

## The Berkeleyacetals, Three Meroterpenes from a Deep Water Acid Mine Waste *Penicillium*

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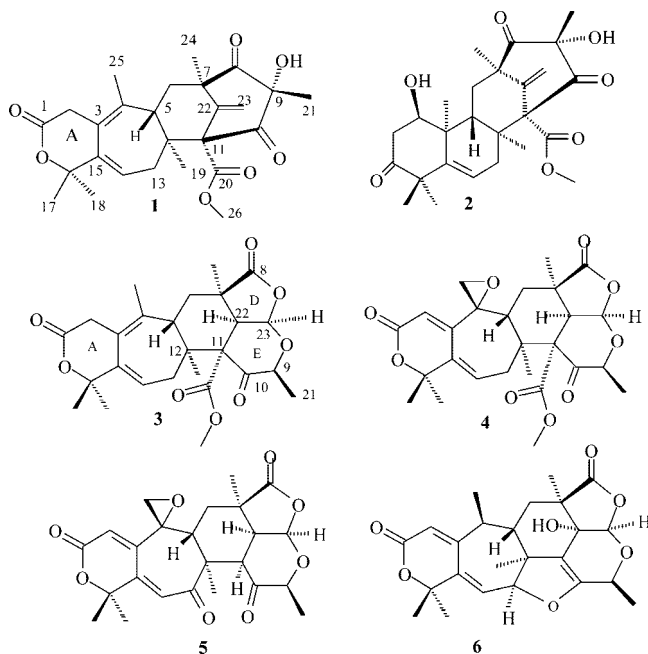
Berkeley Pit Lake is a 1500 ft deep abandoned open-pit copper mine filled with over 1140 billion liters of acidic, metal-sulfate-contaminated water. This harsh environment is proving to be a source of unusual microorganisms that produce novel bioactive compounds. We recently reported the structures of berkeleydione (**1**) and berkeleytrione (**2**), two novel hybrid polyketide-terpenoid metabolites isolated from a deep water *Penicillium* sp. growing in Berkeley Pit Lake. In this paper we report the structures of three new compounds, berkeleyacetals A–C (**3–5**) isolated from extracts of this fungus. The structures of these compounds were deduced by comparison of mass spectral and NMR data to that of berkeleydione (**1**).

The Berkeley Pit Lake system is part of the largest EPA Superfund site in North America. Berkeley Pit Lake contains roughly 1140 billion liters of acidic (pH 2.5), metal-sulfate-rich water that sits poised at the head of the Columbia River ecosystem.<sup>1</sup> We are studying the secondary metabolism of the culturable microbes isolated from Pit water and sediment samples. Several interesting compounds have been isolated from these microbes on the basis of their activities in different bioassay-guided fractionation schemes.<sup>2–6</sup>

One of the first microbes to be studied from this environment was isolated from a depth of 885 ft and was subsequently identified as *Penicillium rubrum* Stoll on the basis of rRNA sequencing alignment data (300 base pairs).<sup>3</sup> The fungus was grown in acidified potato dextrose broth (pH 2.7, still) for 21 days. At harvest time the fungus was killed by the addition of MeOH, the mycelia were removed by gravity filtration, and the filtrate was extracted with CHCl<sub>3</sub>. The CHCl<sub>3</sub> extract inhibited both MMP-3 and caspase-1 in assay systems, so it was fractionated by LH-20 column chromatography followed by HPLC to yield berkeleydione (**1**) and berkeleytrione (**2**).<sup>3</sup> The <sup>1</sup>H NMR spectra from all of the column fractions were examined for evidence of related analogues. Promising candidates were purified and tested for activity. The three new berkeleyacetals **3–5** were isolated on the basis of this “NMR-guided” fractionation as well as their activity in both enzyme bioassays. We report here the isolation and characterization of berkeleyacetals A–C (**3–5**). The structures were established on the basis of extensive spectroscopic data and a single-crystal X-ray structure for **1**.

