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Berkeleydione and Berkeleytrione, New Bioactive Metabolites from an Acid Mine Organism

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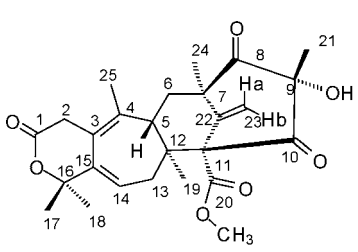
ABSTRACT



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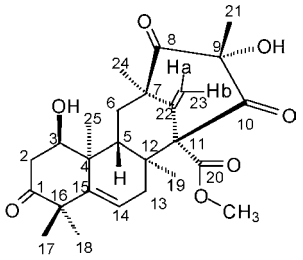

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Two novel hybrid polyketide-terpenoid metabolites were isolated from a *Penicillium* sp. growing in the Berkeley Pit Lake of Butte, Montana. Their structures were deduced by spectroscopic analysis and confirmed by single-crystal X-ray analysis on berkeleydione (1). Both compounds inhibited matrix metalloproteinase-3 and caspase-1, and berkeleydione showed activity toward non-small-cell lung cancer in NCI's human cell line antitumor screen.

The Berkeley Pit mine waste 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. During its operation, the Pit and 2500 miles of underground mine tunnels were dewatered through constant pumping. When mining ceased in 1982 the pumping stopped, and groundwater began to infiltrate the pyrite-rich area, dissolving veins of minerals and 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 metal ions (including 1200 ppm of iron, 240 ppm of copper, 290 ppm of aluminum, and 650 ppm of zinc).[1] 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.

The geology and water chemistry of the Pit Lake system have been studied for over 15 years, but the possibility of life in this toxic lake was not even considered. Seven years ago we collected surface water from the Pit and isolated three fungi: *Penicillium chrysogenum*, *Pichia anomala*, and a *Pithomyces* sp. With the assistance of the Montana Bureau of Mines and Geology, we have collected water samples from the Pit Lake from various water depths to the basal sediment. To date, we have isolated over 42 fungi and bacteria from the Pit Lake and are in the process of isolating and characterizing bioactive metabolites from these organisms. This extreme environment may select for new species or varieties of microbes that could be effective bioremediators of the mine wastewater in which they grow. Their metabolic byproducts could impact the overall ecology of the Pit Lake

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by raising the pH of the water, by providing nutrients for other heterotrophs, or by sorbing metal contaminants. It is also likely that organisms from this poorly to sparsely explored environment will yield previously undescribed small molecules with significant biological activity. Thus, the research potential of this site is tremendous.

The organic extracts of a *Penicillium* species[2] isolated from a depth of 885 ft. were active against *Staphylococcus aureus* and in the brine shrimp lethality screen. These extracts were further tested using enzyme inhibition assays for two different signal transduction enzymes—matrix metalloproteinase-3 (MMP-3) and caspase-1 (Casp-1). MMP inhibitors represent a new therapeutic approach to the treatment of cancers. They block the activity of proteolytic enzymes (matrix metalloproteinases) used by tumor cells to promote metastatic spread. Recent studies show that MMP inhibitors might also halt tumor progression and could be used as low toxicity complements to cytotoxic therapies.[3,4] MMPs are also implicated in the occurrence of rheumatoid arthritis and multiple sclerosis.[4]

Caspase-1 was the first of a novel type of cysteine proteases responsible for converting interleukin-1β to its mature form in monocytes. 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*. Caspase-1 inhibitors have shown some promise in delaying the onset of Huntington's disease and amyotropic lateral sclerosis and in mitigating the effects of stroke.[5,6] In this paper, we report the isolation of berkeleydione (**1**) and berkeleytrione (**2**), two previously undescribed biologically active polyketide-terpenoids that were purified by enzyme assay guided fractionation.

Compounds **1** and **2** were isolated from the $CHCl_3$ extract of a *Penicillium* sp. found growing in Berkeley Pit Lake.[7] Berkeleydione[8] (**1**, 5.5 mg/L) was isolated as a crystalline solid. High-resolution CIMS established the molecular formula of $C_{26}H_{33}O_7$ ($M^+ + H$) with 11 units of unsaturation. The ^{13}C NMR spectrum suggested two ketones, two esters, and three carbon−carbon double bonds (Table 1). Of the 26 carbons, 13 were quaternary. Detailed analysis of the $^1H-^1H$ COSY, HSQC, and HMBC spectra run in different solvents indicated several part structures, but the quantity and proximity of the many quaternary carbons made an unambiguous structural determination impossible. A single crystal was submitted for X-ray crystallographic analysis (Figure 1).

