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3-Nitroasterric Acid Derivatives from an Antarctic Sponge-Derived *Pseudogymnoascus* sp. Fungus

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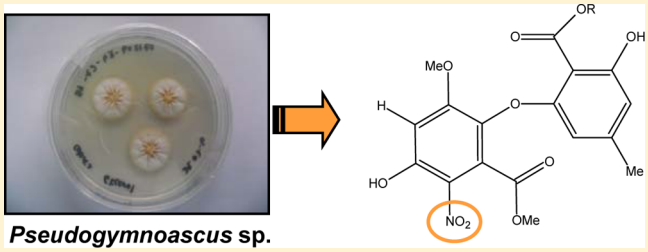
Supporting Information

ABSTRACT: Four new nitroasterric acid derivatives, pseudogymnoascins A–C (**1**–**3**) and 3-nitroasterric acid (**4**), along with the two known compounds questin and pyriculamide, were obtained from the cultures of a *Pseudogymnoascus* sp. fungus isolated from an Antarctic marine sponge belonging to the genus *Hymeniacidon*. The structures of the new compounds were determined by extensive NMR and MS analyses. These compounds are the first nitro derivatives of the known fungal metabolite asteric acid. Several asteric acid derivatives isolated from other fungal strains have shown antibacterial and antifungal activities. However, the new compounds described in this work were inactive against a panel of bacteria and fungi (MIC > 64 μg/mL).



Asteric acid[1] and its derivatives constitute a group of diphenyl ether compounds that have been isolated from fungi belonging to different genera.[2] These secondary metabolites have attracted considerable interest, as asteric acid was the first non-peptide compound to show endothelin-1 (ET-1) binding inhibition.[3] Furthermore, it has been claimed that a number of asteric acid derivatives are useful in the treatment of myocardial infarction and renal insufficiency[4] and, more recently, that the asteric acid analogues geomycins B and C display significant antifungal and antimicrobial activity, respectively.[2]

Fungi from extreme environments have proven to be outstanding producers of unusual chemical structures.[5] In particular, the few chemical studies carried out so far on Antarctic fungi have shown that they are able to produce compounds with atypical structural features that make them particularly interesting.[6] Thus, fungi from terrestrial and maritime Antarctica are attractive sources for the isolation of new secondary metabolites with novel structures.[6]

In the course of our ongoing investigations into new secondary metabolites from fungi isolated from Antarctic marine sponges, we focused our attention on the strain *Pseudogymnoascus* sp. F09-T18-1 isolated from a *Hymeniacidon* sp. sponge.[7] Chromatographic separation of the EtOAc extract obtained from the fermentation broth of this strain resulted in the isolation of four novel nitroasterric acid analogues, pseudogymnoascins A–C (**1**–**3**) and 3-nitroasterric acid (**4**), along with two known compounds, the nitro-diketopiperazine pyriculamide and the anthraquinone questin. Interestingly, this work represents the first description of asteric acid derivatives that contain a nitro group.

Cultures of the fermentation broth of *Pseudogymnoascus* sp. F09-T18-1 were extracted with EtOAc, and the organic extract, after concentration, was fractionated using our standard partition procedure.[8] The CH_2Cl_2 fraction obtained by this procedure was separated by gel-filtration chromatography on Sephadex LH-20 resin eluted with MeOH, followed by semi-preparative HPLC, to give compounds **1**–**4** and the known compounds pyriculamide and questin.

The molecular formula of compound **1** was determined to be $C_{22}H_{23}NO_{12}$ (12 degrees of unsaturation) based on the $[M - H]^-$ ion peak at m/z 492.1130 observed in the (−)-HRESIM spectrum and on NMR data (Table 1). The ^1H NMR spectroscopic data for **1** in CD_3OD (Table 1) showed two *meta*-coupled aromatic protons (δ_H 6.45 and 5.96, each d, J = 1.5 Hz) and one aromatic proton of a pentasubstituted benzene (δ_H 6.84, s). In addition to these aromatic protons, signals for one oxymethylene group (δ_H 4.47 and 4.44), three

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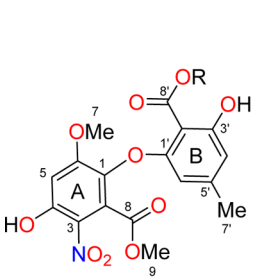

