An Antimicrobial Guanidine-Bearing Sesterterpene from the Cultured Cyanobacterium *Scytonema* sp.

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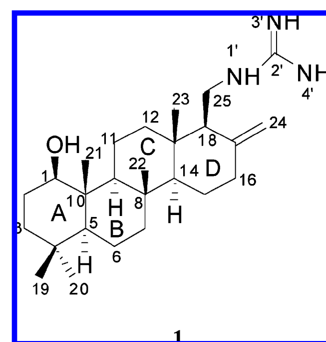
Scytoscalarol (**1**), a antimicrobial sesterterpene bearing a guanidino group, was isolated from the cultured cyanobacterium *Scytonema* sp. (UTEX 1163) by bioassay-guided fractionation. The chemical structure was determined by spectroscopic analysis including MS and 1D and 2D NMR. Scytoscalarol (**1**) showed antimicrobial activities against *Bacillus anthracis*, *Staphylococcus aureus*, *Escherichia coli*, *Candida albicans*, and *Mycobacterium tuberculosis* with MIC values in the range from 2 to 110 μM.

Cyanobacteria are a promising but underexplored source for biologically active secondary metabolites.[1,2] Numerous biologically active natural products have been reported from cyanobacteria, inclusive of alkaloids, polyketides, and linear as well as cyclic peptides.[1−3] However, terpenes are not commonly found in cyanobacteria, and there are only a few reports of cyanobacterial terpenes.[4−7] In our search for antimicrobial compounds from cyanobacteria, a guanidine-bearing sesterterpene, scytoscalarol (**1**), was isolated from the cultured terrestrial cyanobacterium *Scytonema* sp. (UTEX 1163). Scytoscalarol (**1**) has a scalarane skeleton, a class of sesterterpenes commonly found in various species of marine sponges of the order Dictyoceratida.[8] Several scalarane sesterterpenes have been reported to exhibit significant cytotoxicity in human cancer cell lines[9] and antimicrobial activity against various microorganisms.[10] Scytoscalarol (**1**) is the first sesterterpene reported from cyanobacteria and the first guanidine-containing sesterterpene from a natural source. Herein, we reported the isolation, structural elucidation, and biological activity of this compound.

Scytonema sp. was initially acquired from the Culture Collection of Algae at the University of Texas at Austin (UTEX 1163). In our screening, the crude extract of *Scytonema* sp. (UTEX 1163) was found to possess antibacterial activity against *Bacillus anthracis* (MIC 40 μg/mL) and was mass cultured in the laboratory for chemical investigation. The antibacterial MeOH/CH_2Cl_2 extract was fractionated using a diaion HP20SS resin column, and scytoscalarol (**1**) was obtained as a precipitate from fraction 3. The structure was determined by interpretation of MS and 1D and 2D NMR data.

Scytoscalarol (**1**) was obtained as a white, amorphous solid. The molecular formula was determined by HRMS as $C_{26}H_{45}N_3O$ (*m/z* 416.3651 $[M + H]^+$) and implied six degrees of unsaturation. Analysis of ^1H, ^{13}C, COSY, and HSQC NMR spectra suggested the presence of four isolated $CHCH_2CH_2$ spin systems (C-1 to C-3, C-5 to C-7, C-9−C-11−C-12, and C-14 to C-16) and one $CHCH_2$ (C-18−C-25) spin system (Figure 1). Two proton resonances at δ_H 4.94 and 4.48 were assigned to an exomethylene moiety (C-24, δ_C 107.6). The ^1H NMR spectrum also contained resonances for five isolated singlet methyl groups at δ_H 0.82, 0.85, 0.91, 0.88, and 0.77.

The ^{13}C NMR spectrum contained 26 carbon signals, three of which appeared above 100 ppm. Two of these were assigned to the exomethylene moiety (C-17 δ_C 147.8 and C-24 δ_C 107.6). The quaternary carbon at δ_C 158.6 ppm was attributed to a guanidino group on the basis of the chemical shift[11] as well as consideration of the molecular formula. Of the six degrees of unsaturation required by the molecular formula, two were accounted for by the exomethylene moiety and the guanidino group. Hence, scytoscalarol (**1**) was deduced to be tetracyclic.



