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

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Abstract

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, 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.[1] 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 based on their activities in different bioassay guided fractionation schemes.[2-6]

One of the first microbes to be studied from this environment was isolated from a depth of 885 feet and was subsequently identified as *Penicillium rubrum* Stoll based on rRNA sequencing alignment data (300 base pairs).[3] 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 removed by gravity filtration and the filtrate extracted with $CHCl_3$. The $CHCl_3$ 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**).[3] The ^1H NMR spectra from all of the column fractions were examined for evidence of related analogs. Promising candidates were purified and tested for activity. The three new berkeleyacetals **3-5** were isolated based on 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 based upon 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 lab based on these assays have shown activity against non-small cell lung cancer[3] and ovarian cancer[4]. Matrix metalloproteinases (MMP) hydrolyze the extracellular matrix of connective tissues and basement membranes.[7, 8] MMP-3 is up-regulated in many tumors[9, 10] and can induce

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epithelial–mesenchymal transition (EMT) and malignant transformation in cultured cells.[11, 12], 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. [13, 14]

Caspase-1 is a cysteine protease responsible for converting interleukin-1β to its mature form in monocytes.[15] Caspase-1 inhibitors have shown promise in delaying the onset of diseases that exhibit certain autoimmune phenomena including Huntington's disease[16] and amyotropic lateral sclerosis,[17] and in mitigating the effects of stroke[18] and multiple sclerosis.[19, 20] 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.[21] These enzyme assays were used to guide isolation of the compounds described in this paper.

Examination of mass spectra, ^{1}H NMR, ^{13}C NMR, ^{1}H-^{1}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 $C_{26}H_{32}O_8$ with 11 degrees of unsaturation. There were 11 quaternary carbons, 5 methines, 3 methylenes and 7 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 tri-substituted and tetra-substituted 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 δ-lactone A ring similar to that of **1**. Both axial methyl H_3-17 (δ 1.45 ppm) and equatorial H_3-18 (δ 1.67) showed 2-bond connectivity to oxygen-bearing quaternary C-16 (δ 82.9) and 3-bond connectivity to olefinic C-15 (δ 135.5). Olefinic H-14 (δ 6.25 ppm) also showed 3-bond connectivity to both C-16 and olefinic C-3 (δ 127.5) and 2-bond connectivity to methylene C-13 (δ 36.0). H_2-13 showed 3-bond correlations to C-11, C-5 and C-15. Methyl 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 H-6β (δ 2.11) showed 3-bond correlations to C-12 (δ 56.8), C-22 (δ 49.3) and the γ-lactone carbonyl C-8 (δ 176.3), while both H-6 methylene protons were 2-bond coupled to C-5 (δ 38.6) and C-7 (δ42.7). H_3-19 (δ 0.75) also exhibited correlations to carbons in both the B and C rings with 3-bond coupling to C-13 (δ 36.0) and C-11 (δ 63.4) and 2-bond coupling to C-12. H-22 (δ 3.86) provided a plethora of correlation information. It showed 3-bond coupling to both ester carbonyls C-8 (δ 176.3) and C-20 (δ 168.7), ketone C-10 (δ 206.3) and methyl C-24 (δ 29.1). H-22 also showed 2-bond correlations to quaternary C-7 (δ 42.7) and C-11 (δ 63.4), as well as to methine C-23 (δ 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 (δ 98.5) was appropriate for an acetal, while the downfield shift of H-23 (δ 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 (δ 73.6). H-23 showed similar connectivity to quaternary C-11 which helped in establishing the D and E rings. 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}H-^{1}H COSY showed scalar coupling (^{3}J) between acetal H-23 and H-22, between methyl H_3-21 and oxygen-bearing methine H-9, and between H-5 and the mutually geminal (^{2}J) coupled H_2-6. Olefinic H-14 and methylene H-13 were coupled, and although both methylene H_2-2 and methyl H_3-25 appeared as broad singlets in the ^{1}H NMR spectrum, the COSY showed homoallylic (^{5}J) 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 H_3-24 and H-22, of H_3-19 and H-22, and of 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-6α.