Compound	R
1	CO_2Me
2	CO_2H
3	CO_2H
4	H

methoxy groups (δ_H 3.82, 3.77, and 3.57), one methine proton (δ_H 2.90), and two methyl groups (one aromatic at δ_H 2.17 and one aliphatic at δ_H 1.22, which resonates as a doublet with J = 7.2 Hz) were also observed. The DEPT-135 edited HSQC experiment allowed assignment of each proton to its corresponding carbon atom. Analysis of the ^1H, ^{13}C, HSQC, and HMBC NMR spectroscopic data for **1** (Table 1) revealed the presence of structural features similar to those found in ethyl asterrate.[2] The aromatic methyl protons resonating at δ_H 2.17 (H_3-7′) displayed HMBC correlations with the quaternary carbon C-5′ (δ_C 146.8) and the aromatic methine carbons C-4′ (δ_C 107.6) and C-6′ (δ_C 112.5). These correlations, along with the HMBC cross-peaks between the aromatic proton at δ_H 5.96 and carbons C-2′ (δ_C 104.4) and C-6′ (δ_C 112.5), are indicative of an identical B-ring to that of ethyl asterrate. However, significant differences were found in the A-ring. The aromatic proton resonating at δ_H 6.84 was assigned as H-5 according to the HMBC correlations with the nonprotonated aromatic carbons C-1 (δ_C 135.0), C-3 (δ_C 124.4), C-4 (δ_C 156.4), and C-6 (δ_C 160.3), with the latter carbon also showing an HMBC correlation to H_3-7 at δ_H 3.82. The absence of the proton signal corresponding to H-3 and the downfield chemical shift of C-3 (δ_C 124.4) in **1** compared to that (δ_C 108.4) for the same carbon in ethyl asterrate[2] are indicative of the presence of a strong electron-withdrawing group. This strong electron-withdrawing group could be located at the C-3 carbon on the basis of the observed deshielding effect at the C-6 carbon (δ_C 160.3) when compared to the chemical shift (δ_C 153.9) for the same carbon in ethyl asterrate. These spectroscopic data are consistent with the deshielding effect of a nitro group located at C-3. Furthermore, the signals due to the ethoxy unit attached to C-8′ in ethyl asterrate were replaced by signals for a (2-methoxycarbonyl)propoxy moiety in the NMR spectra of **1**. This observation was supported by HMBC correlations from H_3-11′ to C-9′ (δ_C 67.5), C-10′ (δ_C 40.2), and C-12′ (δ_C 174.7) along with the HMBC cross-peak between H_3-13′ (δ_H 3.57) and the carbonyl ester C-12′. On the basis of these data, the structure of pseudogymnoascin A (**1**) was established as (2-methoxycarbonyl)propyl-3- nitroasterrate.

The molecular formula of pseudogymnoascin B (**2**) was established as $C_{21}H_{21}NO_{12}$ (12 degrees of unsaturation) based on the $[M - H]^-$ ion peak observed at m/z 478.0964 in the (−)-HRESIM spectrum and on NMR data (Table 1). These data indicate a decrease of 14 mass units (CH_2) compared to compound **1**. The presence of the nitro moiety in **2** was further confirmed by IR absorptions characteristic for the NO_2 group at 1537 and 1338 cm^{-1}. The absence in the ^1H and ^{13}C NMR spectra of **2** of the signals corresponding to the methoxy group attached to C-12′ in **1** (δ_H/δ_C 3.57/52.3) suggested that **2** must be the demethylated analogue at C-12′ of **1**. This proposal is consistent with the carbon chemical shift of C-12′ at δ_C 177.2, which is characteristic of a carboxylic acid functionality, in the ^{13}C NMR spectrum of **2** as opposed to δ_C 174.7 for the ester carbonyl in that position in compound **1**. Long-range correlations in the HMBC experiment on **2** from the H_3-11′

