Isolation and Structure Determination of an Antimicrobial Ester from a Marine Sediment-Derived Bacterium

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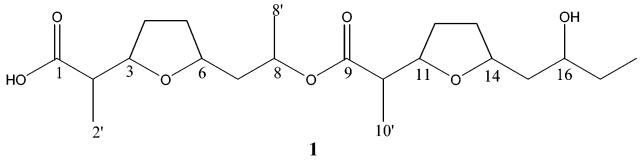
A new compound, assigned the trivial name bonactin (**1**), has been isolated from the liquid culture of a *Streptomyces* sp. BD21-2 obtained from a shallow-water sediment sample collected at Kailua Beach, Oahu, Hawaii. Structure elucidation employed one- and two-dimensional NMR, HRFABMS, IR, and chemical analysis. Bonactin displayed antimicrobial activity against both Gram-positive and Gram-negative bacteria as well as antifungal activity.

Marine bacteria have been the subject of a growing number of natural product studies. Marine microorganisms, like their terrestrial counterparts, are now considered a promising resource of biologically active compounds.[1] Interesting compounds include the cytotoxic and antiviral macrolactins,[2] the cytotoxic octalactins,[3] the cytotoxic caprolactins,[4] the antimicrobial wailupemycins,[5] and the antimicrobial kanchanamycins.[6] In the course of screening for antimicrobial agents, a new antifungal and antimicrobial metabolite has been isolated from a *Streptomyces* sp. BD21-2 cultured from a shallow-water sediment sample obtained at Kailua Beach, Oahu, Hawaii. We now report the discovery and structure determination of bonactin (**1**).



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The bacterium BD21-2 was grown in liquid culture, and the whole broth was extracted with ethyl acetate. The residue from the evaporated ethyl acetate extract was subjected to silica gel flash chromatography using chloroform/methanol (95:5) followed by column chromatography over LH-20 using methanol/chloroform (50:50). Isolation of bonactin followed a bioassay-guided fractionation, which resulted in the isolation of compound **1** as a pure viscous oil.

The ^{13}C NMR data along with the HRFAB mass spectrum of the sample was consistent with a molecular formula of $C_{21}H_{36}O_7$ ([M + H]$^+$, *m*/*z* 401). The ^{13}C NMR data for **1** (Table 1) included signals assignable to four methyl carbons, seven methylene carbons, eight methine carbons, and two quaternary carbons. The ^{13}C NMR data showed that two of the four sites of unsaturation could be attributed to two carbonyl carbons, a carboxylic acid (177.4 ppm), and an ester (174.4 ppm). The two remaining sites of unsaturation suggested the presence of two rings. The ^1H NMR data for **1** (Table 1) included signals assignable to eight methine protons, seven sets of diastereotopic methylene protons, and 12 methyl protons. Protons were placed on their respective carbons through interpretation of the HMQC NMR data. Partial structures were assembled by interpretation of $^1H-^1H$ COSY data and connected by analysis of the HMBC NMR data (Table 1). Key correlations are shown in Figure 1.

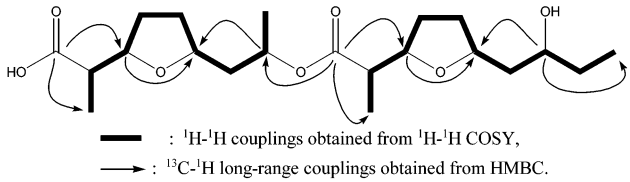

Figure 1. COSY and HMBC correlations.

Saponification followed by acidification of compound **1** resulted in two products whose proton NMR data were consistent with that of nonactic and homononactic acid.[7,8] Nonactic acid and homononactic acid are known to be optically active compounds.[9] Both compound **1** and its saponification products were optically inactive. The lack of optical activity of the saponification products suggests that bonactin consists of a racemic mixture of (±)-nonactic acid and (±)-homononactic acid moieties.