Much of the data for **4** indicated a structure related to that of **3**. HREIMS established the molecular formula of **4** as $C_{26}H_{30}O_9$ with 12 degrees of unsaturation, one more than in compound **3**. There were 11 quaternary carbons, 6 methines, 3 methylenes and 7 methyl carbons. Preliminary inspection of the ^{1}H and ^{13}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 tri-substituted 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 (δ 176.4) and C-20 (δ 167.8), ketone carbon C-10 (δ 208.5), and methyl C-24 (δ 27.5). It also showed 2-bond correlations to quaternary carbons C-7 (δ 44.1) and C-11 (δ 63.0). H_3-24 showed 3-bond connectivities to C-8, C-6 (δ 28.6), and C-22. Acetal H-23 showed 3-bond coupling to C-9 (δ 73.1) and C-11 (δ 63.0) and 2-bond coupling to C-22. These data provided ample evidence for the D and E rings.

The ^{13}C NMR experiment indicated that the A ring was an αβ-unsaturated δ-lactone. This could be deduced from the upfield shift of C-1 (δ 163.7) and the typical skewing of the olefinic carbons resulting from conjugation to a carbonyl (δ 115.9 and δ 150.9). In the HMBC spectrum olefinic proton H-2 (δ 6.05) showed 2-bond coupling to ester carbon C-1 (δ 163.7), and 3-bond coupling to olefinic C-15 (δ 137.8) and quaternary C-4 (δ 59.5). Both methyl H_3-17 (δ 1.40) and H_3-18 (δ 1.59) showed HMBC correlations to C-15 (δ 137.8) and C-16 (δ 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 (δ 2.51) showed HMBC correlations to both olefinic carbons C-14 and C-15 (δ 130.2 and δ 137.8, respectively). ^{1}H-^{1}H COSY also showed ^{3}J scalar coupling between olefinic H-14 (δ 5.97) and methylene H-13.

Methyl H_3-19 (δ 0.75) showed 3-bond correlations to methylene C-13 (δ 34.8) and methine C-5 (δ 45.6), while the correlation between H-5 and C-3 generated the 7-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_2-6 showed HMBC correlations to C-12 (δ 40.8) which established the connection between the B and C rings.

It remained to assign methylene C-25 (δ 56.8), quaternary C-4 (δ 59.5) and a single oxygen to accommodate the last degree of unsaturation. The ^{13}C chemical shifts of C-25 and C-4 were typical of an epoxide moiety. The constraints of requisite methylene and quaternary carbons indicated spiro-oxirane functionality. In support of this assignment both H_2-25 exhibited HMBC correlations to olefinic C-3 and C-5 nicely tying the oxirane ring into the 7-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_3-19 and H_3-24, while H_3-24 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_B-25, H_β-6 and H_β-13, while irradiation of H_3-17 enhance both H_A-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 $C_{24}H_{26}O_8$, with 12 degrees of unsaturation as in compound **4**. There were 10 quaternary carbons, 7 methines, 2 methylenes and 5 methyl carbons. Preliminary inspection of the ^1H and ^{13}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 tri-substituted olefins. The carbon inventory indicated that with two double bonds and four carbonyls compound **5** was hexacyclic.

The spectroscopic data of **5** differed from that 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 (δ 6.06) showed connectivity to C-1 (δ 162.5), C-3 (δ 152.2), C-4 (δ 59.2), and C-15 (141.6). The spiro-oxirane methylene protons showed correlations to C-3, C-4 and C-5 (δ 32.2) tying that functionality into the northern portion of the B ring. H-5 (δ 2.97) showed correlations to the new ketone carbon C-13 (δ 204.8), as well as to C-4, C-6 (δ 27.5), C-12 (δ 47.9), and C-24 (δ 26.8). Both H_2-6 showed correlations to C-4, C-5, C-7 (δ 43.2), and C-8 (δ 176.0), while H_β-6 showed an additional correlation to C-12. Methyl H_3-24 exhibited correlations to C-6, C-8 and C-22 (δ 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, methyl C-24 (δ 26.8), and 2-bond correlations to C-11 (δ 51.5) and ketone C-10 (δ 211.6). Acetal H-23 exhibited 3-bond connectivities to C-8, C-9 (δ 74.1), 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 (δ 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_3$19 (δ 1.12) showed 3-bond connectivities to C-5, C-11, ketone C-13, and a 2-bond correlation to C-12. Olefinic H-14 (δ 6.29) provided connections to the A-B-C rings with correlations to C-3, C-12, C-16 (δ 82.8), C-15 and C-13. Geminal dimethyls H_3-17 and H_3-18 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_3-19, 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**[22] and citreonigrin A[23], 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.[24] Several classes of meroterpenenes have been isolated from both *Penicillium* and *Aspergillus* sp. since 1976.[25-29] 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. [29]