Table 1. NMR Data for Pseudogymnoascins A (1) and B (2) in CD_3OD

	pseudogymnoascin A (**1**)			pseudogymnoascin B (**2**)		
position	δ_C[a] mult	δ_H[b] mult (J in Hz)	HMBC (H→C#)	δ_C[a] mult	δ_H[b] mult (J in Hz)	HMBC (H→C#)
1	135.0, C			135.3, C		
2	127.0, C			127.0, C		
3	124.4, C			124.8, C		
4	156.4, C			156.0, C		
5	104.7, CH	6.84, s	1, 3, 4, 6	104.0, CH	6.86, s	1, 3, 4, 6
6	160.3, C			160.5, C		
7	57.4, CH_3	3.82, s	6	57.5, CH_3	3.82, s	
8	165.4, C			165.6, C		
9	53.6, CH_3	3.77, s	8	53.7, CH_3	3.79, s	
1′	nd[c]			162.6, C		
2′	104.4, C			103.8, C		
3′	nd			159.6, C		
4′	107.6, CH	5.96, d (1.5)	2′, 6′	107.6, CH	5.96, brs	2′, 6′, 7′
5′	146.8, C			146.7, C		
6′	112.5, CH	6.45, d (1.5)	4′	112.6, CH	6.45, brs	2′, 4′, 7′
7′	21.9, CH_3	2.17, s	4′, 5′, 6′	21.8, CH_3	2.17, s	4′, 5′, 6′
8′	nd			170.7, C		
9′	67.5, CH_2	4.47, dd (6.5, 10.9)	12′	67.7, CH_2	4.51, dd (6.1, 10.8)	
		4.44, dd (5.5, 10.9)			4.39, dd (6.1, 10.8)	
10′	40.2, CH	2.90, ddq (7.2, 6.5, 5.5)		40.1, CH	2.85, ddq (7.1, 6.1, 6.1)	
11′	14.0, CH_3	1.22, d (7.2)	9′, 10′, 12′	14.4, CH_3	1.23, d (7.1)	9′, 10′, 12′
12′	174.7, C			177.2, C		
13′	52.3, CH_3	3.57, s	12′			

[a]Recorded at 125 MHz. [b]Recorded at 500 MHz [c]nd = not detected.

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Table 2. NMR Data for Pseudogymnoascin C (3) and 3-Nitroasterric Acid (4)

	pseudogymnoascin C (3)					3-nitroasterric acid (4)	
position	δ_C[a] mult	δ_H[b] mult (J in Hz)	δ_C[c] mult	δ_H[d] mult (J in Hz)	HMBC[e] (H→C#)	δ_C[a] mult	δ_H[b] mult (J in Hz)
1	135.1, C		134.3, C			nd[f]	
2	127.2, C		125.4, C			nd	
3	125.9, C		124.5, C			nd	
4	156.0, C		156.2, C			nd	
5	103.9, CH	6.89, s	102.5, CH	6.70, s	1, 3, 4, 6	103.9, CH	6.88, s
6	160.6, C		159.9, C			nd	
7	57.5, CH_3	3.86, s	57.2, CH_3	3.85, s	6	57.5, CH_3	3.82, s
8	165.4, C		163.4, C			nd	
9	53.6, CH_3	3.81, s	53.6, CH_3	3.81, s	8	53.6, CH_3	3.79, s
1′	162.4, C		163.9, C			nd	
2′	nd		nd			nd	
3′	159.4, C		158.4, C			nd	
4′	107.2, CH	5.93, brs	106.8, CH	5.91, brs		107.1, CH	5.90, brs
5′	146.8, C		147.4, C			nd	
6′	112.7, CH	6.45, brs	112.9, CH	6.53, brs	4′, 7′	112.6, CH	6.42, brs
7′	21.9, CH_3	2.18, s	22.3, CH_3	2.20, s	4′, 5′, 6′	21.8, CH_3	2.16, s
8′	169.7, C		169.7, C			nd	
9′	71.2, CH	5.27, q (7.0)	69.8, CH	5.43, q (7.0)			
10′	17.4, CH_3	1.55, d (7.0)	17.2, CH_3	1.65, d (7.0)	9′, 11′		
11′	174.6, C		171.6, C				
HO-4				11.22, s	3, 4, 5		
HO-11′				10.96, brs			

[a]Recorded at 125 MHz in CD_3OD. [b]Recorded at 500 MHz in CD_3OD. [c]Recorded at 125 MHz in $CDCl_3$. [d]Recorded at 500 MHz in $CDCl_3$. [e]Recorded in $CDCl_3$. [f]nd = not detected.

protons at δ_H 1.23 (d, J = 7.1 Hz) to C-12′ (δ_C 177.2), C-9′ (δ_C 67.7), and C-10′ (δ_C 40.1) confirmed these assignments. The remaining ^{1}H, ^{13}C, and 2D NMR data for **2** were very similar to those of **1** (Table 1). Therefore, the structure of pseudogymnoascin B (**2**) was established as 2-carboxypropyl-3-nitroasterrate.

