Debromosceptrin, an Alkaloid from the Caribbean Sponge *Agelas conifera*

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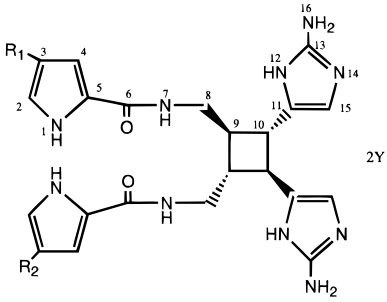
As the result of a structurally guided isolation to identify lead compounds for the treatment of opportunistic infections of AIDS, the dihydrochloride salt of a new symmetrical pyrrole dimer debromosceptrin (**1**), and two known pyrrole analogues (**2** and **3**) were isolated from the Caribbean sponge *Agelas conifera* collected from Belize. The structure of debromosceptrin was identified by analysis of spectral data. ^{15}N spectral data assignments were made for compounds **1**−**3**. Compounds **2** and **3** showed marginal inhibition of *Mycobacterium tuberculosis*.

Bromopyrrole alkaloids are characteristic metabolites of sponges of the genus *Agelas*. Faulkner et al.[1] first identified the water-soluble antimicrobial alkaloid sceptrin from *Agelas sceptrum*. Rinehart et al.[2] reported that an extract of the Caribbean sponge *Agelas conifera* possessed activity in antiviral and antibacterial assays, and a series of sceptrin related bromopyrrole metabolites were isolated as acetate salts.

During our studies of bioactive marine natural products as leads against opportunistic infections of AIDS, we found that the ethanolic extract of *A. conifera* (Schmidt) (order Agelasida, family agelasidae) collected from Belize showed differential cytotoxicity for P-388, A-549, and HT-29 cell lines. In this paper, we describe the isolation and structure elucidation of debromosceptrin (**1**) and the activity of compounds **2** and **3** against *Mycobacterium tuberculosis*.



	R_1	R_2	Y
1	H	H	HCl
2	Br	H	HCl
3	Br	Br	HCl
4	Br	H	AcOH

Purification of the 95% ethanolic extract from *A. conifera* was accomplished by flash chromatography using silica gel and a step gradient from hexane to methanol−water (1:1). The EtOAc−MeOH (1:1) fraction was subjected to reversed-phase C-18 HPLC using water as eluent to obtain the water-soluble alkaloid hydrochloride salts **1**−**3**.

The molecular formulas of compounds **1** ($C_{22}H_{26}N_{10}O_2$), **2** ($C_{22}H_{25}BrN_{10}O_2$), and **3** ($C_{22}H_{24}Br_2N_{10}O_2$), established by HRFABMS, suggested de-, mono-, and dibrominated analogues of the same compound. ^1H and ^{13}C NMR spectral data identified compound **3** as the known compound sceptrin, whose structure was determined by X-ray crystallography.[1] Compound **2** was shown to be the dihydrochloride salt of the previously reported compound **4**.[2]

The ^1H and ^{13}C NMR signals of **1** were nearly identical to those of compounds **2** and **3** with the exception of the pyrrole region. The ^1H NMR spectrum of **1** differed from that of **3** only by an additional resonance at 6.08 ppm (dd, J = 3.8, 2.4 Hz). Comparison of the ^{13}C NMR spectra of **1** and **3** demonstrated that a resonance at 109.6 ppm (d) in **1** instead of the resonance at 96.7 ppm (s) was assigned to the C-3 and C-3′ in pyrrole ring carbons of sceptrin **3**. The DQF−COSY and HMQC spectra of **1** clearly indicated a pyrrole spin system (6.85, 6.07, and 6.49 ppm) with the corresponding carbons (123.5, C-2 and C-2′; 109.6, C-3 and C-3′; 111.4, C-4 and C-4′). The assignment of **1** as debromosceptrin was further confirmed by HMBC experimental data. The H-2, H-2′ resonance (6.85 ppm) was correlated to 109.6 ppm (C-3 and C-3′), 111.4 ppm (C-4 and C-4′), and a quaternary carbon at 124.0 ppm (C-5 and C-5′) in 5-substituted pyrrole ring, as well as H-3, H-3′ (6.07 ppm). The protons H-4, H-4′ (6.49 ppm) correlated to the C-2, C-2′ (123.5 ppm) and the C-5, C-5′ quaternary carbon (124.0 ppm), respectively. The ^1H NMR chemical shift (H-9, H-9′, H-10, H-10′), coupling pattern, and the corresponding ^{13}C data (39.0 and 42.7 ppm) for the cyclobutane are identical in compounds **1**−**3** and support the assignment of all-trans stereochemistry.

Gradient $^1H-^{15}N$ HMQC and $^1H-^{15}N$ HMBC spectral data (Table 2) of compounds **1**−**3** were acquired.[3] The gradient $^1H-^{15}N$ HMBC experiments were optimized at 7 Hz for **1** and **3** and at 10 Hz for **2**.

The symmetric debromosceptrin (**1**) showed three $^1H-^{15}N$ GHMQC correlations. The proton (7.34 ppm) showed a one-bond ^{15}N correlation to 58.4 ppm (N-16 and N-16′) and long-range correlations to 134.3 ppm (N-12 and N-12′) and 137.8 ppm (N-14 and N-14′). These latter two ^{15}N signals both correlated with the H-15 (6.55 ppm). These results are consistent with an aminoimidazoline ring. Two additional protonated ^{15}N signals in the GHMQC spectrum were found (105.4 and 158.8 ppm). The signal at 105.4 ppm correlated with an amide proton (8.34 ppm) and is assigned to N-7 and N-7′. The ^{15}N signal at 158.8 ppm (N-1 and N-1′) has a one-bond correlation to a proton at 11.45 ppm and a long-range correlation to proton at 6.87 (H-2 and H-2′).

