Diterpene Metabolites from Two Chemotypes of the Marine Sponge Myrmekioderma styx

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DITERPENE METABOLITES FROM TWO CHEMOTYPES OF THE MARINE SPONGE *MYRMEKIODERMA STYX*

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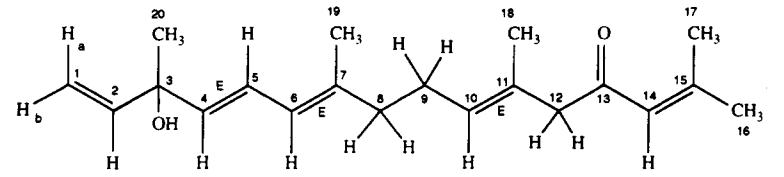
ABSTRACT.—Three apparent chemotypes of the marine sponge *Myrmekioderma styx* exist. An acyclic diterpene, styxenol A [**1**], has been isolated from the shallow water chemotype, while three tricarbocyclic diterpenes **3**, **6**, and **7** have been isolated from the deep chemotype. The structures were elucidated based on spectral methods including homo- and hetero-nuclear 2D nmr experiments. Compounds **1**, **3**, and **6** exhibit moderate cytotoxicity against the P-388 murine leukemia cell line and the A549 human lung tumor cell line.

In a previous report on the potential of tlc as a character for sponge classification, it was shown that at least two depth-dependent chemotypes of *Myrmekioderma styx* de Laubenfels (Halichondriidae) (1) exist (2). In this paper we report the isolation and structure determination of an acyclic diterpene **1** from a shallow water sample of *M. styx* and three tricarbocyclic diterpenes **3**, **6**, and **7** from a deep water collection of *M. styx*. Compound **1** is similar to a series of acyclic diterpenes reported previously from the sponge *Didiscus* (3), while the three metabolites isolated from the deep water sample are reminiscent of the tricyclic diterpenes previously reported from a *Higginsia* sp. collected in western Australia (4). Compounds with the same tricarbocyclic skeleton have been previously reported from the bird's nest fungus *Cyathus* sp. (Basidiomycetes) (5). The bicyclic reiswigins reported previously from *Epipolasis reiswigi* (6), a sponge which has recently been synonymized with *M. styx* (2), may be derived biosynthetically from **1** by cyclization of C-1 to C-7 and C-6 to C-10. Rearrangement of the carbon skeleton is necessary for the formation of the tricarbocyclic compounds from **1**. Compounds **1**, **3**, and **6** exhibit moderate cytotoxic activity against the P388 murine leukemia cell line and the A549 human lung tumor cell line (7).

RESULTS AND DISCUSSION

A shallow water sample of *M. styx* was collected at Fernandez Bay, San Salvador, Bahamas by scuba at 23 m and frozen at −20° for transport to the laboratory. The



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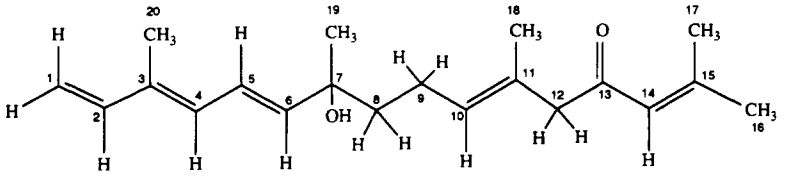

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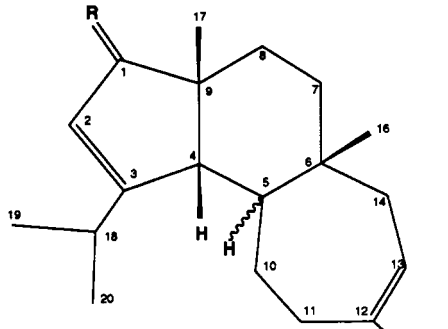
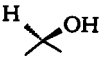
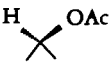


