Chemical Constituents of the Deep Reef Caribbean Sponges *Plakortis angulospiculatus* and *Plakortis halichondrioides* and Their Anti-inflammatory Activities

Sridevi Ankisetty,*[,†] Deborah J. Gochfeld,[‡] M. Cristina Diaz,[§] Shabana I. Khan,[†,‡] and Marc Slattery*[,†]

Department of Pharmacognosy, School of Pharmacy, The University of Mississippi, University, Mississippi 38677, National Center for Natural Products Research, Research Institute of Pharmaceutical Sciences, The University of Mississippi, University, Mississippi 38677, and Museo Marino de Margarita, Nueva Esparta, Venezuela

Received April 7, 2010

Chemical investigations of two collections of the deep reef Caribbean sponge *Plakortis angulospiculatus* resulted in the isolation of a new compound (**1**) along with the known compound spiculoic acid B (**2**) belonging to the spiculoic acid class and four other new compounds (**3**−**6**) belonging to the zyggomphic acid class. Three new aromatic compounds (**7**−**9**) were isolated from the Caribbean sponge *Plakortis halichondrioides*. The structural determination of the compounds was based on extensive NMR and mass spectroscopic analysis. The isolated compounds **1**−**7** were tested for their anti-inflammatory activity using *in vitro* assays for inhibition of inducible nitric oxide synthase and nuclear factor kappa B (NFκB) activity, as well as inhibition of intracellular reactive oxygen species generation as a result of oxidative stress. The cytotoxicity of these compounds was also evaluated to determine the selectivity index of their bioactivity with respect to cytotoxicity. Compounds **1** and **4** were more potent than the positive control in inhibiting NFκB activity and had IC_{50} values of 0.47 and 2.28 μM, respectively.

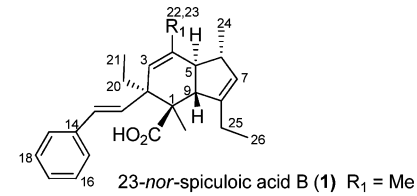
Marine sponges continue to attract attention as a source of structurally diverse secondary metabolites. Sponges belonging to the family Plakinidae are known to be rich sources of polyketides,[1] cyclic peroxides,[2] peroxylactones,[3] and aliphatic peroxy esters.[4] Sponges of the genus *Plakortis* have proven to be a prolific source of polyketides, formed by the combination of acetyl-, propionyl-, and/or butyryl-CoA units.[5] A new family of polyketides with an uncommon spiculane skeleton was described from the Caribbean sponge *Plakortis angulospiculatus* by Andersen et al.[6] Later, zyggomphic and *nor*-zyggomphic acids were reported from the sponge *Plakortis zyggompha.*[7] Cyclic peroxides are the common constituents of the sponge *Plakortis halichondrioides*, but aromatic compounds were also previously reported.[8]

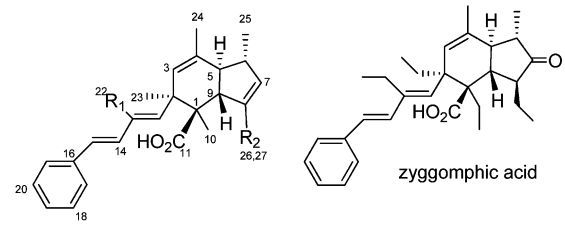
The diversity of reef-building corals decreases with depth and light, whereas many sponges increase in abundance on reefs from shallow to deeper depths.[9] The transition between shallow and deep reef coral communities in the tropics is characterized by reduced light and consequently less photosynthesis. As part of our ongoing studies on chemistry of deep reef sponges, and to compare their secondary metabolites to shallow reef sponges, we investigated the chemical constituents of CH_2Cl_2−MeOH (1:1) extracts of the sponges *P. angulospiculatus* and *P. halichondrioides* collected from the Bahamas and Cayman Islands and also evaluated their biological activities. Herein we report the isolation and structure elucidation of the polyketides **1**−**6** and aromatic constituents **7**−**9** along with the anti-inflammatory activities of compounds **1**−**7**.

Results and Discussion

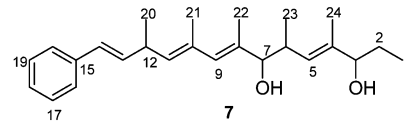
The CH_2Cl_2−MeOH extract of the freeze-dried sponge *P. angulospiculatus* collected from a depth of 61.6 m from Bock Wall, Bahamas, was subjected to C_{18} flash chromatography followed by RP HPLC to yield the new compound 24-*nor*-spiculoic acid B (**1**) and the known compound spiculoic acid B (**2**).

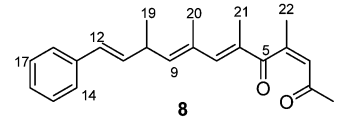
Compound **1** was obtained as an optically active white solid with molecular formula $C_{25}H_{32}O_2$ as determined by HRESIMS {[M − H]$^-$ at *m*/*z* 363.2340}, indicating 10 degrees of unsaturation. Interpretation of the ^{1}H−^{1}H COSY, HSQC, and HMBC experiments identified a carbonyl resonance (δ_C 180.8, C-11), one monosubstituted phenyl group (δ_C 137.8, C-14; 126.3, C-15/19; 128.4, C-16/18; 126.9, C-17), one disubstituted *trans* double bond [δ_C 136.6, C-12; 131.7, C-13; δ_H 5.95 (1H, d, J = 15.8 Hz, H-12); 6.33 (1H,

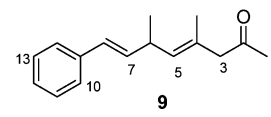




	R_1	R_2
zyggomphic acid B (**3**)	Me	Et
27-*nor*-zyggomphic acid B (**4**)	Me	Me
22-*nor*-zyggomphic acid B (**5**)	H	Et
22,27-*dinor*-zyggomphic acid B (**6**)	H	Me







* To whom correspondence should be addressed. (S.A.) Tel: +1-662-915-1706. Fax: +1-662-915-6975. E-mail: ankisett@olemiss.edu. (M.S.) Tel: +1-662-915-1053. E-mail: slattery@olemiss.edu.
† Department of Pharmacognosy, University of Mississippi.
‡ National Center for Natural Products Research, University of Mississippi.
§ Museo Marino de Margarita.