The molecular formula of pseudogymnoascin C (**3**) was determined as $C_{20}H_{19}NO_{10}$ (12 degrees of unsaturation) on the basis of the $[M - H]^-$ ion peak observed at m/z 464.0833 in the (−)-HRESIM spectrum and on NMR data (Table 2). The lack of one methylene unit in **3** was revealed by comparison of this molecular formula with that of **2**. This was confirmed by the absence of signals in the ^{1}H and ^{13}C NMR spectra in CD_3OD of **3** corresponding to the C-9′ oxymethylene group in **2** (δ_H/δ_C 4.51 and 4.39/67.7). Instead, a signal for an oxymethine proton was present in the ^{1}H NMR spectrum of **3** at δ_H 5.27, and this resonated as a quartet with a coupling constant of 7.0 Hz, which means that it is coupled to an aliphatic methyl group at δ_H 1.55 (d, J = 7.0 Hz). These data indicate the presence of a 1-carboxyethyl group in **3** instead of the 2-carboxypropyl group in **2**. The presence of this moiety in **3** was confirmed on acquiring the NMR spectrum in $CDCl_3$. Thus, an HMBC experiment on **3** in $CDCl_3$ showed long-range correlations between the H_3-10′ protons at δ_H 1.65 (d, J = 7.0 Hz) and the oxymethine carbon at δ_C 69.8 (C-9′) and the carboxylic acid signal at δ_C 171.6 (C-11′). Furthermore, the ^{1}H NMR spectrum of **3** in $CDCl_3$ showed the presence of an OH group (δ_H 11.22) attached to C-4, which in the HMBC experiment displayed correlations to C-5 (δ_C 102.5), C-4 (δ_C 156.2), and C-3 (124.5). The downfield shift of this OH signal was attributed to strong hydrogen bonding between its proton and the oxygen of the nitro group linked to C-3. Additionally, proton assignments in **3** were confirmed by a ROESY experiment on **3** in $CDCl_3$, which showed NOE correlations between H-5 at δ_H 6.70 and H_3-7 OMe protons at δ_H 3.85, between the H_3-7′ Me protons at δ_H 2.20 and H-4′ at δ_H 5.91 and H-6′ at δ_H 6.53 aromatic protons, and between the OH of the C-11 carboxylic acid at δ_H 10.96 and the H_3-10′ Me protons at δ_H 1.65 (d, J = 7.0 Hz). With these data, the structure of pseudogymnoascin C (**3**) was established as 1-carboxyethyl-3-nitroasterrate.

Finally, the $[M - H]^-$ ion peak at m/z 392.0642 observed in the HRESIM spectrum of compound **4** suggested that its molecular formula is $C_{17}H_{15}NO_{10}$ (11 degrees of unsaturation). Comparison of the NMR data of **4** with those of **1**−**3** revealed the absence of signals corresponding to the fragment attached to C-8′ in **1**−**3**, indicating that this fragment was not present. The structure of **4** was consistent with the 3-nitro analogue of asterric acid, and therefore it was named 3-nitroasterric acid.

The presence of the same set of m/z peaks in the (−)-LRESIMS of compounds **1**−**4** allowed us to propose a mass fragmentation pathway for these types of compounds, as displayed in Figure 1. The molecular formula of the most prominent fragments were confirmed by the (−)-HRESIMS of compound **2**. Thus, the presence of a diagnostically important peak at m/z 242 in the (−)-LRESI mass spectra of **2**−**4** is consistent with nitro and methoxycarbonyl groups linked to ring A.