3 R= 

4 R= 

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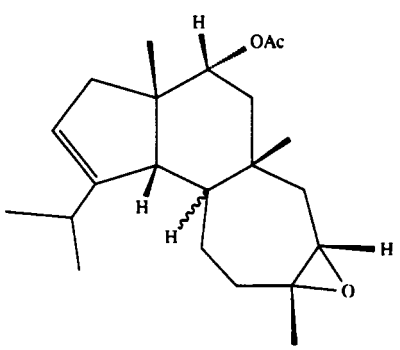
EtOH extract from 190 g of frozen sponge was chromatographed on Si gel using a step gradient of EtOAc in heptane. Further separation by hplc on a Lichrosorb-CN bonded Si gel column (EM Science) with 2.5% IPA in heptane yielded 302 mg of pure compound **1**, which we call styxenol A. ^{13}C nmr (DEPT) and eims suggested a molecular formula of $C_{20}H_{30}O_2$ for compound **1**, which is the same as that of the acyclic diterpene **2** reported previously from a *Didiscus* sp. (3). Compound **1** contained all of the proton spin systems present in **2**; however, differences in proton chemical shifts, coupling constants, and the uv spectra suggested that the compounds were not identical. A 2D-INADEQUATE (8) experiment allowed for the assignment of the highlighted bonds shown in Figure 1. C-5 and C-6 were connected based upon ^1H-^1H coupling information while the position of the ketone carbon C-13 was assigned based upon HMBC (9) correlations, as were the C-18, C-19, and C-20 methyl groups (Table 1, Figure 1). The geometry of the Δ^4, Δ^6, and Δ^{10} double bonds was confirmed by nOe difference experiments, which also allowed the assignment of the C-5–C-6 bond to be *S* trans. NOe difference experiments also allowed for the assignment of protons H_a-1 and H_b-1 and the C-16 and C-20 methyl groups.

Repeated Si gel chromatography of an EtOH extract of a deep water sample of *M. styx* collected by scuba at a depth of 33 m near Islas Los Roques, Venezuela resulted in



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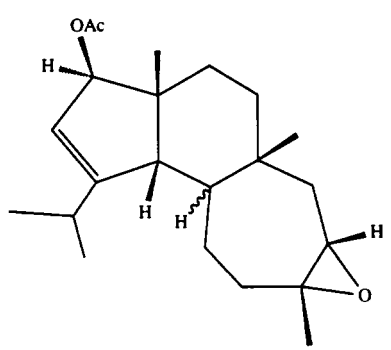

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TABLE 1. ^{1}H- and ^{13}C-nmr Data for Compound **1** in $CDCl_3$.

Position	^{13}C δ (mult)[a]	Attached ^{1}H δ[b]	Long Range Correlation[c]	^{13}C-^{13}C Correlation[d]
1a[e]	111.0 t	5.25 d, J = 17.8		C-2
1b		5.10 dd, J = 10.5, 1.1		
2	143.7 d	5.97 dd, J = 17.8, 10.5	H-20	C-1, C-3
3	72.3 s		H-4, H-5, H-20	C-2, C-4
4	136.3 d	5.65 d, J = 15.3	H-20	C-3, C-5
5	123.5 d	6.42 dd, J = 15.3, 10.9	H-6	C-4
6	124.0 d	5.88 d, J = 10.5	H-5	C-7
7	137.4 s		H-5, H-19	C-6, C-8
8	38.9 t	2.14 m (2H)	H-19	C-7, C-9
9	25.9 t	2.14 m (2H)		C-8, C-10
10	128.1 d	5.16 s	H-12, H-18	C-19, C-11
11	129.2 s		H-12, H-18	C-10, C-12
12	54.6 t	2.99 s (2H)	H-18	C-11
13	198.3 s		H-12, H-14	
14	122.1 d	6.07 t, J = 1.1	H-16, H-17	C-15
15	154.7 s		H-16, H-17	C-14, C-16, C-17
16	27.0 q	1.86 s (3H)	H-14	C-15
17	19.9 q	2.14 s (3H)	H-16, H-20	C-15
18	15.6 q	1.57 s (3H)	H-12	
19	15.9 q	1.72 s (3H)		
20	27.4 q	1.33 s (3H)		

[a]From DEPT sequence.
[b]J values reported in Hz.
[c]From HMBC.
[d]From INADEQUATE.
[e]In order of decreasing δ values.