10.1021/np100233d © 2010 American Chemical Society and American Society of Pharmacognosy
Published on Web 08/25/2010

Table 1. ^{1}H and ^{13}C NMR Spectroscopic Data for Compound **1** in $CDCl_3$

C#	δ_C, mult.	δ_H (*J* in Hz)
1	49.9, C	
2	49.8, C	
3	123.5, CH	5.15, br s
4	135.6, C	
5	53.2, CH	1.86, m
6	39.6, CH	2.36, m
7	132.2, CH	5.32, br s
8	147.2, C	
9	51.8, CH	3.09, d (11.6)
10	14.7, CH_3	1.22, s
11	180.8, C	
12	136.6, CH	5.95, d (15.8)
13	131.7, CH	6.33, d (15.8)
14	137.8, C	
15/19	126.3, CH	7.35, m
16/18	128.4, CH	7.27, m
17	126.9, CH	7.18, m
20	27.0, CH_2	1.64, m 1.85 m
21	9.1, CH_3	0.88, t (7.4)
22	22.6, CH_3	1.95, s
23		
24	20.2, CH_3	1.27, d (6.8)
25	22.3, CH_2	1.86, m 2.05, m

d, J = 15.8 Hz, H-13)], and two trisubstituted double bonds [δ_C 123.5, C-3; 135.6, C-4; δ_H 5.15 (1H, br s, H-3); δ_C 132.2, C-7; 147.2, C-8; δ_H 5.32 (1H, br s, H-7)]. Comparison of ^{1}H and ^{13}C NMR spectroscopic data of compound **1** (Table 1) with those for spiculoic acid B (**2**) immediately identified it to be similar to the spiculoic acid class of polyketides.[6] DEPT 135, ^{13}C NMR, and mass spectrometric analysis indicated lack of a methylene group in comparison to **2**. The ^{1}H NMR spectrum indicated the presence of an olefinic methyl singlet at δ_H 1.95 (3H, s) and the absence of an ethyl group in comparison to spiculoic acid B. The position of the methyl group could be on either C-4 or C-8. HMBC, COSY, and HSQC correlations were analyzed, and HMBC correlations observed from H_3-22/C-3, C-5 fixed the position of the methyl group on C-4 rather than on C-8. Thus the structure of compound **1** was established as 23-*nor*-spiculoic acid B. Compound **2** was isolated as an optically active pale yellow solid and had the molecular formula $C_{26}H_{34}O_2$. Comparison of the spectroscopic data with the literature values[6] readily identified compound **2** as spiculoic acid B. NOESY correlations confirmed the relative configurations of compounds **1** and **2**.

Another sample of the sponge *P. angulospiculatus* was collected from a depth of 57.9 m on Little Cayman Islands. The CH_2Cl_2−MeOH extract of the freeze-dried sample was subjected to a series of reversed-phase HPLC to afford four new compounds, **3**−**6**. Compound **3**, which was isolated as an optically active colorless solid, had a molecular formula of $C_{27}H_{34}O_2$, deduced by HRESIMS {$[M - H]^-$ at *m*/*z* 389.2497}, indicating 11 degrees of unsaturation, one more than the spiculoic acids. Twenty-seven resonances (6×C; 14×CH; 1×CH_2; 6×CH_3) were observed in the ^{13}C NMR spectrum of **3** (Table 2). The ^{1}H NMR spectrum (Table 3) revealed the presence of five deshielded signals (7.46−7.17 ppm), which indicated the presence of a monosubstituted phenyl ring. The UV maximum at 290 nm, which indicated a bathochromic shift, coupled with three deshielded protons δ_H 6.67 (1H, d, J = 16.0, H-15), 7.09 (1H, d, J = 16.0 Hz, H-14), and 5.88 (1H, s, H-12), suggested the presence of an additional conjugation in the *trans*-styryl unit when compared to the spiculoic acids. The deshielded proton value of the methyl group and a missing proton signal from the *exo* conjugated double bond system indicated a methyl substitution on one of the double bonds. COSY correlations between H-12/H_3-22 and HMBC correlations between H-12/C-22 and H-14/C-22 confirmed the position of the methyl group at C-13. Also observed were two trisubstituted double bonds [δ_C 130.3, C-3; 132.7, C-4; δ_H 5.16 (1H, br s, H-3); δ_C 132.4, C-7; 147.7, C-8; δ_H 5.40 (1H, s, H-7)] and a carboxylic group at δ_C 181.4 (C-11). Comparison of the spectroscopic data with literature values indicated that compound **3** belonged to the indane-type polyketide class of compounds, of which the zyggomphic acids were previously reported.[7] The spectroscopic data were similar to those of zyggomphic acids in which the ethyl groups on C-1, -2, and -13 were replaced by methyl groups. However the ^{13}C spectrum of **3** was also missing the ketone carbonyl resonance present in zyggomphic acid. Instead it contained two additional olefinic resonances at δ_C 132.4 (C-7) and 147.7 (C-8). Analysis of the COSY, HMQC, and HMBC data coupled with MS data indicated the presence of a $\Delta^{7,8}$ double bond in place of the C-7 ketone. The relative configuration of **3** was determined by analysis of NOESY and coupling constant data. The vicinal coupling constant between H-9 and H-5 was 11.6 Hz, requiring that both the protons are axial, and therefore the ring junction is *trans*. NOESY correlations were observed between H-9/

