. Dicyclohexylcarbodiimide (DCC) (206 mg, 1 mmol) was added to the mixture at 0 °C over 2 min and stirred for another 5 min at 0 °C. The reaction mixture was then removed from the ice bath and stirred under N_2 for 3 h at room temperature. The precipitated dicyclohexyl urea was removed by filtration and the filtrate was dried invacuo. The dried material was then dissolved in dichloromethane, washed with two portions of 0.5 N HCl followed by partitioning with two portions of saturated sodium bicarbonate solution. Further precipitation of cyclohexyl urea occurred during this process, which was removed by filtration. The resulting organic phase was dried over anhydrous sodium sulfate and dried invacuo to obtain a crude product, which was purified by RP HPLC (20−100 % MeCN−H_2O over 35 min, 10 mg/mL) using a Phenomenex Luna semi-prep phenyl-hexyl column (250 × 10 mm, 5 μm) to obtain **3** [7.0 mg (3%), t_R 18 min] as a white solid.[13] UV (MeOH) λ_{max} (log ε) 232 (4.08), 254 (3.91) nm; IR (ATR) ν_{max} 3340, 3143, 2364, 2343, 1715, 1612, 1593, 1555, 1427, 1284 cm^{-1}; ^1H NMR data for **3** (methanol-d_4, 500 MHz) δ_H 8.45 (1H, d, J = 2.0 Hz), 7.68 (1H, dd, J = 8.4, 2.0 Hz), 6.88 (1H, d, J = 8.4 Hz), 3.84 (3H, s), 2.18 (3H, s); HRESIMS m/z 232.0587 $[M + Na]^+$ (calcd for $C_{10}H_{11}NO_4Na$, 232.0581).

3-acetamido-4-hydroxyoctylbenzoate (4).—Compound **4** was synthesized following the same procedure described for **3** except that octanol (627 μL, 4 mmol) was used in place of methanol. The same work-up described for **3** was performed to obtain the crude product, which was purified by RP HPLC (20−100% MeCN−H_2O over 35 min, 10 mg/mL) using a

Phenomenex Luna semi-prep phenyl-hexyl column (250 × 10 mm, 5 μm) to obtain **4** [9.1 mg (2.8%), t_R 26.9 min] as a white solid.[13] UV (MeOH) λ_{max} (log ε) 234 (4.11), 254 (3.92), 264 (3.93) nm; IR (ATR) ν_{max} 3660, 3457, 2980, 2886, 1739, 1692, 1587, 1548, 1680, 1231 cm^{-1}; ^1H NMR data for **4** (methanol-d_4, 500 MHz) δ_H 8.43 (1H, d, J = 2.0 Hz), 7.68 (1H, dd, J = 8.4, 2.0 Hz), 6.90 (1H, d, J = 8.4 Hz), 4.25 (2H, t, J = 6.7 Hz), 2.18 (3H, s), 1.74 (2H, br p, J = 6.7, 13.9 Hz), 1.44 (2H, br p, J = 6.7, 13.9 Hz), 1.25–1.40 (8H, m), 0.89 (3H, t, J = 7.1 Hz); HRESIMS m/z 330.1672 $[M + Na]^+$ (calcd for $C_{17}H_{25}NO_4Na$, 330.1673).

3-acetamido-4-hydroxydecylbenzoate (1).—Compound **1** was synthesized following the same procedure described for **3** except that decanol (763 μL, 4 mmol) was used in place of methanol.[11] The same work-up described for **3** was performed to obtain the crude product, which was purified by RP HPLC (70−100% MeCN−H_2O over 35 min, 10 mg/mL) using a Phenomenex Luna semi-prep phenyl-hexyl column (250 × 10 mm, 5 μm) to obtain **1** [14.2 mg (4%), t_R 20.7 min] as a white solid.[13] UV (MeOH) λ_{max} (log ε) 236 (3.93), 254 (3.83), 264 (3.82) nm; IR (ATR) ν_{max} 3659, 2980, 2888, 2350, 1738, 1693, 1590, 1380, 1231 cm^{-1}; ^1H NMR data for **1** ($CDCl_3$, 500 MHz) δ_H 7.80 (1H, dd, J = 2.0, 8.4 Hz), 7.75 (1H, d, J = 2.0 Hz), 7.02 (1H, d, J = 8.4 Hz), 4.26 (2H, t, J = 6.6 Hz), 2.30 (3H, s), 1.73 (2H, br p, J = 6.8, 14.0 Hz), 1.41 (2H, br p, J = 6.8, 14.0 Hz), 1.21–1.37 (12H, m), 0.87 (3H, t, J = 7.1 Hz); HRESIMS m/z 358.1984 $[M + Na]^+$ (calcd for $C_{19}H_{29}NO_4Na$, 358.1985).