Table 1. NMR Data of Berkeleydione (**1**) and Berkeleytrione (**2**) in $CDCl_3$

	δ_C **1**	δ_H **1**	δ_C **2**	δ_H **2**
1	169.9, s		214.6	
2	34.1, t	β-3.32 bd, 21.1	42.9	β-2.65 bd, 19.7.6.1
		α-3.23 bd, 21.1		α-2.47 bd, 19.7
3	126.1, s		69.6	3.68 bs
4	135.2, s		43.6	
5	44.4, d	1.9 m	37.8	1.77 bd, 13.9
6	41.8, t	2.0 m (1H)	40.3	1.95 bd, 11.0
		1.9 m		1.70 dd, 13.9,11.0
7	51.0, s		51.0	
8	207.5, s		207.6	
9	79.5, s		79.6	
10	204.1, s		204.1	
11	71.2, s		71.7	
12	67.0, s		45.3	
13	36.3, t	β-3.04 dd, 14.5, 8.4	31.2	β-2.95 bd, 14.5
		α-1.9 m		α-2.55 dd, 14.5, 5.9
14	129.6, d	5.91 dd, 8.4,5.2	124.9	5.84 dd, 5.9,2.4
15	139.9, s		140.5	
16	82.3, s		48.2	
17	29.5, q	1.25 s, 3H	29.2	1.25 s, 3H
18	26.7, q	1.50 s, 3H	28.9	1.24 s, 3H
19	19.9, q	1.25 s, 3H	16.9	1.25 s, 3H
20	169.0, s		168.6	
21	14.9, q	1.28 s, 3H	15.3	1.35 s, 3H
22	145.3, s		144.8	
23	113.1, t	Ha 5.40 bs	112.7	Ha 5.39 bs
		Hb 4.88 bs		Hb 4.89 bs
24	22.1, q	1.47 s, 3H	22.2	1.50 s, 3H
25	15.5, q	1.64 bs, 3H	17.4	.78 s, 3H
OCH_3	52.6, q	3.75 s, 3H	52.6	3.72 s, 3H

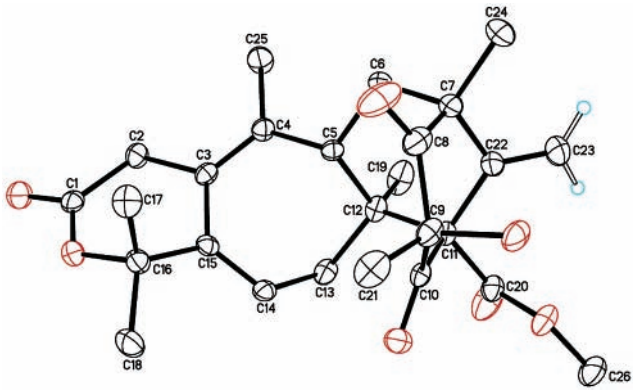

Figure 1. ORTEP drawing of **1**.

With the structure in hand, we could make the spectral assignments which were largely straightforward based on carbon chemical shifts, HSQC correlations, and proton chemical shifts. The two quaternary carbons at δ 71.2 and

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(7) The collection and isolation of Berkeley Pit fungi has previously been described: Stierle, A. A.; Stierle, D. B.; Goldstein, E.; Parker, K.; Bugni, T.; Baarson, C.; Gress, J.; Blake, D. *J. Nat. Prod.* **2003**, *66*, 1097−1100.
(8) Berkeleydione (**1**): white crystalline solid; $[\alpha]^{20}_D = +124$ ($c = 0.013$, MeOH); UV (MeOH) λ_{max} (log ϵ) 210 (2.55) nm; IR (neat) 3364, 2981, 2925, 1737, 1708, 1373, 1219, 1120 cm^{-1}; EIMS *m/z* 456 (5), 413 (20), 231 (60), 177 (95), 119 (100), 91 (95); HRCIMS *m/z* $[M + H]^+$ 457.2222 (calcd for $C_{26}H_{33}O_7$, 457.2226).

67.0 ppm, however, were problematic. In the HMBC spectrum, the carbon resonating at δ 71.2 showed correlations to the methylene protons at C-13, the methyl protons at C-19, and the exocyclic methylene protons at C-23. The carbon resonating at δ 67.0 showed correlations to the methylene protons at C-13 and the methyl protons at C-19. If the HMBC experiment selects for two- and three-bond long-range couplings, the carbon shift of δ 71.2 must be assigned to C-11 and that of δ 67.0 to C-12. Indeed, all correlation data supported these assignments, but the chemical shifts were unusually lowfield for carbons not attached to an electronegative substituent. We explored the use of several computational techniques to calculate these ^{13}C chemical shifts.[9,10] ChemWindow^{13}C chemical shift prediction program[11] gave values for C-11 and C-12 as δ 39.7 and 39.1, respectively. Upstream Solutions TOPNMR[12] gave values of δ 69.7 and 31.5, respectively. ACD[13] calculated the resonance of C-12 as δ 47.0. Unfortunately, the predictions made in these programs do not approximate the observed carbon shifts in this molecule. Chemical shift simulation programs are usually empirically based, and chemical shift predictions for fused ring systems reflect the shielding phenomena inherent in terpenoid systems.[13]