The known metabolites questin and pyriculamide were easily identified by comparison of the NMR and MS data with those reported in the literature. Questin has previously been isolated from numerous *Aspergillus* species,[9] as well as *Eurotium*,[9] *Chrysosporium*,[10] and *Microascus tardifaciens* isolates.[11] Pyriculamide, which was isolated as a racemic mixture in this work ($[\alpha]^{23}_D$ +0.6 (*c* 0.25, MeOH)), is an unusual nitrated 2,5-diketopiperazine produced by the fungus *Pyricularia oryzae*[12] and by an Arctic ice *Salegentibacter* sp. bacterium.[13]

Taking into account the antibacterial and antifungal activities displayed by some asterric acid derivatives,[2] the new metabolites

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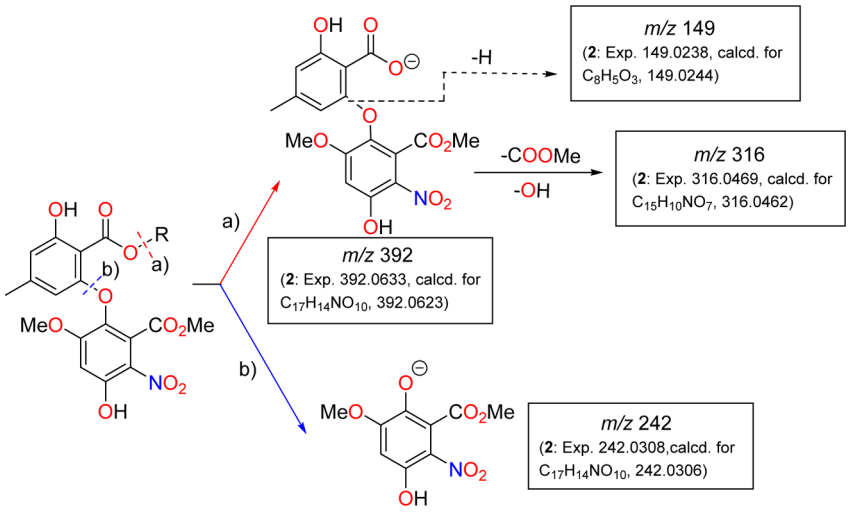

Figure 1. Proposed (−)-ESIMS fragmentation pathway of compounds **1**−**4** and (−)-HRESIMS of the fragments in compound **2**.

1−**4** were tested for their antibacterial and antifungal activities against a panel of bacteria (*Pseudomonas aeruginosa* PAO1, *Acinetobacter baumannii* CL 5973, *Escherichia coli* MB2884, and two *Staphylococcus aureus* strains: a methicillin-sensitive *S. aureus* EPI167 and methicillin-resistant *S. aureus* MB5393) and against three fungal strains (*Candida albicans* MY1055, *C. albicans* ATCC64124, and *Aspergillus fumigatus* ATCC46645). Unfortunately, the compounds did not show activity against any of the microorganisms tested at MIC > 64 μg/mL.

The lack of antibacterial and antifungal activities for 3-nitroasterric acid derivatives **1**−**4** in relation to bioactive asterric acid derivatives[2] suggests that the size of the substituent at C-8′ and/or the presence of a nitro group in the molecule have a marked influence on the activity of these compounds.

A study of the triacylglyceride composition and fatty acyl saturation profile of *Pseudogymnoascus destructans* was published recently.[14] Until this study, there was no work published in the literature on secondary metabolites from the genus *Pseudogymnoascus*. However, it is important to note that the taxonomic position of *Pseudogymnoascus* fungi and their relatives *Geomyces* is currently a matter of debate. Recently, several species formerly recognized as *Geomyces* were transferred to the genus *Pseudogymnoascus*.[15] For example, the substituted *cis*-decalin pannomycin was isolated from *G. pannorum*,[16] which was later identified as *Pseudogymnoascus pannorum*.[15] Interestingly, another chemical investigation into a *Geomyces* strain isolated from an Antarctic soil sample concerned the isolation of asterric acid and several derivatives.[2] While the purpose of this article is not to discuss the taxonomy of the genus *Pseudogymnoascus*, the isolation of these nitroasterric acid derivatives could contribute to the proper classification of these genera in the future through chemotaxonomy.