We are exploring the possibility of isolating compounds with anticancer potential using *in vitro* signal transduction bioassay systems. Two enzyme inhibition schemes have shown promising correlation to the National Cancer Institutes Developmental Therapeutics Program human cancer cell line assay. To date, compounds isolated in our laboratory on the basis of these assays have shown activity against non-small-cell lung cancer<sup>3</sup> and ovarian cancer.<sup>4</sup> Matrix metalloproteinases (MMP) hydrolyze the extracellular matrix of connective tissues and basement membranes.<sup>7,8</sup> MMP-3 is up-regulated in many tumors<sup>9,10</sup> and can induce epithelial-mesenchymal transition (EMT) and malignant transformation in cultured cells.<sup>11,12</sup> Specific MMP inhibitors represent a new therapeutic approach to the treatment of cancers that act by blocking the activity of these proteolytic enzymes used by tumor cells to promote metastatic spread.<sup>13,14</sup>

Caspase-1 is a cysteine protease responsible for converting interleukin-1 $\beta$  to its mature form in monocytes.<sup>15</sup> Caspase-1



inhibitors have shown promise in delaying the onset of diseases that exhibit certain autoimmune phenomena including Huntington’s disease<sup>16</sup> and amyotrophic lateral sclerosis<sup>17</sup> and in mitigating the effects of stroke<sup>18</sup> and multiple sclerosis.<sup>19,20</sup> It has also been implicated in the physiological production of interferon-gamma-inducing factor (IGIF). It therefore appears to play a critical role in the regulation of multiple proinflammatory cytokines. Specific caspase-1 inhibitors might provide a new class of anti-inflammatory drug with multipotent action.<sup>21</sup> These enzyme assays were used to guide isolation of the compounds described in this paper.

Examination of mass spectra and <sup>1</sup>H NMR, <sup>13</sup>C NMR, <sup>1</sup>H–<sup>1</sup>H COSY, HSQC, and HMBC spectra (Table 1) provided the necessary information to determine the structures and the relative configurations of **3–5**. HRESIMS established the molecular formula of compound **3** as C<sub>26</sub>H<sub>32</sub>O<sub>8</sub> with 11 degrees of unsaturation. There were 11 quaternary carbons, five methines, three methylenes, and seven methyl carbons. Preliminary inspection of NMR spectra of **3** suggested the following structural units: a saturated ketone (C-10), a methyl ester (C-20 and C-26), two lactones (C-1 and C-8), and both trisubstituted and tetrasubstituted olefins. As the two double bonds and four carbonyls required 6 degrees of unsaturation, **3** was determined to be a pentacyclic compound.

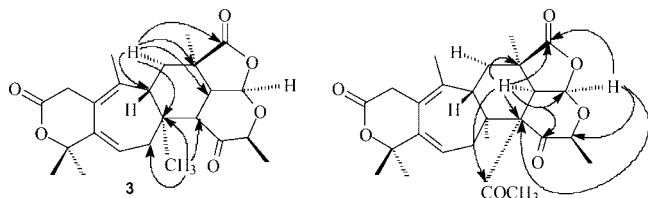
NMR data indicated that **3** possessed a saturated  $\delta$ -lactone A ring similar to that of **1**. Both axial methyl H<sub>3</sub>-17 ( $\delta$  1.45 ppm)

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**Table 1.**  $^1\text{H}$  and  $^{13}\text{C}$  NMR Data for Berkeleyacetals A–C, **3–5** ( $\text{CDCl}_3$ )<sup>a</sup>