3-acetamido-4-hydroxyundecylbenzoate (2).—Compound **2** was synthesized following the same procedure described for **3** except that undecanol (830 μL, 4 mmol) was used in place of methanol. The same work-up described for **3** was performed to obtain the crude product, which was purified by RP HPLC (70−100 % MeCN−H_2O over 35 min, 10 mg/mL) using a Phenomenex Luna semi-prep phenyl-hexyl column (250 × 10 mm, 5 μm) to obtain **2** [10.8 mg (3 %), t_R 21.9 min] as a white solid.[13] UV (MeOH) λ_{max} (log ε) 236 (3.81), 254 (3.61), 264 (3.60) nm; IR (ATR) ν_{max} 3659, 2981, 2888, 2350, 1738, 1692, 1587, 1377, 1230 cm^{-1}; ^1H NMR data for **2** ($CDCl_3$, 500 MHz) δ_H 7.81 (1H, dd, J = 2.0, 8.4 Hz), 7.67 (1H, d, J = 2.0 Hz), 7.03 (1H, d, J = 8.4 Hz), 4.27 (2H, t, J = 6.7 Hz), 2.31 (3H, s), 1.74 (2H, br p, J = 6.8, 14.0 Hz), 1.42 (2H, br p, J = 6.8, 14.0 Hz), 1.21–1.38 (14H, m), 0.87 (3H, t, J = 7.1 Hz); HRESIMS m/z 372.2143 $[M + Na]^+$ (calcd for $C_{20}H_{31}NO_4Na$, 372.2141).

3-acetamido-4-hydroxytetradecylbenzoate (5).—Compound **5** was synthesized following the same procedure described for **3** except that tetradecanol (858 mg, 4 mmol) was used in place of methanol. The same work-up described for **3** was performed to obtain the crude product, which was purified by RP HPLC (70−100 % MeCN−H_2O over 35 min, 10 mg/mL) using a Phenomenex Luna semi-prep phenyl-hexyl column (250 × 10 mm, 5 μm) to obtain **5** [24.7 mg (6%), t_R 24.8 min] as a white solid.[13] UV (MeOH) λ_{max} (log ε) 237 (3.89), 254 (3.76), 264 (3.76) nm; IR (ATR) ν_{max} 3659, 2981, 2971, 2350, 1738, 1693, 1591, 1366, 1228, 1216 cm^{-1}; ^1H NMR data for **5** ($CDCl_3$, 500 MHz) δ_H 7.80 (1H, dd, J = 2.0, 8.4 Hz), 7.71 (1H, d, J = 2.0 Hz), 7.03 (1H, d, J = 8.4 Hz), 4.26 (2H, t, J = 6.7 Hz), 2.30 (3H, s), 1.74 (2H, br p, J = 6.8, 14.0 Hz), 1.42 (2H, br p, J = 6.8, 14.0 Hz), 1.21–1.37 (20H, m), 0.88 (3H, t, J = 7.0 Hz); HRESIMS m/z 414.2616 $[M + Na]^+$ (calcd for $C_{23}H_{37}NO_4Na$, 414.2609).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGMENTS

This work was funded by the National Institutes of Health grants U19AI109673 and U19AI142720. We thank the Analytical Instrumentation Center at the University of Wisconsin-Madison School of Pharmacy for the facilities to acquire spectroscopic data, especially NMR and MS. This study made use of the National Magnetic Resonance Facility at Madison (NMRFAM), which is supported by NIH grant P41GM103399 (NIGMS). Equipment was purchased with funds from the University of Wisconsin-Madison, the NIH P41GM103399, S10RR02781, S10RR08438, S10RR023438, S10RR025062, S10RR029220), the NSF (DMB-8415048, OIA-9977486, BIR-9214394), and the USDA. We also thank Mr. Don DeMaria (Key West, FL) for the collection and photography of *Ecteinascidia turbinata* used in the TOC graphic.