Natural products that include a nitro group are rather unusual in nature,[17] and, in particular, they have rarely been isolated as fungal metabolites. Among the few examples of nitrated compounds reported from fungal strains are the aforementioned pyriculamide, psycrophilins A−C produced by the psychrotolerant fungus *Penicillium ribeum*,[18] four nitrated naphthalene derivatives produced by the endophytic fungus *Coniothryium* sp.,[19] and nitrobenzyl derivatives produced by an unidentified mangrove endophytic fungus.[20] Thus, the isolation of compounds **1**−**4** represents the first description of nitrated asterric acid derivatives in nature and as filamentous fungal metabolites.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured on a JASCO DIP-1000 polarimeter, with a Na (589 nm) lamp and filter. UV spectra were measured on a JASCO V-650 spectrophotometer. IR spectra were measured on FTIR Bruker Vector 22 spectrometer. ^{1}H, ^{13}C, and 2D NMR spectra were recorded on a Bruker Avance 500 spectrometer at 500.13 and 125.0 MHz, respectively, using CD_3OD or $CDCl_3$ as solvents. LRESIMS and HRESIMS experiments were performed on an Applied Biosystems QSTAR Elite system. HPLC separations were performed on an Agilent 1100 liquid chromatography system equipped with a solvent degasser, quaternary pump, and diode array detector (Agilent Technologies, Waldbronn, Germany). Precoated silica gel plates (Merck, Kieselgel 60 F254, 0.25 mm) were used for TLC analysis, and the spots were visualized with UV light (254 nm) and/or by heating the plate pretreated with H_2SO_4/H_2O/AcOH (1:4:20).

Fungal Material. The fungal strain *Pseudogymnoascus* sp. F09-T18-1 used in this work was isolated from a sample of an Antarctic marine sponge collected at Fildes Bay, King George Island, and identified according to its morphological traits and by the amplification and sequencing of the DNA of the ITS region of the rRNA gene as described previously.[7] The sequence is available in GenBank with accession number JX845296.

Fermentation and Extraction. The fungal strain was cultured on slants with potato dextrose agar (PDA) at 22 °C for 10 days. After this time, mycelia and spores from a PDA plate were harvested and grown in 100 mL of CYA medium for 48 h at 22 °C and 200 rpm. Aliquots (20 mL each) of this seed culture were inoculated in 36 1-L Erlenmeyer flasks, each containing 200 mL of medium (0.3% $NaNO_3$; 3.0% glucose; 0.5% yeast extract; 0.13% $K_2HPO_4{\cdot}3H_2O$; 0.05% $MgSO_4{\cdot}7H_2O$; 0.05% KCl; 0.001% $FeSO_4{\cdot}7H_2O$; 0.0005% $CuSO_4{\cdot}5H_2O$; 0.001% $ZnSO_4{\cdot}7H_2O$; 5% NaCl; pH 6.3). The samples were incubated at 15 °C and 100 rpm for 12 days. The filtered fungal broth (7.2 L) was extracted exhaustively with EtOAc (3 × 2 L) at room temperature. The organic phase was concentrated under reduced pressure to give 3.1 g of a red oil.

Isolation and Purification. The oily residue (3.1 g) was partitioned between EtOAc/H_2O (1:1 v/v). The organic phase was concentrated under reduced pressure and partitioned between 10% aqueous MeOH (400 mL) and hexane (2 × 400 mL). The H_2O content (% v/v) of the methanolic fraction was adjusted to 50% aqueous MeOH, and this mixture was extracted with CH_2Cl_2 (100 mL). The fraction that was soluble in CH_2Cl_2 (739 mg) was subjected to gel filtration chromatography on Sephadex LH-20 (eluted with MeOH) to obtain eight fractions (DCF1−DCF8). Fraction DCF6 (115 mg) was again submitted to gel filtration chromatography on Sephadex LH-20 (eluted with *n*-hexane/CH_2Cl_2/MeOH, 1:3:2) to afford seven

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fractions (DCF6-1 to DCF6-7). These fractions were separated by semipreparative HPLC using a Discovery HS F5 (250 mm × 10 mm, 5 μm) column from Supelco with a mobile phase consisting of a 30 min gradient from 45% to 75% MeOH in H_2O (v/v, each containing 0.1% formic acid) followed by 20 min isocratic at 75% MeOH in H_2O at a flow rate of 1.7 mL/min. Separation of fraction DCF6-3 (20 mg) by semipreparative HPLC under these conditions yielded the compounds pyriculamide (1.0 mg; t_R = 21.4 min), **2** (2.8 mg; t_R = 43.0 min), and **1** (0.9 mg; t_R = 52.4 min). Fraction DCF6-4 (29 mg) was purified by HPLC to yield compounds **4** (0.8 mg; t_R = 39.4 min) and **3** (1.0 mg; t_R = 42.9 min). Separation of fraction DCF7 (163 mg) by HPLC yielded questin (14 mg; t_R = 29.1 min).

