Serrulatane Diterpenes with Antimycobacterial Activity Isolated from the West Indian Sea Whip *Pseudopterogorgia elisabethae*[1]

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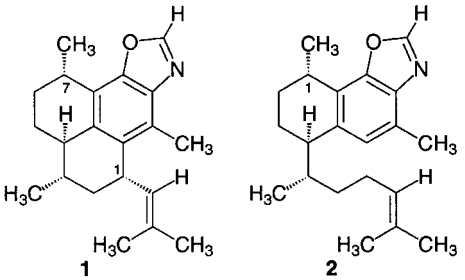
Two new antimycobacterial serrulatane diterpenes, erogorgiaene (**3**) and 7-hydroxyerogorgiaene (**4**), and a novel C_{40} bisditerpene (**5**), have been isolated from the West Indian gorgonian octocoral *Pseudopterogorgia elisabethae*. The structures of compounds **3**−**5** were determined by spectral (1D and 2D NMR, IR, UV, and HREIMS) analysis.

Marine organisms have attracted considerable attention as a source of novel natural products with intriguing structures and useful biological activities.[3] A recent bioassay-guided search for new antituberculosis agents from the West Indian gorgonian octocoral *Pseudopterogorgia elisabethae* Bayer (order Gorgonacea, family Gorgoniidae, phylum Cnideria) led to the discovery of two benzoxazole alkaloids, pseudopteroxazole (**1**) and *seco*-pseudopteroxazole (**2**).[4,5] Compound **1** was found to effect potent inhibitory activity (97%) against *Mycobacterium tuberculosis* $H_{37}Rv$ at a concentration of 12.5 μg/mL, whereas **2** inhibited 66% of mycobacterial growth. In this paper, we wish to describe the identification and antimycobacterial properties of two serrulatane-based diterpenes (also known as biflo-ranes),[6] erogorgiaene (**3**) and 7-hydroxyerogorgiaene (**4**), obtained from the hexane extract of the coral. This paper also describes the isolation and structure elucidation of a novel C_{40} bisditerpenoid, namely, bis-7-hydroxyerogorgiaene (**5**).



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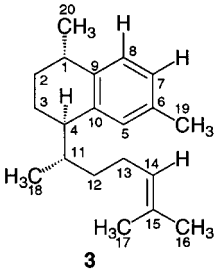

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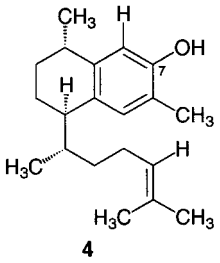

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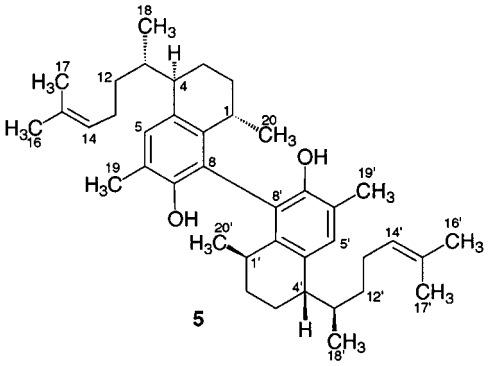
Erogorgiaene (**3**) was obtained as an optically active colorless oil, $[\alpha]^{25}_D$ +24.4°. HREIMS established a molecular formula of $C_{20}H_{30}$. The UV maximum at 280 nm and IR bands at 1600 and 1496 cm^{-1} indicated the presence of an aromatic ring. The ^1H NMR spectrum of **3** showed two



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doublets at δ 7.13 and 6.94 (1H, J = 7.8 Hz each) and a broad one-proton singlet at δ 7.02, indicating the presence of a 1,2,4-trisubstituted benzene ring. Other features of the spectrum included a broad triplet at δ 5.17 (1H, J = 6.9 Hz) and two broad methyl singlets at δ 1.72 and 1.64 indicative of a $(CH_3)_2C{=}CH-$ group, a sharp three-protons singlet at δ 2.30 indicating an aromatic methyl, two methyl doublets at δ 1.27 (3H, J = 7.0 Hz) and 0.64 (3H, J = 6.8 Hz), and two complex multiplets at δ 2.88 and 2.72 (1H each) suggesting two benzylic hydrogens.