Table 2. ^{13}C NMR Spectroscopic Data (100 MHz) of **3**−**6**

C #	**3**[a]	**4**[a]	**5**[a]	**6**[b]
1	47.1, C	47.0, C	46.4, C	49.3, C
2	50.9, C	50.7, C	49.7, C	46.2, C
3	130.3, CH	130.4, CH	128.9, CH	128.3, CH
4	132.7, C	132.7, C	134.0, C	134.3, C
5	53.4, CH	53.1, CH	53.1, CH	53.1, CH
6	39.4, CH	39.4, CH	39.8, CH	39.6, CH
7	132.4, CH	135.4, CH	132.4, CH	135.2, CH
8	147.7, C	138.3, C	147.3, C	141.1, C
9	52.8, CH	52.5, CH	51.9, CH	51.3, CH
10	15.1, CH3	15.1, CH_3	15.0, CH_3	14.9, CH_3
11	181.4, C	181.5, C	180.5, C	180.5, C
12	140.0, CH	139.9, CH	141.7, CH	141.6, CH
13	134.0, C	141.5, C	128.8, CH	128.5, CH
14	136.8, CH	136.8, CH	129.6, CH	129.3, CH
15	126.4, CH	126.4, CH	131.6, CH	131.3, CH
16	138.3, C	134.2, C	137.8, C	137.6, C
17/21	126.6, CH	126.6, CH	126.4, CH	126.4, CH
18/20	128.6, CH	128.6, CH	128.6, CH	128.5, CH
19	127.0, CH	127.1, CH	127.2, CH	127.1, CH
22	13.2, CH3	13.1, CH_3		
23	24.8, CH3	24.8, CH_3	21.1, CH_3	21.2, CH_3
24	21.9, CH3	21.8, CH_3	22.0, CH_3	22.1, CH_3
25	20.2, CH3	19.9, CH_3	20.2, CH_3	20.0, CH_3
26	23.0, CH2	16.6, CH_3	22.8, CH_2	16.2, CH_3
27	11.9, CH3		11.8, CH_3	

[a] Data recorded in C_6D_6. [b] Data recorded in $CDCl_3$.

Table 3. ^{1}H NMR Spectroscopic Data (400 MHz) of Compounds **3**−**6**

C#	**3**[a]	**4**[a]	**5**[a]	**6**[b]
1				
2				
3	5.16, br s	5.12, br s	4.89, br s	4.70, br s
4				
5	2.06, m	2.07, m	2.04, m	1.88, m
6	2.47, m	2.39, m	2.46, m	2.36, m
7	5.40, s	5.36, s	5.43, s	5.32, br s
8				
9	3.53, d (11.6)	3.47, d (11.2)	3.50, d (11.3)	3.06, d (11.6)
10	1.34, s	1.34, s	1.34, s	1.16, s
11				
12	5.88, s	5.82, s	6.13, d (15.4)	5.83, d (15.6)
13			6.42, dd (15.4, 10.4)	6.14, dd (15.6, 10.4)
14	7.09, d (16.0)	6.92, d (16.0)	6.98, dd (15.4, 10.4)	6.74, dd (15.6, 10.4)
15	6.67, d (16.0)	6.64, d (16.0)	6.54, d (15.4)	6.48, d (15.6)
16				
17/21	7.46, m	7.47, m	7.39, m	7.37, m
18/20	7.28, m	7.20, m	7.25, m	7.35, m
19	7.17, m	7.16, m	7.14, m	7.19, m
22	2.17, s	2.14, s		
23	1.43, s	1.45, s	1.34, s	1.16, s
24	1.83, s	1.81, s	1.86, s	1.80, s
25	1.27, d (6.8)	1.23, d (6.8)	1.26, d (6.4)	1.21, d (6.8)
26	2.06, 2.47, m	1.85, s	2.04, 2.44, m	1.62, s
27	1.09, t (7.0)		1.13, t (7.2)	

[a] Data recorded in C_6D_6. [b] Data recorded in $CDCl_3$.

Table 4. ^{1}H and ^{13}C NMR Spectroscopic Data for Compounds **7**−**9**[a] in C_6D_6