Bonactin displayed antimicrobial activity against both Gram-negative and Gram-positive bacteria as well as antifungal activity (Table 2). This is the first acyclic ester related to the nonactins reported to have antimicrobial activity. This finding is surprising since dimeric nonactic acid, which consists of (+)-nonactic acid and a (−)-nonactic acid moiety[10] (difference of a single methylene from compound **1**), is reported to display neither antibacterial nor antifungal activity in concentrations < 2000 μg/mL.

Both bonactin and dinactin are comprised of nonactic acid and homononactic acid units. Dinactin is a member of the macrotetrolide antibiotics known as the nonactins. The nonactins possess the ability to form cation complexes with alkali metal ions.[11,12] This type of complexation involves chelation of four carbonyl oxygens on the macrotetrolide ring with alkali metal ions to form a tetradentate complex. It has been suggested that chelation is a prerequisite for antimicrobial activity of the nonactins.[13] Our findings show that antimicrobial activity is possible without a macrotetrolide ring system.

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Table 1. ^{13}C, ^{1}H, COSY, and HMBC NMR Data for Compound **1** (500 MHz in $CDCl_3$)[a]

carbon	δ_C	δ_H	COSY correlations	HMBC correlations
1	177.4	C		3
2	45.0	CH; 2.50 (bq, J = 7.1 Hz)	2′, 3	4a, 4b
2′	13.4	CH_3; 1.15 (d, J = 6.7 Hz)	2	3
3	80.1	CH; 3.98 (m)	2, 3a, 3b	5a, 5b, 6
4	31.1	CH_2; 1.60 (m)	3, 5a, 5b	2, 6
		2.02 (m)	3, 5a, 5b	2, 6
5	29.0	CH_2; 2.03 (m)	4a, 4b, 6	3, 7
		1.60 (m)	4a, 4b, 6	3, 7
6	76.8	CH; 3.99 (m)	5a, 5b, 7	3, 4, 8
7	42.3	CH_2; 1.79 (m)	6, 8	5, 8′
8	68.9	CH; 5.25 (dq, J = 6.4, 2.9 Hz)	7, 8′	6
8′	20.4	CH_3; 1.24 (d, J = 6.4 Hz)	8	7
9	174.4	C		8, 10′, 11
10	45.5	CH; 2.45 (dq, J = 7.1, 1.1 Hz)	10′, 11	12a, 12b
10′	13.2	CH_3; 1.07 (d, J = 7.1 Hz)	10	9, 11
11	80.3	CH; 4.00 (m)	10, 12a, 12b	10′, 13a, 13b, 14
12	28.3	CH_2; 1.60 (m)	11, 13a, 13b	10, 14
		1.93 (m)	11, 13a, 13b	10, 14
13	31.3	CH_2; 1.40 (m)	12a, 12b, 14	11, 15
		1.94 (m)	12a, 12b, 14	11, 15
14	76.4	CH; 3.85 (m)	13a, 13b, 15	11, 16
15	40.0	CH_2; 1.71 (m)	14, 16	13a, 13b, 17
16	73.4	CH; 4.88 (ddt, J = 5.5, 1.5, 1.3 Hz)	15, 17	14, 18
17	27.4	CH_2; 1.60 (m)	16, 18	15
18	9.28	CH_3; 0.89 (t, J = 7.5 Hz)	17	16

Table 2. Antimicrobial Activity of Bonactin (**1**)

test organism	zone of inhibition
Bacillus megaterium	8
Micrococcus luteus	8
Kleibsiella pneumoniea	8.5
Staphylococcus aureas	7
Alicagenes faecalis	10
Escherichia coli	9
Saccharomyces cerevisiae	7.5

[a] Bonactin concentrations were 1000 μg/mL. Inhibition zones are in mm.

