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ARTICLE *in* JOURNAL OF NATURAL PRODUCTS · JUNE 2015

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Soudanones A–G: Antifungal Isochromanones from the Ascomycetous Fungus *Cadophora* sp. Isolated from an Iron Mine

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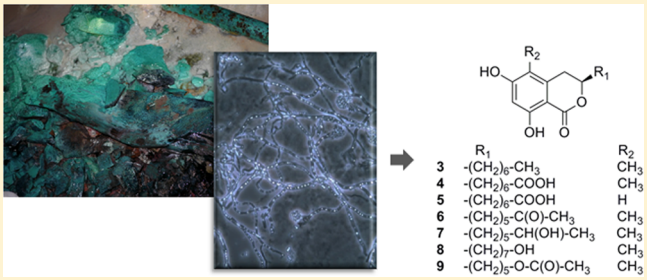
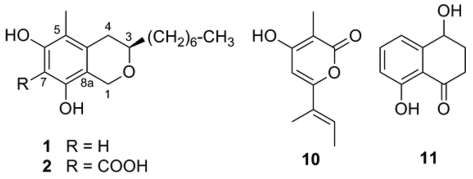
Supporting Information

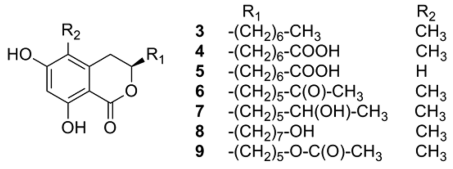
ABSTRACT: One new isochromane (pseudoanguillosporin C, **2**), seven isochromanones (soudanones A–G, **3**–**9**), and six known analogues including **10** and **11** were isolated from a culture of the fungus *Cadophora* sp. 10-5-2 M, collected from the subterranean 10th level of the Soudan Underground Iron Mine in Minnesota. All of the compounds were tested against a panel of microbial pathogens, and **2**, **3**, **10**, and **11** were found to have activity against *Cryptococcus neoformans* (MIC = 35, 40, 20, and 30 μg/mL, respectively). Compound **11** was also active against *Candida albicans*, with an MIC of 40 μg/mL.



Subterranean caves and mines are unique environments that host diverse microbial species.[1,2] Some of the features that shape the microbial communities in these areas include high levels of salts or metals as well as limited access to plant- or animal-based nutrients. As part of a project to identify new antifungal natural products, we have been characterizing compounds and their activities from the cultivable bacteria and fungi from the Soudan Underground State Park Iron Mine in northern Minnesota. Herein we report the structures and biological activities of one new isochromane (pseudoanguillosporin C, **2**) and seven new isochromanones (soudanone A–G, **3**–**9**) together with six previously reported congeners, pseudoangillosporin A (**1**),[3] nectriapyrone (**10**),[4] isosclerone (**11**),[5] 3,8-dihydroxy-3-hydroxymethyl-6-methoxy-4,5-dimethylisochroman-1-one,[6] 7-hydroxy-3-(1-hydroxyethyl)-5-methoxy-3,4-dimethylisobenzofuran-1(3*H*)-one,[7] and 3-acetyl-7-hydroxy-5-methoxy-3,4-dimethylisobenzofuran-1(3*H*)-one,[8] from *Cadophora* sp. (Ascomycota, Helotiales, GenBank No. KP708756) isolated from copper-encrusted wood timbers on the 10th level of the mine, approximately 220 m below ground. The structures of the previously reported compounds were determined by comparison of NMR and MS data to literature values,[3−8] and the new compounds were identified as described below.

Compound **1** was isolated as a yellow solid with a molecular formula of $C_{17}H_{26}O_3$ (5 degrees of unsaturation) based on HRAPCIMS. Analysis of the NMR and CD data (Supporting Information, Figures S1–S6) led to the identification of compound **1** as pseudoanguillosporin A, a previously reported and structurally characterized antibacterial compound isolated from *Pseudoanguillospora* sp.[3] Comparison of the data for analogues **2**–**9** to those for **1** allowed for rapid determination of the new structures.