	7		**8**		**9**	
C#	δ_C, mult.	δ_H (J in Hz)	δ_C, mult.	δ_H (J in Hz)	δ_C, mult.	δ_H (J in Hz)
1	10.3, CH_3	1.02, t (7.3)	29.4, CH_3	1.74, s	28.1, CH_3	1.80, s
2	27.3, CH_2	1.56, m	194.4, C		204.8, C	
3	80.0, CH	4.05, t (6.7)	125.9, CH	5.74, s	54.3, CH_2	2.83, s
4	139.2, C		154.5, C		129.3, C	
5	130.4, CH	5.34, d (9.6)	200.8, C		133.0, CH	5.14, d (8.8)
6	35.9, CH	2.78, m	134.6, C		35.4, CH	3.20, m
7	83.8, CH	3.76, d	143.0, CH	6.86, s	134.2, CH	6.18, dd (16.0, 6.4)
8	136.2, C		132.3, C		132.9, CH	6.50, d (16.0)
9	132.1, CH	6.01, s	140.0, CH	5.62, d (8.8)	138.1, C	
10	131.6, C		36.2, CH	3.24, m	128.5, CH	7.26, m
11	134.2, CH,	5.49, d (9.0)	133.2, CH	6.08, dd (16.0, 6.8)	126.3, CH	7.40, m
12	36.2, CH	3.35, m	128.8, CH	6.43, d (16.0)	127.0, CH	7.16, m
13	134.3, CH	6.25, dd (15.8, 6.6)	137.6, C		128.5, CH	7.26, m
14	128.0, CH	6.54, d (15.8)	126.3, CH	7.44, m	126.3, CH	7.40, m
15	137.9, C		128.5, CH	7.35, m	20.7, CH_3	1.17, d (6.8)
16	126.0, CH	7.38, m	127.2, CH	7.27, m	16.3, CH_3	1.67, s
17	128.5, CH	7.26, m	128.5, CH	7.35, m		
18	127.0, CH	7.15, m	126.3, CH	7.44, m		
19	128.5, CH	7.26, m	20.5, CH_3	1.12, d (6.8)		
20	126.0, CH	7.38, m	16.2, CH_3	1.79, s		
21	20.9, CH_3	1.26, d (6.6)	12.7, CH_3	2.38, s		
22	17.0, CH_3	1.86, s	22.0, CH_3	1.87, s		
23	12.5, CH_3	1.98, s				
24	17.4, CH_3	0.93, d (6.6)				
25	10.9, CH_3	1.85, s				

[a] Chemical shifts were deduced on the basis of cross-peaks in the COSY and HMQC spectra.

H-6, H-5/CH_3-25, and CH_3-10/H-5, thus establishing the relative configuration of compound **3**, which is same as the previously reported compounds.[7] Thus compound **3** was established as zyggomphic acid B. The naming was done in analogy with spiculoic acids A and B.

Compound **4**, obtained as an optically active, colorless solid, had the molecular formula $C_{26}H_{32}O_2$, as determined by HRESIMS {[M − H]$^-$ at *m*/*z* 375.2344}, and differed from compound **3** by one methylene unit (Δm = 14 amu). The ^{13}C NMR (Table 2) and ^{1}H NMR data (Table 3) were similar to those of compound **3** except that there was an additional olefinic methyl group at δ_C 16.6, C-26; δ_H 1.85 (3H, s) and a missing C-8 ethyl group. HMBC correlations were observed from H-7 and H-9 to C-26, thus positioning the methyl group on C-8. Thus the structure of compound **4** was established as 27-*nor*-zyggomphic acid B.

Compound **5** was isolated as an optically active, colorless solid with a molecular formula of $C_{26}H_{32}O_2$, identical to that of **4**, as indicated by HRESIMS {[M − H]$^-$ at *m*/*z* 375.2323}. The spectroscopic data were similar to **3** except for a missing methyl group, as indicated by the molecular formula and the DEPT 135 spectrum. Analysis of the ^{13}C and ^{1}H NMR spectroscopic data (Tables 2 and 3) indicated a missing olefinic methyl signal from the *exo* conjugated double-bond system. COSY and HMBC correlations unambiguously established the presence of the conjugated styryl system, as indicated by the presence of four deshielded signals at δ_H 6.54 (1H, d, J = 15.4, H-15), 6.98 (1H, dd, J = 15.4, 10.4 Hz, H-14), 6.42 (1H, dd, J = 15.4, 10.4 Hz, H-13), and 6.13 (1H, d, J = 15.4 Hz, H-12). Thus the structure of compound **5** was established as 22-*nor*-zyggomphic acid B.

Compound **6**, obtained as an optically active, white solid, had a molecular formula of $C_{25}H_{30}O_2$, deduced by HRESIMS {[M − H]$^-$ at *m*/*z* 361.2197}. It differed from compound **5** by one methylene unit (Δm = 14 amu). The spectroscopic data were similar to those for compound **5** except for the absence of a −CH_2 unit, as indicated by the DEPT 135 spectrum, and the appearance of a new olefinic methyl at δ_C 16.2, C-26; δ_H 1.62 (3H, s, H-26). HMBC correlations observed from H-7 and H-9 to C-26 confirmed the position of the methyl group at C-8, thus establishing the structure of compound **6** as 22,27-*dinor*-zyggomphic acid B. The geometry of the *exo* disubstituted double bonds was assigned as *E* for all compounds on the basis of the large values of the coupling constants. In the case of compounds **3** and **4**, strong NOESY correlations between H-12/H-14 also required an *E* configuration for the *exo* trisubstituted double bond.

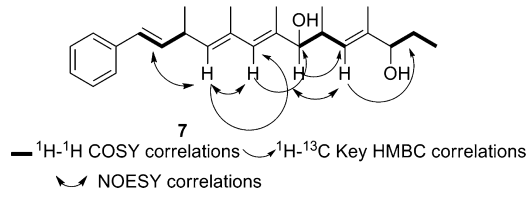


Figure 1. HMBC, COSY, and NOESY correlations of compound **7**.

The sponge *P. halichondrioides* was harvested by hand from Bock Wall, Bahamas, at a depth of 91.4 m. The CH_2Cl_2−MeOH extract of the freeze-dried sponge was subjected to C_{18} flash column chromatography followed by normal-phase HPLC, which led to the isolation of new compounds **7**, **8**, and **9**.