Experimental Section

General Experimental Procedures. Infrared spectra were obtained using a Perkin-Elmer 1600 FT-IR spectrometer. All ^{1}H and ^{13}C NMR data were recorded in $CDCl_3$ on a GE Omega-500 instrument at 500 and 125 MHz operating frequencies, respectively. Chemical shifts are referenced to solvent peaks: δ_H 7.26 (residual $CHCl_3$) and δ_C 77.0. Mass spectral data were obtained using a Gemini 3000BB spectrometer.

Taxonomic Study. A physiological examination of the bacterium BD21-2 showed that it contained white aerial mycelium and yellow substrate mycelium. A fatty acid methyl ester (FAME) analysis of BD21-2 performed by Microbial ID, Inc., identified our isolate as *Streptomyces* "sp".

Culture Conditions. The bacterium, designated BD21-2, was obtained from a shallow-water sediment sample collected at Kailua Beach, Oahu, Hawaii. The sample of sediment was diluted 100:1 with sterile artificial seawater and plated onto marine agar 2216 (Difco) Petri plates. The isolated culture was grown in 20 2-L flasks containing 1 L of marine broth 2216 (Difco) for 3 days at 23−25 °C on an orbital shaker at 250 rpm.

Bioassays. The crude extract of BD21-2 (10 mg/mL in MeOH) was tested for antimicrobial activity against *Bacillus subtilis* and *Escherichia coli*, while pure bonactin was tested for antimicrobial activity against *B. subtilis*, *Staphylococcus aureas*, *Micrococcus luteus*, *Kleibsiella pneumoniea*, *Bacillus megaterium*, *Alicagenes faecalis*, and *Saccharomyces cerevisiae* using the paper disk diffusion method.[14] In this method, each paper disk (6 mm) containing 100 μg of the extract was placed directly onto an agar plate that had been freshly inoculated with a lawn of the test organism. After 24 h of incubation, the diameters of the inhibition zones were measured.

Extraction and Purification. The liquid culture was extracted with ethyl acetate, yielding 0.534 mg of organic soluble material. Chromatography of the ethyl acetate extract on Sephadex LH-20 (110 × 2 cm column) using CH_2Cl_2/MeOH (1:1) as eluant yielded aliquots that were combined into three fractions. The middle fraction contained 56 mg of **1**.

Spectral Data of 1: $[\alpha]^{25}_D$ 0° (*c* 0.84, CH_2Cl_2); IR (film) ν 3433, 2970, 2938, 2878, 1730, 1738, 1456, 1379, 1267, 1196, 1060, 758 cm^{-1}; ^{1}H NMR data, see Table 1; ^{13}C NMR data, see Table 1; HRFABMS $(M + H)^+$ 401.25394 ($C_{21}H_{37}O_7$, Δ +3.8 mmu).

Saponification of 1. To 10 mg (0.025 mol) of **1** was added 0.1 N KOH, and the resulting mixture was stirred at room temperature for 24 h. Acid workup followed by extraction with ethyl acetate resulted in a mixture of acids, which were separated using column chromatography (silical gel: 80:5 CH_2Cl_2/CH_3OH w/10% TFA), affording nonactic acid (4.7 mg, 0.023 mmol, 93%) and homononactic acid (5.6 mg, 0.024 mmol, 100%). Homononactic acid: $[\alpha]^{25}_D$ 0° (*c* 0.8, CH_3OH); ^{1}H NMR ($CDCl_3$) δ 0.94 (3H, t, J = 7.5 Hz), 1.18 (3H, d, J = 6.9 Hz), 1.56 (2H, m), 1.70 (4H, m), 2.35 (2H, m), 2.50 (1H, m), 3.78 (1H, m), 4.00 (1H, m), 4.23 (1H, m). Nonactic acid: $[\alpha]^{25}_D$ 0° (*c* 0.7, CH_3OH); ^{1}H NMR ($CDCl_3$) δ 1.16 (3H, d, J = 6.9 Hz), 1.24 (3H, d, J = 6.0 Hz), 1.74 (4H, m), 2.18 (2H, m), 2.50 (1H, m), 4.00 (1H, m), 4.12 (1H, m), 4.24 (1H, m).

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