The planar structure of **1** was determined by analysis of HMBC data (Table 1 and Figure 1). The isolated $CHCH_2CH_2$ spin system from C-1 to C-3 was connected to the C-5 to C-7 spin system via a *gem*-dimethyl group at C-4 based on the correlations from both H_3-19 and H_3-20 to C-3 and C-5. HMBC correlations from H_3-21 to C-10, C-1, and C-5, as well as from H-5 to C-1, connected C-1 and C-5 to C-10 to form a six-membered ring (A). HMBC correlations from H_3-21 to C-9 also connected the C-9−C-11−C-12 spin system to C-10. HMBC correlations from H_3-22 to C-8, C-9, and C-7 connected both C-7 and C-9 to C-8 and formed a

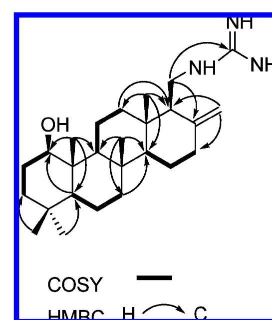


Figure 1. Key COSY and HMBC correlations of scytoscalarol (**1**).

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Table 1. NMR Data of Scytoscalarol (**1**) in MeOD-d_4[a]

position	δ_C, mult.	δ_H, mult., J (Hz)	COSY	HMBC[b]	NOE
1	81.0, CH	3.32, m	H-2, OH	9, 10, 21	H-2_{eq}, 3_{ax}, 5, 9, 11
2_{ax}	30.3, CH_2	1.69, ddd (12.7, 11.8, 3.8)	H-1, 3	1, 3	H-3, 19, 21
2_{eq}		1.57, m			H-1, 20
3_{ax}	41.1, CH_2	1.29, ddd (12.7, 12.7, 3.8)	H-3	1	H-1, 5, 20
3_{eq}		1.38, m			H-19
4	34.0, qC				
5	56.4, CH	0.81, dd (11.8, 2.7)	H-6	1, 9, 10, 19, 20, 21	H-1, 3_{ax}, 9, 20
6	19.6, CH_2	1.54, m	H-5, 7	5, 7, 10	H-19, 20
7_{ax}	43.2, CH_2	0.95, ddd (12.7, 12.5, 4.5)	H-6	5, 8, 9, 14, 22	H-14
7_{eq}		1.79, d (12.7)			H-22
8	40.1, qC				
9	63.2, CH	1.06, d (10.7)	H-11	1, 10, 11, 12, 21, 22	H-1, 5, 11_{eq}, 12_{ax}, 14
10	44.8, qC				
11_{ax}	21.6, CH_2	1.50, m	H-9, 12	13	H-21, 22, 23
11_{eq}		2.63, d (12.7)			H-9
12_{ax}	42.1, CH_2	1.33, ddd (13.3, 12.7, 3.6)	H-11	11, 13, 18, 23	H-9, 11_{eq}, 18
12_{eq}		1.83, d (12.7)			H-11_{ax}, 23, 25
13	40.0, qC				
14	61.6, CH	1.16, dd (12.1, 1.1)	H-15	8, 13,18, 23	H-7, 9, 16_{ax}, 18
15_{ax}	24.1, CH_2	1.37, m	H-14, 16	13, 14, 17	H-22, 23, 24 H-14
15_{eq}		1.76, m			
16_{ax}	38.6, CH_2	2.04, ddd (12.7, 12.7, 4.4)	H-15	14, 15, 17, 18, 24	H-14 (strong)
16_{eq}		2.44, d (12.0)			H-14 (weak), 24
17	147.8, qC				
18	57.3, CH	1.96, d (10.3)	H-19	16, 17, 23, 24, 25	H-12_{eq}, 14, 24, 25
19	21.5, CH_3	0.82, s		3, 4, 5, 20	H-2_{ax}, 3_{eq}
20	33.3, CH_3	0.85, s		3, 4, 5, 19	H-2_{eq}, 3_{ax}, 5
21	13.0, CH_3	0.91, s		1, 9, 5, 10	H-2_{ax}, 11_{ax}
22	18.3, CH_3	0.88, s		7, 8, 9, 14	H-7_{eq}, 11_{ax}, 15_{ax}, 23
23	16.1, CH_3	0.77, s		12, 13, 14, 18	H-11_{ax}, 12_{eq}, 15_{ax}, 24, 25
24	107.6, CH_2	4.94, s		16, 17, 18	H-16_{eq}
		4.49, s			H-15ax, 23, 25
25	38.8, CH_2	3.38, d (10.3)	H-18	17, 18, 2′	H-12_{eq}, 18 H-24
		3.22, t (11.6)			
OH [c]		4.04, d (5.0)[c]	H-1[c]	1, 2, 10[c]	H-19, 21[c]
NH-1′ [c]		6.94, s[c]	H-25[c]		H-18, 24, NH_2-3′ and 4′[c,d]
2′	158.6, qC				
NH_2-3′ [c,d]		7.29, brs[c,d]			NH-1′[c]
NH_2-4′ [c,d]		6.60, brs[c,d]			NH-1′[c]

