

Caspase-1 and -3 Inhibiting Drimane Sesquiterpenoids from the Extremophilic Fungus *Penicillium solitum*

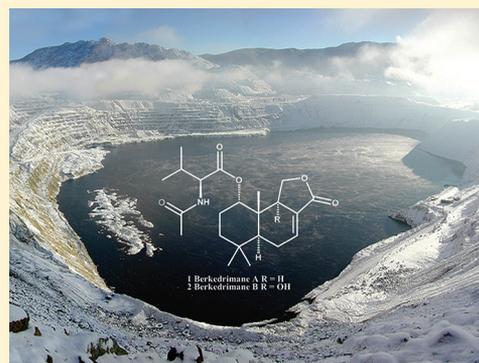
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S Supporting Information

ABSTRACT: Two new drimane sesquiterpene lactones and one new tricarboxylic acid derivative were isolated from the Berkeley Pit extremophilic fungus *Penicillium solitum*. The structures of these compounds were deduced by spectroscopic analysis. Berkedrimanes A and B inhibited the signal transduction enzymes caspase-1 and caspase-3 and mitigated the production of interleukin 1- β in the induced THP-1 (pro-monocytic leukemia cell line) assay.



Microorganisms isolated from the acidic, metal-rich waters of the Berkeley Pit Lake System in Butte, Montana, have been a rich source of interesting and biologically active compounds.^{1,2a-f} We recently isolated a filamentous fungus from surface waters of the Pit Lake, which was identified as *Penicillium solitum*. The organic extracts of broth cultures of this fungus exhibited potent activity in caspase-1, caspase-3, and matrix metalloproteinase-3 enzyme inhibition assays. These enzyme assays were used to guide the isolation of two new drimane sesquiterpenes, berkedrimanes A and B. Their isolation, characterization, inhibition of the caspases, and mitigation of interleukin-1 β production in induced THP-1 cells will be described in this report.

The role of caspase-1 in inflammation is complex. Caspase-1 is activated upon binding to the inflammasome, a multiprotein complex that plays a key role in innate immunity by activating the proinflammatory cytokines interleukin 1- β and IL-18.³ There is a strong correlation between dysregulated inflammasome activity and both inherited and acquired inflammatory diseases.³ Caspase-1 inhibitors have shown promise in either delaying the onset or mitigating the effects of a number of diseases, including Huntington's disease,⁴ amyotrophic lateral sclerosis,⁵ stroke,⁶ and multiple sclerosis.^{7,8} Caspase-1 inhibitors have also been proposed as potential therapies for osteoarthritis and rheumatoid arthritis,⁹ Alzheimer's disease,¹⁰ and brain and nerve trauma.^{6,8}

There is a complex relationship between different members of the caspase enzyme class. Caspase-3 is an executioner caspase and an important component of apoptosis, which can be activated by either extrinsic or intrinsic factors.¹¹ High levels of caspase-3 have been associated with Alzheimer's disease, particularly when coupled with high levels of caspase-1.

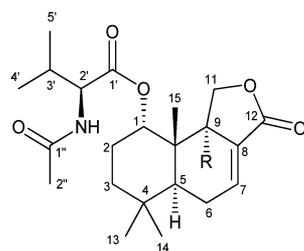
The pathological hallmarks of Alzheimer's disease include neuronal loss, extracellular senile plaques containing the peptide amyloid- β , and neurofibrillary tangles (NFTs).¹⁰ Although amyloid plaques and NFTs have been largely regarded as independent neuropathologic phenomena, they may be functionally linked, and caspases, particularly caspase-3 and -1, may play an important role in their development.¹⁰ We have begun exploring the implications of compounds with activity against both of these caspases. The development of new enzyme inhibitors will not only provide potential chemotherapeutics or pharmacophore models but also provide tools for the investigation of the intricacies of signal transduction.