no.	berkeleyacetal A, <b>3</b>					berkeleyacetal B, <b>4</b>					berkeleyacetal C, <b>5</b>				
	$\delta_{\text{C}}$	$\delta_{\text{H}}$				$\delta_{\text{C}}$	$\delta_{\text{H}}$				$\delta_{\text{C}}$	$\delta_{\text{H}}$			
1	170.6	C				163.7	C				162.5	C			
2	35.1	$\text{CH}_2$	3.37	bs	2H	115.9	CH	6.05	bs		117.6	CH	6.06	d	$J = 1.5$
3	127.5	C				150.9	C				152.2	C			
4	135.1	C				59.5	C				59.2	C			
5	38.6	CH	2.51	dd	$J = 13.7, 4.6$	45.6	CH	1.75	dd	$J = 13.8, 3.2$	32.2	CH	2.97	dd	$J = 12.9, 4.9$
6	30.2	$\text{CH}_2$	$\beta$ 2.11 $\alpha$ 1.67	dd m	$J = 14.4, 4.6$	28.6	$\text{CH}_2$	$\beta$ 2.42 $\alpha$ 1.45	dd m	$J = 13.8, 3.2$	27.5	$\text{CH}_2$	$\beta$ 1.97 $\alpha$ 1.46	dd dd	$J = 14.6, 4.9$ $J = 14.6, 12.9$
7	42.7	C				44.1	C				43.2	C			
8	176.3	C				176.4	C				176.0	C			
9	73.6	CH	4.32	q	$J = 7.1$	73.1	CH	4.27	q	$J = 7.0$	74.1	CH	4.22	q	$J = 7.2$
10	206.3	C				208.5	C				211.6	C			
11	63.4	C				63.0	C				51.5	CH	3.61	d	$J = 9.9$
12	56.8	C				40.8	C				47.9	C			
13	36.0	$\text{CH}_2$	$\beta$ 2.42 $\alpha$ 1.57	dd m	$J = 14.1, 8.3$	34.8	$\text{CH}_2$	$\beta$ 3.66 $\alpha$ 2.51	d dd	$J = 18.2$ $J = 18.2, 8.5$	204.8	C			
14	129.6	CH	6.25	dd	$J = 8.3, 5.0$	130.2	CH	5.97	d	$J = 8.5$	130.0	CH	6.29	d	$J = 1.5$
15	135.5	C				137.8	C				141.6	C			
16	82.9	C				83.2	C				82.8	C			
17	29.1	$\text{CH}_3$	1.45	s	3H	28.6	$\text{CH}_3$	1.40	s	3H	26.6	$\text{CH}_3$	1.55	s	3H
18	26.3	$\text{CH}_3$	1.67	s	3H	26.9	$\text{CH}_3$	1.59	s	3H	26.2	$\text{CH}_3$	1.61	s	3H
19	20.9	$\text{CH}_3$	0.77	s	3H	17.5	$\text{CH}_3$	0.75	s	3H	17.4	$\text{CH}_3$	1.12	s	3H
20	168.7	C				167.8	C								
21	18.6	$\text{CH}_3$	1.34	d	3H, $J = 7.1$	18.4	$\text{CH}_3$	1.34	d	3H, $J = 7.0$	17.1	$\text{CH}_3$	1.26	d	3H, $J = 7.2$
22	49.3	CH	3.86	d	$J = 6.0$	50.3	CH	3.99	d	$J = 6.5$	45.8	CH	3.10	dd	$J = 9.9, 5.1$
23	98.5	CH	6.10	d	$J = 6.0$	97.7	CH	6.08	d	$J = 6.5$	98.7	CH	6.08	d	$J = 5.1$
24	29.1	$\text{CH}_3$	1.41	s	3H	27.5	$\text{CH}_3$	1.41	s	3H	26.8	$\text{CH}_3$	1.29	s	3H
25	16.3	$\text{CH}_3$	1.76	s	3H	56.8	$\text{CH}_2$	$\beta$ 2.92 $\alpha$ 2.58	d s	$J = 5.4$ $J = 5.4$	55.0	$\text{CH}_2$	$\beta$ 3.14 $\alpha$ 2.58	d d	$J = 5.0$ $J = 5.0$
26	53.0	$\text{CH}_3$	3.84	s	3H	53.3	$\text{CH}_3$	3.85	s	3H					

<sup>a</sup> All assignments are based on COSY, NOE, HSQC, and HMBC experiments.