[a] Frequency: 900 MHz for ^{1}H and 226 MHz for ^{13}C. [b] HMBC correlations are from proton(s) stated to the indicated carbon(s). [c] ^{1}H, COSY, HMBC, and NOESY data of both hydroxy and guanidine groups were observed in the NMR spectra acquired in DMSO-d_6. The complete NMR data in DMSO-d_6 are included in the Supporting Information. [d] Assignments of proton signals of the guanidine zwitterion may be reversed.

second six-membered ring (B). In addition, a correlation from H_3-22 to C-14 as well as from H-7_{ax} to C-14 indicated that the C-14 to C-16 spin system was also connected to C-8. HMBC correlations from H_3-23 to C-13, C-12, and C-14 completed ring C. Correlations from both H_3-23 and H-12_{ax} to C-18 placed the C-18−C-25 spin system adjacent to C-13. The final ring (D) was deduced by HMBC correlations from H_2-24 to C-16, C-17, and C-18, thus connecting C-18 and C-16 via C-17 of the exomethylene group and establishing a tetracyclic scalarene-type sesterterpene skeleton. The chemical shift of C-1 (δ_c 81.0) combined with one oxygen atom required by the molecular formula placed a hydroxy group at C-1. This was confirmed by a HMBC correlation from the OH proton (δ_H 4.04, observed in DMSO-d_6) to C-1. The guanidino moiety at C-25 was confirmed by a HMBC correlation from H_2-25 to the guanidine carbon C-2′, as well as the COSY correlation between NH-1′ and H_2-25.

The relative configuration was determined by analysis of the 2D NOESY spectrum (Figure 2 and Table 1). NOESY correlations observed between H-11_{ax} and H_3-21, H_3-22, and H_3-23 as well as between H_3-23 and H_2-25 indicated all these groups to be in the β-plane. This led to the determination of the relative configuration as 8*S**, 10*S**, and 13*S**. Correlations between H-9 and both H-5 and H-14 indicated H-5, H-9, and H-14 all to be in the α-plane. NOESY correlations between H-5 and H-1, as well as between H-14 and H-18, indicated H-1 and H-18 to be in the α-plane as well and determined the relative configuration as 1*R**, 5*S**, 9*S**, 14*S**, and 18*S**. The rings A, B, C, and D were all-*trans*, and the relative configuration of scytosclarol was determined as 1*R**, 5*S**, 8*S**, 9*S**, 10*S**, 13*S**, 14*S**, and 18*S** as shown above.

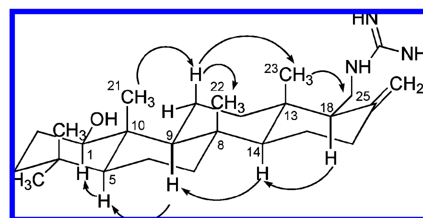

Figure 2. Key NOESY correlations of scytoscalarol (**1**).

Scytoscalarol (**1**) has a scalarane skeleton and showed antimicrobial activity against *Bacillus anthracis* (MIC 6 μM), *Staphylococcus aureus* (MIC 2 μM), *Escherichia coli* (MIC 30 μM), *Candida albicans* (MIC 4 μM), and *Mycobacterium tuberculosis* (MIC 110 μM). Scytoscalarol was also weakly cytotoxic (IC_{50} 135 μM) in a Vero cell assay.

This is the first report of a scalarane sesterterpene from cyanobacteria. Scalaranes represent a common class of sesterterpenes found in various species of marine sponges of the order Dictyoceratida.[8,12] 12-Keto-scalarane derivatives have also been found in sponge-feeding *Glossodoris* nudibranchs. It was assumed that certain *Glossodoris* nudibranchs can produce these compounds by enzymatic oxidation of dietary scalaranes.[13] A biosynthetic study of scalarane sesterterpenes suggested that this class of compounds might be of microbial origin.[14] Several previously reported

metabolites from sponges have been found to be produced by symbiotic microorganisms.[15] However, there are no reports of the cyanobacterium *Scytonema* associated with sponges of the order Dictyoceratida. More research is needed to determine if cyanobacteria are involved in the production of the scalarane-type sesterterpenoids in marine sponges.