P. solitum, a surface isolate of acidic Berkeley Pit Lake (pH 2.7), was grown in mycological broth for 22 days. At time of harvest, the mycelium was removed by filtration and the filtrate was extracted with CHCl₃. When compared to other fungal extracts from Pit microbes in our study, the organic extract of *P. solitum* demonstrated excellent inhibitory activity in the signal transduction assays. Flash silica gel chromatography followed by silica gel HPLC yielded the two new drimane derivatives 1 and 2, which contributed to the observed enzyme inhibitory activity. A known tricarboxylic acid (3) and its lactone (5) and the new tricarboxylic acid derivative compound 7 were also isolated from this extract.

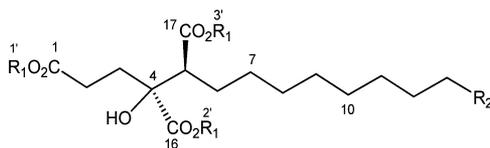
The major drimane derivative, 1, isolated as a colorless oil, had a molecular formula of C₂₂H₃₃NO₅ as deduced by HRCIMS [M + H]⁺, with seven degrees of unsaturation. The IR spectrum indicated the presence of an α,β -unsaturated γ -butyrolactone and both ester and amide functionalities (1756,

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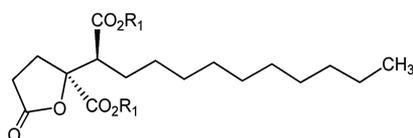
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- 1 R=H
2 R=OH



- 3 R₁ = H R₂ = CH₂CH₃ 4 R₁ = CH₃ R₂ = CH₂CH₃
7 R₁ = H R₂ = CH=CH₂ 8 R₁ = CH₃ R₂ = CH=CH₂



- 5 R₁ = H 6 R₁ = CH₃

1733, and 1675 cm⁻¹, respectively).¹² All 22 carbons were observed in the ¹³C NMR spectrum, and analysis of this spectrum along with the DEPT and HMQC spectra indicated the presence of three ester/amide carbonyls (δ_C 171.5, 170.2, 169.7), one trisubstituted double bond (δ_C 135.7, 126.9), two oxygen-bearing carbons (δ_C 75.5, 66.7), one nitrogen-bearing carbon (δ_C 57.4), two quaternary carbons (δ_C 37.5, 33.0), three methine carbons (δ_C 44.3, 42.9, 31.1), three methylene carbons (δ_C 34.7, 25.1, 22.7), and six methyl carbons (δ_C 32.7, 23.3, 21.5, 19.5, 17.3, 13.6). An *N*-acetyl valine residue was deduced from the following data: the ¹H-¹H COSY spectrum indicated the presence of the spin system NH-CH-CH-(CH₃)₂, and the HMBC spectrum showed a correlation from both NH and H-2' to carbonyl C-1' (δ_C 171.5) and from NH and methyl singlet H₃-2'' (δ_H 2.08) to carbonyl C-1'' (δ_C 170.2). These assignments accommodated C₇H₁₂NO₂ and both the amide and the ester functionalities. There remained to assign C₁₅H₂₁O₃, which suggested a sesquiterpene that included three singlet methyls (δ_H 1.00, 0.99, 0.92) and the α,β -unsaturated γ -butyrolactone, which accommodated three degrees of unsaturation. The remaining two degrees required two additional rings, suggesting a fused tricyclic carbon skeleton.