Analysis of the HRESIMS data for **2** revealed a pseudomolecular ion peak at *m*/*z* 321.1703 $[M - H]^-$ consistent with a molecular formula of $C_{18}H_{26}O_5$ (6 degrees of unsaturation). Comparison of the ^{1}H NMR spectra suggested a close resemblance to compound **1**, except for the absence of the downfield aromatic proton (Table 1). An extra carbonyl at δ_C 161.0 in the ^{13}C spectrum and an increase of 44 mass units compared to **1** provided evidence for a carboxylic acid substituent. The chemical shift of an sp^2 quaternary carbon at δ_C 102.8 allowed placement of COOH at the C-7 position (Table 2). HMBC correlations of an isolated oxy-methylene at position 1 (two doublets, H-1a/b) at δ_H 4.85 and 4.50, as well as the correlations of H-4a/b (δ_H 2.62 and 2.34) to C-3 (δ_C 76.7), C-4a (δ_C 138.7), and C-8a (δ_C 112.8) identified the isochromane subunit as the core of this compound. A long continuous spin system consisting of six aliphatic methylenes, one methyl group, and one proton attached to an oxygenated

Received: March 5, 2015

DOI: 10.1021/acs.jnatprod.5b00204
J. Nat. Prod. XXXX, XXX, XXX–XXX

Table 1. ^{1}H NMR Data of 2−9 (600 MHz, CD_3OD, δ in ppm, mult, J in Hz)

position	2	3	4	5	6	7	8	9
1	a 4.85 d (14.6)							
	b 4.50 d (14.6)							
3	3.56 m	4.47 m	4.47 m	4.53 m	4.47 m	4.47 m	4.47 m	4.47 m
4	a 2.62 d (16.7)	a 3.02 dd (16.8, 3.2)	a 3.02 dd (16.8, 3.2)	a 2.90 dd (16.5, 3.6)	a 3.02 dd (16.8,3.2)	a 3.02 dd (16.8, 3.2)	a 3.02 dd (16.8,3.2)	a 3.02 dd (16.8,3.2)
	b 2.34 dd (16.7, 10.6)	b 2.68 dd (16.8, 11.5)	b 2.68 dd (16.8, 11.5)	b 2.85 dd (16.5, 10.7)	b 2.68 dd (16.8, 11.5)	b 2.68 dd (16.8, 11.5)	b 2.68 dd (16.8, 11.5)	b 2.68 dd (16.8, 11.5)
5				6.23 s				
7		6.25 s	6.25 s	6.20 s	6.25 s	6.25 s	6.25 s	6.25 s
1′	a 1.63 m	a 1.83 m	a 1.83 m	a 1.83 m	a 1.83 m	a 1.83 m	a 1.83 m	a 1.83 m
	b 1.60 m	b 1.76 m	b 1.76 m	b 1.76 m	b 1.76 m	b 1.76 m	b 1.76 m	b 1.76 m
2′	a 1.63 m	a 1.60 m	a 1.60 m	a 1.60 m	a 1.60 m	a 1.60 m	a 1.60 m	a 1.60 m
	b 1.44 m	b 1.49 m	b 1.49 m	b 1.49 m	b 1.49 m	b 1.49 m	b 1.49 m	b 1.49 m
3′	1.33 m	1.33 m	1.38 m	1.38 m	1.39 m	a 1.49 m	1.38 m	1.35 m
						b 1.47 m		
4′	1.33 m	1.33 m	1.38 m	1.38 m	1.60 m	a 1.44 m	1.38 m	a 1.69 m
						b 1.39 m		b 1.44 m
5′	1.33 m	1.33 m	1.60 m	1.60 m	2.51 t (7.2)	a 1.47 m	1.38 m	4.08 t (5.2)
						b 1.39 m		
6′	1.33 m	1.33 m	2.28 t (7.0)	2.28 t (7.0)		3.72 m	1.54 m	
7′	0.90 t (6.4)	0.90 t (6.4)			2.14 s	1.15 d (6.2)	3.54 t (6.5)	
8′								2.01 s
5-Me	1.97 s	2.03 s	2.03 s		2.03 s	2.03 s	2.03 s	2.03 s

Table 2. ^{13}C NMR Data of 2−9[a] (CD_3OD, δ in ppm, mult)

