Bioactive Asterric Acid Derivatives from the Antarctic Ascomycete Fungus *Geomyces* sp.

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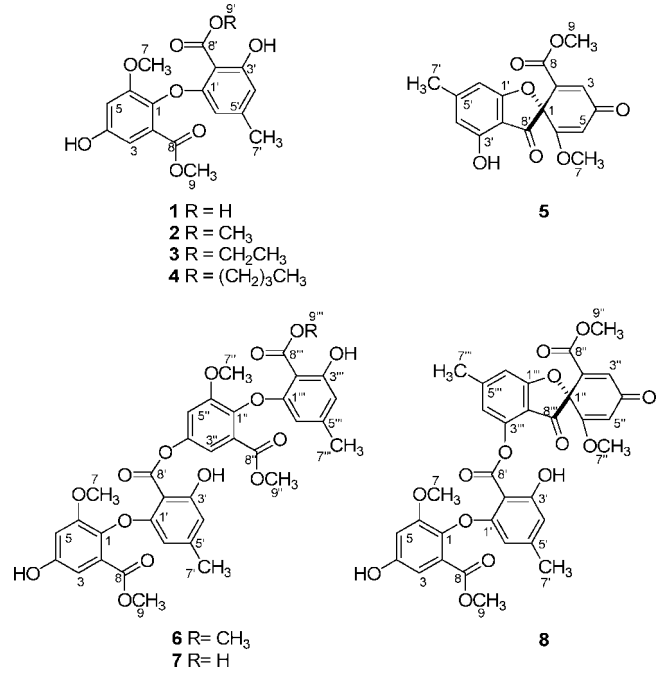
Five new asterric acid derivatives, ethyl asterrate (**3**), *n*-butyl asterrate (**4**), and geomycins A−C (**6**−**8**), have been isolated from cultures of an isolate of the Antarctic ascomycete fungus *Geomyces* sp. The structures of these metabolites were elucidated by NMR spectroscopy. The absolute configuration of **8** was determined by application of the CD excitation chirality method. Compound **7** displayed antifungal activity against *Aspergillus fumigatus*, whereas **8** showed antimicrobial activities against Gram-positive and Gram-negative bacteria.

Asterric acid,[1] a diphenyl ether isolated from *Aspergillus terreus*, *Oospora sulfurea-ochracea*, *Penicillium frequentans*, *Scytalidium* spp., and *Phoma* sp.,[1−8] is the first nonpeptide endothelin (ET) binding inhibitor discovered from natural sources.[1] It is also an inhibitor of vascular endothelial growth factor (VEGF) and an endothelin receptor antagonist for the treatment of early stage idiopathic pulmonary fibrosis.[2,3] Although a number of asterric acid derivatives have been claimed to be useful in the treatment of myocardial infarction and renal insufficiency,[4] none of them has been reported to display antifungal activity.[5,6]

As part of an ongoing search for new antifungal and antibacterial natural products from fungal species inhabiting unique environments, a subculture of an isolate of *Geomyces* sp., obtained from a soil sample that was collected at the Fildes Peninsula of King George Island, Antarctica, was grown in solid-substrate fermentation culture. Its organic solvent extract displayed significant antifungal activity against *Aspergillus fumigatus* (ATCC 10894) and antibacterial activities against *Staphylococcus aureus* (ATCC 6538), *Streptococcus pneumoniae* (CGMCC 1.1692), and *Escherichia coli* (CGMCC 1.2340). Bioassay-directed fractionation of this extract afforded two new diphenyl ethers, ethyl asterrate (**3**; synthetically known) and *n*-butyl asterrate (**4**), and three other new complex asterric acid derivatives, which we named geomycins A−C (**6**−**8**). In addition, three known compounds, asterric acid (**1**),[7] methyl asterrate (**2**),[7,8] and bisdechlorogeodin (**5**),[9−11] were also isolated from the crude extract. Details of the isolation, structure elucidation, and biological activities of these compounds are reported herein.