The ¹H-¹H COSY spectrum indicated three isolated spin systems: OCH₂-CH, =CH-CH₂-CH, and OCH-CH₂-CH₂. The HMBC spectrum provided key correlations to connect these systems and to assemble the molecule. The α,β -unsaturated γ -butyrolactone provided an appropriate starting point. Oxygen-bearing methylene H-11 α (δ_H 4.23) exhibited three-bond correlations to olefinic C-8 (δ_C 126.9) and carbonyl C-12 (δ_C 169.7), and H-11 β (δ_H 4.02) had a similar correlation to C-10 (δ_C 37.5). Both protons showed two-bond correlations to C-9 (δ_C 42.9). H-9 (δ_H 3.18) showed three-bond

correlations to methyl C-15 (δ_C 13.6) and olefinic C-7 (δ_C 135.7). H-7 (δ_H 6.88) exhibited three-bond correlations to C-9, -12, and -5 (δ_C 44.3) connecting the B and C rings to the A ring. H-5 (δ_H 1.74) exhibited three-bond correlations to C-1 (δ_C 75.5), C-15 (δ_C 13.6), and C-9 (δ_C 42.9). Oxygen-bearing methine H-1 (δ_H 4.78) exhibited three-bond correlations to both valine C-1' (δ_C 171.5) and C-3 (δ_C 34.7), linking the tricyclic moiety to valine. These data fit the drimane skeleton and generated the structure of berkedrimane A (**1**) as shown.

Berkedrimane A (**1**) is isomeric with the known compound purpuride, isolated from *Penicillium purpurogenum*¹³ (the double bond is Δ^8 rather than Δ^7) and has the same sesquiterpene skeleton as nebularilactone B isolated from *Lepista nebularis*.¹⁴ Comparison of the NMR data of berkedrimane A with those of nebularilactone B provided additional support for the structure of compound **1** as shown.

The relative configuration of **1** was established by consideration of coupling constants and from 1-D difference NOE studies. H-1 appeared as a broad singlet, indicating that it occupied an equatorial position in the cyclohexane ring and that the ester substituent must therefore be axial. Irradiation of H-9 (δ_H 3.18) enhanced the intensity of H-11 α (4.23) as well as that of H-5 (δ_H 1.74), indicating a 1,3-syn-diaxial relationship between H-5 and -9. In C₆D₆ the methyl singlets of H-13, H-14, and H-15 shifted from δ_H 1.00, 0.99, and 0.92 ppm, respectively, to δ_H 0.55, 0.66, and 0.27 ppm. This greater signal dispersion in C₆D₆ allowed selective irradiation of the methyl resonances to observe the resultant NOE enhancements. A large NOE enhancement was observed between methyl H-14 and methyl H-15, indicating a 1,3-diaxial relationship. Irradiation of H-15 also enhanced the absorption of methine H-1. These data were sufficient to generate the relative configuration of berkedrimane A (**1**), which was consistent with that of purpuride and nebularilactone B.

The molecular formula of **2** was C₂₂H₃₃NO₆, established by HRCIMS [M + H]⁺. The IR spectrum again indicated the presence of α,β -unsaturated γ -butyrolactone, ester, and amide functionalities as in compound **1**, but compound **2** also showed an additional -OH stretch at 3436 cm⁻¹. The ¹H NMR and ¹³C NMR spectra (Table 1) for **1** and **2** were very similar, with the following key exceptions: methine C-9 of compound **1** (δ_C 42.9) was replaced with a quaternary oxygen-bearing carbon in compound **2** (δ_C 78.0); methylene H₂-11 in compound **1** appeared as two triplets (δ_H 4.23 and 4.02, *J* = 9.2 Hz), whereas the analogous protons appeared as geminally coupled doublets at (δ_H 4.40 and 4.25, *J* = 10.1 Hz) in compound **2**. These data suggested that the hydroxy moiety in **2** was at position C-9. All of the HMBC correlations were similar to those of **1** and were in accord with this proposed structure. Key NOE correlations were also similar to those of **1**, indicating the same relative stereoconfiguration and the structure of berkedrimane B (**2**) as shown.

