Novel Sesquiterpenoid Matrix Metalloproteinase-3 Inhibitors from an Acid Mine Waste Extremophile†

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Berkeley Pit Lake is a 1500 ft deep abandoned open-pit copper mine filled with 30 billion gallons of acidic, metal-contaminated water. This harsh environment is proving to be a source of unusual, biologically active microorganisms. Bioassay-guided fractionation using signal transduction enzyme assays led to the isolation of three novel bisabolane sesquiterpenes and a novel coumarin. The isolation and characterization of these compounds are reported here.

In 1984 we began our investigation of marine microorganisms, inspired by the pioneering work of D. John Faulkner in this field a decade earlier.[1] While Faulkner's earliest ventures into marine microbes involved a seawater sample from an intertidal pool at La Jolla Shores, we targeted endosymbionts of marine sponges. Our early results were promising, but the logistics of marine research from a land-locked Montana School of Mines proved difficult.[2−4]

We did not abandon our study of aquatic microbes, however. Instead, we changed our focus to microbes in a lake system located less than one mile from our laboratory. The Berkeley Pit Lake system in Butte, Montana, is part of the largest EPA Superfund site in North America. It includes Berkeley Pit Lake, an abandoned open-pit copper mine, 1500 feet deep and one mile across. As infiltrating groundwater seeps into the Pit, rich veins of pyrite and other minerals dissolve, generating acid in the process. There are currently 30 billion gallons of water in the Pit, with an inflow rate of 4 million gallons/day. The water is acidic (pH 2.7) and contaminated with high concentrations of metal sulfates (including 1200 ppm iron, 240 ppm copper, 290 ppm aluminum, and 650 ppm zinc).[5] Unfortunately, the Pit Lake system sits at the headwaters of the Columbia River. If the water rises another 200 feet, it will reach the critical overflow level. At the current rate of rise, the critical level will be reached in approximately 10 years.

Although the chemistry and possible remediation strategies of the Pit Lake have been studied for 20 years, the microbial ecology has been neglected. With its low pH and high metal content, it was considered too toxic to support life. Since 1995, however, with colleague Grant Mitman, we have isolated over 40 fungi, protists, algae, protozoans, and bacteria.[6] Although conditions within the Pit Lake system are toxic for "normal" aquatic biota, these same conditions represent an ideal environment for extremophiles. This hostile environment may also select for new species that may produce novel secondary metabolites. It is the unique challenge of drug discovery to find methods for targeting the bioactive components in these organisms.

Enzyme-specific assays provide effective and efficient means for screening large numbers of crude extracts and then subsequently guiding isolation of pure, active compounds. We are focusing on the isolation and characterization of compounds that inhibit two different signal transduction enzymes: matrix metalloproteinase-3 (MMP-3) and caspase-1. The matrix metalloproteinases are a family of zinc-containing, calcium-dependent endopeptidases that hydrolyze the extracellular matrix of connective tissues and basement membranes.[7,8] Matrix metalloproteinase inhibitors represent a new therapeutic approach to the treatment of cancers.[9,10] They block the activity of specific proteolytic enzymes (matrix metalloproteinases) used by tumor cells to promote metastatic spread. Recent studies show that these inhibitors might also halt tumor progression and could be used as low-toxicity complements to cytotoxic therapies. Elevated levels of MMP-3 and MMP-1 have also been found in the synovium and cartilage of osteoarthritis and rheumatoid arthritis patients and have been implicated in the occurrence of rheumatoid arthritis and multiple sclerosis.[11−14]

Caspase-1 was the first of a novel type of cysteine protease responsible for the conversion of interleukin-1β to its mature form in monocytes.[15] Mature IL-1β is a key mediator of inflammation. Caspase-1 is believed to be analogous to CED-3, a cell death protein in *Caenorhabitis elegans*.[16] Caspase-1 inhibitors have shown promise in delaying the onset of Huntington's disease and amyotropic lateral sclerosis and in mitigating the effects of stroke.[17] These two assays were used in tandem to guide isolation and purification of the compounds reported here.