Debromosceptrin (**1**) is the first sceptrin analogue reported without bromine substitution. The $^1H-^{15}N$ GHMQC and GHMBC spectra and its corresponding 1D ^{15}N spectrum proved valuable for the structure elucidation of this new marine alkaloid. Sceptrin **2** was reported to exhibit antimicrobial activity and sceptrin **4** was reported as actively antimicrobial/antiviral with low cytotoxicity.[1,2] Compounds **1**−**3** showed no activity in anti-HIV, anti-

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Table 1. ^{1}H and ^{13}C NMR Data for Debromosceptrin (1)

	^{1}H		^{13}C
position	500 MHz, D_2O	400 MHz, DMSO-d_6	100 MHz, D_2O
N-1,1′		11.45 (s)	
2, 2′	6.85 (dd, 2.6, 1.4)	6.87 (br s)	123.5 (d)
3, 3′	6.07 (dd, 3.6, 2.6)	6.08 (dd, 3.8, 2.4)	109.6 (d)
4, 4′	6.49 (dd, 3.6, 1.4)	6.86 (br s)	111.4 (d)
5, 5′			124.0 (s)
6, 6′			163.5 (s)
N-7,7′		8.34 (br.s)	
8, 8′	3.34 (dd, 6.2, 4.0)	3.07 (m)	41.8 (t)
9,9′	2.31 (m)	2.52 (m)	39.0 (d)
10,10′	2.84 (d, 9.0)	2.26 (d, 6.2)	42.7 (d)
11,11′			127.0 (s)
N-12, 12′		12.21 (br s)	
13,13′			148.7 (s)
15,15′	6.36 (s)	6.55 (s)	109.8 (d)
N-16, 16′		7.34 (br.s)	

Table 2. ^{15}N (50 MHz, DMSO-d_6) NMR Data for Compounds 1−3

position	1	2	3
1,1′	158.8	161.3, 158.7	161.3
7, 7′	105.4	105.5, 105.3	106.4
12, 12′	134.3	134.4	134.1
14, 14′	137.8	137.9	137.9
16, 16′	58.4	58.5	58.3

malarial, and cytotoxicity assays, but compounds **2** and **3** showed marginal inhibition of *M. tuberculosis* (35% and 14% at the MIC 12.5 μg/mL, respectively). The result suggests that the debromopyrrole alkaloid **1** may provide improved activity that could be optimized by synthesis for potential treatment of mycobacterial infections. Insufficient quantity of the natural product preclude examination against *M. tuberculosis*.

Experimental Section

General Experimental Procedures. IR and UV spectra were obtained using an AATI Mattson Genesis Series FTIR and a Perkin-Elmer Lambda 3B UV/vis spectrophotometers. Optical rotations were measured with a JASCO DIP-370 digital polarimeter. NMR spectra were recorded on Bruker DRX 500 and DRX 400 spectrometers using the solvent peak as the internal standard. The two-bond HMBC correlation of nitromethane was calibrated to a chemical shift of 380.2 ppm. The reference value from this spectrum was then used for the unknown compounds acquired under the same conditions. HRFABMS spectra were conducted on a Fisons/VG Autospec Q mass spectrometer. Semipreparative HPLC was carried out on Waters 510 model system.

Animal Material. The sponge, *A. conifera* (Schmidt) (order Agelasida, family Agelasidae), was collected from Belize in March 1997 from reef slopes at −20 m, where it was relatively common. A voucher specimen has been deposited in the Natural History Museum, London (BMNH 1997.11.11.7).

Extraction and Isolation. Sponge material (1.25 kg wet wt) was extracted 3× with 95% EtOH. The extract (13.2 g) was purified by flash chromatography using silica gel and a step gradient from hexane to MeOH−H_2O (1:1). The EtOAc-MeOH (1:1) fraction was purified on a preparative reversed-phase HPLC column (Ultracarb 5 μm ODS-30, 10 × 250 mm, Phenomenex) using H_2O as an eluent (flow rate of 10 mL/min and UV detection at 254 nm). The water-soluble alkaloids were obtained with the following yields: **1** (0.0018%), **2** (0.018%), and **3** (0.0052%).

Debromosceptrin (1): light yellow amorphous powder; $[\alpha]_D$ +53.8 (*c* 0.36, EtOH); IR (MeOH) ν_{max} 3282, 3168, 2778, 1679, 1525 cm^{-1}; UV (EtOH) λ_{max} (log ϵ) 267 nm (3.5); HRFABMS *m/z* 463.2305 $[MH]^+$ (calcd for $C_{22}H_{27}N_{10}O_2$, 463.2240), and electrospray mass spectrometry, *m/z* 463 $[MH]^+$; ^{1}H, ^{13}C, and ^{15}N NMR data are shown in Tables 1 and 2.

Monobromosceptrin (2): light yellow solid; ^{1}H NMR and ^{13}C NMR same as that reported in ref 2; HRFABMS *m/z* 543.1337 $[MH]^+$, calcd for $C_{22}H_{26}BrN_{10}O_2$ 541.1423.

Sceptrin (3): light yellow solid; ^{1}H NMR and ^{13}C NMR same as that reported in ref 1; HRFABMS *m/z* 620.3959 $[MH]^+)^+$, calcd for $C_{22}H_{25}Br_2N_{10}O_2$ 619.0528.

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