The magnitude of the scalar coupling constants (³*J*) associated with olefinic H-7 also supported the assigned configuration at C-9. In the energy-minimized model (Molecular Dynamics, MM2 force field calculations) of berkedrimane B as shown, the dihedral angles of H-7 to H _{α} -6 and H _{β} -6 were -55° and 58°, respectively, which would indicate that H-7 was a doublet of doublets (*J* = 4.0, 3.9). In the MM2 energy-minimized model of 9-epi-berkedrimane, the dihedral angles of H-7 were 100° relative to H _{β} -6 and -7.5° relative to H _{α} -6. If this were the case, H-7 would be a doublet of doublets (*J* = 12, 1). In fact, H-7 is a triplet (*J* = 3.5), which supports the

Table 1. ^{13}C and ^1H NMR Data for Compounds 1, 2, and 8 in CDCl_3 ^a

no.	1		2		8	
	δ_{C} , mult. ^d	δ_{H} , mult. (J in Hz) ^b	δ_{C} , mult. ^d	δ_{H} , mult. (J in Hz) ^b	δ_{C} , mult. ^d	δ_{H} , mult. (J in Hz) ^c
1	75.5, CH	4.78, bs	76.3, CH	5.01, bs	173.4, C	
2	22.7, CH ₂	1.85, m 1.76, m	23.5, CH ₂	1.92, dt (14.5, 3.1) 1.76, dq (14.5, 3.5)	28.6, CH ₂	2.46, m 2.11, m
3	34.7, CH ₂	1.69, m 1.32, dt (13.7, 3.2)	34.8, CH ₂	1.61, td (13.7, 3.4) 1.35, dt (13.7, 3.7)	32.3, CH ₂	2.17 1.97
4	33.0, C		32.6, C		77.7, C	
5	44.3, CH	1.74, dd (11.5, 6.0)	37.9, CH	2.51, dd (15.3, 5.8)	53.8	2.76
6	25.1, CH ₂	2.50, m 2.21, m	25.5, CH ₂	2.58, ddd (20.1, 5.7, 3.9) 2.24, m	27.7, CH ₂	1.82, m 1.35, m
7	135.7, CH	6.88, bq (3.5)	139.9, CH	7.01, t (3.5)	27.9, CH ₂	1.22, bs
8	126.9, C		130.4, C		29.5, CH ₂	1.22, bs
9	42.9, CH	3.18, m	78.0, C		29.3, CH ₂	1.22, bs
10	37.5, C		41.0, C		29.5, CH ₂	1.22, bs
11	66.7, CH ₂	α 4.23, t (9.2) β 4.02, t (9.2)	73.9, CH ₂	4.40, d (10.1) 4.25, d (10.1)	29.3, CH ₂	1.22, bs
12	169.7, C		168.5, C		31.9, CH ₂	1.22, bs
13	32.7, CH ₃	1.00, s	33.0, CH ₃	1.04, s	33.8, CH ₂	2.03, bq 1.95, m
14	21.5, CH ₃	0.98, s	21.6, CH ₃	1.03, s	139.2, CH	5.78, ddt (17.1, 10.2, 6.8)
15	13.6, CH ₃	0.92, s	18.6, CH ₃	0.92, s	114.1, CH ₂	4.91, ddt (10.2, 2.0, 1.2) 4.97, ddt (17.1, 2.0, 1.6)
16					174.4, C	
17					174.4, C	
1'	171.5, C		170.9, C		51.9, CH ₃	3.64, s
2'	57.4, CH	4.62, m	58.9, CH	4.36, dd (7.4, 5.8)	51.8, CH ₃	3.79, s
3'	31.1, CH	2.21, m	29.9, CH	2.20, m	53.1, CH ₃	3.70, s
4'	19.5, CH ₃	1.02, d (6.8)	19.4, CH ₃	1.02, d (7.0)		
5'	17.3, CH ₃	0.93, d (7.0)	17.9, CH ₃	0.98, d (6.8)		
Ac	170.2, C		169.9, C			
Ac CH ₃	23.3, CH ₃	2.08, s	23.2, CH ₃	2.07, s		
NH		5.90, bd (8.4)		5.94, bd (6.8)		

^aAll assignments are based on COSY, HSQC, HMBC, and NOE experiments. ^b300 MHz for ^1H NMR, HMBC, COSY. ^c500 MHz for ^1H NMR, HMBC, COSY. ^d75 MHz for ^{13}C NMR.

structure of compound 2 as shown. Similar results were obtained when the minimum energy geometries were determined using Hartree–Fock (3-21G) calculations for compound 2 and its C-9 epimer.