The ^{13}C NMR spectrum exhibited 20 signals (five CH_3, four CH_2, seven CH, and four C) whose chemical shift values and multiplicity confirmed the presence of a 1,2,4-trisubstituted aromatic ring [δ 140.4 (s), 139.9 (s), 134.7 (s), 128.1 (d), 126.4 (d), 126.0 (d)] and a trisubstituted olefin [δ 131.3 (s), 124.8 (d)]. Spectral evidence thus demanded that compound **3** is bicyclic with one olefin and a benzene ring. Thus, in common with *seco*-pseudopteroxazole (**2**) and other *P. elisabethae* metabolites, it appeared that compound **3** posseses a serrulatane skeleton.[7] The structure of **3** was easily determined by 2D NMR spectra, including COSY, NOESY, HMQC, and HMBC (Table 1). The ^1H and ^{13}C NMR (Table 1) chemical shifts of **3** were very similar to those of **2**, except for some signals that displayed minor variations due to the disappearance of the oxazole ring. The relative position of such atoms was clearly supported by $^1H-^1H$ COSY, NOESY, and HMBC results (Table 1).

A molecular formula of $C_{20}H_{30}O$ was established for 7-hydroxyerogorgiaene (**4**) from HREIMS plus ^1H and ^{13}CNMR data. The IR band contained a strong hydroxyl stretching band at 3405 cm^{-1}. The ^1H NMR spectrum of compound **4** was almost identical with that of compound **3**, with the exception that one of the aromatic proton resonances in the latter was replaced by an exchangeable one-proton singlet at δ 4.50. The replacement of the H-7

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Table 1. ^{1}H NMR (300 MHz), ^{13}C NMR (75 MHz), and HMBC Spectral Data for Compounds **3**−**5** in $CDCl_3$[a]

	erogorgiaene (**3**)			7-hydroxyerogorgiaene (**4**)			*bis*-7-hydroxyerogorgiaene (**5**)		
atom	δ_H, mult, intgr (*J*)	δ_C (mult)	HMBC[b]	δ_H, mult, intgr (*J*)	δ_C (mult)	HMBC[b]	δ_H, mult, intgr (*J*)	δ_C (mult)	HMBC[b]
1	2.72, m, 1H	32.8 (d)	H8, H20	2.68, m, 1H	32.9 (d)	H8, H20	2.52, br m, 1H	30.1 (d)	H20
2α	1.28, m, 1H	31.8 (t)	H20	1.30, m, 1H	31.8 (t)	H3α, H20	1.42, br m, 1H	17.8 (t)	H20
2*β*	1.79, m, 1H			1.90, m, 1H			1.78, br m, 1H		
3α	1.78, m, 1H	21.5 (t)	H2α*β*	1.78, m, 1H	21.6 (t)	H2α*β*	1.78, br m, 1H	28.1 (t)	
3*β*	1.52, m, 1H			1.47, m, 1H			1.40, br m, 1H		
4	2.88, m, 1H	41.4 (d)	H5, H12α*β*, H18	2.81, m, 1H	40.8 (d)	H5, H12α*β*, H18	2.74, br m, 1H	38.9 (d)	H5, H18
5	7.02, br s, 1H	128.1 (d)	H7, H19	6.94, br s, 1H	129.9 (d)	H19	7.02, br s, 1H	132.0 (d)	H19
6		134.7 (s)	H8, H19		120.7 (s)	7-OH, H8, H19		122.0 (s)	H19
7	6.94, d, 1H (7.8 Hz)	126.0 (d)	H5, H19		151.2 (s)	H5, 7-OH, H8, H19		149.6 (s)	H5, H19
8	7.13, d, 1H (7.8 Hz)	126.4 (d)		6.66, br s, 1H	112.7 (d)	7-OH		117.6 (s)	
9		140.4 (s)	H5, H7, H20		142.4 (s)	H5, H20		140.9 (s)	H5, H20
10		139.9 (s)	H3α, H5, H8		132.2 (s)	H8		132.1 (s)	
11	2.13, m, 1H	36.9 (d)	H12α*β*, H18	2.09, m, 1H	36.9 (d)	H12α*β*, H18	1.98, br m, 1H	39.8 (d)	H18
12α	1.43, m, 1H	35.2 (t)	H13α*β*, H18	1.40, m, 1H	35.2 (t)	H18	1.45, br m, 1H	35.8 (t)	H13α*β*, H18
12*β*	1.34, m, 1H			1.32, m, 1H			1.36, br m, 1H		
13α	2.08, m, 1H	26.3 (t)	H12α*β*, H14	2.08, m, 1H	26.3 (t)	H12α*β*	2.08, br m, 1H	26.3 (t)	H12α*β*
13*β*	2.00, m, 1H			2.00, m, 1H			2.04, br m, 1H		
14	5.17, br t, 1H (6.9 Hz)	124.8 (d)	H12α*β*, H16, H17	5.16, br t, 1H (7.0 Hz)	124.9 (d)	H12α*β*, H16, H17	5.17, br s, 1H	124.9 (d)	H16, H17
15		131.3 (s)	H13α*β*, H16, H17		131.2 (s)	H13α, H16, H17		131.3 (s)	H16, H17
16	1.72, br s, 3H	25.8 (q)	H14, H17	1.72, br s, 3H	25.8 (q)	H14, H17	1.73, br s, 3H	25.8 (q)	H17
17	1.64, br s, 3H	17.7 (q)	H14, H16	1.64, br s, 3H	17.7 (q)	H14, H16	1.65, br s, 3H	17.7 (q)	H16
18	0.64, d, 3H (6.8 Hz)	14.5 (q)	H12α*β*	0.64, d, 3H (6.9 Hz)	14.4 (q)	H12α*β*	0.72, br d, 3H (6.9 Hz)	16.4 (q)	
19	2.30, s, 3H	21.2 (q)	H5, H7	2.21, s, 3H	15.5 (q)	H5	2.26, br s, 3H	16.2 (q)	H5
20	1.27, d, 3H (7.0 Hz)	21.8 (q)		1.24, d, 3H (6.9 Hz)	21.8 (q)		0.72, br d, 3H (6.9 Hz)	21.5 (q)	H2α*β*
7-OH				4.50, br s, 1H			4.74, br s, 1H		