REFERENCES

(1). Timmermans ML; Paudel YP; Ross AC Mar. Drugs 2017, 15, 235–270.

(2). Desriac F; Jegou C; Balnois E; Brillet B; Le Chevalier P; Fleury Y Mar. Drugs 2013, 11, 3632–3660. [PubMed: 24084784]

(3). Stackebrandt E; Murray RGE; Truper HG Int. J. Syst. Bacteriol. 1988, 38, 321–325.

(4). Woese CR Microbiol. Rev. 1987, 51, 221–271. [PubMed: 2439888]

(5). Gupta RS FEMS Microbiol. Rev. 2000, 24, 367–402. [PubMed: 10978543]

(6). Stevens H; Stubner M; Simon M; Brinkhoff T FEMS Microbiol. Ecol. 2005, 54, 351–365. [PubMed: 16332333]

(7). Eilers H; Pernthaler J; Glockner FO; Amann R Appl. Environ. Microbiol. 2000, 66, 3044–3051. [PubMed: 10877804]

(8). Cottrell MT; Kirchman DL Appl. Environ. Microbiol. 2000, 66, 5116–5122. [PubMed: 11097877]

(9). Llobet-Brossa E; Rossello-Mora R; Amann R Appl. Environ. Microbiol. 1998, 64, 2691–2696. [PubMed: 9647850]

(10). Glockner FO; Fuchs BM; Amann R Appl. Environ. Microbiol. 1999, 65, 3721–3726. [PubMed: 10427073]

(11). Cottrell MT; Kirchman DL Appl. Environ. Microbiol. 2000, 66, 1692–1697. [PubMed: 10742262]

(12). Bhatnagar I; Se-Kwon K Mar. Drugs 2010, 8, 2673–2701. [PubMed: 21116414]

(13). King GM; Smith CB; Tolar B; Hollibaugh JT Front. Microbiol. 2012, 3, 438–451. [PubMed: 23346078]

(14). Jamieson RE; Rogers AD; Billett DSM; Smale DA; Pearce DA FEMS Microbiol. Ecol. 2012, 80, 452–468. [PubMed: 22273466]

(15). Lau SCK; Zhang R; Brodie EL; Piceno YM; Andersen G; Liu W-T FEMS Microbiol. Ecol. 2013, 84, 259–269. [PubMed: 23237658]

(16). Gilbert JA; Field D; Swift P; Newbold L; Oliver A; Smyth T; Somerfield PJ; Huse S; Joint I Environ. Microbiol. 2009, 11, 3132–3139. [PubMed: 19659500]

(17). Oh DC; Strangman WK; Kauffman CA; Jensen PR; Fenical W Org. Lett. 2007, 9, 1525–1528. [PubMed: 17373804]

(18). Brinkhoff T; Bach G; Heidorn T; Liang L; Schlingloff A; Simon M Appl. Environ. Microbiol. 2004, 70, 2560–2565. [PubMed: 15066861]

(19). Shiozawa H; Kagasaki T; Kinoshita T; Haruyama H; Domon H; Utsui Y; Kodama K; Takahashi SJ Antibiot. 1993, 46, 1834–1842.

(20). Tsukimoto M; Nagaoka M; Shishido Y; Fujimoto J; Nishisaka F; Matsumoto S; Harunari E; Imada C; Matsuzaki TJ Nat. Prod. 2011, 74, 2329–2331.

(21). Gonzales JM; Mayer F; Moran MA; Hodson RE; Whitman WB Int. J. Syst. Bacteriol. 1997, 47, 369–376. [PubMed: 9103623]

(22). Yoon J-H; Kim I-G; Shin D-Y; Kang KH; Park Y-H Int. J. Syst. Evol. Microbiol. 2003, 53, 53–57. [PubMed: 12656152]

(23). Baba A; Miyazaki M; Nagahama T; Nogi Y Int. J. Syst. Evol. Microbiol. 2011, 61, 2215–2220. [PubMed: 20935083]

(24). Miyazaki M; Nogi Y; Ohta Y; Hatada Y; Fujivara Y; Ito S Int. J. Syst. Evol. Microbiol. 2008, 58, 1128–1133. [PubMed: 18450701]

(25). Vashist P; Nogi Y; Ghadi SC; Verma P; Shouche YS Int. J. Syst. Evol. Microbiol. 2013, 63, 2532–2537. [PubMed: 23243090]