The absolute configuration of the valine residue in berkedrimane B (2) was established by Marfey's analysis.¹⁵ Compound 2 was hydrolyzed in boiling 6 M HCl and treated with 2,4-dinitro-5-fluoro-L-alanamide (Marfey's reagent). The resulting derivative was analyzed by LC-MS. Authentic samples of D- and L-valine were derivatized under identical conditions. The valine derivative from the hydrolysis of 2 was identical to the L-valine stereoisomer.

We also isolated two tricarboxylic acids and a tricarboxylic acid lactone along with the two drimane derivatives. These acids were difficult to purify, but were easily separated following methylation. A mixture of acids 3, 5, and 7 was treated with diazomethane and separated by HPLC to yield the methyl esters 4, 6, and 8. Analysis of NMR and mass spectral data indicated that compound 4 was the methyl ester of secospiculisporic acid (3),¹⁶ and compound 6 was the methyl ester of the tricarboxylic acid lactone, spiculisporic acid (5).¹⁷ Both of these acids have been previously described as metabolites of *Penicillium spiculisporum*.¹⁷

The HRCIMS $[M + H]^+$ of the trimethyl ester 8 established a molecular formula of $\text{C}_{20}\text{H}_{34}\text{O}_7$. The ^1H and ^{13}C NMR spectra of compounds 4 and 8 were almost identical, with one key exception; the ^1H NMR of trimethyl ester 4 indicated the presence of a terminal methyl group, and this methyl triplet was not present in the ^1H NMR spectrum of compound 8. Instead, both the ^1H and ^{13}C NMR spectra indicated the presence of a terminal monosubstituted double bond [δ_{C} 114.1, δ_{H} 4.97 (ddt, $J = 17.1, 2.0, 1.6$), 4.91 (ddt, $J = 10.2, 2.0, 1.2$) and δ_{C} 139.2, δ_{H} 5.78 (ddt, $J = 17.1, 10.2, 6.7$)]. This placed the double bond at the end of the straight chain and established the structure of triacid 7 as the previously undescribed 4-hydroxy-4,5-dicarboxy-14-pentadecenoic acid.

Secospiculisporic acid 3 and spiculisporic acid 5 have received much attention because of their amphiphilic nature and their ability to aggregate as liposome-like vesicles. They act as bio-soaps and biosurfactants and can associate with metals in solution and precipitate these metal salts.¹⁸ According to the NCBI PubChem Web site, spiculisporic acid was evaluated in 63 different biological assays, including several enzyme assays, and was inactive in each test.¹⁹

Berkedrimanes A and B were evaluated for their ability to inhibit caspase-1 and caspase-3 *in vitro* in fluorometric assays. Berkedrimane A (1) exhibited an IC_{50} value of 100 μM against

caspase-1 and 200 μM against caspase-3. It maintained moderate inhibition of caspase-3 with an IC_{25} value of 15 μM . Berkedrimane B (2) was slightly more potent, with IC_{50} values of 90 μM against caspase-1 and 50 μM against caspase-3. It maintained moderate inhibition of caspase-3 with an IC_{25} value of 10 μM . The three methyl esters were inactive against the two caspases and MMP-3.

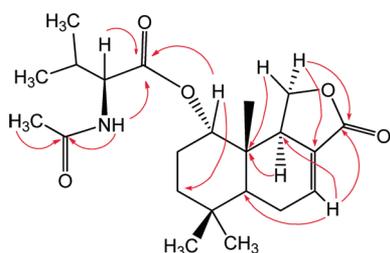


Figure 1. Key HMBC correlations for berkedrimane 1.