the isolation of 93 mg, 45 mg, and 1.7 mg of **3**, **6**, and **7**, respectively. High resolution eims of the major metabolite, **3**, was consistent with a molecular formula of $C_{20}H_{32}O$. The ^{1}H- and ^{13}C-nmr spectra suggested the presence of two trisubstituted olefins [δ 159.7 (s), 124.7 (d), 140.6 (s), 122.8 (d)], a secondary alcohol [δ 78.8 (d)], and an isopropyl group [δ 2.34, (m); 1.06, (3H, d, J = 6.7); 0.96, (3H, d, J = 6.7)]. Reaction of **3** with Ac_2O in pyridine led to the formation of monoacetate **4** confirming the presence of alcohol functionality in **3**. Significant overlap of resonances in the upfield region of the ^{1}H-nmr spectrum of **3** made it difficult to assign the ^{1}H-^{1}H couplings observed in the homonuclear proton COSY experiment. Fortunately, sufficient material (93 mg) was available to run a 2D ^{13}C-^{13}C correlation experiment (INADEQUATE), which allowed for the assignment of the bonds highlighted in Figure 2. Correlations between the double bond carbons were not observed in the INADEQUATE experiment, but the results of a ^{1}H-^{13}C 2D HMBC experiment allowed for further definition

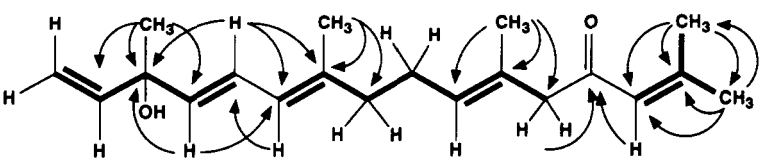

FIGURE 1. Structure of compound **1**, with INADEQUATE ^{13}C-^{13}C connectivities represented by bold-faced bonds and long-range ^{1}H-^{13}C HMBC correlations indicated by arrows.

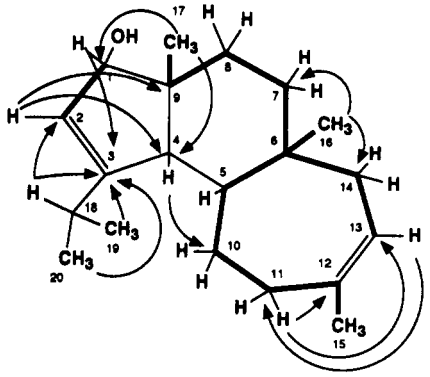

FIGURE 2. Structure of compound **3**, with INADEQUATE connectivities represented by bold-faced bonds and long range ^{1}H-^{13}C HMBC correlations indicated by arrows.

of the olefins as follows: A correlation observed between H-13 (δ 5.32 brs) and C-11 (δ 33.6 t) allowed a bond to be drawn between C-12 and C-13 closing the seven-membered ring. A bond was drawn between C-3 and C-2 based upon a correlation observed between H-1 (δ 4.78) and C-3 (δ 159.7 s). A bond can be drawn between C-1 and C-9 based upon HMBC correlations observed between the H-17 methyl protons (δ 0.82) and C-1 as well as HMBC correlations observed between H-2 and C-9. The isopropyl group was placed at C-3 based upon HMBC correlations observed between the isopropyl methine, H-18, and the two olefinic carbons C-2 and C-3. Similarly, the methyl protons of the isopropyl group, Me-19 and Me-20, both showed HMBC correlations to the olefinic carbon C-3. HMBC correlations observed between the methine carbon observed at δ 55.5 (d, C-4) and both the methyl group, H-17, and the olefinic proton H-2 allowed for the placement of C-4 and closure of the five-membered ring. The remaining connection between C-4 and C-5 closes the six-membered ring and is supported by the observation of an HMBC correlation between H-4 and C-10 completing the planar structure of **3**. Oxidation of **3** with PCC led to formation of the α,β-unsaturated ketone **5** as evidenced by the observation of a ketone carbonyl resonance at 192.8 ppm in the ^{13}C-nmr spectrum and an absorption at 1700 cm^{-1} in the ir spectrum consistent with an α,β-unsaturated ketone in a five-membered ring (10).