Compound **7** was isolated as an optically active, pale yellow, viscous oil, and its molecular formula was deduced as $C_{25}H_{36}O_2$ by HRESIMS {[M + Na]$^+$ at *m*/*z* 391.2629}. An IR absorption at 3385 cm^{-1} indicated the presence of a hydroxy functionality. Analysis of the ^{1}H and ^{13}C NMR spectroscopic data (Table 4) and the DEPT 135 spectrum revealed the presence of 25 carbon atoms (6×CH_3, 1×CH_2, 14×CH, and 4×C). Eight degrees of unsaturation as required by the molecular formula were attributed to the presence of the styryl unit and three trisubstituted double bonds. The ^{1}H and ^{13}C NMR data revealed resonances for two methyl doublets (δ 20.9/1.26, 17.4/0.93), three olefinic methyl singlets (δ 1.86/17.0, 1.98/12.5, 1.85/10.9), one ethyl group [δ (1.56, 2H)/27.3, (1.02, 3H)/10.3)], two hydroxylated methines (δ 3.76/83.8, 4.05/80.0), a styryl group [δ (7.38−7.15, 5H)/137.9−126.0, 6.54/128.0, 6.25/134.3], and three olefinic methines (5.49/134.2, 6.01/132.1, 5.34/130.4). ^{1}H−^{1}H COSY and HMQC experiments allowed the assembly of the C-13/C-11, C-7/C-5, and C-3/C-1. The structure of compound **7** was further established by key HMBC correlations (Figure 1) between H-11/C-9, H-9/C-7, H-7/C-5, and H-5/C-3, thus confirming the positions of the two hydroxy moieties. The terminal methyl group showed HMBC correlations to C-3, thus placing one of the hydroxy groups at C-3. NOESY correlations were observed between H-13/H-11, H-11/H-9, H-9/H-7, and H-5/H-7,6,3, thus

establishing the *E*-configurations of the double bonds. Thus the structure was established as **7**.

Compound **8** was obtained as a pale yellow oil, which analyzed for the molecular formula $C_{22}H_{26}O_2$ by HRESIMS {$[M + Na]^+$ at *m/z* 345.1825}. Analysis of the ^1H and ^{13}C NMR data revealed the presence of a styryl unit and a slight modification in the side chain pattern compared to **7**, in which the hydroxy groups were replaced by two carbonyl groups. Eight degrees of unsaturation out of 10 required by the molecular formula were attributed to the presence of the styryl unit and three trisubstituted double bonds. IR absorptions at 1658 and 1612 cm^{-1} indicated the presence of an α,β-unsaturated ketone with possible further conjugation, which was supported by the presence of two carbonyl functionalities (δ_C 194.4, 200.8), thus satisfying the rest of the double-bond equivalents. Evidence for an enone with extended conjugation also came from the UV absorption at 294 nm. The ^1H NMR data (Table 4) revealed the presence of two conjugated olefinic α,β-methines [δ_H 6.86 (s), 5.74(s)], three olefinic methyl singlets (δ_H 1.79, 2.38, 1.87), and a methyl group (δ_H 1.74) attached to a ketone. COSY correlations were observed between H-9/H-10, H-10/H-11, and H-11/H-2, thus establishing the major skeletal fragment from C-9 to C-18. HMBC correlations were observed from H-7/C-5,C-21,C-9, CH_3-22/C-5,C-3, and H-3/C-5,C-2,CH_3-22. NOESY correlations were observed between H-11/H-9, H-9/H-7, and H-3/CH_3-22, thus establishing *E*-configurations for Δ^{11}, Δ^8, and Δ^6 and a *Z*-configuration for Δ^3 double bonds. Thus the structure of the new compound was established as **8**.

Compound **9** was obtained as a colorless oil, and the molecular formula was established as $C_{16}H_{20}O$ by HRESIMS {$[M + Na]^+$ at *m/z* 251.1406}. Analysis of the ^1H and ^{13}C NMR data revealed the presence of a styryl unit, a $-CH_2$ (δ 54.3/2.83), and a ketone functionality at δ_C 204.8. COSY correlations were observed between H-8/H-7, H-7/H-6, and H-6/H-5. Key HMBC correlations were observed between H-7/C-5, CH_3-15, H-5/C-3, CH_3-15, CH_3-16, H-3/C-16, and H-1/C-2. The skeletal fragment C-4 to C-14 was similar to that of **7**. A NOESY correlation between H-5/H-3 and the large coupling constant value between H-7/H-8 established the *E*-configuration of the double bonds. Thus the structure was established as **9**. All of these compounds were unstable when the spectroscopic data were acquired in slightly acidic media such as $CDCl_3$, which was attributed to a slow polymerization process, which is a quite common phenomenon for styryl derivatives. They were quite stable, however, when the spectroscopic data were measured in d_6-benzene.