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}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). Mass spectra were provided by the University of Montana Mass Spectrometer facility in Missoula, Montana. 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] The fungus was identified as *Penicillium rubrum* by Microbial Identification, Inc. The fungus was grown in 3 × 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 $CHCl_3$, and the extract was reduced *in vacuo* to an oil (0.3591 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.

The $CHCl_3$ extract was fractionated on a flash Si gel column using hexanes, hexane/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 × 200 mL cultures in 500 mL Roux bottles as still cultures for 21 days and extracted with $CHCl_3$ as described above (0.460 g). The $CHCl_3$ 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)—$[\alpha]^{20}_{D}$ +109 (*c* 0.0015, MeOH); UV ($CHCl_3$) λ_{max} (log ε) 227 (2.94); IR ($CHCl_3$) λ_{max} 2986, 1787, 1749, 1720, 1354, 1120, 946 $cm^{-1;}$ ^1H NMR and ^{13}C NMR ($CDCl_3$) see Table 1; APCIMS (+ ions) *m/z* 473; APCIMS (- ions) *m/z* 471; HRESIMS *m/z* 473.2158, $[M+H]^+$ (calcd for $C_{26}H_{33}O_8$, 473.2175).

Berkeleyacetal B (4)—$[\alpha]^{20}_{D}$ +59° (*c* 0.0014, MeOH); UV ($CHCl_3$) λ_{max} (log ε) 275 (3.20), 224 (3.16); IR ($CHCl_3$) λ_{max} 2956, 1790, 1715, 1698, 1455, 1154, 979 $cm^{-1;}$ ^1H NMR and ^{13}C NMR ($CDCl_3$) see Table 1; APCIMS (+ ions) *m/z* 487; APCIMS (- ions) *m/z* 485; HRESIMS *m/z* 487.1977 $[M+H]^+$ (calcd for $C_{26}H_{31}O_9$, 486.1968).

Berkeleyacetal C (5)—$[\alpha]^{20}_{D}$ +80.7° (*c* 0.0115, MeOH); UV ($CHCl_3$) λ_{max} (log ε) 269 (3.87), 203 (3.89); IR (neat) λ_{max} 3368, 2954, 2922, 1780, 1714, 1652, 1291, 1160, 1120, 980 $cm^{-1;}$ ^1H NMR and ^{13}C NMR ($CDCl_3$) see Table 1; EIMS *m/z* 442 (8), 427 (100), 398 (70), 91 (74), 55(65); HREIMS *m/z* 442.1661 $[M]^+$ (calcd for $C_{24}H_{26}O_8$, 442.1627).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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The views and conclusions contained in this document are those of the authors and should not be interpreted as representing the official policies, either expressed or implied, of NIH or of USGS.

References

1. Montana Bureau of Mines and Geology. Berkeley Pit and Butte Mine-Flooding Operable Unit. [January 10, 2006]. website: http://www.mbmg.mtech.edu/env-berkeley.htm
2. Stierle AA, Stierle DB, Parker K, Goldstein E, Bugni T, Baarson C, Gress J, Blake D. J Nat Prod 2003;66:1097–1100. [PubMed: 12932132]
3. Stierle DB, Stierle AA, Hobbs JD, Stokken J, Clardy J. Org Lett 2004;6:1049–1052. [PubMed: 15012097]
4. Stierle AA, Stierle DB, Kelly K. J Org Chem 2006;71:5357–5360. [PubMed: 16808526]
5. Stierle AA, Stierle DB, Kemp K. J Nat Prod 2004;67:1392–1395. [PubMed: 15332861]
6. Stierle, AA.; Stierle, DB. Bioactive Natural Products. Atta-Ur-Rahman, editor. 32. Elsevier Science; Amsterdam: 2005.
7. Murphy G, Docherty AJP. Am J Respir Cell Mol Biol 1992;7:120–125. [PubMed: 1497900]
8. Docherty AJP, O' Connell J, Crabbe T, Angal S, Murphy G. Trends Biotechnol 1992;10:200–207. [PubMed: 1368394]
9. Sternlicht MD, Werb Z. Annu Rev Cell Dev Biol 2001;17:463–516. [PubMed: 11687497]
10. Sternlicht MD, Lochter A, Sympson CJ, Huey B, Rougier JP, Gray JW, Pinkel D, Bissell MJ. Cell 1999;98:137–146. [PubMed: 10428026]
11. Radisky DC, Levy DD, Littlepage LE, Liu Hong, Nelson CM, Fata JE, Leake D, Godden EL, Albertson DG, Nieto MA, Werb Z, Bissell MJ. Nature 2005;436:123–127. [PubMed: 16001073]
12. Bissell MJ, Radisky D. Nature Rev Cancer 2001;1:46–54. [PubMed: 11900251]
13. Brown PD. Adv Enzyme Regul 1995;35:293–301. [PubMed: 7572350]
14. Liedtke W, Cannella B, Mazzaccaro RJ, Clements JM, Miller KM, Wucherpfennig KW, Gearing AJ, Raine CS. Ann Neurol 1998;44:35–46. [PubMed: 9667591]