The relative stereochemistry of **3** was determined through a series of nOe difference experiments (Table 2). The most important enhancements are as follows: cis configuration was assigned at the ring junction of the five- and six-membered rings based upon observation of a strong enhancement of H-4 when the H-17 methyl protons were irradiated. Irradiation of the H-16 methyl protons enhanced the resonances observed for H-4, H-15, and H-17 allowing for the assignment of the relative stereochemistry at these centers. Enhancement of H-1 was observed when H-2 was irradiated. However, no enhancement of H-1 was observed when the H-17 methyl protons were irradiated, suggesting that H-1 is in the α position. Additional evidence of this assignment is based on comparison with the coupling constant observed between H-1 and H-2 and those derived from PC Model (11). The calculated coupling for the α-hydroxy isomer was larger than that for the β-hydroxy isomer. The observed coupling constant is negligible, suggesting it is the β-hydroxy isomer. No enhancement of H-5 was observed upon irradiation of either the H-16 or H-4, which suggests a trans ring junction at C-5,

TABLE 2. NOe Data for Compound **3**.

Proton	^{1}H Irradiated	^{1}H Enhanced
H-1	4.78	H-2, H_a-10
H-2	5.24	H-1, H-19
H-4	1.82	H_a-11, H-16, H-17
H_b-7	1.15	H_a-7
H_a-10	1.42	H-16
H-13	5.32	H-15
H-15	1.67	H_a-11, H-16
H-16	0.70	H-4, H-15, H-17
H-17	0.82	H-4, H-16
H-18	2.34	H-4
H-20	0.96	H-16, H-17, H-18

C-6. A weak nOe observed between H-4 and H_a-11 also suggests this relative stereochemistry. A trans ring junction at C-5 would lead to a less sterically hindered structure.

High resolution fabms suggested a molecular formula of $C_{22}H_{34}O_3$ for **6**. Compound **6** had resonances attributable to two olefinic carbons and one acetate carbonyl in the ^{13}C nmr spectrum. No further unsaturation was suggested by the ^{13}C-nmr or ir spectra; therefore compound **6** should have 4 rings. The ^{1}H- and ^{13}C-nmr spectra of **6** were similar to those observed for **3**. The primary difference was greater dispersion of the upfield region of the ^{1}H-nmr spectrum. This allowed for the unambiguous assignment of the ^{1}H COSY and HMBC experiments, which confirmed that **6** has the same carbocyclic skeleton as **3** but with a different oxidation pattern. The primary differences were replacement of the olefinic resonances observed for H-13, C-12, and C-13 in **3** with a ^{1}H resonance observed at δ 2.72 (t, $J = 7.3$ Hz) and ^{13}C resonances observed at δ 60.2 and 60.1, which suggested replacement of the Δ^{12} olefin by epoxide functionality. Additionally, the H-15 methyl resonance was shifted to higher field [δ 1.32 (3H, s)] as would be expected if the olefin had been epoxidized. All of the HMBC correlations observed for these atoms are consistent with the proposed epoxidation (Table 3). The presence of a ^{1}H-nmr resonance observed at δ 2.01 (3H, s) and the ^{13}C-nmr resonances observed at δ 170.9 (s) and 21.2 (q) suggested the presence of acetate functionality. Analysis of the 2D HMBC experiment suggested that the acetate in **6** is located at C-8 rather than C-1 as found for compound **3**. The evidence for this is the long range ^{1}H-^{13}C correlations observed between H-8 and C-1, C-6, C-7, C-9, C-17, and C-21, as well as the HMBC correlation observed between H_a-7, H_b-7, and C-8. NOe enhancements observed between the olefinic proton H-2 and the methylene protons H_a-1 and H_b-1 also support this assignment.

The relative stereochemistry of **6** was determined through a series of difference nOe experiments (Table 4). As for compound **3**, cis configuration was assigned at the ring junction of the five- and six-membered rings based upon observation of a strong enhancement of H-4 when the H-17 methyl protons are irradiated. An enhancement of the resonance observed for H-4 when H-8 was irradiated assigned the relative stereochemistry between these two centers. Irradiation of the H-16 methyl protons enhanced the resonances observed for H-4, H-8, H-13, and H-15, allowing for the assignment of the relative stereochemistry at these centers. A trans ring junction at C-5 is again suggested, as this leads to the less sterically hindered structure.

