Cembranoid Diterpenes from the Caribbean Sea Whip *Eunicea knighti*

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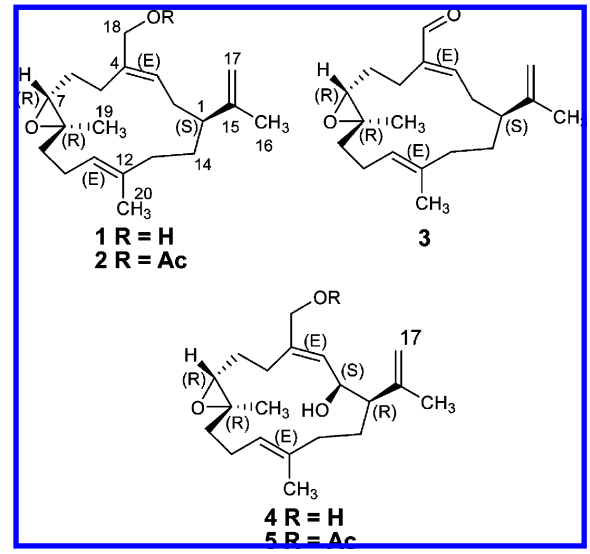
Three new cembranoid diterpenes, knightol (**1**), knightol acetate (**2**), and knightal (**3**), along with the known asperdiol (**4**) and asperdiol acetate (**5**), were isolated as major compounds from the sea whip *Eunicea knighti* collected from the Colombian Caribbean. The structures and absolute configurations of **1**−**5** were determined on the basis of spectroscopic analyses and by a combination of chemical and NMR methods, multiple correlations observed in a ROESY experiment, and using the modified Mosher method. Additionally, five semisynthetic compounds, **6**−**10**, obtained during the chemical transformations of the natural compounds are here reported for the first time. All compounds were tested for antimicrobial activity against marine bacteria associated with heavily fouled surfaces and were also screened for antiquorum sensing (QS) activity. Compounds **1**, **3**, and **8** showed significant antimicrobial activity against bacterial isolates, and **1**, **3**, **7**, and **8** showed excellent anti-QS inhibition activity measured by means of bioluminescence inhibition with biosensor model systems.

Among the gorgonian octocorals, *Eunicea Lamoroux* is the most abundant and diverse genus present in coral reefs and on the rocky coast of the Caribbean sea.[1] These animals, usually referred to as sea whips, have proven to be a rich source of compounds with novel structures,[2−4] particularly cembranolide diterpenes displaying remarkable arrays of functional groups, many of them exhibiting potent biological activities, i.e., antitumor, antiplasmodial, anticancer, and anti-inflammatory.[2−4] Although this genus has been the subject of numerous chemical and biological studies, many *Eunicea* species still remain to be investigated. Considering these factors, we undertook the study of *Eunicea knighti* from the Colombian Caribbean, which has been the subject of only limited investigation.[5] On the other hand, our continuous search for useful compounds from marine invertebrates[6−10] and our screenings for antifouling activity[10] pointed to this species as a promising source of antifoulant compounds. Thus, we here describe the isolation and structure elucidation of knightol (**1**), knightol acetate (**2**), and knightal (**3**), previously unreported cembranolide-type diterpenoids, together with the known asperdiol (**4**) and asperdiol acetate (**5**) from specimens of *E. knighti* collected off Santa Marta Bay, Colombian Caribbean, as well as five new semisynthetic cembranolides (**6**−**10**) obtained by chemical transformations of the isolated natural compounds. Furthermore we report here the antimicrobial activities of **1**−**10** against marine bacteria associated with heavily fouled surfaces and whether those compounds have the ability to interfere with bacterial signal-mediated quorum sensing systems, which modulate between microbial surface colonization and biofilm maturation, the first steps of the fouling process.