We isolated a filamentous fungus from the surface waters of the Berkeley Pit that was identified as a *Penicillium* species.[18] The *Penicillium* sp. isolate was grown in six different broths, including unmodified (pH 5.1) and acidified potato dextrose broth (pH 2.7) as still cultures for 21 days. The cultures were killed by the addition of MeOH, the mycelia were removed by gravity filtration, and the filtrates were extracted with $CHCl_3$. The $CHCl_3$ extract of this *Penicillium* sp. was active in both the matrix metalloproteinase-3 and caspase-1 inhibition assays when grown in acidified potato dextrose broth. These assays were used to guide flash silica gel column chromatography and silica

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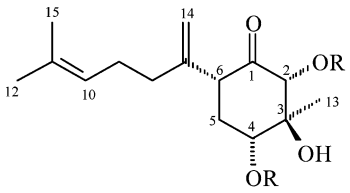
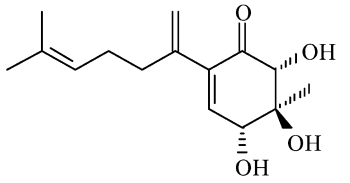
Table 1. NMR Data for Sesquiterpene **1** ($CDCl_3$)

position	δ_H	$^1H-^1H$ COSY	δ_C	HMBC
1			206.6	
2	4.13 (bs)	H-6	80.8	C-1, C-3, C-13
3			79.3	
4	4.14 (ddd, 11.7, 4.7, 1.0)	H-5a,b, H-13	72.2	C-13
5a	1.74 (m)	H-5b, H-4, H-6	32.6	C-4, C-6
5b	2.21 (m)	H-5a, H-4, H-6		C-1, C-4. C-3, C-6
6	3.18 (dd, 13.6, 9.3)	H-5a,b, H-2	51.4	C-1, C-7, C-5, C-8, C-14
7			144.0	
8	2.00 (2H, m)	H-9, H-14a,b	34.8	C-6, C-7, C-9, C-13
9	2.10 (2H, m)	H-8, H-10	26.3	C-8, C-10
10	5.06 (bt)	H-9, H-12, H-15	123.6	C-12, C-15
11			132.1	
12	1.65 (3H, bs)	H-10, H-15	25.7	C-10, C-11, C-15
13	0.98 (3H, s)	H-4	14.0	C-2, C-3, C-4
14	5.05 (1H, bs)		113.1	C-6, C-8
	4.85 (1H, bs)			C-6, C-7, C-8
15	1.58 (3H, bs)	H-10, H-12	17.7	C-10, C-11, C-12

gel HPLC that yielded sesquiterpenes **1**, **3**, and **4** and coumarin derivative **5**.



1 R = H
2 R = Ac



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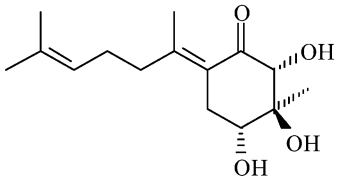

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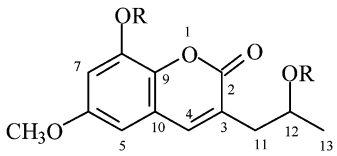