The known metabolites asterric acid (**1**) and methyl asterrate (**2**) were isolated as the major components from the crude extract, and their structures were readily identified by comparison of the NMR and MS data with those reported.[5] Methyl asterrate (**2**) was previously isolated from *Oospora* sp. and also named as trimethylosoic acid,[8] whereas bisdechlorogeodin (**5**), initially identified from both *Oospora* sp. and *Chrysosporium* sp., was believed to be an important precursor in the biosynthesis of asterric acid (**1**).[9,11]

The molecular formula of compound **3** was determined to be $C_{19}H_{20}O_8$ (10 degrees of unsaturation) on the basis of its HRESIMS (*m*/*z* 399.1017 $[M + Na]^+$) and NMR data (Table 1). Analysis of the ^1H, ^{13}C, and HMQC NMR spectroscopic data of **3** revealed the presence of structural features similar to those found in **1**, except that the hydroxy group attached to C-8′ (δ_C 171.1) was replaced by signals for an ethoxy unit (δ_H/δ_C 4.41, 1.34/61.3, 14.2) in the NMR spectra of **3**, and this observation was supported by relevant $^1H-^1H$ COSY correlations, as well as HMBC cross-peaks from H_3-10′ to C-9′ and from H_2-9′ to C-8′ and C-10′. On the basis of these data, the structure of **3** was established as ethyl asterrate.



Compound **4** was assigned a molecular formula of $C_{21}H_{24}O_8$ by analysis of its HRESIMS (*m*/*z* 427.1373 $[M + Na]^+$) and NMR data (Table 1), and comparison of this formula with that of **3** revealed the presence of two more methylene units. Analysis of the $^1H-^1H$ coupling patterns for these methylene protons suggested the presence of a butoxy unit attached to C-8′ in **4**, instead of an ethoxy group in **3**. $^1H-^1H$ COSY NMR data verified the identity of the *n*-butyl group, and HMBC correlations from the newly observed oxygenated methylene protons (H_2-9′) to C-8′ further confirmed this assignment. Therefore, the structure of **4** was established as *n*-butyl asterrate.

The molecular formula of geomycin A (**6**) was established as $C_{35}H_{32}O_{15}$ (20 degrees of unsaturation) on the basis of HRESIMS analysis (*m*/*z* 715.1678 $[M + Na]^+$) and the NMR data (Table 2). Interpretation of the ^1H, ^{13}C, and HMQC NMR spectroscopic data of **6** revealed the presence of three phenolic protons (two of which are intramolecularly hydrogen-bonded, as evidenced by their downfield ^1H NMR chemical shift values), seven methyl groups

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Table 1. NMR Data of Ethyl Asterrate (**3**) and Butyl Asterrate (**4**) in $CDCl_3$

	ethyl asterrate (**3**)			butyl asterrate (**4**)	
position	δ_H[a] (*J* in Hz)	δ_C,[b] mult.	HMBC (H → C#)	δ_H[a] (*J* in Hz)	δ_C,[b] mult.
1		136.4, qC			135.7, qC
2		125.8, qC			125.6, qC
3	6.96, d (2.5)	108.4, CH	1, 4, 5, 8	6.96, d (2.5)	108.5, CH
4		153.5, qC			154.5, qC
5	6.71, d (2.5)	105.0, CH	1, 3, 4, 6	6.70, d (2.5)	105.2, CH
6		153.9, qC			153.6, qC
7	3.75, s	56.4, CH_3	6	3.71, s	56.3, CH_3
8		166.0, qC			165.9, qC
9	3.68, s	52.2, CH_3	8	3.65, s	52.0, CH_3
1′		159.9, qC			160.2, qC
2′		101.0, qC			100.9, qC
3′		163.0, qC			162.9, qC
4′	6.41, s	110.9, CH	2′, 3′, 6′, 7′	6.38, s	110.7, CH
5′		145.8, qC			145.7, qC
6′	5.76, s	105.8, CH	1′, 2′, 4′, 7′, 8′	5.75, s	105.8, CH
7′	2.13, s	22.0, CH_3	4′, 5′, 6′	2.11, s	22.0, CH_3
8′		171.1, qC			171.2, qC
9′	4.41, d (7.0)	61.3, CH_2	8′, 10′	4.33, s	64.9, CH_2
10′	1.34, t (7.0)	14.2, CH_3	9′	1.66, m	30.5, CH_2
11′				1.40, m	18.9,CH_2
12′				0.85, t (7.5)	13.5, CH_3
OH-4	6.06, s			8.40, s	
OH-3′	11.56, s		2′, 3′, 4′	11.56, s	

[a] Recorded at 500 MHz. [b] Recorded at 100 MHz.