[a] Assignments were aided by ^{1}H−^{1}H COSY, spin splitting patterns, DEPT, HMBC, HMQC, and NOESY experiments, and chemical shift values. The δ values are in ppm and are referenced to either the residual $CHCl_3$ (7.26 ppm) or $CDCl_3$ (77.0 ppm) signals. [b] Protons correlated to carbon resonances in atom column. Parameters were optimized for $^{2,3}J_{CH} = 6$ and 8 Hz.

aryl proton by a hydroxyl group was supported by the presence in the ^{13}C NMR spectrum of a quaternary resonance at δ 151.2 (s) assigned to C-7 that showed strong HMBC couplings, with the proton signals ascribable to 7-OH (δ_H 4.50), H-8 (δ_H 6.66), and Me-19 (δ_H 2.21). This was further confirmed by complementary HMBC correlations between 7-OH and C-6 (δ_C120.7) and C-8 (δ_C112.7).

Conventional chromatographic methods yielded pure compound **5**, $[\alpha]^{25}_D$ +61.5° (*c* 0.7, $CHCl_3$). This colorless oil presented at first a number of puzzling features. The ^{1}H and ^{13}C NMR spectra (Table 1), which appear to indicate a C_{20} compound, suggested a close structural similarity with 7-hydroxyerogorgiaene (**4**); but the signals in the ^{1}H NMR spectrum were, however, unusually broad. The IR spectrum of **5** indicated that this compound contained the same functional groups as **4**, that is, olefin, hydroxyl, and benzene moieties. Furthermore, because there were no significantly large differences in both ^{1}H and ^{13}C chemical shift values at most positions in **5** when compared to **4**, it was quickly thought that **5** could be a symmetrical bis-diterpene. An explanation for these unusual features was provided when it was shown by HREIMS that **5** analyzed for $C_{40}H_{58}O_2$, corresponding to two diterpenoid units minus two hydrogens. Thus, the NMR spectra and the UV and optical activity established that the two C_{20} units were structurally and configurationally identical. The broadening of the ^{1}H signals can be attributed to the reduced rate of tumbling of this rather large molecule in solution. Although the ^{1}H and ^{13}C NMR data for **5** were very similar to those of **4**, there were some significant variations. The ^{1}H NMR chemical shift of Me-20 (δ 0.72 versus 1.24) and the ^{13}C NMR chemical shift of C-2 (δ 17.8 versus 31.8) indicated that these atoms, which appear unusually shielded in **5**, are forced into the π-cloud of the nearby biaryl system. Compound **4** is assumed to be a logical biogenetic precursor to bis-7-hydroxyerogorgiaene (**5**) upon undergoing further de-hydrogen coupling with another molecule through C-8.