**Figure 1.** Key HMBC correlations for berkeleyacetal A, **3**.

and equatorial  $\text{H}_3$ -18 ( $\delta$  1.67) showed 2-bond connectivity to oxygen-bearing quaternary C-16 ( $\delta$  82.9) and 3-bond connectivity to olefinic C-15 ( $\delta$  135.5). Olefinic H-14 ( $\delta$  6.25 ppm) also showed 3-bond connectivity to both C-16 and olefinic C-3 ( $\delta$  127.5) and 2-bond connectivity to methylene C-13 ( $\delta$  36.0).  $\text{H}_2$ -13 showed 3-bond correlations to C-11, C-5, and C-15. Methyl  $\text{H}_3$ -25 showed connectivity to C-3 (3-bond) and to C-4 (2-bond). These data generated A and B rings consistent with that of berkeleydione (**1**) and initiated the connection of the B ring to the C ring (Figure 1).

Equatorial  $\text{H}-6\beta$  ( $\delta$  2.11) showed 3-bond correlations to C-12 ( $\delta$  56.8), C-22 ( $\delta$  49.3), and the  $\gamma$ -lactone carbonyl C-8 ( $\delta$  176.3), while both H-6 methylene protons were 2-bond coupled to C-5 ( $\delta$  38.6) and C-7 ( $\delta$  42.7).  $\text{H}_3$ -19 ( $\delta$  0.75) also exhibited correlations to carbons in both the B and C rings with 3-bond coupling to C-13 ( $\delta$  36.0) and C-11 ( $\delta$  63.4) and 2-bond coupling to C-12. H-22 ( $\delta$  3.86) provided a plethora of correlation information. It showed 3-bond coupling to both ester carbonyls C-8 ( $\delta$  176.3) and C-20 ( $\delta$  168.7), ketone C-10 ( $\delta$  206.3), and methyl C-24 ( $\delta$  29.1). H-22 also showed 2-bond correlations to quaternary C-7 ( $\delta$  42.7) and C-11 ( $\delta$  63.4), as well as to methine C-23 ( $\delta$  98.5). These correlation data for both H-6 and H-22 were instrumental in generating the C ring and its three exocyclic carbonyl carbons. We were surprised to find that despite differences in certain chemical shifts, the A–C rings of **1** and **3** were identical.

It only remained to assign three carbons and to establish the last two rings of the pentacycle. The chemical shift of C-23 ( $\delta$  98.5) was appropriate for an acetal, while the downfield shift of H-23 ( $\delta$

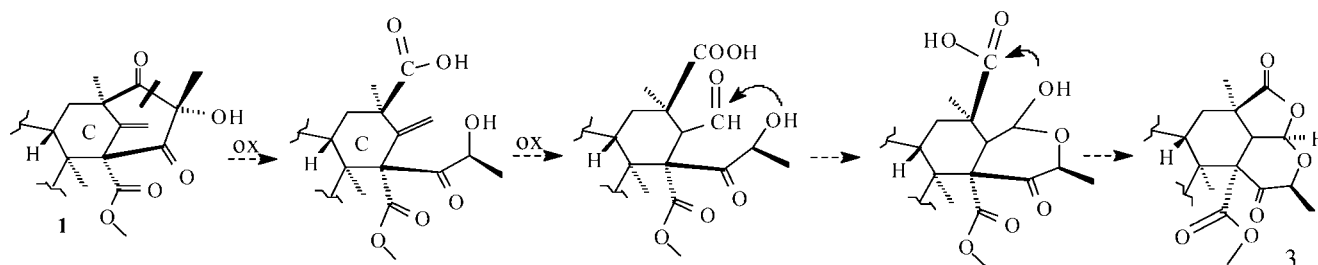
6.10) suggested that the acetal was esterified. This assumption was confirmed by the 3-bond correlation of H-23 to lactone C-8 and the as yet unassigned oxygen-bearing methine C-9 ( $\delta$  73.6). H-23 showed similar connectivity to quaternary C-11, which helped in establishing the D and E rings.  $\text{H}_3$ -21 showed 3-bond coupling to ketone C-10 and 2-bond coupling to C-9, completing the D and E rings and establishing berkeleyacetal A (**3**) as shown.