15. Marcus ME, Heufelder AE, Hengartner MO. Proc Natl Acad Sci USA 1997;94:12736–12737. [PubMed: 9398063]
16. Ona VO, Li M, Vonsattel JPG, Andrews LJ, Khan SQ, Chung WM, Frey AS, Menon AS, Li XJ, Stieg PE, Yuan J, Penney JB, Young AB, Cha JHJ, Friedlander RM. Nature 1999;399:263–267. [PubMed: 10353249]
17. Li M, Ona VO, Guegan C, Chen M, Jackson V, Andrews LJ, Olszewski AJ, Stieg PE, Lee J, Przedborski S, Friedlander RM. Science 2000;288:335–339. [PubMed: 10764647]
18. Rabuffetti M, Sciorati C, Tarozzo G, Clementi E, Manfredi AA, Beltramo M. J Neurosci 2000;20:4398–4404. [PubMed: 10844008]
19. Ming X, Li W, Maeda Y, Blumberg B, Raval S, Cook SD, Dowling PC. J Neurol Sci 2002;197:9–18. [PubMed: 11997061]
20. Furlan R, Martino G, Galbiati F, Poliani PL, Smiroldo S, Bergami A, Desina G, Comi G, Flavell R, Su MS, Adorini L. J Immunol 1999;163:2403–2409. [PubMed: 10452974]
21. Ghayur T, Banerjee S, Hugunin M, Butler D, Herzog L, Carter A, Quintal L, Sekut L, Talanian R, Paskind M, Wong W, Kamen R, Tracey D, Allen H. Nature 1997;386:619–23. [PubMed: 9121587]
22. Okuyama E, Yamazaki M, Kobayashi K, Sakurai T. Tet Lett 1983;24(30):3113–3114.
23. Ebel, R.; Rusman, Y.; Brauers, G.; Proksch, P.; Frank, W.; Wray, V. Planta Med; 54th Annual Congress On Medicinal Plant Research; 2006.
24. Rusman, Y. Ph D Thesis. University of Dusseldorf; Dusseldorf, Germany: 2006. Isolation of New Secondary Metabolites from Sponge-Associated and Plant-Derived Endophytic Fungi; p. 99
25. Dunn AW, Johnstone RAW, Sklarz B, King TJJ. Chem Soc, Chem Commun 1976:270–271.
26. Dunn AW, Johnstone RAW, Sklarz B, Lessinger L, King TJ. J Chem Soc, Chem Commun 1978:533–534.
27. Simpson TJ. J Chem Soc, Perkin Trans 1 1979:2118–2121.
28. Santos RMG, Rodrigues-Fo E. Phytochemistry 2002;61:907–912. [PubMed: 12453515]
29. Simpson TJ, Ahmed SA, Mclntyre CR, Scott FE, Sadler IH. Tetrahedron 1997;53(11):4013–4034.