The isolated compounds **1**−**7** were tested in a series of *in vitro* cell-based assays for their effects on selected targets involved in the process of inflammation. Inflammation and oxidative stress are known to be linked to the development of many disorders such as cancer, organ damage, and neurodegenerative conditions. The NFκB family of transcription factors plays a key role in inflammation, cell cycle regulation, apoptosis, and oncogenesis by controlling gene network expression.[10,11] The activation of NFκB involves many cellular processes leading to inflammation and development of cancer.[12,13] In the assay for NFκB activity, a luciferase construct with binding sites for specificity protein (SP-1) was used as a control because this transcription factor is relatively unresponsive to inflammatory mediators. Inducible nitric oxide synthase (iNOS) plays a key role in regulation of blood pressure, the immune system, infection, and inflammation.[14] Overproduction of nitric oxide (NO) by iNOS has been implicated in various pathological processes such as septic shock, inflammation, rheumatoid arthritis, cancer, and tissue damage.[15,16] The NO response is caused mainly by endotoxins and proinflammatory mediators. Thus an iNOS inhibitor could be considered a potential anti-inflammatory agent. A strong inhibition of the activity of NFκB in SW1353 cells was observed for compounds **1** and **4** (Table 5) with IC_{50} values of 0.47 and 2.28 μM, respectively. Other compounds inhibited the NFκB activity to a much lesser extent, and their IC_{50} values were in the range 25−28 μM. Compound **5** was inactive. None of the compounds inhibited the activity of SP-1 up to the highest concentration tested, indicating that they did not have any cytotoxicity or nonspecific inhibition of transcription mediated by control plasmid. Inhibition of lipopolysaccharide (LPS)-induced nitric oxide synthase activity in RAW 264.7 cells was determined (Table 5) by measuring the decrease in nitrite production in cells treated with compounds **1**−**7** in comparison to untreated vehicle controls. Compounds **4** and **5** were more active than compound **6**. Compounds **1** and **7** were moderately active, while compound **2** was inactive. Inhibition of iNOS activity in LPS-induced RAW 264.7 cells indicates a protective effect against inflammation. Compounds **8** and **9** were not tested in the above assays due to their limited amounts, and they decomposed in $CDCl_3$ and could not be reisolated due to lack of starting material. The effect on the production of reactive oxygen species (ROS) as a result of oxidative stress in PMA-treated HL-60 cells was examined to determine the antioxidant activity of these compounds. None of the compounds showed any inhibition of ROS generation, indicating no antioxidant effect. No cytotoxicity was observed toward RAW 264.7 and HL-60 cells up to the highest tested concentration (25 μg/mL) in the iNOS and antioxidant assays. In addition, no effect was seen on the growth of solid tumor cell lines and kidney epithelial and kidney fibroblast cells, ruling out the possibility of any anti-cell proliferation activity of these compounds. Evaluation of the biological activity of these compounds in the present study establishes their potential as anti-inflammatory agents. In conclusion, the deep reef sponges are potential sources of bioactive metabolites, and our future studies will also focus on the ecological significance of these compounds.

Table 5. Inhibition of Inducible Nitric Oxide Synthase (iNOS) and Nuclear Factor Kappa B (NFκB) Activities of Compounds **1**−**7**

compound	iNOS IC_{50} (μM)	NFκB IC_{50} (μM)
1	54.9	0.47
2	NA	27.2
3	>64.1	25.8
4	18.6	2.28
5	19.9	NA
6	30.4	27.9
7	64.1	25.6
L-NMMA[a]	>100.8	
parthenolide		3.3

[a] L-NMMA, N^G-monomethyl-L-arginine acetate (iNOS selective inhibitor). NA = not active.

Experimental Section

General Experimental Procedures. Optical rotations were measured using a JASCO DIP-370 digital polarimeter. UV spectra were recorded on a Hewlett-Packard 8452A diode array spectrometer. IR spectra were recorded on an ATI Mattson Genesis series FTIR spectrometer. NMR spectra were measured on a Bruker Advance DRX-400 spectrometer. ^1H and ^{13}C NMR spectra were measured and reported in ppm by using the benzene-d_6 solvent peak (δ_H 7.16 and δ_C 128.3) as an internal standard. ESI-FTMS analyses were measured on a Bruker Magnex BioAPEX 30es ion cyclotron HR HPLC-FT spectrometer by direct injection into an electrospray interface. HPLC was carried out on a Waters 2695 model system. Reversed-phase HPLC separations were performed using a preparative column (Phenomenex, Luna, $C_{18}(2)$, 10 μm, 250 × 21.2 mm; flow rate, 10 mL/min; Phenomenex PhenylHexyl, 5 μm, 250 × 10 mm; flow rate, 3 mL/min; detector wavelength, 254 nm).

Animal Material. The sponge *Plakortis angulospiculatus* (KY10507006) was collected by mixed gas scuba at a depth of 57.9 m from Rock Bottom Wall, Little Cayman Island. The sponges *P. angulospiculatus* (BA10704007) and *Plakortis halichondrioides* (BA10704008) were collected by mixed gas scuba from Bock wall, Exuma Cays, Bahamas, at depths of 61.6 and 91.4 m, respectively. Voucher specimens of the samples were deposited at the NOAA-NIUST Ocean Biotechnology Center and Repository, Oxford, MS.

Taxonomic Identification. Even though there exists phenotypic plasticity in *Plakortis* species, external morphological characters are

sufficient to distinguish most of the species as indicated below. In the literature, there are three recognized species or major morphotypes of *Plakortis* in the Caribbean.[17] *P. angulospiculatus* (Carter, 1882) is a smooth massive encrusting sponge, brown to gray in external color, light internal color, with large (1−2 cm) oscules, with relatively large diods, (100−180) × (2−6) μm, and no triods. *P. halichondrioides* (Wilson, 1902) is a thin to thick encrusting sponge, brown externally and internally with large diods, (120−180) × (2−7) μm, and triods. *Plakortis zyggompha* (De Laubenfels, 1936) is a thick encrusting greenish sponge internally and externally with diods present in a shorter length range, (35−110) × (2−4) μm, and with triods (rays 20−55 μm).