All of these assignments were supported by examination of other NMR data.  $^1\text{H}$ – $^1\text{H}$  COSY showed scalar coupling ( $^3J$ ) between acetal H-23 and H-22, between methyl  $\text{H}_3$ -21 and oxygen-bearing methine H-9, and between H-5 and the mutually geminal ( $^2J$ ) coupled  $\text{H}_2$ -6. Olefinic H-14 and methylene H-13 were coupled, and although both methylene  $\text{H}_2$ -2 and methyl  $\text{H}_3$ -25 appeared as broad singlets in the  $^1\text{H}$  NMR spectrum, the COSY showed homoallylic ( $^5J$ ) coupling between these protons.

The relative stereochemistry of **3** for all positions except C-11 was established by NOE difference spectroscopy. Irradiation of H-23 resulted in enhanced signals for both H-9 and H-22. Mutual enhancement of  $\text{H}_3$ -24 and H-22, of  $\text{H}_3$ -19 and H-22, and of  $\text{H}_3$ -19 and H-26 indicated their *cis* relationships as well. Both H-19 and H-24 were also enhanced by irradiation of H-6 $\alpha$ .

Much of the data for **4** indicated a structure related to that of **3**. HREIMS established the molecular formula of **4** as  $\text{C}_{26}\text{H}_{30}\text{O}_9$  with 12 degrees of unsaturation, one more than in compound **3**. There were 11 quaternary carbons, six methines, three methylenes, and seven methyl carbons. Preliminary inspection of the  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra of **4** suggested the presence of the following structural units: a saturated ketone (C-10), a methyl ester (C-20), two lactones (C-1 and C-8), and two trisubstituted olefins. As the two double bonds and four carbonyls required 6 degrees of unsaturation, **4** was determined to be a hexacyclic compound.

Much of the NMR data associated with the D and E rings of **3** were present in the NMR spectra of **4**. As in **3**, H-22 provided much correlation data with 3-bond couplings to ester carbons C-8 ( $\delta$  176.4) and C-20 ( $\delta$  167.8), ketone carbon C-10 ( $\delta$  208.5), and methyl C-24 ( $\delta$  27.5). It also showed 2-bond correlations to



**Figure 2.** Proposed correlation of carbon skeletons of berkeleydione and berkeleyacetals.

quaternary carbons C-7 ( $\delta$  44.1) and C-11 ( $\delta$  63.0). H<sub>3</sub>-24 showed 3-bond connectivities to C-8, C-6 ( $\delta$  28.6), and C-22. Acetal H-23 showed 3-bond coupling to C-9 ( $\delta$  73.1) and C-11 ( $\delta$  63.0) and 2-bond coupling to C-22. These data provided ample evidence for the D and E rings.

The <sup>13</sup>C NMR experiment indicated that the A ring was an  $\alpha,\beta$ -unsaturated  $\delta$ -lactone. This could be deduced from the upfield shift of C-1 ( $\delta$  163.7) and the typical skewing of the olefinic carbons resulting from conjugation to a carbonyl ( $\delta$  115.9 and 150.9). In the HMBC spectrum olefinic proton H-2 ( $\delta$  6.05) showed 2-bond coupling to ester carbon C-1 ( $\delta$  163.7) and 3-bond coupling to olefinic C-15 ( $\delta$  137.8) and quaternary C-4 ( $\delta$  59.5). Both methyl H<sub>3</sub>-17 ( $\delta$  1.40) and H<sub>3</sub>-18 ( $\delta$  1.59) showed HMBC correlations to C-15 ( $\delta$  137.8) and C-16 ( $\delta$  83.2), connecting the two methyl groups to methine C-16 and providing connection of the A ring to the B ring through olefin 14–15. Methylene H-13 ( $\delta$  2.51) showed HMBC correlations to both olefinic carbons C-14 and C-15 ( $\delta$  130.2 and 137.8, respectively). <sup>1</sup>H–<sup>1</sup>H COSY also showed <sup>3</sup>J scalar coupling between olefinic H-14 ( $\delta$  5.97) and methylene H-13.