5 R = H
6 R = Ac

A molecular formula of $C_{15}H_{24}O_4$ with four degrees of unsaturation was established for **1** by HREIMS. The IR spectrum showed strong hydroxyl and ketone absorbances. The ^{13}C NMR spectrum (Table 1) indicated a ketone carbon (δ_C 206.6), a trisubstituted double bond (δ_C 123.6 and 132.1), a disubstituted vinylic methylene (δ_C 144.0 and 113.1), and three carbons attached to oxygen (δ_C 80.8, 79.3, and 72.2). One ring was required for the remaining degree of unsaturation. Both ^1H and ^{13}C NMR spectra indicated an isoprenoid tail.[19] The HMBC spectrum (Table 1) supported this observation, with correlations from olefinic proton H-10 (δ_H 5.06) to the terminal methyl carbons C-12 (δ_C 25.7) and C-15 (δ_C 17.7). C-10 (δ_C 123.6) showed correlations (HMBC) to methylene protons H-9 (2H, δ_H 2.10) and H-8 (2H, δ_H 2.00). The protons at δ_H 2.00 also showed correlations to olefinic methylene C-14 (δ_C 113.1) and quaternary carbon C-7 (δ_C 144.0), which was further correlated to methine proton H-6 (δ_H 3.18). C-6 (δ_C 51.4) showed correlations to olefinic methylene protons H-14a (δ_H 5.05) and H-14b (δ_H 4.85). Ketone carbon C-1 (δ_C 206.6) showed two-bond correlations to methine protons H-6 (δ_H 3.18) and H-2 (δ_H 4.13) and a three-bond correlation to methylene proton H-5b (δ_H 2.21). H-2 and H-5b also showed correlations to quaternary carbon C-3 (δ_C 79.3), which was further correlated to methyl proton H-13 (δ_H 0.98). H-5b, H-5a (δ_H 1.74), and H-13 showed correlations to C-4 (δ_C 72.2).

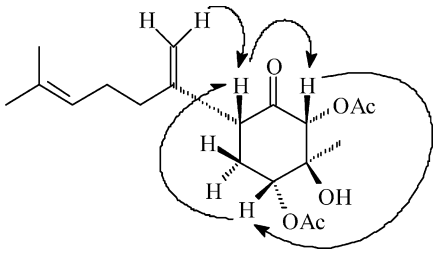

Figure 1. Relevant NOE data for diacetate **2**.

The $^1H-^1H$ COSY spectrum supported these correlations with clear couplings between H-6 and H-5a and b, which were further coupled to H-4. Consideration of a typical sesquiterpene framework generated the bisabolane **1**. Although NOE studies showed correlations between H-5a and methyl H-13, the overlapping chemical shifts of protons H-2 and H-4 precluded NOE studies to establish the relative stereochemistry of **1**. Fortunately, acetylation of **1** yielded the diacetate **2**, in which the chemical shifts of all of the ring protons were sufficiently resolved for NOE studies. 1D NOE difference experiments on **2** are shown in Figure 1 and established the relative stereochemistry of **1**. Bisabolane sesquiterpenes are not typical microbial metabolites. Most compounds of this skeletal class have been isolated from numerous terrestrial plants, a basidiomycete,[20] sponges,[21,22] octocoral,[23,24] and red algae.[25] To our knowledge there have been two reports of bisabolanes from fungi. The first report in 1989 was of a mycotoxin from *Fusarium sambucinum*.[26] More recently, mass spectral analysis of the volatile constituents of toxigenic *Penicillium roqueforti* strains yielded β-bisabolene.[27]

The ^1H NMR and ^{13}C NMR spectra of **3** were similar to that of **1**. Its molecular formula of $C_{15}H_{22}O_4$ (HREIMS and CI mass spectrometry) required one more unit of unsaturation that was established as an additional trisubstituted double bond from the NMR spectra (Table 2). $^1H-^1H$ COSY clearly showed coupling between the olefinic protons H-5a and b and H-4, and HMBC generated the structure of **3** as shown. NOE studies indicated the same relative stereochemistry as that of **1**.

Sesquiterpene **4** was isomeric with **1**. Both the ^1H and ^{13}C NMR spectra (Table 2) indicated that the disubstituted double bond was replaced by a tetrasubstituted alkene that was conjugated with the ketone (IR 1685 cm^{-1}). NOE studies again established the same relative stereochemistry as that of sesquiterpene **1**.