The relative configurations for the stereocenters of serrulatanes **3**−**4** (i.e., C-1, C-4, and C-11) were assigned primarily on the basis of ^{1}H NMR decoupling experiments, NOESY NMR data, and a molecular modeling study acquired with 7-hydroxyerogorgiaene (**4**). Irradiation of the protons with almost co-incident chemical shifts at δ 1.24 (Me-20) and 1.30 (H-2α) collapsed the multiplet at δ 2.68 (H-1) to a broad doublet (J = 8.1 Hz). The large coupling between H-1 and H-2*β* (δ_H 1.90) showed that these protons were pseudoaxial. The very intense NOE correlation of Me-20 and δ_H 6.66 (H-8), together with the conspicuous absence of NOE between H-1 and H-8, suggested that the methyl group at C-1 is pseudoequatorial and that H-1 is pseudoaxial. This assumption was confirmed by a strong NOESY correlation between H-1 and H-3*β* (δ_H 1.47) establishing the spatial proximity of these protons on the top face of the molecule. A series of complementary NOE correlations showed the proximity of H-2α, H-3α (δ_H 1.78), and H-4 (δ_H 2.81) on the α-face of the molecule. Additionally, irradiation of the proton at δ 2.09 (H-11) collapsed the multiplet at δ 2.81 (H-4) to a broad doublet of doublets, showing the pseudodiaxial coupling between H-3*β* and H-4 (8.4 Hz) and the pseudoaxial−pseudoequatorial coupling between H-3α and H-4 (6.6 Hz). Furthermore, a strong NOESY correlation between H-3*β* and Me-18 (δ_H 0.64) also allowed the assignment of H-4 to the bottom face of the molecule (i.e., cis to Me-20). That the C_8H_{15} alkenyl side chain at C-4 is pseudoequatorial was also consistent with the strong NOESY correlation between δ_H 6.94 (H-5) and δ_H 2.09 (H-11) but not H-4. Additional NOESY correlations were observed between H-4 and H-11, H-2α and H-4, H-14

and Me-16, and H-5 and Me-19. The distance between protons experiencing all these NOEs in **4** lie within 2.1−2.6 Å, according to a molecular modeling study. Thus, the overall relative stereochemistry for serrulatanes **3** and **4** was assigned as 1*S**, 4*R**, and 11*S**.

Erogorgiaene (**3**) induced 96% growth inhibition for *Mycobacterium tuberculosis* $H_{37}Rv$ at a concentration of 12.5 μg/mL. On the other hand, 7-hydroxyerogorgiaene (**4**) inhibited 77% of mycobacterial growth at a concentration of 6.25 μg/mL, which indicates that C-7 hydroxylation apparently does not reduce the activity.[8,9] Since *seco*-pseudopteroxazole (**2**), erogorgiaene (**3**), and pseudopteroxazole (**1**) induced 66, 96, and 97% inhibition of *M. tuberculosis* growth at 12.5 μg/mL, respectively, clearly the benzoxazole moiety is not essential for activity, as illustrated by compound **3**. Follow-up biological screening of **4** in the National Cancer Institute's (NCI) 60-cell-line tumor panel indicated no significant in vitro cancer cell cytotoxicity, suggesting that erogorgiaene (**3**), which could not be tested due to scarcity of material, will probably display minimal toxicity.