^{13}C-nmr spectral data suggested a molecular formula of $C_{22}H_{34}O_3$ for compound **7**. Comparison of spectral data obtained for **7** with that of **4** and **6** suggested that com-

TABLE 3. ^{1}H- (360 MHz) and ^{13}C- (90 MHz) Nmr Data in $CDCl_3$ for **3** and **6**.

Position	Compound						
	3			**6**			
	δ ^{13}C mult	δ ^{1}H (*J*, Hz)	HMBC	δ ^{13}C mult	δ ^{1}H (*J* = Hz)	^{1}H-^{1}H COSY	HMBC
1	78.8 d	a 4.78 s	H-4	36.9 t	2.57 bd (16.2)	H_a-1, H-2	H-2, H-4, H-17
					1.68 dd (16.2, .63)	H_b-1, H-2, H-4, H-18	
2	124.7 d	5.24 s	H-1, H-4, H-18	121.0 d	5.34 bt (1.5)	H_a-1, H_b-1, H-18	H-1, H-4, H-18
3	159.7 d		H-1, H-4, H-18, H-19, H-20	156.8 s			H-1, H-4, H-18, H-19, H-20
4	55.5 d	1.82 m	H-2, H-17	56.3 d	1.89 d (10)	H-2, H-5	H-2, H-17
5	55.6 d	1.09		55.2 d	1.07 dt (11.0, 3.6)	H-4, H_a-10, H_b-10	H-4, H_a-7, H_b-10, H_a-11, H_b-14, H_a-7, H-8, H_a-10, H_a-14, H-16
6	34.4 s		H-8	37.8 s			
7	38.7 t	a 1.43 s	H-16	42.7 t	1.56 dd (12.6, 5.0)	H_b-7, H-8	H-8, H-16
		b 1.13 m			1.37 m	H_a-7, H-8	
8	28.2 t	a 1.65 m		75.5 d	5.05 dd (12.6, 5.0)	H_a-7, H_b-7	H_a-7, H_b-7, H-17
		b 1.40 m					
9	48.6 s		H-2	46.7 s			H-2, H-4, H_a-7, H-8, H-17
10 . . .	26.4 t	a 1.51 m	H-4	25.3 t	1.78 m	H-5, H_b-10, H_a-11	H-4, H_a-11
		b 1.06 m				H-5, H_a-10, H_a-11	
11 . . .	33.6 t	a 2.12 m	H-15	35.3 t	1.92 m	H_a-10, H_b-10, H_b-11	H_a-10, H-15
		b 1.83 m			1.39 m	H_b-10, H_a-11	
12 . . .	140.6 s		H-11	60.1 s			H_a-10, H_a-11, H-13, H_a-14, H-15
13 . . .	122.8 d	5.32 brs	H-11	60.2 d	2.72 t (7.3)	H_a-14, H_b-14	H-15
14 . . .	43.2 t	a 1.95 m	H-16	44.2 t	1.95 m	H_b-14, H-13	H_a-7, H_b-11, H-13, H-16
		b 1.68 s			1.26 m	H-13, H_a-14	
15 . . .	25.0 q	1.67 3H, s		22.2 q	1.32 3H, s		H_a-11, H_b-11, H-13
16 . . .	15.8 q	0.70 3H, s	H-7	15.8 q	1.02 3H, s		H_a-7, H_b-14
17 . . .	23.7 q	0.82 3H, s		26.4 q	0.93 3H, s		H_b-1, H-4, H-8
18 . . .	31.1 d	2.34 m	H-19, H-20	31.0 d	2.40 m	H_b-1, H-2, H-19, H-20	H-2, H-19, H-20
19 . . .	21.1 q	1.06 d (6.7)		21.2 q	1.10 3H, d (6.6)	H-18	H-18, H-20
20 . . .	22.0 q	0.96 d (6.7)	H-18, H-19	22.6 q	0.97 3H, d (6.6)	H-18	H-18, H-19
21 . . .				170.9 s			H-8, H-22
22 . . .				21.2 q	2.01 3H, s		

TABLE 4. NOe Data for Compound **6**.