Table 2. NMR Data for Sesquiterpenes **3** and **4** ($CDCl_3$)

	3		**4**	
position	δ_C	δ_H (mult, *J* Hz)	δ_C	δ_H (mult, *J* Hz)
1	197.1		200.0	
2	80.1	4.09 (bs)	81.3	3.96 (s)
3	81.2		76.9	
4	73.9	4.67 (bs)	71.7	3.99 (dd, 11.6, 6.1)
5	146.0	6.72 (bs)	32.0	3.07 (dd, 15.2, 6.1) 2.10 (m)
6	138.1		125.5	
7	143.0		152.5	
8	34.9	2.30 (m, 2H)	36.3	2.20 (m, 2H)
9	26.6	2.03 (m, 2H)	25.9	2.20 (m, 2H)
10	123.3	5.03 (bt, 6.5)	122.7	5.09 (bt)
11	132.2		133.0	
12	25.7	1.65, (bs, 3H)	25.7	1.67 (bs, 3H)
13	13.7	1.03 (s, 3H)	14.0	1.02 (s, 3H)
14	116.7	5.21 (bs) 5.11 (bs)	21.4	1.98 (bs, 3H)
15	17.7	1.55 (bs, 3H)	17.7	1.57 (bs, 3H)

Compound **5** had a molecular formula of $C_{13}H_{14}O_5$ from the HREIMS. The ^{1}H NMR of this compound showed two *meta* coupled aromatic protons (δ 6.66 and 6.41, $J = 2.8$ Hz), an isolated olefinic proton (δ 7.53, s), a methoxyl signal (δ 3.77, s, 3H), and the coupled system $CH_2-CHOH-CH_3$ (δ 2.80, dd, $J = 14.2$, 3.4; δ 2.57, dd, $J = 14.2$, 8.2; δ 4.19, m; and δ 1.30, d, $J = 6.3$). Acetylation of **5** gave the diacetate **6**. The molecular formula and collective NMR data seemed to fit nicely into the structure of the isochromenone, orthosporin, which was previously reported from the fungus *Rhynchosporium orthosporum*.[28] More careful comparison of the data, however, showed some striking differences. Of particular note were the chemical shifts of the C-3 and C-4 olefinic carbons. In orthosporin, quaternary C-3 resonated at δ 156.3 and tertiary C-4 at δ 106.3. In **5**, however, C-3 resonated at δ 119.5 and C-4 at δ 141.6 (CH). This indicated a reversal in polarity and showed that **5** fit into a coumarin skeleton. The position of the substituents and assignment of all protons were established by NOE difference studies, and the relevant results are found in Figure 2. There are few 3-alkyl-6,8-dioxy coumarins known from fungal sources. These include 3-hydroxymethyl-6,8-dimethoxy coumarin from *Talaromyces flavus*.[29]

The three sesquiterpenes showed moderate inhibitory activity against both MMP-3 and caspase-1. Each compound was tested in triplicate at concentrations from 300 μM to 300 nM. All three compounds showed MIC_{50}'s in the 30 μM range against caspase-1 and in the 300 nM range against MMP-3. Compound **1** showed the greatest potency and **3** the least inhibitory potential. These compounds were sent to the Developmental Therapeutics Program at NCI/NIH for evaluation in human cancer cell lines.

Experimental Section

General Experimental Procedures. ^{1}H and ^{13}C NMR spectra were run on a Bruker DPX-300 spectrometer. ^{1}H NMR spectra were recorded at 300 MHz, and the ^{13}C NMR spectra were recorded at 75 MHz unless otherwise noted. All of the chemical shifts were recorded with respect to the deuterated solvent shift ($CDCl_3$, δ 7.24 for the proton resonance and δ 77.0 for the carbon). IR spectra were recorded on a Nicolet NEXUS 670 FT-IR spectrometer. Optical rotations were recorded on a Perkin-Elmer 241 MC polarimeter using a 1 mL cell. Mass spectral data were provided by the Montana State Mass Spectrometer Facility at Montana State University. All solvents used were spectral grade.