position	2[b]	3	4	5	6	7	8	9
1	66.3, CH_2	172.2, C	172.2, C	172.2, C	172.2, C	172.2, C	172.2, C	172.2, C
3	76.7, CH	80.3, CH	80.3, CH	79.4	80.3, CH	80.3, CH	80.3, CH	80.3, CH
4	34.3, CH_2	31.6, CH_2	31.6, CH_2	32.5, CH_2	31.6, CH_2	31.6, CH_2	31.6, CH_2	31.6, CH_2
4a	138.7, C	140.8, C	140.8, C	142.2, C	140.8, C	140.8, C	140.8, C	140.8, C
5	112.6, C	115.4, C	115.4, C	106.5, CH	115.4, C	115.4, C	115.4, C	115.4, C
6	159.2, C	164.9, C	164.9, C	164.5, C	164.9, C	164.9, C	164.9, C	164.9, C
7	102.8, C	101.6, CH	101.6, CH	100.7, CH	101.6, CH	101.6, CH	101.6, CH	101.6, CH
8	156.8, C	163.9, C	163.9, C	164.5, C	163.9, C	163.9, C	163.9, C	163.9, C
8a	112.8, C	101.4, C	101.4, C	101.0, C	101.4, C	101.4, C	101.4, C	101.4, C
1′	37.7, CH_2	36.1, CH_2	36.1, CH_2	36.8, CH_2	36.1, CH_2	36.1, CH_2	36.1, CH_2	36.1, CH_2
2′	27.1, CH_2	26.7, CH_2	26.7, CH_2	26.7, CH_2	26.0, CH_2	26.8, CH_2	26.0, CH_2	25.9, CH_2
3′	31.2, CH_2	30.7, CH_2	30.6, CH_2	30.6, CH_2	30.7, CH_2	30.7, CH_2	30.7, CH_2	27.1, CH_2
4′	30.9, CH_2	30.6, CH_2	28.9, CH_2	28.9, CH_2	24.6, CH_2	27.5, CH_2	30.6, CH_2	29.9, CH_2
5′	33.5, CH_2	28.4, CH_2	24.8, CH_2	24.8, CH_2	44.3, CH_2	40.2, CH_2	27.0, CH_2	65.9, CH_2
6′	24.2, CH_2	21.8, CH_2	33.9, CH_2	33.9, CH_2	212.3, C	68.7, CH	33.8, CH_2	
7′	14.9, CH_3	13.8, CH_3	177.0, C	177.0, C	31.7, CH_3	23.6, CH_3	63.2, CH_2	173.3, C
8′								21.0, CH_3
5-Me	10.4, CH_3	10.7, CH_3	10.7, CH_3		10.7, CH_3	10.7, CH_3	10.7, CH_3	10.7, CH_3
7-COOH	161.0, C							

[a]Collected from HMQC and HMBC experiment data (600 MHz). [b]Combined with ^{13}C experiment data (151 MHz).

carbon (H-3, δ_H 3.56) confirmed the presence of the heptyl side chain at C-3. This assignment was also corroborated by HMBC correlations of H-1′a/b (δ_H 1.63 and 1.60) to C-4 (δ_C 34.3) and H-2′a/b (δ_H 1.63 and 1.44) to C-3 (δ_C 76.7) (Figure 1). The configuration of C-3 was determined by observation of the large coupling constant between H-3 and H-4b (10.6 Hz, pseudoaxial) and NOE correlation between H-3 and H-4a. Moreover, the presence of NOE correlations of H-4b (pseudoaxial position) with H-1′a and H-1′b indicated the pseudoequatorial orientation of the heptyl side chain at C-3 (Figure 1). The well-characterized chiroptical properties of substituted isochromane (isocoumarin) compounds in the literature allowed us to determine the absolute configuration of

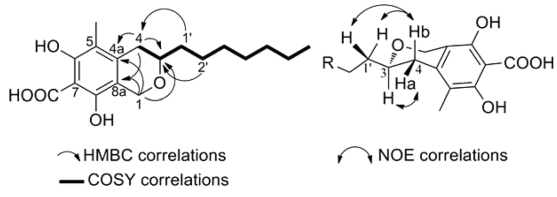


Figure 1. Select COSY, HMBC, and NOE correlations of compound **2** in CD_3OD.

the C-3 chiral center as *R* by analysis of the CD spectrum, which showed a characteristic negative Cotton effect at 270 nm (Supporting Information, Figure S14).[9,10] Compound **2** was

DOI: 10.1021/acs.jnatprod.5b00204
J. Nat. Prod. XXXX, XXX, XXX−XXX

therefore identified as 3(*R*)-heptyl-6,8-dihydroxy-5-methylisochromane-7-carboxylic acid and given the trivial name pseudoanguillosporin C.