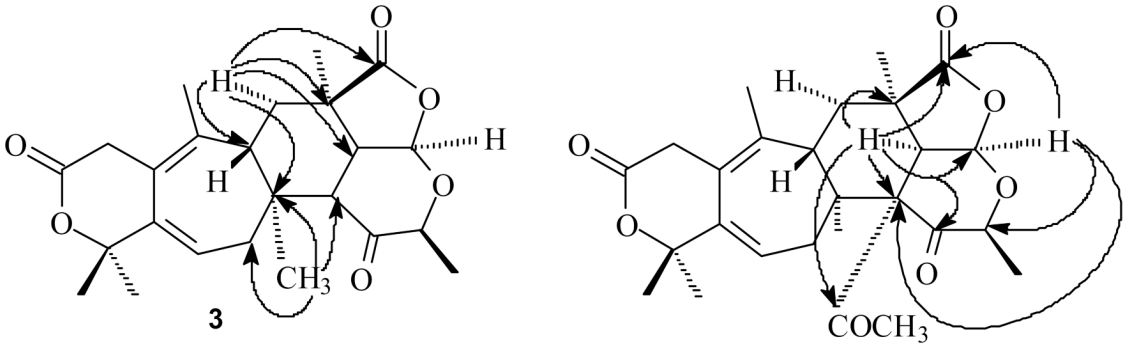

Figure 1.
Key HMBC correlations for berkeleyacetal A **3**.

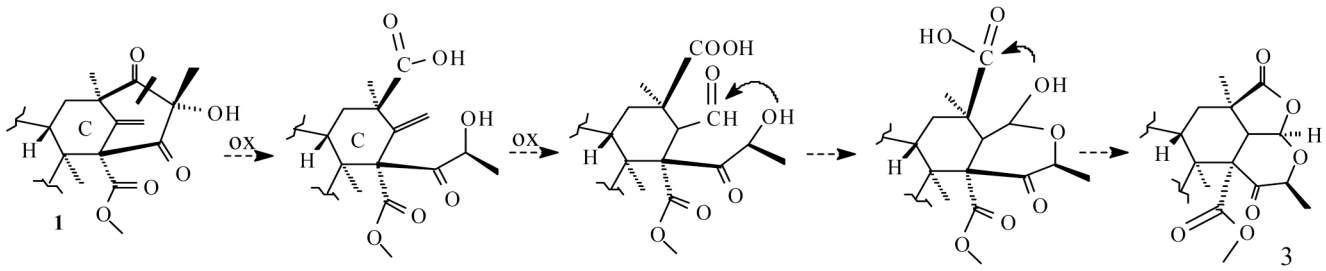

Figure 2.
Proposed correlation of carbon skeletons of berkeleydione and berkeleyacetals.