Collection, Extraction, and Isolation Procedures. The collection of water samples from the Berkeley Pit, the isolation of the various organisms, and the pilot growths and biological testing of the extracts have been previously described.[30] Fungus *Pitna4* was identified as a *Penicillium* sp. by Microbial Identification, Inc. The $CHCl_3$ extract of the *Pitna4* pilot culture showed weak antimicrobial activity and some brine shrimp lethality. The fungus was regrown in 32 × 200 mL of DIFCO potato dextrose broth (acidified to pH 2.7 with sulfuric acid) in 500 mL Erlenmeyer flask still cultures for 21 days. At harvest time, the fungus was killed with the addition of 20 mL of MeOH/flask. The culture was filtered through cheesecloth to remove the mycelial mat. The filtrate was extracted three times with 1 L of $CHCl_3$, and the extract was reduced in vacuo to an oil (1.3761 g). This $CHCl_3$ extract demonstrated inhibition of caspase-1 and MMP-3, antimicrobial activity against *Staphylococcus aureus* and *Escherichia coli*, and brine shrimp lethality.

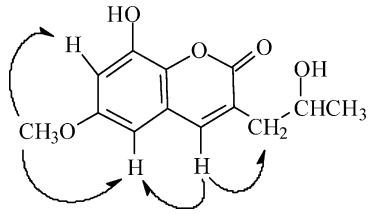

Figure 2. Relevant NOE data for **5**.

The $CHCl_3$ extract was fractionated on a flash Si gel column using hexane, hexane/IPA mixtures to IPA/MeOH mixtures. The large flash fractions were further fractionated by preparative HPLC on a Rainin 21 mm preparative Si gel column with a hexane/IPA gradient to give pure sesquiterpenes **1** (7.6 mg), **3** (0.5 mg), and **4** (5.9 mg) and coumarin **5** (5.2 mg).

Compound 1: clear oil; $[\alpha]^{20}_D$ +5.5° (*c* 0.011, MeOH); IR ($CHCl_3$) ν_{max} 3449, 3007, 2966, 2931, 1720, 1457, 1375, 1107, 1025 cm^{-1}; ^{1}H NMR and ^{13}C NMR, see Table 1; EIMS *m*/*z* 268 (0.5), 225 (7), 189 (11), 109 (41), 69 (100); HREIMS *m*/*z* 268.1675 (calcd for $C_{15}H_{24}O_4$, 268.1675).

Acetylation of Compound 1. Compound **1** (0.5 mg) was dissolved in pyridine (50 μL) and Ac_2O (50 μL) and stirred for 24 h. After that time the solvents were removed in vacuo to give **2** as an oil (0.5 mg): ^{1}H NMR ($CDCl_3$) δ 5.31 (dd, 11.7,4.6, H-4), 5.20 (bs, H-2), 5.06 (bt, H-10), 5.02 (bs, H-14), 4.80 (bs, H-14), 3.47 (bs, OH), 3.25 (dd, 13.5, 5.8, H-6), 2.20 (3H, Ac), 2.14 (3H, Ac), 2.00 (m, 4H), 1.65 (bs, 3H, H-12), 1.58 (bs, 3H, H-13), 1.18 (s, 3H, H-15).

Compound 3: oil, $[\alpha]^{20}_D$ +9.8° (*c* 0.014, MeOH); UV (MeOH) λ_{max}(log ϵ) 203 (4.14), 264 (s) (3.56) nm; IR (neat) ν_{max} 3429, 3007, 2968, 2932, 1685, 1453, 1382, 1344, 1111, 1037 cm^{-1}; ^{1}H NMR and ^{13}C NMR, see Table 2; EIMS *m*/*z* 266 (0.1), 248 (2), 223 (10), 109 (18), 69 (100); CIMS *m*/*z* 267 ($M^+ + 1$) (0.5), 248 (3), 205 (14), 177 (16), 149 (25), 69 (100); HREIMS *m*/*z* found 248.1396 (calcd for $C_{15}H_{20}O_3$ ($M^+ - H_2O$), 248.1412).

Compound 4: oil, $[\alpha]^{20}_D$ +9.8° (*c* 0.014, MeOH); UV (MeOH) λ_{max} (log ϵ) 201 (3.88), 254 (3.68) nm; IR ($CHCl_3$) ν_{max} 3429, 3007, 2968, 2932, 1685, 1453, 1382, 1344, 1111, 1037 cm^{-1}; ^{1}H NMR and ^{13}C NMR, see Table 2; EIMS *m*/*z* 268 (0.3), 250 (0.1), 234 (4), 189 (6), 164 (15), 87 (80), 69 (100); HREIMS *m*/*z* found 268.1669, calcd for $C_{15}H_{24}O_4$, 268.1675.