Compound **3** was the most abundant compound in the extract (158 mg) and was isolated as a colorless crystal with a molecular formula of $C_{17}H_{24}O_4$ (6 degrees of unsaturation) determined by HRESIMS. The ^{1}H NMR, COSY, and TOCSY spectra indicated the presence of one continuous spin system incorporating one multiplet proton attached to an oxygenated carbon at δ_H 4.47 (H-3), six methylenes in the aliphatic region, and one triplet methyl signal at δ_H 0.90, consistent with the heptyl side chain as observed in compounds **1** and **2** (Table 1, Supporting Information, Figure S16–S18). The presence of one aromatic singlet at δ_H 6.25 (H-7) and one methyl at δ_H 2.03 with HMBC correlations to C-5 (δ_C 115.4) and C-6 (δ_C 164.9) indicated the presence of the same polysubstituted aromatic subunit as for **1**. However, HMBC correlations of H-7 to a new carbonyl signal at C-1 (δ_C 172.2), C-8 (δ_C 163.9), and C-8a (δ_C 101.4), H_2-4 (δ_H 3.02 and 2.68) to C-5 (δ_C 115.4) and C-8a (δ_C 101.4), and H-3 (δ_H 4.47) to C-1 (δ_C 172.2) led to the identification of the core isochromanone system with a cyclic ester (Figure 2). The NOE correlations of this

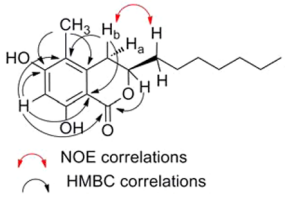


Figure 2. Select HMBC and NOE correlations for compound **3**.

compound were nearly identical to those for compound **2**, suggesting the same configuration for the C-3 heptyl side chain (Figure 2). Additionally, the CD spectrum showed a negative Cotton effect in the 270 nm range, indicating a 3-*R* configuration (Supporting Information, Figure S23).[9,10] The trivial name assigned to **3** is soudanone A, as the first compound identified from a microbe isolated from the Soudan Mine.

HRESIMS analysis of soudanone B (**4**) showed a pseudomolecular ion peak at *m/z* 321.1323 $[M - H]^-$, consistent with a formula of $C_{17}H_{22}O_6$ (7 degrees of unsaturation). Comparison of the 2D NMR data suggested a structure with the same isochromanone subunit found in **3**. However, the aliphatic side chain at C-3 was slightly different from **3** due to the presence of a terminal carboxylic acid moiety as indicated by an increase of 30 mass units and HMBC correlations of two methylenes in the upfield region (H_2-5′, δ_H 1.60 and H_2-6′, δ_H 2.28) to a carbonyl signal at δ_C 177.0. The remaining HMBC, COSY, and NOE correlations of this compound were otherwise similar to those for compound **3**.

The molecular formula of soudanone C (**5**) was calculated as $C_{16}H_{20}O_6$ (7 degrees of unsaturation) from HRESIMS data and found to differ from **4** by 14 mass units (CH_2). The ^{1}H NMR spectrum of **5** differed from **4** by two aromatic singlets at δ_H 6.23 and 6.20 and the absence of a singlet methyl signal (C-5 Me) (Table 1 and Supporting Information, Figure S32). HMBC correlations of the aromatic protons to C-6 (δ_C 164.5) and C-8a (δ_C 101.0), as well as the correlations of δ_H 6.23 to C-4 (δ_C 32.5) and δ_H 6.20 to C-8 (δ_C 164.5), established the position of these two protons at C-5 and C-7 in the isochromanone subunit, respectively. The HMBC, COSY, and NOE data for the side chain were identical to those for **4**, confirming a heptanoic acid moiety at C-3.

HRESIMS analysis of soudanone D (**6**) revealed a pseudomolecular ion peak at *m/z* 305.1387 $(M - H)^-$, consistent with a a molecular formula of $C_{17}H_{22}O_5$ (7 degrees of unsaturation). The ^{1}H and NOE NMR spectra closely resembled those of **4**, and the 2D heteronuclear correlation data indicated the same isochromanone core structure. However, the data for **6** differed by the presence of an extra methyl signal at δ_H 2.14 and absence of one methylene in the long spin system of the side chain in the COSY spectrum (Supporting Information, Figure S38). HMBC correlations of the terminal methyl group at δ_H 2.14 (7′-Me) and two methylenes at δ_H 2.51 (H_2-5′) and 1.60 (H_2-4′) to a carbonyl group at δ_C 212.3 (C-6′) established a keto functional group at this position and revealed the C-3 side chain as heptan-2-one.

Soudanone E (**7**) was obtained as a yellowish-green solid with a molecular formula of $C_{17}H_{24}O_5$ (6 degrees unsaturation) derived from HRESIMS. The NMR spectra of this compound were similar to those of **6** and indicated the identical isochromanone core structure (Tables 1 and 2). The primary difference was identified in the HMQC spectrum as a methine proton at δ_H 3.72 attached to a hydroxylated carbon at δ_C 68.7 (Supporting Information, Figure S45). In the COSY spectrum, this proton is incorporated into the long spin system heptyl side chain. The position of the methine proton (H-6′) was supported by HMBC correlations to the terminal methyl carbon (Me-7′, δ_C 23.6), and between the methyl protons (H_3-7′, δ_H 1.15) and a carbon signal at δ_C 68.7 (C-6′), confirming the C-3 side chain as heptan-2-ol. The configuration of the chiral center at C-6 was not determined due to the limited quantity of material.