Results and Discussion

Frozen material of *E. knighti*, collected at Santa Marta Bay, Colombian Caribbean, was extracted with CH_2Cl_2−MeOH (1:1 v/v), and the resulting extract was subjected to vacuum chromatography on silica gel, to give six fractions, of which 2, 3, and 4 were active in the antimicrobial bioassay used. The active fractions were subjected to CC on silica gel using a mobile phase of increasing polarity and finally purified by preparative reversed-phase HPLC to afford compounds **1**−**5**.



Compound **1** (knightol) was isolated as a colorless oil. The molecular formula of **1** was assigned as $C_{20}H_{32}O_2$ on the basis of HRESIMS (*m*/*z* 327.2301 $[M + Na]^+$) and ^{13}C NMR spectra, requiring five degrees of unsaturation. Its IR spectrum revealed the presence of hydroxy, epoxide, and olefinic bands at 3420, 1224, 3070, and 1643 cm^{-1}, respectively. The ^{13}C NMR data of **1** (Table 1) revealed the presence of 20 carbon signals of a cembrene-type diterpenoid,[11,12] which were assigned using DEPT to three methyls, nine methylenes, four methines, and four quaternary carbons. Their chemical shifts and multiplicities indicated the presence of an epoxide [δ 64.5, d, (C-7) and δ 60.5, s, (C-8)], two trisubstituted olefins [δ 139.3, s, (C-4) and δ 125.4, d, (C-3); δ 135.2, s, (C-12) and δ 124.8, d, (C-11)], a terminal olefin [δ 148.3, s, (C-15) and δ 110.5, t, (C-17)], and a hydroxy-bearing methylene carbon [δ 66.6, t, (C-18)]. The ^1H NMR spectrum of **1** (Table 1) showed three methyl groups (δ 1.21, s; 1.61, br s; and δ 1.68, br s), the first two as substituents of the olefins and the third one as a substituent of the epoxide (assigned by its HMBC correlation with the carbon signal at δ 60.5). Moreover, the ^1H NMR spectrum revealed the presence of two olefinic protons as broad triplets at δ 5.19 (br t, J = 6.6 Hz) and δ 5.51 (br t, J = 7.8 Hz), one proton signal at δ 2.76 (dd, J = 7.5, 3.4 Hz), which correlated in the HMQC spectrum with the carbon signal at δ 64.5, two broad singlets corresponding to the terminal methylene protons at δ 4.61

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Table 1. NMR Spectroscopic Data[a] (400 MHz, $CDCl_3$) for Compounds **1**−**3**

	1		**2**		**3**	
position	δ_C, mult.[b]	δ_H, (J inHz)	δ_C, mult.[b]	δ_H, (J in Hz)	δ_C, mult.[b]	δ_H (J in Hz)
1	45.3, CH	1.97, m	45.1, CH	1.96, m	45.1, CH	2.09, m
2a	29.5, CH_2	2.37, m	29.7, CH_2	2.38, m	30.4, CH_2	2.70, m
2b		1.82, m		1.82, m		2.10, m
3	125.4, CH	5.51, br t (7.8)	129.2, CH	5.56, t (7.9)	153.4, CH	6.49, dd (9.8, 5.0)
4	139.3, qC		134.4, qC		143.3, qC	
5a	24.6, CH_2	2.33, m	24.9, CH_2	2.30, m	21.8, CH_2	2.45, dt (14.3, 7.4)
5b		2.22, m		2.23, m		2.33, dt (13.7, 6.9)
6a	26.6, CH_2	2.02, m	26.5, CH_2	1.88, m	26.6, CH_2	1.96, m
6b		1.49, m		1.46, m		1.47, m
7	64.5, CH	2.76, dd (7.5, 3.4)	64.3, CH	2.74, dd (7.4, 3.4)	63.8, CH	2.65, dd (7.3, 4.1)
8	60.5, qC		60.5, qC		60.4, qC	
9a	37.2, CH_2	2.04, m	37.2, CH_2	2.00, m	37.2, CH_2	1.88, m
9b		1.42, m		1.42, m		1.46, m
10a	23.8, CH_2	2.16, m	23.8, CH_2	2.16, m	23.6, CH_2	2.13, m
10b		1.59, m		1.59, m		
11	124.8, CH	5.19, br t (6.6)	124.8, CH	5.16, t (6.7)	125.5, CH	5.21, br t (6.6)
12	135.2, qC		135.2, qC		134.6, qC	
13a	36.4, CH_2	2.22, m	36.3, CH_2	2.22, m	36.6, CH_2	2.14, m
13b		2.17, m		2.14, m		2.07, m
14a	30.5, CH_2	1.59, m	30.4, CH_2	1.58, m	30.5, CH_2	1.64, m
14b		1.45, m		1.44, m		1.53, m
15	148.3, qC		148.0, qC		146.8, qC	
16	19.4, CH_3	1.68, s	19.4, CH_3	1.66, s	18.9, CH_3	1.69, s
17a	110.5, CH_2	4.73, br s	110.7, CH_2	4.71, br s	111.8, CH_2	4.78, br s
17b		4.61, br s		4.60, br s		4.64, br s
18a	66.6, CH_2	4.09, d (12.6)	68.1, CH_2	4.54, d (12.3)	195.1, CH	9.33, s
18b		4.03, d (12.6)		4.43, d (12.3)		
19	16.9, CH_3	1.21, s	16.9, CH_3	1.20, s	17.2, CH_3	1.14, s
20	15.4, CH_3	1.61, s	15.4, CH_3	1.59, s	15.4, CH_3	1.59, s
21			170.8, qC			
22			21.0, CH_3	2.06, s		