Experimental Section

General Experimental Procedures. IR spectra were recorded with a FT-IR spectrophotometer. ^1H and ^{13}C NMR spectral data and $^1H-^1H$ COSY, NOESY, DEPT, HMQC, and HMBC experiments were measured with a 300 MHz FT-NMR spectrometer. Column chromatography was performed on Si gel (35−75 mesh) or bonded C_{18} Si gel (35−75 mesh). TLC analyses were carried out using glass precoated Si gel plates. All solvents used were either spectral grade or were distilled from glass prior to use. The percentage yield of each compound is based on the weight of the dry gorgonian MeOH−$CHCl_3$ extract.

Extraction and Isolation. A voucher specimen of *P. elisabethae* (no. PESAI-01) is stored at the Chemistry Department of the University of Puerto Rico. The extraction scheme has been previously described.[10] A portion of the hexane extract (50 g) was dissolved in a small volume of toluene, filtered, and loaded onto a large Bio-Beads SX-3 column with toluene as eluent. Fractions were pooled based on their TLC and NMR profile to yield four primary fractions, designated as I−IV. Fraction III (15.1 g) was further chromatographed by Si gel (270 g) column chromatography using 10% EtOAc in hexane as eluent. Eighteen tertiary fractions were obtained (IIIA−IIIR). Fraction F (363 mg) was purified repeatedly by reversed-phase column chromatography over C_{18} Si gel (20 g) using 3% water in methanol and normal-phase column chromatography over Si gel (5.0 g) using 5% ethyl acetate in hexane to yield pseudopteroxazole (**1**) (15.0 mg; 1.88×10^{-2}% yield). Fraction H (182 mg) was purified by column chromatography on Si gel (8.5 g) using 5% acetone in hexane to yield several fractions. The first two fractions to elute (20.2 mg and 60.5 mg, respectively) were chromatographed separately over C_{18} Si gel (3 g each) using 15% water in methanol to furnish, respectively, bis-7-hydroxyerogorgiaene (**5**) (ca. 1.0 mg; 1.25×10^{-3}% yield) and *seco*-pseudopteroxazole (**2**) (ca. 2.0 mg; 2.51×10^{-3}% yield). Fraction I (171.4 mg) was repeatedly chromatographed over Si gel (6.4 g) using 25% hexane in $CHCl_3$ and then C_{18} Si gel (1.6 g) with 3% water in methanol to afford 7-hydroxyerogorgiaene (**4**) (5.3 mg; 6.64×10^{-3}% yield). Fractions K and L were combined (4.30 g) and purified by column chromatography on Si gel (150 g) using a step gradient of 10−20% acetone in hexane. The least polar fraction obtained was rechromatographed on Si gel (23 g) using 3% acetone in hexane to yield erogorgiaene (**3**) (7.6 mg; 9.53×10^{-3}% yield).

Erogorgiaene (3): colorless oil; $[\alpha]^{25}_D$ +24.4° (*c* 3.2, $CHCl_3$); UV (MeOH) λ_{max} 208 nm (ε 16 000), 280 nm (ε 1500); IR (film) 2954, 2922, 2853, 1600, 1496, 1457, 1376, 1323, 1261, 806 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz), see Table 1; EIMS *m*/*z* $[M]^+$ 270 (12), 199 (6), 186 (19), 160 (14), 159 (100), 157 (9), 144 (7), 129 (8), 105 (6), 69 (7); HREIMS *m*/*z* $[M]^+$ 270.2337 (calcd for $C_{20}H_{30}$, 270.2347).

7-Hydroxyerogorgiaene (4): colorless oil; $[\alpha]^{25}_D$ +25.8° (*c* 3.8, $CHCl_3$); UV (MeOH) λ_{max} 208 nm (ε 22 000), 284 nm (ε 1800); IR (film) 3405, 2965, 2929, 2846, 1512, 1465, 1383, 1264 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz), see Table 1; EIMS *m*/*z* $[M]^+$ 286 (12), 215 (3), 202 (7), 176 (13), 175 (100), 173 (3), 160 (5), 69 (6); HREIMS *m*/*z* $[M]^+$ 286.2293 (calcd for $C_{20}H_{30}O$, 286.2297).

bis-7-Hydroxyerogorgiaene (5): colorless oil; $[\alpha]^{25}_D$ +61.5° (*c* 0.7, $CHCl_3$); UV (MeOH) λ_{max} 210 nm (ε 41 600), 286 nm (ε 5800); IR (film) 3525, 3434, 2956, 2926, 2857, 1652, 1457, 1375, 1315, 1223, 1177, 1108, 1027, 896 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz), see Table 1; EIMS *m*/*z* $[M]^+$ 570 (36), 568 (21), 512 (8), 460 (34), 459 (100), 458 (17), 457 (45), 175 (15), 69 (20); HREIMS *m*/*z* $[M]^+$ 570.4443 (calcd for $C_{40}H_{58}O_2$, 570.4437).