Soudanone F (**8**) was identified as an isomer of **7** ($C_{17}H_{24}O_5$), determined by the HRESIMS pesudomolecular ion peak at *m/z* 307.1537 $[M - H]^-$. Analysis of the NMR data provided evidence for the same isochromanone subunit as in compounds **3**, **4**, **6**, and **7**, but differences in the signals associated with the end of the side chain were observed. In contrast to the data for compound **7**, the ^{1}H NMR spectrum of **8** included a signal for an oxygenated methylene at δ_H 3.54 (triplet, *J* 6.5 Hz) as part of the side chain aliphatic spin system (Table 1). HMBC correlations of these methylene protons to two adjacent carbons at δ_C 33.8 (C-6′) and 27.0 (C-5′) indicated their location next to a terminal hydroxyl group and established the C-3 side chain as heptanol.

The molecular formula of soudanone G (**9**) was determined as $C_{17}H_{22}O_6$ (7 degrees of unsaturation) from the HRESIMS data showing a pseudomolecular ion at *m/z* 321.1337 $[M - H]^-$. The ^{1}H NMR and 2D spectra suggested a close structural relationship to compound **6**. However, a difference in mass due to one additional oxygen atom (16 amu) and HMBC correlations of the methyl singlet at δ_H 2.01 (8′-Me) and a methylene triplet at δ_H 4.08 (t, 5.2 Hz, H_2-5′) to the same carbonyl signal at δ_C 173.3 (C-7′) revealed pentyl acetate as the C-3 side chain.

Due to the structural similarities of compounds **2**–**9**, the configurations of the C-3 chiral center for isochromanones **4**–**9** were determined by comparison of their optical rotations. The negative value of the optical rotation measurements ($[\alpha]^{23}_D$: −40 to −55) for the six compounds matched those for compounds **2** and **3** and were therefore tentatively assigned as 3-*R*.

DOI: 10.1021/acs.jnatprod.5b00204
J. Nat. Prod. XXXX, XXX, XXX−XXX

All isolated compounds were evaluated for their antibacterial and antifungal activities. Among the new compounds, only compounds **2** and **3** showed moderate activity against *Cryptococcus neoformans*, with MICs of 35 and 40 μg/mL, respectively (Table 3). The compounds nectriapyrone (**10**) and isosclerone (**11**) were previously reported to have moderate antibacterial activities.[4,11] However, in our study these compounds did not inhibit bacterial growth and exhibited antifungal activity against *C. neoformans* with MIC values of 20 and 30 μg/mL, respectively (Table 3). Isosclerone (**11**) was the only analogue in the suite with additional antifungal activity against *C. albicans* (MIC 40 μg/mL). Pseudoanguillosporin A (**1**) was previously reported to have activity against *Bacillus megaterium*, *Microbotryum violaceum*, and *Chlorella fusca* using disk diffusion assays,[3] but was not active against any of the pathogens tested in the broth dilution assay panel.

Table 3. Inhibitory Activities of Antifungal Compounds[a] (MIC, μg/mL)

	C. neoformans	*C. albicans*
2	35	>100
3	40	>100
10	20	>100
11	30	40
amphotericin B	0.2	0.2

[a]Compounds **1** and **4**–**9** were inactive against all microbial strains tested (MIC > 100 μg/mL).

The structures of the new compounds identified in this study are similar to previously reported antimalarial dihydroisocoumarin (isochromanone) analogues containing alkyl side chains at both the 7-C and 3-C positions, isolated from an endophytic *Geotrichum* sp. fungus (IC_{50} = 2.6–4.7 μg/mL against multidrug-resistant *Plasmodium falciparum* K1).[9] A subset of the 3-alkyl isochromanones identified in this study (**2**, **3**, **6**, **9**) were therefore tested against drug-sensitive *P. falciparum* NF_{54}, but none were active at 10 μg/mL. These data suggest the importance of the saturated 7-C alkyl chain and/or a shorter 3-C alkyl chain (n = 5) for antimalarial activity.[9]