(26). Lee H-Y; Lee Y-S; Choi Y-L Biotechnol. Biofuels 2018, 11, 1–14. [PubMed: 29321810]

(27). Lee Y-S Front. Microbiol. 2016, 7, 1–13. [PubMed: 26834723]

(28). Xue P; Kyoko A; Choryu C; Hiroaki K; Kaneo K; Yoshikazu S; Norihiko M Appl. Environ. Microbiol. 2006, 72, 5556–5561. [PubMed: 16885309]

(29). Elodie Q; Isabelle D-C; Mathieu P; Marie-Lise B-K. Environ. Microbiol. 2009, 11, 1527–1539. [PubMed: 19226298]

(30). Nagao, T.; Adachi, K.; Nishida, F.; Nishijima, M.; Mochida, K. JP 2000016976, 2000.

(31). Dimitrijevich SD; Verheyden JPH; Moffatt JG J. Org. Chem. 1979, 44, 400–406.

(32). Martínez-Montero S; Fernandez S; Rodríguez-Perez T; Sanghvi YS; Wen K; Gotor V; Ferrero M Eur. J. Org. Chem. 2009, 3265–3271.

(33). Kyeremeh K; Acquah KS; Sazak A; Houssen W; Tabudravu J; Deng H; Jaspars M Mar. Drugs 2014, 12, 999–1012. [PubMed: 24534843]

(34). Mao Y; Zhu W; Kong X; Wang Z; Xie H; Ding J; Terrett NK; Shen J; Shen J Bioorg. Med. Chem. 2013, 21, 3090–3104. [PubMed: 23602524]

(35). Neises B; Steglich W Angew. Chem. Int. Ed. Engl. 1978, 17, 522–524.

(36). Zhang F; Adnani N; Vazquez-Rivera E; Braun DR; Tonelli M; Andes DR; Bugni TS J. Org. Chem. 2015, 80, 8713–8719. [PubMed: 26273993]

(37). National Committee for Clinical Laboratory Standards Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically, 7th ed.; NCCLS: Villanova, PA, 2006; Approved standard M7-A7.

Table 1.

^{1}H NMR (600 MHz) and ^{13}C NMR (125 MHz) spectral data for compounds **1** and **2** in $CDCl_3$

position	1		2	
	δ_C, type	δ_H (*J* in Hz)	δ_C, type	δ_H (*J* in Hz)
1	125.9, C		125.6, C	7.70, d (1.8)
2	124.5, CH	7.70, d (2.0)	124.2, CH	
3	122.4		122.5	
4	153.6, C		153.5, C	
4-OH		8.17, br s		8.17, br s
5	119.9, CH	7.03, d (8.5)	120.3, CH	7.03, d (8.5)
6	129.0, CH	7.80, dd (8.5, 2.0)	129.1, CH	7.81, dd (8.5, 1.8)
7	171.5, C		171.4, C	
8	166.7, C		166.2, C	
9	22.9, CH_3	2.30, s	22.9, CH_3	2.30, s
1′	65.5, CH_2	4.27, t (6.6)	65.4, CH_2	4.27, t (6.7)
2′	29.4, CH_2	1.74, quin (6.6)	29.5, CH_2	1.74, quin (6.6)
3′	26.2, CH_2	1.42, quin (6.6)	26.2, CH_2	1.42, quin (6.6)
4′	29.8*, CH_2	1.24–1.40	29.8*, CH_2	1.24–1.40
5′	29.7*, CH_2	1.24–1.40	29.8*, CH_2	1.24–1.40
6′	29.5*, CH_2	1.24–1.40	29.7*, CH_2	1.24–1.40
7′	28.9*, CH_2	1.24–1.40	28.5*, CH_2	1.24–1.40
8′	23.8*, CH_2	1.24–1.40	28.9*, CH_2	1.24–1.40
9′	32.1*, CH_2	1.24–1.40	23.8*, CH_2	1.24–1.40
10′	14.3, CH_3	0.88, t (7.2)	32.1*, CH_2	1.24–1.40
11′			14.3, CH_3	0.88, t (7.2)

*these values are interchangeable

Table 2.

Bioactivity data for compounds 1–5.

Compound	*E. coli* (inhibition zone diameter in mm)	MSSA (inhibition zone diameter in mm)
1	6	10
2	5	5
3	no activity	7
4	no activity	5
5	not determined	not determined