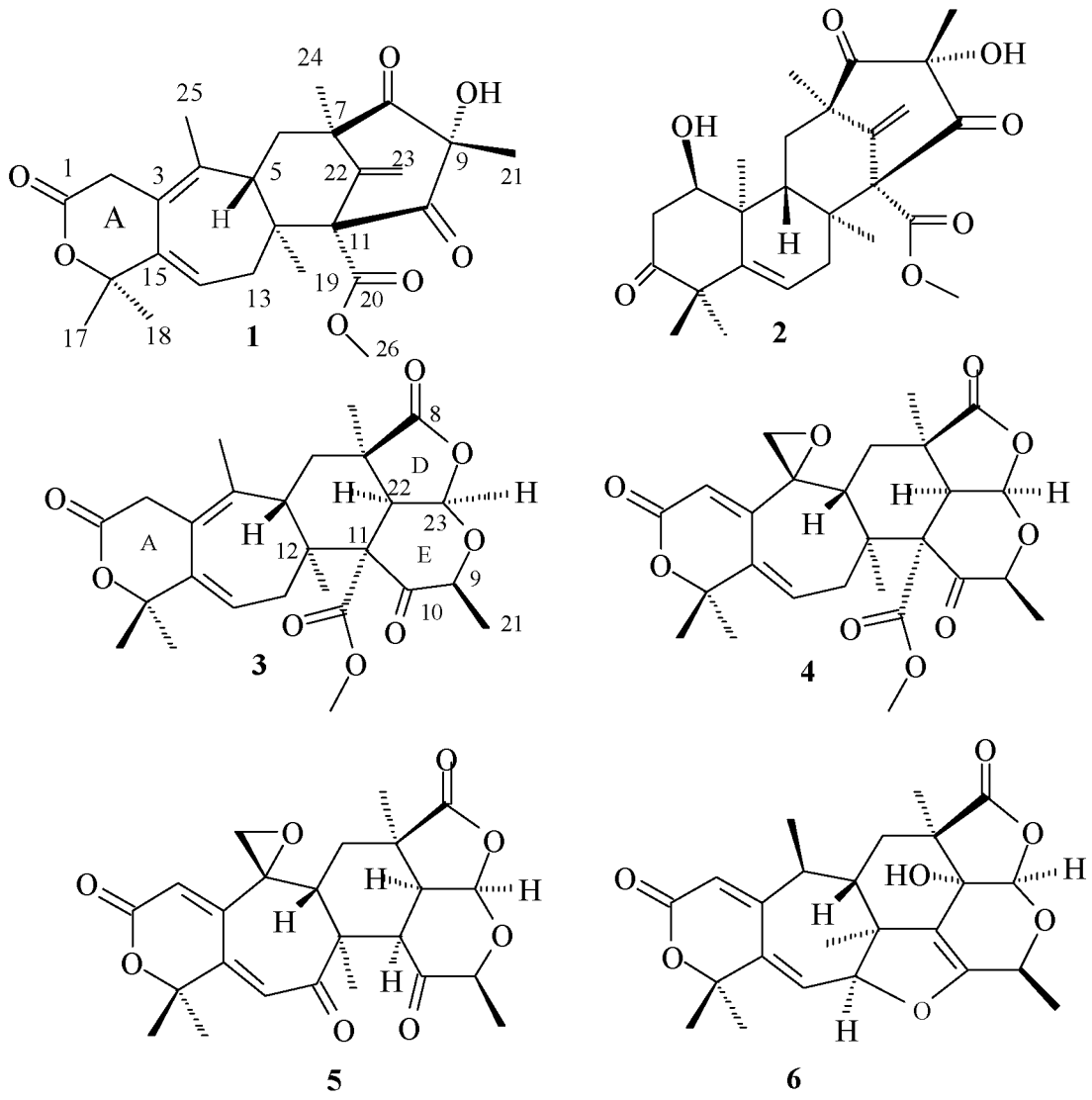

Table 1

^{1}H and ^{13}C NMR data for berkeleyacetals A-C, **3-5** ($CDCl_3$).[a]

#	Berkeleyacetal A 3 δc	δ_H	Berkeleyacetal B 4 δc	δ_H	Berkeleyacetal C 5 δc	δ_H
1	170.6 C		163.7 C		162.5 C	
2	35.1 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	d *J* = 12.9, 2.97 d 4.9
6	30.2 CH_2	β 2.11 dd *J* =14.4, 4.6	28.6 CH_2	β 2.42 dd *J* =13.8, 3.2	27.5 CH_2	d *J* =14.6, β 1.97 d 4.9
		α 1.67 m		α 1.45 m		d *J* =14.6, A 1.46 d 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 CH_2	β 2.42 dd *J* = 14.1, 8.3	34.8 CH_2	β 3.66 d *J* = 18.2	204.8 C	
		α 1.57 m		α 2.51 dd *J* = 18.2, 8.5		
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 CH_3	1.45 s 3H	28.6 CH_3	1.40 s 3H	26.6 CH_3	1.55 s 3H
18	26.3 CH_3	1.67 s 3H	26.9 CH_3	1.59 s 3H	26.2 CH_3	1.61 s 3H
19	20.9 CH_3	0.77 s 3H	17.5 CH_3	0.75 s 3H	17.4 CH_3	1.12 s 3H
20	168.7 C		167.8 C			
21	18.6 CH_3	1.34 d 3H, *J* = 7.1	18.4 CH_3	1.34 d 3H, *J* = 7.0	17.1 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	d *J* = 9.9, 3.10 d 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 CH_3	1.41 s 3H	27.5 CH_3	1.41 s 3H	26.8 CH_3	1.29 s 3H
25	16.3 CH_3	1.76 s 3H	56.8 CH_2	β 2.92 d *J* = 5.4	55.0 CH_2	β 3.14 d *J* = 5.0
				α 2.58 d *J* = 5.4		α 2.58 d *J* = 5.0
26	53.0 CH_3	3.84 s 3H	53.3 CH_3	3.85 s 3H		

[a] All assignments are based on COSY, NOE, HSQC and HMBC experiments.