Extraction and Isolation. Freeze-dried material of the sponge *P. angulospiculatus* (BA10704007) was extracted by ASE (accelerated solvent extraction) using 1:1 CH_2Cl_2−MeOH to yield 1.15 g of the extract, which was subjected to C_{18} flash column chromatography using H_2O and MeOH mixtures. The fraction eluted with 100% MeOH was further subjected to RP HPLC (Phenomenex, Luna, C_{18} (2), 250 × 21.2 mm, 10 μm) with a gradient solvent system using H_2O and CH_3CN (0−35 min; 80−100% CH_3CN) to yield new compound 23-*nor*-spiculoic acid B (**1**, 5.2 mg) and the known spiculoic acid B (**2**, 6.2 mg) with elution times of 35.3 and 37.8 min, respectively. The freeze-dried material of the sponge *P. angulospiculatus* (KY10507006), 4.875 g, was extracted by ASE with 1:1 CH_2Cl_2−MeOH to give 501.5 mg of the extract. The extract was subjected to RP HPLC (Phenomenex, Luna, C_{18} (2), 250 × 21.2 mm, 10 μm) using a gradient (80:20 CH_3CN−H_2O to 100% CH_3CN over 20 min; 100% CH_3CN over 40 min) to give six peaks. Peaks 3 and 5, eluted at 21.2 and 25.4 min, were identified as compounds 22,27-*dinor*-zyggomphic acid B (**6**, 5.6 mg) and zyggomphic acid B (**3**, 3.4 mg), respectively, while peak 4, which eluted at 23.3 min, was a mixture of two compounds, which was further separated on a PhenylHexyl column (Phenomenex, Luna, 250 × 10 mm, 5 μm) using an isocratic elution with 34:66 H_2O−CH_3CN to yield the compounds 22-*nor*-zyggomphic acid B (**5**, 2.3 mg) and 27-*nor*-zyggomphic acid B (**4**, 2.2 mg). The extract (1.5 g) of the sponge *P. halichondrioides* (BA10704008) was subjected to C_{18} flash column chromatography using gradient elution of H_2O and MeOH, and fraction D, which eluted with 100% MeOH, was further subjected to normal-phase HPLC (Waters Spherisorb, 250 × 20 mm, 10 μm) using 100% hexane to 100% EtOAc in 45 min, which afforded nine peaks. Peak 1 (20.8 min) was identified as compound **9** (1.0 mg), peak 6 (35.2 min) was identified as compound **8** (1.5 mg), and peak 9 (49.4 min) was identified as compound **7** (3.2 mg).

23-*nor*-Spiculoic acid B (1): white solid; $[\alpha]^{25}_D$ −18 (*c* 0.23, CH_2Cl_2); UV (MeOH) λ_{max} (log ε) 251 (3.89) nm; IR (NaCl) ν_{max} 2960, 2924, 2362, 2345, 1693 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; HRESIMS *m/z* 363.2340 [M − H]$^-$ (calcd for $C_{25}H_{31}O_2$, 363.2324).

Spiculoic acid B (2): white solid; $[\alpha]^{25}_D$ −21 (*c* 0.23, CH_2Cl_2); $[\alpha]_D$ −22 (*c* 0.1, CH_2Cl_2);[6] HRESIMS *m/z* 377.2484 [M − H]$^-$ (calcd for $C_{26}H_{33}O_2$ 377.2481).

Zyggomphic acid B (3): white solid; $[\alpha]^{25}_D$ −19 (*c* 0.15, CH_2Cl_2); UV (MeOH) λ_{max} (log ε) 290 (3.51) nm; IR (NaCl) ν_{max} 2952, 2944, 2362, 1675 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 2 and 3; HRESIMS *m/z* 389.2497 [M − H]$^-$ (calcd for $C_{27}H_{33}O_2$, 389.2480).

27-*nor*-Zyggomphic acid B (4): colorless solid; $[\alpha]^{25}_D$ −19 (*c* 0.15, CH_2Cl_2); UV (MeOH) λ_{max} (log ε) 289 (3.45) nm; IR (NaCl) ν_{max} 2954, 2945, 2356, 1670 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 2 and 3; HRESIMS *m/z* 375.2344 [M − H]$^-$ (calcd for $C_{26}H_{31}O_2$, 375.2324).

22-*nor*-Zyggomphic acid B (5): colorless solid; $[\alpha]^{25}_D$ −17 (*c* 0.15, CH_2Cl_2); UV (MeOH) λ_{max} (log ε) 290 (3.82) nm; IR (NaCl) ν_{max} 2950, 2943, 2360, 1677 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 2 and 3; HRESIMS *m/z* 375.2323 [M − H]$^-$ (calcd for $C_{26}H_{31}O_2$, 375.2324).

22,27-*dinor*-Zyggomphic acid B (6): white solid; $[\alpha]^{25}_D$ −12 (*c* 0.11, CH_2Cl_2); UV (MeOH) λ_{max} (log ε) 289 (3.63) nm; IR (NaCl) ν_{max} 2954, 2930, 2360, 1689 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 2 and 3; HRESIMS *m/z* 361.2197 [M − H]$^-$ (calcd for $C_{25}H_{29}O_2$, 361.2167).

Compound 7: pale yellow oil; $[\alpha]^{25}_D$ +7 (*c* 0.11, CH_2Cl_2); UV (MeOH) λ_{max} (log ε) 257 (4.24) nm; IR (NaCl) ν_{max} 3385, 2961, 2924, 2360, 2358, 1598, 1450 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 4; HRESIMS *m/z* 391.2629 [M + Na] (calcd for $C_{25}H_{36}O_2Na$, 391.2613).

Compound 8: pale yellow oil; $[\alpha]^{25}_D$ +20 (*c* 0.05, CH_2Cl_2); UV (MeOH) λ_{max} (log ε) 294 (3.18), 254 (3.30) nm; IR (NaCl) ν_{max} 2928, 1692, 1658, 1612, 1357, 1188 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 4; HRESIMS *m/z* 345.1825 [M + Na]$^+$ (calcd for $C_{22}H_{26}O_2Na$, 345.1830).

Compound 9: colorless oil; $[\alpha]^{25}_D$ +58 (*c* 0.05, CH_2Cl_2); UV (MeOH) λ_{max} (log ε) 254 (2.56), 207 (2.58) nm; IR (NaCl) ν_{max} 2923, 2360, 2341, 1716, 1597, 1457, 1121 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 4; HRESIMS *m/z* 251.1406 [M + Na]$^+$ (calcd for $C_{16}H_{20}ONa$, 251.1411).