We then explored ab initio calculational methods of chemical shift determination. Single-point energy calculations of the ORTEP structure of **1** (Hartree−Fock with STO-3G) indicated unusually positive Mulliken charges for C-12. Relative and isotropic proton-decoupled ^{13}C NMR shifts were calculated for geometry optimized structures using a restricted Hartree−Fock SCF method with a 6-31G** basis set available in Spartan'04.[14] Structures were previously geometry optimized at both the 3-21G** and 6-31G** level; however, no significant differences in the ^{13}C NMR shifts were observed for spectra calculated at these different geometries. Using this methodology, the chemical shift for C-11 was δ 64.4 and that of C-12 was δ 55.7. Although few compounds in the literature have such aberrant ^{13}C chemical shift data, paraherquonin has similar chemical shift anomalies.[15] We are continuing to explore carbon tensors derived from higher order ab initio calculations to explain ^{13}C chemical shifts in both of these systems.[16]

Berkeleytrione[17] (**2**, 3.4 mg/L) was isolated as an amorphorus solid. High-resolution EIMS established the molecular formula of $C_{26}H_{34}O_7$ with 10 units of unsaturation. The ^{13}C NMR spectrum suggested three ketones, one ester, and two carbon−carbon double bonds (Table 1). Comparison to the ^{1}H and ^{13}C NMR spectra of **1** suggested the C and D rings of **2** were identical. Consideration of the COSY and HMBC spectra (Figure 2) indicated a 6−6, A and B ring system

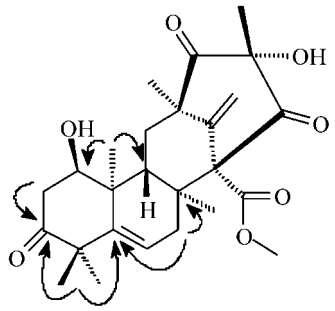

Figure 2. HMBC (H → C) correlations in berkeleytrione (**2**).

typical of many bicylic sesquiterpenes.[18] The relative stereochemistry of **2** could be established by NOESY and NOE difference spectroscopy. These experiments were run in C_6D_6 which gave much better signal dispersion than $CDCl_3$. The relative stereochemistry, and presumably the absolute stereochemistry of the C and D rings, was identical to that of **1**. Key NOE difference interactions for the A and B rings are given in Figure 3 and indicate the relative stereochemistry shown.

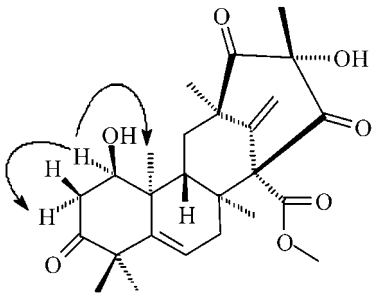

Figure 3. Key NOE interactions in berkeleytrione (**2**).

Several hybrid sesquiterpene−dimethyl orsellinate metabolites are known from *Aspergillus* sp.[19−22] All of these

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(17) Berkeleytrione (**2**): white solid; $[\alpha]^{20}_D = -18.5$ (c = 0.0034, MeOH); IR ($CHCl_3$) 3544, 2980, 2932, 1754, 1738, 1709, 1456, 1379, 1130, 1031, 909 cm^{-1}; EIMS m/z 458 (0.5), 440 (1), 416 (3), 370 (3), 209 (40), 160 (100), 119 (25); HREIMS m/z 458.2294 (calcd for $C_{26}H_{34}O_7$, 458.2304).
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are highly oxygenated and have undergone rearrangements. Biosynthetic studies have demonstrated that the precursor of the terpenoid portion is farnesyl pyrophosphate and of the nonterpenoid portion is a bis-C-methylated polyketide.[23] Our studies on the berkeleyones indicate similar biosynthetic origins with the addition of a methyl 4,6-dimethylorsellinate to form the C6−C7 and C11−C12 bonds of both **1** and **2**.

Berkeleydione (**1**) and berkeleytrione (**2**) effectively inhibited both MMP-3 and caspase-1 in the micromolar range.[24] Berkeleydione (**1**) was tested in NCI's antitumor screen against 60 human cell lines. It showed selective activity toward nonsmall cell lung cancer NCI-H460 with a $\log_{10}$ GI_{50} of −6.40.

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Supporting Information Available: One- and two-dimensional NMR spectra for **1** and **2** and crystallographic data. This material is available free of charge via the Internet at http://pubs.acs.org.

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