Table 2. NMR Data of Geomycins A−C (**6**−**8**) in $CDCl_3$

	geomycin A (**6**)			geomycin B (**7**)		geomycin C (**8**)		
position	δ_H[a] (*J* in Hz)	δ_C,[b] mult.	HMBC (H → C#)	δ_H[a] (*J* in Hz)	δ_C[c], mult.	δ_H[a] (*J* in Hz)	δ_C,[b] mult.	HMBC (H → C#)
1		135.6, qC			136.6, qC		136.6, qC	
2		125.5, qC			125.2, qC		126.2, qC	
3	6.98, d (3.0)	108.6, CH	1, 5, 8	6.97, d (3.0)	108.4, CH	6.93, d (3.0)	108.4, CH	1, 4, 5, 8
4		154.8, qC			153.9, qC		153.2, qC	
5	6.73, d (3.0)	105.1, CH	1, 3, 4, 6	6.75, d (3.0)	104.9, CH	6.69, d (3.0)	104.0, CH	1, 3, 4
6		153.5[d], qC			153.4[d], qC		154.0, qC	
7	3.76, s	56.3, CH_3	6	3.81, s	56.4, CH_3	3.74, s	56.5, CH_3	6
8		165.5, qC			165.2, qC		166.0, qC	
9	3.74, s	52.2, CH_3	8	3.80, s	52.9, CH_3	3.67, s	52.4, CH_3	8
1′		160.4, qC			160.2, qC		160.0, qC	
2′		100.1, qC			100.0, qC		100.4, qC	
3′		163.5, qC			163.5, qC		162.8, qC	
4′	6.46, s	111.3, CH	2′, 3′, 6′, 7′	6.50, s	111.4, CH	6.46, s	111.2, CH	2′, 3′, 6′, 7′
5′		147.4, qC			147.8, qC		147.0, qC	
6′	5.86, s	106.3, CH	1′, 2′, 4′, 7′	5.86, s	106.3, CH	5.81, s	106.0, CH	1′, 2′, 4′, 8′
7′	2.13, s	22.2, CH_3	4′, 5′, 6′	2.20, s	22.3, CH_3	2.16, s	22.2, CH_3	4′, 5′, 6′
8′		169.7, qC			169.5, qC		167.6, qC	
1″		140.8, qC			138.5, qC		84.1, qC	
2″		125.9, qC			125.6, qc		138.2, qC	
3″	7.38, d (2.5)	115.9, CH	1″, 4″, 5″, 8″	7.51, d (2.0)	116.5, CH	7.09, s	137.0, CH	1″, 5″, 8″
4″		147.4, qC			148.7, qC		185.4, qC	
5″	7.07, d (2.5)	111.1, CH	1″, 3″, 4″, 6″	7.18, d (2.0)	111.7, CH	5.77, s	100.4, CH	1″, 3″, 4″, 6″
6″		153.6[d], qC			153.5[d], qC		169.0, qC	
7″	3.77, s	56.6, CH_3	6″	3.82, s	56.6, CH_3	3.68, s	56.8, CH_3	6″
8″		164.8, qC			164.1, qC		163.3, qC	
9″	3.69, s	52.1, CH_3	8″	3.77, s	52.3, CH_3	3.66, s	52.9, CH_3	8″
1‴		159.3, qC			157.3, qC		147.0, qC	
2‴		100.9, qC			99.9, qC		112.3, qC	
3‴		163.1, qC			163.7, qC		173.4, qC	
4‴	6.42, s	111.2, CH	2‴, 3‴, 6‴, 7‴	6.54, s	113.1, CH	6.86, s	110.9, CH	1‴, 2‴, 3‴, 6‴, 7‴
5‴		146.1, qC			146.9, qC		151.4, qC	
6‴	5.78, s	106.0, CH	1‴, 2‴, 4‴, 7‴	5.76, s	104.5, CH	6.80, s	117.0, CH	1‴, 2‴, 4‴, 7‴
7‴	2.18,s	22.0, CH_3	4‴, 5‴, 6‴	2.17, s	22.2, CH_3	2.46, s	22.7, CH_3	4‴, 5‴, 6‴
8‴		171.6, qC			170.5, qC		189.9, qC	
9‴	3.92, s	52.4, CH_3	8‴					
OH-4	8.40, s		3, 4, 5	5.31, s		5.22, s		
OH-3′	11.07, s		2′, 3′, 4′	11.00, s		10.62, s		2′, 3′, 4′
OH-3‴	11.54, s		2‴, 3‴, 4‴	11.91, s				
OH-9‴				11.07, br				

[a] Recorded at 500 MHz. [b] Recorded at 100 MHz. [c] Recorded at 150 MHz. [d] These assignments are interchangeable.