Scytoscalarol (**1**) is the first guanidine-bearing sesterterpene isolated from natural sources. Many guanidine-containing polyketides, peptides, and alkaloids have been reported from terrestrial, freshwater, and marine microganisms and invertebrates.[16] Most reports of cyanobacterial guanidine-bearing compounds are from arginine-containing linear or cyclic peptides as well as alkaloids.[3] Some terpenoid guanidine alkaloids have been identified from higher plants, such as the galegines from *Galega officinalis*[17] and pterogynidine and the nitensidines from *Pterogyne nitens*.[18,19]

Experimental Section

General Experimental Procedures. The optical rotation was determined on a Perkin-Elmer 241 polarimeter. IR spectra were obtained on a Jasco FTIR-410 Fourier transform infrared spectrometer. ^{1}H and ^{13}C NMR spectra were obtained on a Bruker Avance AVII900 MHz NMR spectrometer with a 5 mm ATM CPTCI Z-gradient probe, referenced to the corresponding solvent peaks. Low-resolution ESI mass spectra were obtained on a ThermoFinnigan TSQuantum Triple Quadrupole mass spectrometer. High-resolution ESI mass spectra were obtained on a Thermo Electron LTQ-FT-ICR mass spectrometer.

Biological Material. *Scytonema* sp., a terrestrial cyanobacterium, was acquired from the Culture Collection of Algae at the University of Texas at Austin (UTEX 1163). The cyanobacterium was grown in 13 L of aerated inorganic media (Z media).[20] Cultures were illuminated with fluorescent lamps at 1.93 klx with an 18/6 h light/dark cycle. The temperature of the culture room was maintained at 22 °C. After 4 weeks, the biomass of cyanobacteria was harvested by centrifugation and freeze-dried.

Extraction and Isolation. The freeze-dried biomass (2.88 g) was extracted by repeated maceration with CH_2Cl_2/MeOH (1:1) to yield 597.6 mg of crude extract. The crude extract showed inhibitory activity against *B. anthracis* (MIC 40 μg/mL). A portion of the crude extract (572 mg) was fractionated on a diaion HP20SS column using a gradient with increasing amount of 2-propanol in water to afford nine fractions. Fraction 3 (eluted with 40% 2-propanol) was the most active fraction against both *B. anthracis* (MIC 2.5 μg/mL) and *M. tuberculosis* (MIC 10.8 μg/mL). A white, amorphous powder precipitated from fraction 3 upon drying under air flow. The precipitate was purified by filtration to afford **1** (15.4 mg).

Scytoscalarol (1): white, amorphous powder; $[\alpha]_D$ +6 (*c* 0.41 MeOH); IR (neat) ν_{max} 3341, 3189, 2935, 2848, 1666, 1388, 1339, 1035 cm^{-1}; ^{1}H NMR and ^{13}C (see Table 1); HRESIMS *m/z* 416.3651 $[M + H]^+$ (calcd for $C_{26}H_{46}N_3O$, 416.3641).

Antimicrobial Assays. *Bacillus anthracis*: The fractions and compound **1** were tested with concentrations from 100 μg/mL to 48.8 ng/mL using the previously described method.[21] The minimal inhibitory concentration (MIC) sample was calculated as the lowest concentration that prevents visible bacterial growth. *Mycobacterium tuberculosis*: The inhibitory activity of fractions and compound **1** against *M. tuberculosis* was performed using the microplate Alamar Blue assay (MABA).[22] Virulent H37Rv strain was used in the assay. The MIC value was determined as the lowest sample concentration effecting an inhibition of $\geq$90%. *Other organisms*: The broth microdilution MIC method was used to test the activity of **1** against bacteria[23] and *Candida albicans*.[24]

Cytotoxicity. The cytotoxicity of **1** was evaluated using green monkey kidney cells (Vero).[25] Cell viability was measured using the CellTiter 96 aqueous nonradioactive cell proliferation assay.

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Supporting Information Available: 1D NMR spectra in MeOD-d_4 and DMSO-d_6, as well as a table of NMR data acquired in DMSO-d_6. This material is available free of charge via the Internet at http://pubs.acs.org.

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