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References and Notes

(1) Taken in part from the Ph.D. Dissertation of C. Ramírez, University of Puerto Rico, 2000.
(2) Graduate student sponsored by the NIH-MBRS Program of the University of Puerto Rico.
(3) (a) Faulkner, D. J. *Nat. Prod. Rep.* **2000**, *17*, 7−55, and previous reports in this series. (b) Rodríguez, A. D. *Tetrahedron* **1995**, *51*, 4571−4618 and references therein.
(4) For isolation and original structure determination of benzoxazole alkaloids **1** and **2**, see: Rodríguez, A. D.; Ramírez, C.; Rodríguez, I. I.; González, E. *Org. Lett.* **1999**, *1*, 527−530.
(5) In this paper, the original stereochemical assignment for pseudopteroxazole (**1**) at the C-1 and C-7 stereocenters and for *seco*-pseudopteroxazole (**2**) at C-1, which was based primarily on spectral comparisons with pseudopterosins G−J and helioporin E, has been modified. Recent independent work by the groups of Schmalz and of Corey has provided sound evidence for the stereochemical reassignment of these amphilectane-based compounds at C-1 and C-7. Thus, it follows that compounds **1** and **2** may also require revision in line with the recent evidence. Synthetic studies are ongoing in Corey's group to clarify this matter. See: (a) Lazerwith, S. E.; Johnson, T. W.; Corey, E. J. *Org. Lett.* **2000**, *2*, 2389−2392. (b) Geller, T.; Schmalz, H.-G.; Bats, J. W. *Tetrahedron Lett.* **1998**, *39*, 1537−1540. (c) Geller, T.; Jakupovic, J.; Schmalz, H.-G. *Tetrahedron Lett.* **1998**, *39*, 1541−1544. (d) Hörstermann, D.; Schmalz, H.-G.; Kociok-Köhn, G. *Tetrahedron* **1999**, *55*, 6905−6916. For work on the isolation and original structure determination of pseudopterosins G−J, see Roussis et al.[7b] For the isolation and original structure determination of the helioporins, see: Tanaka, J.; Ogawa, N.; Liang, J.; Higa, T.; Gravalos, D. G. *Tetrahedron* **1993**, *49*, 811−822.
(6) Biflorin represents the first natural product with this skeleton: Comin, J.; Gonçalves de Lima, O.; Grant, H. N.; Jackman, L. M.; Keller-Schierlein, W.; Prelog, V. *Helv. Chim. Acta* **1963**, *46*, 409−415.
(7) (a) Look, S. A.; Fenical, W.; Matsumoto, G. K.; Clardy, J. *J. Org. Chem.* **1986**, *51*, 5140−5145. (b) Roussis, V.; Wu, Z.; Fenical, W.; Strobel, S. A.; Van Duyne, G. D.; Clardy, J. *J. Org. Chem.* **1990**, *55*, 4916−4922. (c) Harvis, C. A.; Burch, M. T.; Fenical, W. *Tetrahedron Lett.* **1988**, *29*, 4361−4364. (d) Look, S. A.; Fenical, W. *Tetrahedron* **1987**, *43*, 3363−3370.
(8) Presumably, the 19% decrease in inhibitory activity observed for **4** when compared to erogorgiaene (**3**) is due, not to C-7 hydroxylation, but to a 2-fold decrease in concentration. Such concentration (6.25 μg/mL) is currently used by the TAACF in their primary (Level I) screening assay.
(9) El Sayed, K. A.; Bartyzel, P.; Shen, X.; Perry, T. L.; Zjawiony, J. K.; Hamann, M. T. *Tetrahedron* **2000**, *56*, 949−953.
(10) Rodríguez, A. D.; González, E.; Huang, S. D. *J. Org. Chem.* **1998**, *63*, 7083−7091.

NP000196G