(five *O*-methyls), 24 aromatic carbons (10 of which are oxygenated), and four carboxyl carbons. Comparison of the NMR data of **6** with those of **1** and **2** indicated that **6** could be the condensation product resulting from the esterification or etherification of compounds **1**

and **2**. In addition, the molecular formula of **6** ($C_{35}H_{32}O_{15}$) is equivalent to the summation of those for **1** ($C_{17}H_{16}O_8$) and **2** ($C_{18}H_{18}O_8$) subtracting one H_2O molecule, suggesting the formation of an ether/ester linkage between **1** and **2** to satisfy its structural requirement. The connectivity between the two substructures of **6** was established by analysis of its HMBC data (Table 2). HMBC correlations from the phenolic proton at δ_H 8.40 (OH-4) to C-3, C-4, and C-5, from the phenolic proton at δ_H 11.07 (OH-3′) to C-2′, C-3′, and C-4′, and from the third phenolic proton at δ_H 11.54 (OH-3‴) to C-2‴, C-3‴, and C-4‴ indicated that C-4, C-3′, and C-3‴ are all attached to hydroxy groups. These results required the formation of an ester linkage between C-4′ and C-4″ to complete the gross structure of geomycin A as **6**.

Geomycin B (**7**) was assigned the molecular formula $C_{34}H_{30}O_{15}$ (20 degrees of unsaturation) on the basis of its HRESIMS (*m/z* 701.1443 $[M + Na]^+$) and NMR data (Table 2), which reflected a 14 mass unit (CH_2) decrease compared to that of **6**. Analysis of the ^1H and ^{13}C NMR data of **7** indicated that the methoxy group (δ_H/δ_C 3.92/52.4) attached to C-8‴ in **6** was replaced by the signal for a hydroxy group (δ_H 11.07) in the NMR spectra of **7**, suggesting geomycin B (**7**) is the demethyl analogue of geomycin A (**6**).

The molecular formula of geomycin C (**8**) was established as $C_{34}H_{28}O_{14}$ (20 degrees of unsaturation) by analysis of its HRESIMS (*m/z* 683.1350 $[M + Na]^+$) and NMR data (Table 2). Interpretation of the ^1H, ^{13}C, and HMQC NMR spectroscopic data of **8** revealed the presence of two phenolic protons (one of which is intramolecularly hydrogen-bonded), six methyl groups (four *O*-methyls), one oxygenated sp^3 quaternary carbon, two monosubstituted olefin units, 18 aromatic carbons, three carboxyl carbons, and two carbonyl carbons. Comparison of the ^1H and ^{13}C NMR spectra of **8** with those of compounds **1**−**7** revealed that **8** displayed signals characteristic of the known compounds asterric acid (**1**) and bisdechlorogeodin (**5**), suggesting that **8** could be a metabolite derived from **1** and **5** through an ester/ether linkage. The molecular formula of **8** ($C_{34}H_{28}O_{14}$) was again in agreement with that resulting from a combination of **1** ($C_{17}H_{16}O_8$) and **5** ($C_{17}H_{14}O_7$). The HMBC data for **8** (Table 2) established the partial structures of **1** and **5**. Key HMBC correlations from the phenolic proton at δ_H 10.62 (OH-3′) to C-2′, C-3′, and C-4′ allowed assignment of one hydroxy group to C-3′ and the other phenolic proton to the hydroxy group attached to C-4 on the basis of its ^1H NMR chemical shift value (δ_H 5.22). Therefore, an ester linkage was established between C-2′ and C-3‴ in **8** by its structural requirement. On the basis of these considerations, the gross structure of geomycin C was determined as **8**.

The absolute configuration at C-1″ for geomycin C (**8**) was assigned by application of the CD excitation chirality method.[12] The CD spectrum of **8** showed a positive Cotton effect at 217 ($\Delta\varepsilon$ +1.3) nm and a negative Cotton effect at 278 ($\Delta\varepsilon$ −1.5) nm, which reflected the dissymmetric chromophore of the spirotricyclic moiety, and is similar to that of the known compound (−)-bisdechlorogeodin (**5**),[11,12] suggesting the *R* absolute configuration at C-1″.