Proton	^{1}H Irradiated	^{1}H Enhanced
H_a-1	2.57	H_b-1, H-2
H_b-1	1.68	H_a-1
H-2	5.34	H_a-1, H_b-1, H-19
H_a-7	1.56	H_b-7
H-8	5.05	H-4, H_a-7, H-16, H-17
H_a-10	1.78	H-18
H-13	2.72	H_a-11, H-15, H-16
H-15	1.32	H-13, H_a-14, H-16
H-16	1.02	H-4, H-8, H-13, H-15
H-17	0.93	H_b-1, H-4, H-8
H-18	2.40	H_a-10, H-19
H-19	1.10	H-2, H_a-10, H-18, H-20
H-20	0.97	H-4, H-18, H-19

pound **7** should contain an acetate at C-1, and an epoxide at C-12, C-13. In addition, these comparisons suggested that the relative stereochemistry of **7** is the same as that observed in compounds **4** and **6**. Compound **7** decomposed rapidly, and we were unable to determine the relative stereochemistry directly. Epoxidation of compound **4** yielded a compound spectroscopically identical to **7**.

The marine sponges *M. styx* and *Didiscus oxeata* Hechtal (Halichondriidae) (12) are morphologically similar and virtually impossible to distinguish during collection (13) and therefore it is important to keep all individuals separate until microscopic examination is possible. A diagnostic feature which distinguishes the two sponges is the presence of discorhabds, a type of spicule present in *D. oxeata* but not found in *M. styx*. Discorhabds are not distributed evenly throughout the ectosome of *D. oxeata*; therefore several pieces of the sponge should be analyzed for their presence. A series of acyclic diterpenes (3) and the sesquiterpenes curcuphenol and curcudiol (14) have been reported previously from the sponge *Didiscus* while the reiswigins have been reported from *E. reiswigi* (=*M. styx*) (6).

Styxenol A is closely related to the acyclic diterpenes previously reported from *Didiscus* sp. (4). Cyclizations between C-1 and C-7 and between C-6 and C-10, of styxenol A would lead to the reiswigin skeleton. It is interesting to note that all of the shallow-water (<33 m depth) samples that we have examined to date ($n = 9$) contain acyclic diterpenes, while analysis of 15 deep-water samples of *M. styx* suggested that, although there may be some overlap of depths at which the bicyclic and tricyclic chemotypes occur, samples collected between 33 and 66 m depth contain predominantly the tricarbocyclic metabolites reported here while those occurring at depths greater than 66 m contain predominantly the bicyclic diterpenes. The tlc patterns of curcuphenol and related sesquiterpenes from *D. oxeata* are distinctly different from those of *M. styx* and were not observed in any of the *M. styx* samples analyzed. These results suggest that tlc may be used to distinguish the three *M. styx* chemotypes from one another and from *D. oxeata*. Reciprocal transplant experiments are planned to investigate what role, if any, depth has in the type of metabolite produced by the sponge.

Styxenol A exhibits moderate cytotoxicity against the P388 murine leukemia cell line and the A549 human lung tumor cell line (7) with IC_{50}'s of 5.0 and 6.1 μg/ml, respectively. Deep-water metabolites **3** and **6** have activity against the P-388 human leukemia cell line with IC_{50}'s of 11.2 and 5.6 μg/ml, respectively, and against the A549 human lung tumor cell line with IC_{50}'s of 4 and 7 μg/ml, respectively. Compounds **4**, **5**, and **7** were not tested.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Spectral data were measured on the following instruments: uv/vis, Perkin-Elmer Lambda 3B; nmr, Bruker AM 360 with the Aspect 3000 computer; hrcims, eims, or fabms on a Kratos MS-80RFA (Chemical Instrumentation Center, Yale University); [α], Jasco DIP-360 Digital Polarimeter. ^{1}H chemical shifts are reported as δ values in ppm relative to TMS (0 ppm). ^{13}C chemical shifts are reported as δ values in ppm relative to $CDCl_3$ (77.0 ppm). ^{13}C multiplicities were measured using the DEPT sequence. One-bond ^{1}H-^{13}C correlations were detected using an XHCORR experiment, and long range ^{1}H-^{13}C couplings were detected using the proton-detected HMBC experiment. The INADEQUATE spectrum of **1** was obtained on a 290 mg sample in 20 h. The INADEQUATE spectrum of **3** was obtained on a 93 mg sample in 63 h.

COLLECTION OF SHALLOW *M. STYX*.—The shallow water chemotype, sample number 27-VI-87-2-003, was collected by scuba at 23 m depth in Fernandez Bay, San Salvador, Bahamas. A voucher specimen is deposited in the Harbor Branch Oceanographic Museum (003:00067).