When induced with titanium nanowires and bacterial lipopolysaccharide (LPS), THP-1 cells produce large numbers of inflammasomes, which in turn activate pro-IL-1 β to the active form, IL-1 β . Induced THP-1 cells were exposed to compounds 1 and 2 at concentrations of 100, 10, and 1 μM , and the concentrations of IL-1 β postexposure were determined (Figure 2). Both berkedrimanes effectively inhibited the

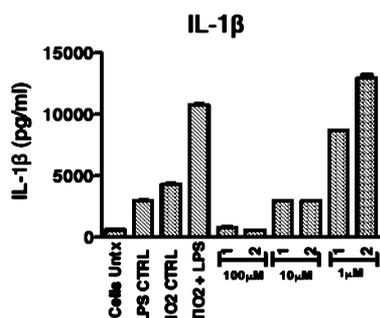


Figure 2. Production of IL-1 β in THP-1 cells induced with TiO₂ and bacterial LPS following treatment with compounds 1 and 2.

production of IL-1 β by intact inflammasomes at low micromolar concentrations, showing greater potency in the cell line assay than in the enzyme assay. Further work is under way to determine the relevance of these observations.

The inflammasome is becoming an important therapeutic target as studies continue to link inflammation and disease. In mice, potential therapeutic agents that target the inflammasome and mitigate the production of IL-1 β have been shown to reduce the inflammatory response following thromboembolic stroke²⁰ and to improve histopathology following traumatic brain injury.²¹ The biological activity of the berkedrimanes is similar to that of parthenolide, an anti-inflammatory sesquiterpene lactone isolated from feverfew, *Tanacetum parthenium*.²²

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were recorded on a Perkin-Elmer 241 polarimeter using a 1 mL cell. IR spectra were recorded on a Nicolet NEXUS 670 FT-IR spectrometer. ¹H NMR and ¹³C NMR spectra were obtained on Bruker DPX-300 or Varian 500 NMR spectrometers. ¹H NMR spectra were recorded at

300 or 500 MHz, and the ¹³C NMR spectra were recorded at 75 MHz unless otherwise noted. All of the chemical shifts were recorded with respect to the deuterated solvent shift (CDCl₃, δ_{H} 7.24, δ_{C} 77.0 or C₆D₆, δ_{H} 7.18, δ_{C} 128.0). Mass spectral data were provided by the University of Montana Mass Spectrometer Facility. All solvents used were spectral grade or distilled prior to use.

Collection, Extraction, and Isolation Procedures. The collection and isolation of Berkeley Pit fungi have previously been described.^{2e} *Penicillium solitum* was grown in potato dextrose broth (8 flasks with 500 mL of broth per flask, in 1 L Erlenmeyer flasks) for 22 days (still culture). At time of harvest MeOH (50 mL per flask) was added to each flask, the mycelium was removed by filtration, and the filtrate was extracted with CHCl₃ (3 \times 1 L). Removal of the CHCl₃ *in vacuo* yielded 0.4128 g of total crude extract. This extract showed potent enzyme inhibition in the MMP-3, caspase-1, and caspase-3 assays. It was chromatographed on a flash Si gel column in a gradient fashion, starting with hexane, then with increasing amounts of IPA. The column was washed with 100% IPA and finally with MeOH. The fraction that eluted with 50% IPA/hexane was active in both the caspase-1 and caspase-3 enzyme inhibition assays. It was further purified with Si gel HPLC in an isocratic mode using 20% IPA/hexane to yield berkedrimanes A (1) (11.6 mg) and B (2) (5.1 mg). The two berkedrimanes, which were 2.8% and 1.2% of the crude CHCl₃, respectively, were active in the enzyme inhibition assays, although several of the other column fractions exhibited activity as well. The fraction that eluted with 20% IPA (180.2 mg) was a mixture of acids (3, 5, and 7) that was difficult to separate. The acid fraction was methylated with diazomethane and then chromatographed on Sigel HPLC to give methyl esters of the known 4-hydroxy-4,5-dicarboxypentadecanoic acid (3) (40.3 mg), the previously undescribed 4-hydroxy-4,5-dicarboxy-9-pentadecenoic acid (7) (75.3 mg), and spiculisporic acid (5) (60.2 mg).