Methyl H<sub>3</sub>-19 ( $\delta$  0.75) showed 3-bond correlations to methylene C-13 ( $\delta$  34.8) and methine C-5 ( $\delta$  45.6), while the correlation between H-5 and C-3 generated the seven-membered B ring typical of this family of compounds. Methyl H-19 also showed HMBC correlation to C-11, which helped to establish the path from the B–C–E ring connectivity. Both H-19 and methylene H<sub>2</sub>-6 showed HMBC correlations to C-12 ( $\delta$  40.8), which established the connection between the B and C rings.

It remained to assign methylene C-25 ( $\delta$  56.8), quaternary C-4 ( $\delta$  59.5), and a single oxygen to accommodate the last degree of unsaturation. The <sup>13</sup>C chemical shifts of C-25 and C-4 were typical of an epoxide moiety. The constraints of requisite methylene and quaternary carbons indicated a spiro-oxirane functionality. In support of this assignment both H<sub>2</sub>-25 exhibited HMBC correlations to olefinic C-3 and C-5, nicely tying the oxirane ring into the seven-membered B ring and completing the structure of berkeleyacetal B (**4**) as shown.

NOE difference data again established the relative stereochemistry of **4**. H-22 was enhanced by the irradiation of both H<sub>3</sub>-19 and H<sub>3</sub>-24, while H<sub>3</sub>-24 also showed NOE effects on H-23, confirming the *cis* relationships of these protons. On the other face of the molecule, H-5 showed NOE correlations to spiro-oxirane methylene H<sub>B</sub>-25, H<sub>B</sub>-6, and H<sub>B</sub>-13, while irradiation of H<sub>3</sub>-17 enhanced both H<sub>A</sub>-25 and olefinic H-2. These data established the relative stereochemistry of **4**, which was consistent with the other berkeleyacetals.

HREIMS established the molecular formula of **5** as C<sub>24</sub>H<sub>26</sub>O<sub>8</sub>, with 12 degrees of unsaturation as in compound **4**. There were 10 quaternary carbons, seven methines, two methylenes, and five methyl carbons. Preliminary inspection of the <sup>1</sup>H and <sup>13</sup>C NMR spectra of **5** suggested the presence of the following structural units: two ketones (C-10 and C-13), two lactones (C-1 and C-8), and two trisubstituted olefins. The carbon inventory indicated that with two double bonds and four carbonyls compound **5** was hexacyclic.

The spectroscopic data of **5** differed from those of **4** in two main respects: the loss of the methyl ester functionality and the addition

of a ketone functionality. The lack of the methyl ester was immediately apparent by the absence of both carbon signals associated with that functionality. The loss of these two carbons did not affect the skeletal integrity of the berkeleyacetals. Quaternary C-11 was replaced by a methine, consistent with these data. The additional ketone functionality was tentatively assigned to C-13, indicated both by the absence of the methylene peaks from the NMR data and the downfield shift of olefinic H-14, typical of ketone influence. It remained to support these assignments through correlation data.

All of the key HMBC correlations could be found for this compound. Olefinic H-2 ( $\delta$  6.06) showed connectivity to C-1 ( $\delta$  162.5), C-3 ( $\delta$  152.2), C-4 ( $\delta$  59.2), and C-15 (141.6). The spiro-oxirane methylene protons showed correlations to C-3, C-4, and C-5 ( $\delta$  32.2), tying that functionality into the northern portion of the B ring. H-5 ( $\delta$  2.97) showed correlations to the new ketone carbon C-13 ( $\delta$  204.8), as well as to C-4, C-6 ( $\delta$  27.5), C-12 ( $\delta$  47.9), and C-24 ( $\delta$  26.8). Both H<sub>2</sub>-6 showed correlations to C-4, C-5, C-7 ( $\delta$  43.2), and C-8 ( $\delta$  176.0), while H<sub>B</sub>-6 showed an additional correlation to C-12. Methyl H<sub>3</sub>-24 exhibited correlations to C-6, C-8, and C-22 ( $\delta$  45.8). All of these HMBC data supported the smooth transition from the A–B–C–D rings.