Compound 5: oil, $[\alpha]^{20}_D$ +21.4° (*c* 0.0029, MeOH); UV (MeOH) λ_{max} (log ϵ) 208 (4.55), 224 (4.18), 294 (4.11); IR ($CHCl_3$) ν_{max} 3352, 2968, 2928, 1714, 1701, 1594, 1497, 1193, 1156, 1042, 839 cm^{-1}; ^{1}H NMR δ 7.53 (bs, H-4), 6.66 (d, 2.8, H-5), 6.41 (d, 2.8, H-7), 4.19 (m, H-12), 3.77 (s, 3H, OCH_3), 2.80 (dd, 14.2, 3.4, H-11), 2.57 (dd, 14.2, 8.2, H-11), 1.30 (d, 6.3, H-13); ^{13}C NMR δ 161.6 (C-2), 156.5 (C-6), 143.8 (C-8), 141.6 (C-4), 135.8 (C-9), 126.6 (C-10), 119.5 (C-3), 104.9 (C-5), 101.3 (C-7), 66.4 (C-12), 55.6 (OCH_3), 40.7 (C-11), 23.5 (C-13); EIMS *m*/*z* 250 (25), 206 (100), 177 (10), 163 (11), 69 (8); HREIMS *m*/*z* found 250.0838, calcd for $C_{13}H_{14}O_5$ (M+), 250.0841.

Acetylation of Compound 5. Compound **5** (1.0 mg) was dissolved in pyridine (50 μL) and Ac_2O (50 μL) and stirred for 24 h. After that time the solvents were removed to give **6** as an oil (0.9 mg): ^{1}H NMR ($CDCl_3$) δ 7.44 (s, H-4), 6.83 (d, 2.8, H-5), 6.74 (d, 2.8, H-7), 5.18 (m, H-12), 3.80 (s, 3H, OCH_3),

2.83 (dd, 14.2, 3.4, H-11), 2.70 (dd, 14.2, 8.2, H-11), 2.38 (s, 3H, OAc), 1.96 (s, 3H, OAc), 1.28 (d, 6.3, H-13).