New metabolites **3**, **4**, and **6**−**8** were tested for their antibacterial and antifungal activities against a panel of bacteria and fungi. Geomycin B (**7**) showed significant antifungal activity against *A. fumigatus* (ATCC 10894), with IC_{50}/MIC values of 0.86/29.5 μM (the positive control fluconazole showed IC_{50}/MIC values of 7.35/163.4 μM). Geomycin C (**8**) displayed antimicrobial activities against the Gram-positive bacteria *Staphylococcus aureus* (ATCC 6538) and *Streptococcus pneumoniae* (CGMCC 1.1692), with IC_{50}/MIC values of 17.3/75.8 and 36.2/151.5 μM, respectively (the positive control antimicrobial peptide AMP showed IC_{50}/MIC values of 1.40/4.50 and 7.20/18.0 μM, respectively). Geomycin C (**8**) also exhibited activity against the Gram-negative bacterium *Escherichia coli* (CGMCC 1.2340), with IC_{50}/MIC values of 12.9/30.3 μM (the positive control streptomycin showed IC_{50}/MIC values of 1.05/11.2 μM). In our assays, compounds **3**, **4**, and **6** did not show noticeable *in vitro* antibacterial or antifungal activities against the above-mentioned organisms ($IC_{50} > 50$ μM).

To verify that the new metabolites **3**, **4**, and **6**−**8** isolated from the crude extract of *Geomyces* sp. are authentic natural products, a portion of freeze-dried fermented rice substrate was extracted with distilled, HPLC grade EtOAc, and the resulting extract was subjected to reversed-phase HPLC analysis using distilled, HPLC grade H_2O and MeOH as solvents. Compounds **1**−**8** were all identified on the HPLC chromatogram of the crude extract by comparison of their retention times with those of the pure compounds, indicating that these compounds are indeed naturally occurring metabolites. Ethyl and butyl asterrates (**3** and **4**) are new analogues of asterric acid (**1**) and methyl asterrate (**2**). Biogenetically, this class of compounds could be considered to originate from the anthraquinone emodin, via sulochrin, and bisdechlorogeodin (**5**).[5,13,14] The tetraphenyl core structure with two diphenyl ethers connected through an ester linkage, like that in geomycins A (**6**) and B (**7**), has not been previously reported. Geomycin C (**8**) is also a structurally unique metabolite that is derived from a diphenyl ether and a spirocoumaranone through an ester linkage. Although natural products derived from a combination of two substructures through an ester linkage have been encountered frequently, such as the rubupungenosides A and B,[15] this is the first report of the isolation of metabolites resulting from esterification of asterric acid, methyl asterrate, and bisdechlorogeodin.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a Perkin-Elmer 241 polarimeter, and UV data were recorded on a Shimadzu Biospec-1601 spectrophotometer. The CD spectrum was recorded on a JASCO J-815 spectropolarimeter, using CH_3OH as solvent. IR data were recorded using a Nicolet Magna-IR 750 spectrophotometer. ^1H and ^{13}C NMR data were acquired with Varian Mercury-400, -500, and -600 spectrometers using solvent signals ($CDCl_3$; δ_H 7.26/δ_C 77.7) as references. The HMQC and HMBC experiments were optimized for 145.0 and 8.0 Hz, respectively. ESIMS data were recorded on a Bruker Esquire 3000^{plus} spectrometer, and HRESIMS data were obtained using Bruker APEX III 7.0T and APEXII FT-ICR spectrometers, respectively.

Fungal Material. The culture of *Geomyces* sp. (previously classified as *Chrysosporium* sp.)[16] was isolated by Dr. Haiying Wang from a soil sample collected at the Fildes Peninsula of King George Island, Antarctica, in January 2007. The isolate was identified by one of the authors (B.S.) and assigned the accession number 2481 in China General Microbial Culture Collection (CGMCC) at the Institute of Microbiology, Chinese Academy of Sciences, Beijing. The fungal strain was cultured on slants of potato dextrose agar (PDA) at 25 °C for 10 days. Fermentation was carried out in five 500 mL Fernbach flasks each containing 80 g of rice. Spore inoculum was prepared by suspension in sterile, distilled H_2O to give a final spore/cell suspension of 1×10^6/mL. Distilled H_2O (100 mL) was added to each flask, and the contents were soaked overnight before autoclaving at 15 lb/in.^{2} for 30 min. After cooling to room temperature, each flask was inoculated with 5.0 mL of the spore inoculum and incubated at 25 °C for 40 days.