*Pseudogymnoascin A (**1**):* yellow powder; $[\alpha]^{23}_D$ −18 (*c* 0.05, MeOH); UV (MeOH) λ_{max} (log ε) 209 (3.42), 270 (2.86), 301 (2.72), 359(2.60) nm; ^{1}H, ^{13}C, and HMBC NMR data in Table 1; (−)-HRESIMS *m/z* 492.1130 $[M - H]^-$ (calcd for $C_{22}H_{22}NO_{12}$, 492.1147, Δ 1.7 mmu).

*Pseudogymnoascin B (**2**):* yellow powder; $[\alpha]^{23}_D$ +25 (*c* 0.15, MeOH); UV (MeOH) λ_{max} (log ε) 210 (3.91), 254(3.81), 312 (3.61), 358 (3.44) nm; IR (neat) ν_{max} 2926, 2853, 1738, 1657, 1615, 1586, 1537, 1440, 1395, 1338, 1257, 1202, 1119, 1064, 1028, 752 cm^{-1}; ^{1}H, ^{13}C, and HMBC NMR data in Table 1; (−)-HRESIMS *m/z* 478.0964 $[M - H]^-$ (calcd for $C_{21}H_{20}NO_{12}$, 478.0991, Δ 2.7 mmu).

*Pseudogymnoascin C (**3**):* yellow powder; $[\alpha]^{23}_D$ +17 (*c* 0.08, MeOH); UV (MeOH) λ_{max} (log ε) 209 (3.23), 241 (2.84), 302(2.60), 359 (2.51) nm; ^{1}H, ^{13}C, and HMBC NMR data in Table 2; (−)-HRESIMS *m/z* 464.0833 $[M - H]^-$ (calcd for $C_{20}H_{17}NO_{10}$, 464.0834, Δ 1.0 mmu).

*3-Nitroasterric acid (**4**):* UV (MeOH) λ_{max} (log ε) 209 (3.28), 249 (2.80), 301 (2.56), 351 (2.46) nm; ^{1}H and ^{13}C NMR data in Table 2; (−)-HRESIMS *m/z* 392.0642 $[M - H]^-$ (calcd for $C_{17}H_{15}NO_{10}$, 392.0623, Δ 1.9 mmu).

Antimicrobial Assays. Antibacterial susceptibility was tested against *Pseudomonas aeruginosa* PAO1, *Acinetobacter baumannii* CL5973, *Escherichia coli* MB2884, and two *Staphylococcus aureus* strains, a methicillin-sensitive *S. aureus* EPI 167 and methicillin-resistant *S. aureus* MB5393. Antifungal susceptibility was tested against two *Candida* strains, *C. albicans* MY1055 and *C. albicans* ATCC 64124, and also against *Aspergillus fumigatus* ATCC46645. Previously described methods were used to test for antimicrobial and antifungal activities.[21] Each compound was serially diluted in DMSO with a dilution factor of 2 to provide 10 concentrations starting at 64 μg/mL for all the assays. The MIC was defined as the lowest concentration of an antimicrobial or antifungal compound that inhibited ≥95% of the growth of a microorganism after overnight incubation. The Genedata Screener software (Genedata, Inc.) was used to process and analyze the data and also to calculate the RZ′ factor, which predicts the robustness of an assay.[22] In all experiments performed in this work the RZ′ factor obtained was between 0.89 and 0.97.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

^{1}H, ^{13}C, HSQC, and HMBC NMR spectra and (−)-HRESIMS data of pseudogymnoascins A−C (**1**−**3**) and 3-nitroasterric acid (**4**). FT-IR data of pseudogymnoascin B (**2**). ROESY spectrum of pseudogymnoascin C (**3**). This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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