Biological Assays. Inhibition of NF-κB-mediated transcription was determined in SW 1353 cells by a reporter gene assay as described by Ma et al.[18] using parthenolide as a positive control. Inhibition of iNOS activity assay[19] with L-NMMA as positive control and cytotoxicity assays[20] were performed in mouse macrophages (RAW 264.7). Inhibition of intracellular generation of ROS was assayed in myelomonocytic HL-60 cells as described by Reddy et al.[21]

Acknowledgment. This work was supported by the National Oceanic and Atmospheric Association's National Undersea Research Program [NOAA, NIUST (NA16RU1496)]. The authors thank Mr. F.Wiggers for providing NMR data, Dr. B. Avula and Mr. J. Bowling for mass spectrometric data, and Mr. J. Trott, Mr. P. Bates, and Ms. K. Martin, National Center for Natural Products Research, for performing bioassays.

Supporting Information Available: ^{1}H and ^{13}C NMR spectra for compounds **1**−**5** and **7**−**9**. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) (a) Qureshi, A.; Stevenson, C. S.; Albert, M.; Jacobs, R. S.; Faulkner, D. J. *J. Nat. Prod.* **1999**, *62*, 1205–1207. (b) Berrue, F.; Thomas, O. P.; Laville, R.; Prado, S.; Golebiowski, J.; Fernandez, R.; Amade, P. *J. Nat. Prod.* **2005**, *68*, 547–549.
(2) (a) Rudi, A.; Afanii, R.; Gravals, L. G.; Aknin, M.; Gaydou, E.; Vacelet, J.; Kashman, Y. *J. Nat. Prod.* **2003**, *66*, 682–685. (b) Faulkner, D. J. *Nat. Prod. Rep.* **2001**, *18*, 1–49, and previous reports in this series. (c) Casteel, D. A. *Nat. Prod. Rep.* **1999**, *16*, 55–73.
(3) Davidson, B. S. *Tetrahedron Lett.* **1991**, *32*, 7167–7170.
(4) De Guzman, F. S.; Schmitz, F. J. *J. Nat. Prod.* **1990**, *53*, 926–931.
(5) Faulkner, D. J. *Nat. Prod. Rep.* **1997**, *14*, 259–302, and previous reviews in this series.
(6) Huang, X. H.; Soest, R. V.; Roberge, M.; Andersen, R. J. *Org. Lett.* **2004**, *6*, 75–78.
(7) Berrue, F.; Thomas, O. P.; Laville, R.; Prado, S.; Golebiowski, J.; Fernandez, R.; Amade, P. *Tetrahedron* **2007**, *63*, 2328–2334.
(8) (a) Ravi, B. N.; Armstrong, R. W.; Faulkner, D. J. *J. Org. Chem.* **1979**, *44*, 3109–3113. (b) Phillipson, D. W.; Rinehart, K. L. *J. Am. Chem. Soc.* **1983**, *105*, 7735–7736.
(9) Lesser, M. P.; Slattery, M.; Stat, M.; Ojimi, M.; Gates, R.; Grottoli, A. *Ecology* **2010**, *91*, 990–1003.
(10) Aggarwal, B. B.; Sethi, G.; Nair, A.; Ichikawa, H. *Curr. Signal Transduction Ther.* **2006**, *1*, 25–52.
(11) Melisi, D.; Chiao, P. J. *Expert Opin. Ther. Targets* **2007**, *11*, 133–144.
(12) Brasier, A. R. *Cardiovasc. Toxicol.* **2006**, *6*, 111–130.
(13) Navab, M.; Gharavi, N.; Watson, A. D. *Curr. Opin. Clin. Nutr. Metab. Care.* **2008**, *11*, 459–464.
(14) Jung, Y.; Hyun, J. K.; Kyunghoon, K.; Chang-Jin, L.; Eun-Hee, P. *Ethnopharmacology* **2008**, *120*, 282–286.
(15) Lirk, P.; Hoffman, G.; Rieder, *J. Curr. Drug Targets-Inflamm. Allergy* **2002**, *1*, 89–108.
(16) Kroncke, K. D.; Fehsel, K.; Kolb-Bachofen, V. *Clin. Exp. Immunol.* **1998**, *113*, 147–156.
(17) (a) Zea, S. *Esponjas del Caribe Colombiano*; Editorial Catalágo Científico: Bogotá, 1987. (b) Muricy, G.; Diaz, M. C. In *Systema Porifera: a guide to the classification of sponges*; Hooper, J. N. A.; van Soest, R. W. M., Eds.; Kluwer Academic/Plenum Publishers: New York, 2002; pp 71−82. (c) Diaz, M. C.; Soest, R. W. M. V. *Proceedings of the 4th International Porifera Conference*; University of Amsterdam: Amsterdam, 1993; pp 93−109.
(18) Ma, G.; Khan, S. I.; Gloria, B.; Wolfgang, S.; Fischer, N. H.; Khan, I. A.; Pasco, D. S. *Cancer Chemother. Pharmacol.* **2007**, *60*, 35–43.
(19) Quang, D. N.; Harinantenaina, L.; Nishizawa, T.; Hashimoto, T.; Kohchi, C.; Soma, G.; Asakawa, Y. *Biol. Pharm. Bull.* **2006**, *29*, 34–37.
(20) Mustafa, J.; Khan, S. I.; Ma, G.; Walker, L. A.; Khan, I. A. *Lipids* **2004**, *39*, 167–172.
(21) Reddy, M. K.; Gupta, S. K.; Jacob, M. R.; Khan, S. I.; Ferreira, D. *Planta Med.* **2007**, *73*, 461–467.

NP100233D