ISOLATION OF COMPOUND **1**.—Frozen sponge (190 g) was extracted in 250 ml of 100% EtOH in a Waring blender. The extract was filtered and concentrated by distillation under reduced pressure to yield 7.5 g of crude extract (3.9% of frozen wt). The residue from the EtOH extraction was partitioned between H_2O (100 ml) and EtOAc (3 × 50 ml) to yield 490 mg (6.5% of crude) of organic-soluble material and 7.01 g of H_2O-soluble material. The EtOAc residue was chromatographed on Si gel using a step gradient of EtOAc in heptane. Compound **1** eluted with 30% EtOAc/70% heptane. Hplc of this fraction on a Lichrosorb-CN bonded Si gel column (EM Science) with an eluent of 2.5% IPA in heptane yielded a total of 302 mg of pure compound **1** as an oil: hrcims $[M-H]^+$ *m/z* 301.2152 (calcd 301.2160); $[\alpha]_D$ +15.1; ^{1}H nmr ($CDCl_3$, 360 MHz) δ see Table 1; ^{13}C nmr ($CDCl_3$, 90 MHz) see Table 1; ir (film on KBr) cm^{-1} 3440, 2975, 2915, 1680, 1615, 1440, 1380, 1215, 1110, 970, 920; uv (EtOH) λ max 240 nm (ε 33,255).

COLLECTION OF DEEP *M. STYX*.—The deep water chemotype, sample number 29-V-88-1-014, was collected by scuba at a depth of 33 m at Islas Los Roques, Venezuela. A voucher specimen is deposited in the Harbor Branch Oceanographic Museum (003:00121).

ISOLATION OF **3**, **6**, AND **7**.—Frozen sponge (131 g) was extracted by homogenization with 100% EtOH (2 × 250 ml) followed by homogenization in 250 ml of 100% EtOAc in a Waring blender. The extracts were combined, filtered through Whatman #1 filter paper, and concentrated by distillation under reduced pressure to yield 5.65 g of crude extract. The residue from the extraction was partitioned between H_2O (100 ml) and EtOAc (4 × 50 ml) to yield 510 mg of organic-soluble material and 5.14 g of H_2O-soluble material. The EtOAc residue was chromatographed on Si gel under vlc conditions using a step gradient of EtOAc in heptane as eluent. Compounds **3**, **6**, and **7** eluted with 20% EtOAc in heptane. Hplc of this fraction on a Whatman partisil column with an eluent of 10% EtOAc in heptane yielded 42.3 mg of **3**, 31.8 mg of **6**, and 1.7 mg of **7**.

Compound **3**.—Hreims *m/z* 288.2464 (calcd 288.2454); ^{1}H nmr ($CDCl_3$, 360 MHz) see Table 1; ^{13}C nmr ($CDCl_3$, 90 MHz) see Table 1; ir (deposit on KBr) ν max cm^{-1} 3260, 3040, 2950, 2900, 2610, 2240, 1625, 1430, 1370, 1310, 1265, 1215, 1070, 1010, 830; uv λ max 229.4 (ε = 762); $[\alpha]^{22}_D$ 38.8° (*c* = 0.050, CH_2Cl_2).

Compound **6**.—^{1}H nmr ($CDCl_3$, 360 MHz) see Table 1; ^{13}C nmr ($CDCl_3$, 90 MHz) see Table 1; ir (CH_2Cl_2) ν max cm^{-1} 2920, 2860, 2290, 1715, 1360, 1220, 1085, 1015, 870, 835; uv λ max 227.0 (ε = 494); $[\alpha]^{22}_D$ −87.7° (*c* = 0.018, CH_2Cl_2).