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References and Notes

(1) Wratten, S. J.; Wolfe, M. S.; Andersen, R. J.; Faulkner, D. J. *Antimicrob. Agents Chemother.* **1977**, *11* (3), 411−414.
(2) Stierle, A. C.; Cardellina, J. H., II; Singleton, F. L. *Experientia* **1988**, *44*, 1021.
(3) Stierle, A.; Cardellina, J. H., II. *Tetrahedron Lett.* **1991**, *32*, 4847−4848.
(4) Stierle, D. B.; Stierle, A. A. *Experientia* **1992**, *48* (11−12), 1165−1169.
(5) Dr. William Chatham, Montana Tech of the University of Montana, personal communication, 1997.
(6) Mitman, G. G. *A Final Report: Biological Survey of the Berkeley Pit Lake System;* Mine Waste Technology Program Activity IV, Project 10; USEPA National Risk Management Lab, IAG ID# DW89938513-01-0, 1999.
(7) Murphy, G.; Docherty, A. J. P. *Am. J. Respir. Cell Mol. Biol.* **1992**, *7*, 120−125.
(8) Docherty, A. J. P.; O'Connell, J.; Crabbe, T.; Angal, S.; Murphy, G. *Trends Biotechnol.* **1992**, *10*, 200−207.
(9) Brown, P. D. *Adv. Enzyme Regul.* **1995**, *35*, 293−301.
(10) Liedtke, W.; Cannella, B.; Mazzaccaro, R. J.; Clements, J. M.; Miller, K. M.; Wucherpfennig, K. W.; Gearing, A. J.; Raine, C. S. *Ann Neurol.* **1998**, *44*, 35−46.
(11) Roughley, P. J.; Nguyen, Q.; Mort, J. S.; Hughes, C. E.; Caterson, B. *Agents Actions Suppl.* **1993**, 149−159.
(12) Dean, D. D.; Martel-Pelletier, J.; Pelletier, J. P.; Howell, D. S.; Woessner, F. J. *J. Clin. Invest.* **1989**, *84*, 678−685.
(13) Hasty, K. A.; Reife, R. A.; Kang, A. H.; Stuart, J. M. *Arthritis Rheum.* **1990**, *33*, 388−397.
(14) Okada, Y.; Shinmei, M.; Tanaka, O.; Naka, K.; Kimura, A.; Nakanishi, I.; Bayliss, M. T.; Iwata, K.; Nagase, H. *Lab. Invest.* **1992**, *66*, 680−690.
(15) Chen, M.; Ona, V. O.; Li, M.; Ferrante, R. J.; Fink, K. B.; Zhu, S.; Bian, J.; Guo, L.; Farrell, L. A.; Hersch, S. M.; Hobbs, W.; Vonsattel, J. P.; Cha, J. H. J.; Friedlander, R. M. *Nat. Med.* **2000**, *6*, 797.
(16) Marcus, M. E.; Heufelder, A. E.; Hengartner, M. O. *Proc. Natl Acad. Sci. U.S.A.* **1997**, *94*, 12736−12737.
(17) Ona, V. O.; Li, M.; Vonsattel, J. P. G.; Andrews, L. J.; Khan, S. Q.; Chung, W. M.; Frey, A. S.; Menon, A. S.; Li, X. J.; Stieg, P. E.; Yuan, J.; Penney, J. B.; Young, A. B.; Cha, J. H. J.; Friedlander, R. M. *Nature* **1999**, *399*, 263−267.
(18) Organisms were identified for us by Microbial ID, Inc., Delaware.
(19) Silverstein, R. M.; Webster, F. X. In *Spectroscopic Identification of Organic Compounds*; Swain, E., Rose, N., Eds.; John Wiley and Sons: New York, 1998; pp 208 and 249.
(20) Stadler, M.; Anke, H.; Sterner, J. *J. Antibiot.* **1994**, *47*, 1284.
(21) Sullivan, B. W.; Faulkner, D. J.; Okamoto, K. T.; Chen, M. H. M.; Clardy, J. *J. Org. Chem.* **1986**, *51*, 5134−5136.
(22) Harrison, B.; Crews, P. *J. Org. Chem.* **1997**, *62*, 2646−2648.
(23) McEnroe, F.; Fenical, W. *Tetrahedron* **1978**, *34*, 1661−1664.
(24) D'Armas, H. T.; Mootoo, B. S.; Reynolds, W. F. *J. Nat. Prod.* **2000**, *63*, 1593−1595.
(25) Vazquez, J. T.; Chang, M.; Nakanishi, K.; Martin, J. D.; Martin, V. S.; Perez, R. *J. Nat. Prod.* **1988**, *51* (6), 1257.
(26) Sanson, D. R.; Corley, D. G.; Barnes, C. L.; Searles, S.; Schlemper, E. O.; Tempesta, M. S. *J. Org. Chem.* **1989**, *54*, 4313−4318.
(27) Jelen, H. H. *J. Agric. Food Chem.* **2002**, *50*, 6569−6574.
(28) Ichihara, A.; Hashimoto, M.; Hirai, T.; Tekeda, I.; Sasamura, Y.; Sakamura, S.; Sata, R.; Tajimi, A. *Chem. Lett.* **1989**, 1495−1498.
(29) Ayers, W. A. *Can. J. Chem.* **1990**, *68*, 2085.
(30) Stierle, A.; Stierle, D. B.; Goldstein, E.; Parker, K.; Bugni, T.; Baarson, C.; Gress, J.; Blake, D. *J. Nat. Prod.* **2003**, *66*, 1097−1100.

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