Berkedrimane 1: colorless oil, [α]_D²⁵ +31.4 (c 0.0014, CHCl₃); IR (CHCl₃) ν_{max} 3436, 2966, 1756, 1733, 1675, 1506, 1373, 1016, 892 cm⁻¹; ¹H NMR and ¹³C NMR (CDCl₃) see Table 1; ¹H NMR (C₆D₆, 500 MHz) δ 0.27 (s, 3H, H-15) 0.55 (s, 3H, H-13) 0.66 (s, 3H, H-14) 0.82 (d, *J* = 6.73 Hz, 6H, H-4',5') 1.50 (s, 3H, OAc) 1.89 - 1.99 (m, 1H) 3.11 (m, 1H, H-9) 3.60 (m, 1H, H-11) 4.19 (m, 1H, H-11) 4.56 (m, 1H, H-1) 4.66 (m, 1H, H-2') 5.32 (bd, 1H, NH) 6.61 (m, 1H, H-7); HRCIMS [M + H]⁺ *m/z* 392.2466 (calcd for C₂₂H₃₄NO₅, 392.2437).

Berkedrimane 2: colorless oil, [α]_D²⁵ -15.3 (c 0.0145, CHCl₃); IR (CHCl₃) ν_{max} 3446, 3081, 2966, 1751, 1730, 1670, 1238, 1022, 729 cm⁻¹; ¹H NMR (CDCl₃) see Table 1; ¹³C NMR (CDCl₃) see Table 1; EIMS *m/z* [M]⁺ 407 (2), 390 (10), 230 (55), 78 (100); HRCIMS [M + H]⁺ *m/z* 408.2361 (calcd for C₂₂H₃₃NO₆, 408.2386).

Determination of the Absolute Configuration of Valine. Drimane 2 (1 mg) was suspended in freshly distilled, constant boiling 6 M HCl (100 μL) and heated in a Teflon-sealed glass vial at 110 $^{\circ}\text{C}$ for 15 h. The solution was then concentrated to dryness under a stream of N₂. This solid was treated with a solution of 2,4-dinitro-5-fluoro-L-alanamide (100 μL , 1% w/v in acetone), followed by 1.0 M NaHCO₃ (25 μL), and heated in a Teflon-sealed glass vial at 80 $^{\circ}\text{C}$ for 10 min. The mixture was cooled and quenched with 1.0 M HCl (25 μL). Authentic L- and D-valine standards were derivatized under identical conditions.¹⁵

LC-MS Analysis. The Marfey's derivatives and the standards derivatives (see above) were analyzed by LC-MS using a ZORBAX Extend C-18 (2.1 \times 50 mm, 3.5 μM) column. Chromatography parameters are as follows: initial, 80% solvent A (H₂O + 0.1% formic acid), 20% solvent B (acetonitrile), at 9 min to 60% A, at 10 min to 100% B. PDA parameters were as follows: channel A 340 nm, channel B 254 nm. MS parameters were as follows: APCI source. Retention time for the authentic L-valine derivative was 10.6 min; retention time for the authentic D-valine was 12.6 min. The Marfey's derivative of the hydrolysis of 2 had a retention time of 10.6 min and gave a mass spectrum identical to the authentic valine samples.