Compound **7**.—^{1}H nmr ($CDCl_3$, 360 MHz) 5.77 (s), 5.27 (s), 2.74 (t, *J* = 7.4 Hz), 2.45 (m), 2.06 (3H, s), 1.97 (m), 192 (m), 161 (s), 1.55 (s), 1.41 (m), 1.33 (s), 1.26 (m), 1.11 (3H, d, *J* = 6.5 Hz), 1.07 (3H, d, *J* = 6.5 Hz), 1.03 (3H, d, *J* = 10.5 Hz), 0.94 (3H, s), 0.91 (3H, s); ^{13}C nmr ($CDCl_3$, 90 MHz) 171.4 (s), 160.6 (s), 122.0 (d), 80.6 (s), 60.6 (d), 60.0 (s), 55.3 (d), 55.0 (d), 48.0 (s), 44.2 (t), 38.2 (t), 36.4 (s), 35.2 (t), 31.4 (d), 27.9 (t), 26.1 (t), 24.0 (q), 22.2 (q), 22.0 (q), 21.1 (q), 20.9 (q), 15.2 (q); ir (deposit on KBr) ν max cm^{-1} 2960, 2920, 2860, 1730, 1445, 1370, 1235, 1020, 915, 870; uv λ max 230.3 (ε = 1096); $[\alpha]^{22}_D$ 69.4° (*c* = 0.018, CH_2Cl_2).

PREPARATION OF **4**.—Compound **3** (4.6 mg) was placed into a 5-ml round-bottom flask containing 0.5 ml pyridine and 0.5 ml Ac_2O. The reaction was allowed to proceed overnight at room temperature. Standard workup yielded 3.0 mg of **4**: hreims *m/z* 330.2570 (calcd 330.2560 for $C_{22}H_{34}O_2$); ^{1}H nmr ($CDCl_3$, 360 MHz) 5.82 (s), 5.35 (brs), 5.27 (s), 2.74 (t, *J* = 7.4 Hz), 2.41 (m), 2.17 (m), 2.07 (3H, s), 1.72 (s), 1.51 (m), 1.48 (s), 1.44 (m), 1.33 (s), 1.26 (s), 1.11 (3H, d, *J* = 6.7), 1.01 (3H, d, *J* = 6.7), 0.93 (3H, s), 0.75 (3H, s); ^{13}C nmr ($CDCl_3$, 90 MHz) 171.6, 160.9, 140.6, 122.8, 121.5, 81.0, 55.5, 55.3, 48.0, 43.2, 38.5, 34.4, 34.0, 33.6, 31.3, 28.6, 26.3, 25.0, 21.9, 21.1, 21.0, 16.0; ir (deposit on

KBr) ν max cm^{-1} 3010, 2970, 2910, 1780, 1490, 1420, 1285, 1070; uv λ max 229.5 ($\epsilon = 790$); $[\alpha]^{22}D$ 39.1° ($c = 0.023$, CH_2Cl_2).

PREPARATION OF **5**.—Compound **3** (4.5 mg) was placed into a 5-ml round-bottom flask containing 0.5 ml CH_2Cl_2 and 5 mg PCC. The reaction was allowed to proceed for 1.75 h at room temperature. Standard workup yielded 4.5 mg of **5**: hreims *m/z* 286.2301 (calcd 286.2298 for $C_{20}H_{30}O$); ^1H nmr ($CDCl_3$, 360 MHz) 5.81 (s), 5.34 (brs), 2.73 (m), 2.26 (d, $J = 10$), 2.17 (m), 2.06 (s), 1.73 (s), 1.57 (3H, s), 1.23 (3H, d, $J = 6.7$), 1.13 (3H, d, $J = 6.7$), 1.04 (3H, s), 0.80 (3H, s); ^{13}C nmr ($CDCl_3$, 90 MHz) 192.87, 174.5 (? very weak), 140.0, 123.5, 122.7, 58.3, 55.6, 53.3, 51.4, 43.2, 39.6, 33.8, 32.5, 30.6, 27.4, 27.1, 25.4, 21.8, 20.8, 16.9; ir (deposit on KBr) ν max cm^{-1} 2960, 2920, 2860, 1700, 1595, 1445, 1370, 1255, 1240, 1170; uv λ max 237.4 ($\epsilon = 5014$); $[\alpha]^{22}D$ 12.0° ($c = 0.039$, CH_2Cl_2).

EPOXIDATION OF **4**.—Compound **4** (4.3 mg) was placed into a 5-ml round-bottom flask containing 5 mg of MCPBA and 500 μl of CH_2Cl_2. The reaction was allowed to proceed at 0° for 1 h. Standard workup yielded 1.5 mg of material spectroscopically identical with **7**.

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