Phylogenetic analysis of the fungal isolate indicated that the closest related species is *Cadophora fastigiata* (98% identical over 497 bp of the ITS rRNA gene, Supporting Information, S63). This species and other members in the genus have been frequently isolated from terrestrial polar environments[12,13] and chromated copper arsenate (CCA)-treated wood[14] and have a tolerance to heavy metals,[15] indicating an ability to withstand harsh environments that frequently exclude other fungi. Since the *Cadophora* specimen was isolated from timbers that were brought into the mine when it was operational (before 1962), we presume that the fungus was introduced via these materials and adapted to the high copper environment. We have isolated several distinct species of *Cadophora* from locations throughout multiple levels of the mine and are currently exploring their chemical phenotypes and phylogenetic relationships to other known terrestrial species.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were determined using a Rudolph Research Analytical Autopol III polarimeter, and CD spectra were recorded using a JASCO 200 system. IR spectra were recorded on a JASCO 4100 FT-IR spectrophotometer. Mass analyses were performed using an Agilent TOF II TOF/MS spectrometer with a dual ESI and APCI source. Standard 1D and 2D NMR spectra were recorded on a Varian 600 MHz spectrometer in CD_3OD. Proton and carbon chemical shifts are reported in ppm and referenced to the ^1H and ^{13}C signals of residual methanol (δ_H 3.31 and δ_C 49.9 ppm, respectively). Semipreparative HPLC separations were performed on an Agilent 1200 instrument system equipped with a photodiode array detector. A Teledyne ISCO Combiflash Rf system was used for flash chromatography separations. TLC separations were performed using Whatman silica gel 60 F254 aluminum-backed TLC plates. Sephadex LH-20 (GE Healthcare) and silica gel 60 (230–400 mesh, Merck) were used as the stationary phases for column chromatography.

Isolation and Cultivation of Fungi. Small wood segments were collected from the 10th level of the Soudan Underground Mine in Saint Louis County, Minnesota, placed in sterile bags, and transported to the laboratory. Samples were then aseptically cut into smaller segments and cultured for fungi using several types of growth media: 1.5% Difco malt extract agar (MEA), MEA with 0.1 g/L streptomycin sulfate added after autoclaving, and a semiselective media for Basidiomycetes that included MEA with 2 g/L of yeast, 0.06 g/L of Benlate, 0.1 g/L of streptomycin sulfate, and 2 mL/L of 85% lactic acid added after autoclaving.[16] Samples were incubated at 20 °C. Following incubation, fungi that grew from wood segments were transferred to another plate to obtain the pure culture of 10-5-2 M *Cadophora* sp. (GenBank No. KP708756). Genomic DNA was extracted using a CTAB extraction protocol.[17] The nuc rDNA ITS1-5.8S-ITS2 regions (ITS) were amplified by PCR using the ITS1/ITS4 primer pair using the methods of Blanchette et al.[13,18] Sequencing for all amplified products was done with an ABI 3730xl DNA sequencer (Applied Biosystems, Foster City, CA, USA), and sequences were compared to others in GenBank using the BLASTn program for the best possible match to other known isolates (Supporting Information, Figure S63). For the large-scale cultures for compound isolations, the *Cadophora* isolate was grown on malt agar (15 g/L malt extract and 15 g/L agar) for 7 days, from which small plugs of the culture were transferred into 30 1 L Erlenmeyer flasks containing rice medium (100 g of rice and 100 mL of water) and incubated at room temperature for 4 weeks.

Biological Assays. Microbial susceptibility testing was performed using the microbroth dilution assay[19] with the following strains: methicillin-resistant *Staphylococcus aureus* (MRSA) ATCC 43300, vancomycin-resistant *Enterococcus faecalis* (VRE) ATCC 51299, *Bacillus subtilis* ATCC 6633, *Escherichia coli* ATCC 25922, *Acinetobacter baumannii* ATCC 19606, *Pseudomonas aeruginosa* ATCC 27853, *Klebsiella pneumonia* ATCC 13883, *Cryptococcus neoformans* ATCC 66031, and *Candida albicans* ATCC 10231. The positive control antibiotics for the antibacterial assays were penicillin G (10 μg/mL for *Enterococcus faecalis*) and tetracycline (50 μg/mL for *P. aeruginosa* and 10 μg/mL for all other strains). The control antifungal compound was amphotericin B (2 μg/mL). Compounds were screened against drug-sensitive *Plasmodium falciparum* NF_{54} in vitro using the modified [^{3}H]hypoxanthine incorporation assay with chloroquine and artesunate as the positive controls.[20]