Extraction and Isolation. The fermented rice substrate was extracted repeatedly with EtOAc (4 × 500 mL), and the organic solvent was evaporated to dryness under vacuum to afford 11.50 g of crude extract. The extract was fractionated by silica gel vacuum liquid chromatography (VLC) using *n*-hexane−CH_2Cl_2−MeOH gradient elution. The fraction (2.40 g) eluted with 100:1 CH_2Cl_2−MeOH was fractionated again by silica gel VLC using petroleum ether−EtOAc as the solvent system. The subfraction eluted with 15% EtOAc (85 mg) was further separated by semipreparative reversed-phase HPLC (Agilent Zorbax SB-C_{18} column; 5 μm; 9.4 × 250 mm; 2 mL/min) to afford ethyl asterrate (**3**; 15.0 mg, t_R 20.8 min; 63% CH_3OH in H_2O over 10 min, 63% to 80% CH_3OH in H_2O over 25 min) and butyl asterrate (**4**; 15.0 mg, t_R 33.0 min; same gradient as in purification of **3**). The fractions eluted with 20% (100 mg), 25% (120 mg), and 30% (50 mg) EtOAc were separated again by Sephadex LH-20 column chromatography using 1:1 $CHCl_3$−CH_3OH as eluent. Purification of the resulting subfractions by reversed-phase HPLC with different gradients afforded three known compounds: asterric acid (**1**; 30.0 mg, t_R 9.9 min; 70% CH_3OH in H_2O

over 20 min), methyl asterrate (**2**; 45.0 mg, t_R 9.4 min; 63% CH_3OH in H_2O over 20 min), and (−)-bisdechlorogeodin (**5**; 5.0 mg, t_R 12.6 min; 45% CH_3CN in H_2O over 20 min). The fractions eluted with 100:2 CH_2Cl_2−MeOH (0.96 g) were combined and separated by silica gel VLC using petroleum ether−EtOAc as solvents. The subfractions eluted with 30% (30 mg) and 40% (35 mg) EtOAc were combined and purified by reversed-phase HPLC (40% CH_3CN in H_2O over 15 min, 63% to 80% CH_3CN in H_2O over 20 min) to afford geomycins A (**6**; 4.0 mg, t_R 32.1 min), B (**7**; 5.0 mg, t_R 27.0 min), and C (**8**; 4.5 mg, t_R 23.0 min).

Asterric Acid (1): ^{1}H NMR, ^{13}C NMR, and the ESIMS data were fully consistent with literature values.[5]

Methyl Asterrate (2): ^{1}H NMR, ^{13}C NMR, and the ESIMS data were fully consistent with literature values.[2,5]

Ethyl Asterrate (3): colorless oil; UV (CH_3OH) λ_{max} 214 (ε 25 300), 250 (ε 7100) nm; IR (neat) ν'_{max} 3411 (br), 2983, 2954, 1714, 1655, 1622, 1468, 1441, 1206, 1103, 1064, 1006 cm^{-1}; ^{1}H NMR, ^{13}C NMR, and HMBC data, see Table 1; HRESIMS obsd *m*/*z* 399.1017 [M + Na]$^+$ (calcd for $C_{19}H_{20}O_8Na$, 399.1050).

Butyl Asterrate (4): colorless oil; UV (CH_3OH) λ_{max} 220 (ε 23 300), 250 (ε 12 900) nm; IR (neat) ν'_{max} 3410 (br), 2959, 1714, 1655, 1622, 1468, 1440, 1206, 1105, 1064, 1005 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; HRESIMS obsd *m*/*z* 427.1373 [M + Na]$^+$ (calcd for $C_{21}H_{24}O_8Na$, 427.1363).

(−)-Bisdechlorogeodin (5): white powder; $[\alpha]_D$ −32 (*c* 0.01, CH_3OH); ^{1}H NMR, ^{13}C NMR, and the ESIMS data were fully consistent with literature values.[10]

Geomycin A (6): white powder; UV (CH_3OH) λ_{max} 211 (ε 31 500), 254 (ε 9200) nm; IR (neat) ν'_{max} 3091 (br), 3008, 2952, 2848, 1731, 1660, 1625, 1597, 1469, 1440, 1247, 1203, 1063, 1003 cm^{-1}; ^{1}H NMR, ^{13}C NMR, and HMBC data, see Table 2; HRESIMS obsd *m*/*z* 715.1678 [M + Na]$^+$ (calcd for $C_{35}H_{32}O_{15}Na$, 715.1633).