Methylation of Carboxylic Acid Mixture. The mixture of acids (10.3 mg) was dissolved in diethyl ether (2 mL), and a solution of CH₂N₂ dissolved in ether was added dropwise until the yellow color

remained. After 1 min the solvent was removed *in vacuo* and the mixture subjected to HPLC using 5% EtOAc/hexane. The trimethyl ester of the known tricarboxylic acid, secospiculisporic acid (4-hydroxy-4,5-dicarboxypentadecanoic acid) (4) (5.2 mg), and the dimethyl ester of spiculisporic acid (6) (3.2 mg) were isolated along with the trimethyl ester of the previously unreported 4-hydroxy-4,5-dicarboxy-14-pentadecenoic (8) (2.2 mg).

Trimethyl 4-hydroxy-4,5-dicarboxy-14-pentadecenoate (8): colorless oil, $[\alpha]_D^{25} -5.0$ (*c* 0.0022, CHCl₃); ¹H NMR (CDCl₃, 500 MHz) δ 1.22 (bs, 12H) 1.35 (m, 2H) 1.83 (m, 1H) 1.98 (m, 2H) 2.14 (m, 2H) 2.46 (m, 1H) 2.76 (dd, *J* = 11.8, 3.3 Hz, 1H) 3.64 (s, 3H) 3.70 (s, 3H) 3.79 (s, 3H) 4.91 (dt, *J* = 10.2, 1.0 Hz, 1H) 4.97 (dq, *J* = 17.1, 1.8 Hz, 1H) 5.78 (dd, *J* = 17.0, 10.1 Hz, 1H); ¹³C NMR (CDCl₃, 125 MHz) δ 27.7, 27.9, 28.6, 29.3(3C), 29.5, 31.9, 32.3, 33.8, 51.8, 51.9, 53.1, 53.8, 77.7, 114.1, 139.2, 173.4, 174.1, 174.4; HRCIMS [M + H]⁺ *m/z* 387.2344 (calcd for C₂₀H₃₅O₇, 387.2383).

Signal Transduction Assays. The signal transduction drug discovery kits for caspase-1, caspase-3, and matrix metalloproteinase-3 enzymes were purchased from Enzo Life Sciences. The positive controls (enzyme inhibitors) used in these assays are Ac-YVAD-CHO for the caspase-1 assay, Ac-DEVD-CHO for the caspase-3 assay, and *N*-isobutyl-*N*-(4-methoxyphenylsulfonyl)glycylhydroxamic acid for the MMP-3 assay.

In Vitro THP-1 Assay. Human monocyte cell line THP-1 was purchased from ATCC (#TIB-202). The cells were suspended at (2–4) × 10⁵ viable cells/mL in RPMI media supplemented with 10% fetal bovine serum, 0.05 mM 2-mercaptoethanol, sodium pyruvate, and an antimycotic/antibiotic cocktail containing penicillin, streptomycin, and amphotericin B (Mediatech, VWR). The cells were differentiated into macrophage-like cells by the phorbol ester PMA (1 μg/mL, Sigma) 24 h prior to experimentation. The transformed cells were removed from the flask by scraping and centrifuged at 450g for 5 min. The resulting cell pellet was suspended at 1.0 × 10⁶ cells/mL and exposed to caspase-1 inhibitors at concentrations described below (0.5–0.005%), LPS [20 ng/mL], and TiO₂ nanowires (100 μg/mL). Experiments were conducted in 96-well plates for 24 h in 37 °C water-jacketed CO₂ incubators (ThermoForma).

Toxicity Assay. Cell viability was determined by MTS reagent using the CellTiter96 assay (Promega), according to the manufacturer's protocol. The plate was read at 490 nm.

Cytokine Assays. Human IL-1β DuoSet was obtained from R&D Systems, and ELISA assays were performed according to the manufacturer's protocol. The plate was read at 490 nm.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Experimental details including the ¹H NMR spectrum of berkedrimane A (1) in both CDCl₃ and C₆D₆ and of berkedrimane B (2) in CDCl₃, the ¹³C NMR, COSY, HMBC, and HSQC spectra for both compounds in CDCl₃, and the ¹H NMR and ¹³C NMR spectra of trimethyl ester 8 in CDCl₃. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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