Extraction and Isolation. The cultures in individual flasks were each successively extracted overnight with 300 mL of methanol and ethyl acetate three times. The organic extracts were then combined, dried under reduced pressure, and partitioned using a modified Kupchan method into water, *n*-butanol, ethyl acetate, and *n*-hexane fractions. Preliminary assays indicated that the ethyl acetate fraction was active against *Candida albicans*, *Cryptococcus neoformans*, and methicillin-resistant *Staphylococcus aureus*. The ethyl acetate partition (23.5 g) was fractionated using VLC (170 g silica) with a step gradient of 100% *n*-hexane to 100% ethyl acetate, resulting in active enriched fractions and pure compounds **3** (158.2 mg) and **1** (22.2 mg). Fraction 5 was subjected to size-exclusion chromatography with Sephadex LH-20 followed by semipreparative HPLC (gradient elution of 60% to 100% of acetonitrile in water) to generate 7-hydroxy-3-(1-hydroxyethyl)-5-methoxy-3,4-dimethylisobenzofuran-1(3*H*)-one[7] (2.3 mg), 3,8-dihydroxy-3-hydroxymethyl-6-methoxy-4,5-dimethyl-isochromano-1-one[6] (3.1 mg), and nectriapyrone[4] (**10**, 5.8 mg). Fraction 6 was purified using semipreparative HPLC (gradient elution

DOI: 10.1021/acs.jnatprod.5b00204
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of 45% to 100% of acetonitrile in water) to obtain compounds **6** (1.3 mg), **9** (1.2 mg), **2** (11.3 mg), and isosclerone[5] (**11**, 4.5 mg). Fraction 7 was fractionated by ISCO flash chromatography (silica gel, 97.5% dichloromethane to 100% methanol gradient) followed by two separations using semipreparative HPLC (gradient elution of 15% to 100% acetonitrile in water) to give compounds **4** (1.9 mg), **5** (2.5 mg), **7** (2.4 mg), **8** (1.8 mg), and 3-acetyl-7-hydroxy-5-methoxy-3,4-dimethyl-3*H*-isobenzofuran-1-one[8] (14.0 mg).

*3-(R)-Heptyl-6,8-dihydroxy-5-methylisochromane-7-carboxylic acid (pseudoanguillosporin C, **2**):* yellowish-green solid; $[\alpha]^{23}_{D}$ −116 (*c* 0.065 in MeOH); UV (MeOH) λ_{max} (log ε) 215 (3.25), 250(3.30), 320 (3.30); IR (film) ν_{max} 3798, 3713, 3630, 2971, 2359, 1508, 1456, 1054, 1032, 1013 cm^{-1}; ^{1}H NMR (Table 1, 600 MHz) and ^{13}C NMR (Table 2, 151 MHz); HRESIMS *m*/*z* 321.1703 $[M - H]^-$ (calcd for $C_{18}H_{25}O_5$, 321.1702).

*3-(R)-Heptyl-6,8-dihydroxy-5-methylisochroman-1-one (soudanone A, **3**):* colorless crystalline solid; $[\alpha]^{23}_{D}$ −43 (*c* 0.065 in MeOH); UV (MeOH) λ_{max} (log ε) 210 (3.17), 230 (3.24), 255 (3.24), 320 (3.26); IR (film) ν_{max} 3694, 3680, 3630, 3115, 2971, 2864, 2843, 1646, 1455, 1346, 1054, 1032, 1013 cm^{-1}; ^{1}H NMR (Table 1, 600 MHz) and ^{13}C NMR (Table 2, 151 MHz); HRESIMS *m*/*z* 291.1595 $[M - H]^-$ (calcd for $C_{17}H_{23}O_4$, 291.1596).

*(R)-7-(6,8-Dihydroxy-5-methyl-1-oxoisochroman-3-yl)heptanoic acid (soudanone B, **4**):* dark green, amorphous solid; $[\alpha]^{23}_{D}$ −58 (*c* 0.065 in MeOH); UV (MeOH) λ_{max} (log ε) 215 (3.19), 230 (3.25), 255 (3.25), 295 (3.24); IR (film) ν_{max} 3713, 3679, 3115, 2971, 2864, 2843,1455, 1346, 1054, 1032, 1013 cm^{-1}; ^{1}H NMR (Table 1, 600 MHz) and ^{13}C NMR (Table 2, 151 MHz); HRESIMS *m*/*z* 321.1323 $[M - H]^-$ (calcd for $C_{17}H_{21}O_6$, 321.1338).