Geomycin B (7): white powder; UV (CH_3OH) λ_{max} 211 (ε 20 800), 254 (ε 4900) nm; IR (neat) ν'_{max} 3318 (br), 2952, 2846, 1725, 1682, 1629, 1594, 1574, 1469, 1440, 1246, 1204, 1065, 1004 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 2; HMBC data ($CDCl_3$, 600 MHz) H-3 → C-1, 4, 5, 8; H-5 → C-1, 3, 4; H_3-7 → C-6; H_3-9 → C-8; H-4′ → C-2′, 3′, 6′, 7′, 8′; H-6′ → C-1′, 2′, 4′, 7′; H_3-7′ → C-4′, 5′, 6′; H-3″ → C-1″, 4″, 5″, 8″; H-5″ → C-1″, 3″, 4″, 6″; H_3-7″ → C-6″; H_3-9″ → C-8″; H-4‴ → C-2‴, 3‴, 6‴, 7‴; H-6‴ → C-1‴, 2‴, 4‴, 7‴, 8‴; H_3-7‴ → C-4‴, 5‴, 6‴; OH-3′ → C-2′, 3′, 4′; OH-3‴ → C-2‴, 3‴, 4‴; HRESIMS obsd *m*/*z* 701.1443 [M + Na]$^+$ (calcd for $C_{34}H_{30}O_{15}Na$, 701.1477).

Geomycin C (8): yellow powder; $[\alpha]_D$ −33 (*c* 0.01, CH_3OH); UV (CH_3OH) λ_{max} 217 (ε 32 200), 262 (ε 14 600) nm; CD (*c* 1.0 × 10^{-4} M, CH_3OH) λ_{max} ($\Delta\varepsilon$) 217 (+1.3), 278 (−1.5) nm; IR (neat) ν'_{max} 3433 (br), 2952, 2848, 1727, 1660, 1626, 1602, 1457, 1249, 1202, 1091, 1064, 1039, 1006 cm^{-1}; ^{1}H NMR, ^{13}C NMR, and HMBC data, see Table 2; HRESIMS obsd *m*/*z* 683.1350 [M + Na]$^+$ (calcd for $C_{34}H_{28}O_{14}Na$, 683.1371).

Antibacterial and Antifungal Bioassays. Antibacterial and antifungal bioassays were conducted in triplicate by following the National Center for Clinical Laboratory Standards (NCCLS) recommendations.[17,18] The bacterial strains, *Staphylococcus aureus* (ATCC 6538), *Streptococcus pneumoniae* (CGMCC 1.1692), and *E. coli* (CGMCC 1.2340), were grown on Mueller-Hinton agar, the yeasts, *Candida albicans* (ATCC 10231) and *Geotrichum candidum* (AS2.498), were grown on Sabouraud dextrose agar, and the fungus, *A. fumigatus* (ATCC 10894), was grown on potato dextrose agar. Targeted microbes (3−4 colonies) were prepared from broth culture (bacteria: 37 °C for 24 h; fungus: 28 °C for 48 h), and the final spore suspensions of bacteria (in MHB medium), yeasts (in SDB medium), and *Aspergillis fumigatus* (in PDB medium) were 10^6 and 10^5 cells/mL and 10^4 mycelial fragments/mL, respectively. Test samples (10 mg/mL as stock solution in DMSO and serial dilutions) were transferred to a 96-well clear plate in triplicate, and the suspension of the test organisms was added to each well, achieving a final volume of 200 μL (antimicrobial peptide AMP, streptomycin, and fluconazole were used as the positive controls). After incubation, the absorbance at 595 nm was measured with a microplate reader (TECAN), and the inhibition rate was calculated and plotted versus test concentrations to afford the IC_{50}. The MIC was defined as the lowest test concentration that completely inhibited the growth of the test organisms.[19,20]

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Supporting Information Available: ^{1}H and ^{13}C NMR spectra of ethyl asterrate (**3**), butyl asterrate (**4**), and geomycins A−C (**6**−**8**). This material is available free of charge via the Internet at http://pubs.acs.org.

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