Further support was provided by strong 3-bond correlations of H-22 to C-7, C-8, and methyl C-24 ( $\delta$  26.8) and 2-bond correlations to C-11 ( $\delta$  51.5) and ketone C-10 ( $\delta$  211.6). Acetal H-23 exhibited 3-bond connectivities to C-8, C-9 ( $\delta$  74.1), and C-11 and a 2-bond correlation to C-22. In the E ring, H-9 showed correlations to adjacent ketone C-10 and methyl C-21. The correlations exhibited by H-11 ( $\delta$  3.61) provided ample evidence of bridging of rings C–E. It showed 3-bond correlations to C-5, C-9, and C-7 and 2-bond connections to C-12 and C-22. Methyl H<sub>3</sub>-19 ( $\delta$  1.12) showed 3-bond connectivities to C-5, C-11, and ketone C-13 and a 2-bond correlation to C-12. Olefinic H-14 ( $\delta$  6.29) provided connections to the A–B–C rings with correlations to C-3, C-12, C-16 ( $\delta$  82.8), C-15, and C-13. Geminal dimethyls H<sub>3</sub>-17 and H<sub>3</sub>-18 completed the ring system with correlations to C-16 and C-15.

Relative stereochemistry could be deduced by NOE difference data. *Cis* relationships could be established for H-22, H-23, H-11, H-9, H<sub>3</sub>-19, and H-24, consistent with the other berkeleyacetals. The berkeleyacetals are clearly related to berkeleydione, so we proposed a correlation scheme (not a biosynthetic pathway) of the berkeleydione and berkeleyacetal skeletons to explain the numbering scheme that was used (Figure 2).

*Penicillium* sp. have been extensively studied as a source of bioactive metabolites. We could find only two other reports of the 6–7 A–B ring system found in berkeleydione and the berkeleyacetals: paraherquonin **6**<sup>22</sup> and citreonigrin A,<sup>23</sup> which was reported in a conference abstract. Paraherquonin also possesses a similar acetal moiety, as do additional citreonigrins, which have been reported in a doctoral thesis.<sup>24</sup> Several classes of meroterpenes have been isolated from both *Penicillium* and *Aspergillus* sp. since 1976.<sup>25–29</sup> Several studies have concluded that all of these compounds were derived by alkylation of polyketide intermediate 3,5-dimethylorsellinate by the terpenoid precursor farnesyl pyrophosphate.<sup>29</sup>

Berkeleyacetal C (**5**), berkeleydione (**1**), and berkeleytrione (**2**) inhibited both MMP-3 and caspase-1 in the micromolar range, while berkeleyacetals A and B inhibited these enzymes in the millimolar range. Only berkeleyacetal C was accepted in the NCI Developmental Therapeutics Program for human cell line screening and tested in the single-dose-response assay. It inhibited the growth of non-small-cell lung cancer NCI H460, the same cell line that was targeted by berkeleydione. We are currently awaiting the results of the five-dose-response assay.

## Experimental Section

**General Experimental Procedures.** Optical rotations were recorded on a Perkin-Elmer 241 MC polarimeter using a 1.0 mL cell. UV spectra were recorded on a Hewlett-Packard 8453 spectrophotometer.  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra were run on a Bruker DPX-300 spectrometer.  $^1\text{H}$  NMR spectra were recorded at 300 MHz, and the  $^{13}\text{C}$  NMR spectra were recorded at 75 MHz unless otherwise noted. All of the chemical shifts were recorded with respect to the deuterated solvent shift ( $\text{CDCl}_3$ ,  $\delta$  7.24 for the proton resonance and  $\delta$  77.0 for the carbon). Mass spectra were provided by the University of Montana mass spectrometer facility in Missoula, MT. 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.<sup>3</sup> The fungus was identified as *Penicillium rubrum* by Microbial Identification, Inc. The fungus was grown in  $3 \times 300$  mL of DIFCO potato dextrose broth (acidified to pH 2.7 with sulfuric acid) in 1 L Erlenmeyer flasks (shaken 180 rpm for 4 days, then still for 20 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  $\text{CHCl}_3$ , and the extract was reduced *in vacuo* to an oil (0.3591 g). This  $\text{CHCl}_3$  extract demonstrated inhibition of caspase-1 and MMP-3, antimicrobial activity against *Staphylococcus aureus* and *Escherichia coli*, and brine shrimp lethality.