*(R)-7-(6,8-Dihydroxy-1-oxoisochroman-3-yl)heptanoic acid (soudanone C, **5**):* yellowish-green solid; $[\alpha]^{23}_{D}$ −52 (*c* 0.065 in MeOH); UV (MeOH) λ_{max} (log ε) 215 (3.20), 230 (3.26), 260 (3.26), 280 (3.24), 300 (3.25); IR (film) ν_{max} 3753, 3677, 3135, 2979, 1653,1541, 1456, 1054, 1032, 1013, 669 cm^{-1}; ^{1}H NMR (Table 1, 600 MHz) and ^{13}C NMR (Table 2, 151 MHz); HRESIMS *m*/*z* 307.1177 $[M - H]^-$ (calcd for $C_{16}H_{19}O_6$, 307.1182) .

*(R)-6,8-Dihydroxy-5-methyl-3-(6-oxoheptyl)isochroman-1-one (soudanone D, **6**):* yellowish-brown oil; $[\alpha]^{23}_{D}$ −40 (*c* 0.05 in MeOH); UV (MeOH) λ_{max} (log ε) 215 (3.32), 230 (3.38), 270 (3.36), 315 (3.38); IR (film) ν_{max} 3798, 3713, 2980, 2842, 2362, 1455, 1064, 1032, 1013 cm^{-1}; ^{1}H NMR (Table 1, 600 MHz) and ^{13}C NMR (Table 2, 151 MHz); HRESIMS *m*/*z* 305.1387 $[M - H]^-$ (calcd for $C_{17}H_{21}O_5$, 305.1389).

*(R)-6,8-Dihydroxy-3-(6-hydroxyheptyl)-5-methylisochroman-1-one (soudanone E, **7**):* yellowish-green solid; $[\alpha]^{25}_{D}$ −40 (*c* 0.065 in MeOH); UV (MeOH) λ_{max} (log ε) 215(3.22), 230 (3.28), 255 (3.28), 295(3.26); IR (film) ν_{max} 3833, 3737, 3649, 3629, 2979, 2360, 1508, 1054, 1032, 669 cm^{-1}; ^{1}H NMR (Table 1, 600 MHz) and ^{13}C NMR (Table 2, 151 MHz); HRAPCIMS *m*/*z* 309.1723 $[M + H]^+$ (calcd for $C_{17}H_{25}O_5$ 309.1702); HRESIMS *m*/*z* 307.1540 $[M - H]^-$ (calcd for $C_{17}H_{23}O_5$, 307.1545).

*(R)-6,8-Dihydroxy-3-(7-hydroxyheptyl)-5-methylisochroman-1-one (soudanone F, **8**):* yellowish-brown oil; $[\alpha]^{23}_{D}$ −55 (*c* 0.065 in MeOH); UV (MeOH) λ_{max} (log ε) 220 (3.19), 230 (3.25), 270 (3.23), 310 (3.21); IR (film) ν_{max} 3833, 3738, 3714, 2939, 2363, 1508, 1055, 1032 cm^{-1}; ^{1}H NMR (Table 1, 600 MHz) and ^{13}C NMR (Table 2, 151 MHz); HRESIMS *m*/*z* 307.1537 $[M - H]^-$ (calcd for $C_{17}H_{23}O_5$, 307.1545).

*(R)-5-(6,8-Dihydroxy-5-methyl-1-oxoisochroman-3-yl)pentyl acetate (soudanone G, **9**):* yellowish-brown oil; $[\alpha]^{23}_{D}$ −72 (*c* 0.05 in MeOH); UV (MeOH) λ_{max} (log ε) 220 (3.37), 240 (3.43), 270 (3.40), 315 (3.43); IR (film) ν_{max} 3713, 3678, 3650, 2940, 2842, 2358, 1653, 1456, 1054, 1032 cm^{-1}; ^{1}H NMR (Table 1, 600 MHz) and ^{13}C NMR (Table 2, 151 MHz); HRESIMS *m*/*z* 321.1337 $[M - H]^-$ (calcd for $C_{17}H_{21}O_6$, 321.1338).

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

1D and 2D NMR data for **2**−**9**, CD spectra for compounds **1**−**3**, and phylogenetic data for the fungus. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jnatprod.5b00204.

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We thank H. Irwin and C. Gelbmann for assistance with biological assays. We are grateful to the Minnesota DNR and Soudan Mine Park Staff for assistance with field collections in the Soudan Mine, especially J. Essig and A. Zavodnik. Funding for this work was provided in part by the Minnesota Environment and Natural Resources Trust Fund.

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DOI: 10.1021/acs.jnatprod.5b00204
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