The  $\text{CHCl}_3$  extract was fractionated on a flash Si gel column using hexanes/isopropyl alcohol mixtures to isopropyl alcohol/MeOH mixtures. The large flash fractions were further fractionated by preparative HPLC on a Rainin 21 mm preparative Si gel column with a hexanes/isopropyl alcohol gradient. The fraction eluting with 50% hexanes/isopropyl alcohol was further resolved on a Rainin semi-preparative Si gel HPLC column with 20% isopropyl alcohol/hexanes to give pure berkeleyacetal C **5** (10.2 mg).

The fungus was also grown in  $20 \times 200$  mL cultures in 500 mL Roux bottles as still cultures for 21 days and extracted with  $\text{CHCl}_3$  as described above (0.460 g). The  $\text{CHCl}_3$  extract was also fractionated on a flash Si gel column using hexanes, hexanes/isopropyl alcohol mixtures to isopropyl alcohol/MeOH mixtures. The first flash fraction was further fractionated by preparative HPLC on a Rainin 21 mm preparative Si gel column with a hexanes/isopropyl alcohol gradient to give two fractions that eluted cleanly with 20% isopropyl alcohol/hexanes and yielded berkeleyacetals A (**3**) (3.0 mg) and B (**4**) (1.9 mg). Compounds **3** and **4** were not found in the cultures that were shaken first then grown still, only in the Roux bottle cultures.

**Berkeleyacetal A (3):**  $[\alpha]_D^{20} +109$  (c 0.0015, MeOH); UV ( $\text{CHCl}_3$ )  $\lambda_{\text{max}}$  (log  $\epsilon$ ) 227 (2.94); IR ( $\text{CHCl}_3$ )  $\lambda_{\text{max}}$  2986, 1787, 1749, 1720, 1354, 1120, 946  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ), see Table 1; APCIMS (+ ions)  $m/z$  473; APCIMS (– ions)  $m/z$  471; HRESIMS  $m/z$  473.2158,  $[\text{M} + \text{H}]^+$  (calcd for  $\text{C}_{26}\text{H}_{33}\text{O}_8$ , 473.2175).

**Berkeleyacetal B (4):**  $[\alpha]_D^{20} +59$  (c 0.0014, MeOH); UV ( $\text{CHCl}_3$ )  $\lambda_{\text{max}}$  (log  $\epsilon$ ) 275 (3.20), 224 (3.16); IR ( $\text{CHCl}_3$ )  $\lambda_{\text{max}}$  2956, 1790, 1715, 1698, 1455, 1154, 979  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ), see Table 1; APCIMS (+ ions)  $m/z$  487; APCIMS (– ions)  $m/z$  485; HRESIMS  $m/z$  487.1977  $[\text{M} + \text{H}]^+$  (calcd for  $\text{C}_{26}\text{H}_{31}\text{O}_9$ , 486.1968).

**Berkeleyacetal C (5):**  $[\alpha]_D^{20} +80.7$  (c 0.0115, MeOH); UV ( $\text{CHCl}_3$ )  $\lambda_{\text{max}}$  (log  $\epsilon$ ) 269 (3.87), 203 (3.89); IR (neat)  $\lambda_{\text{max}}$  3368, 2954, 2922, 1780, 1714, 1652, 1291, 1160, 1120, 980  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ) see Table 1; EIMS  $m/z$  442 (8), 427 (100), 398 (70), 91 (74), 55(65); HREIMS  $m/z$  442.1661  $[\text{M}]^+$  (calcd for  $\text{C}_{24}\text{H}_{26}\text{O}_8$ , 442.1627).

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**Supporting Information Available:** Experimental details including  $^1\text{H}$  NMR,  $^{13}\text{C}$  NMR, COSY, HMBC, and HSQC spectra for berkeleyacetals **3–5**. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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