[a] Full assigments were done by DEPT, HMQC, and HMBC experiments. [b] Multiplicity deduced by DEPT.

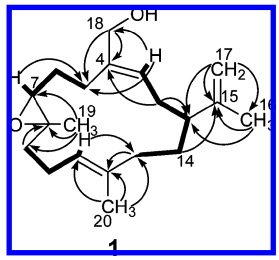

Figure 1. $^1H-^1H$ COSY correlations (bold lines) and key HMBC correlations (arrows) for compound **1**.

and at δ 4.73, and signals corresponding to two diastereotopic protons at δ 4.03 (1H, d, J = 12.6 Hz) and at δ 4.09 (1H, d, J = 12.6 Hz), which correlated in the HMQC spectrum with the carbinolic methylene at δ 66.6. The chemical shifts at δ 66.6 and at δ 24.6 for the methylenes C-18 and C-5, respectively, were in favor of the *E* configuration for the 3,4-trisubstituted double bond. In a similar way, the signals at δ 15.4 for the CH_3-20 and at δ 36.4 for CH_2-13 both allylic to the 11,12-trisubstituted double bond strongly suggested the *E* configuration as well.[13] On the basis of the above results and by the use of $^1H-^1H$ COSY and HMBC experiments, the isolated spin systems were connected, and the planar structure of **1** (Figure 1) could be established.

Compound **2** (knightol acetate) was isolated as a colorless oil. HRESIMS, ^{13}C NMR, and DEPT spectra established the molecular formula of **2** as $C_{22}H_{34}O_3$. Strong IR bands due to epoxide (1228 cm^{-1}) and ester groups (1742 cm^{-1}) were clearly seen. The ^1H NMR spectrum of **2** showed characteristic signals of cembrene-type diterpenes, i.e., three methyl groups, the first two were substituents of double bonds [δ 1.59, br s and δ 1.66, br s] and the third at δ 1.20, a substituent of the epoxide (assigned by the HMBC correlation with the carbon signal at δ 60.5), an epoxymethine proton at δ 2.74 (dd, J = 7.4, 3.4 Hz), a methylene-bearing oxygen at δ 4.43 (d, J = 12.3 Hz) and at δ 4.54 (d, J = 12.3 Hz), a terminal methylene at δ 4.60 (br s) and at δ 4.71 (br s), and two olefinic protons at δ 5.16 (br t, J = 6.7 Hz) and δ 5.56 (br t, J = 7.9 Hz). The ^{13}C NMR spectrum exhibited 22 signals ($4CH_3$, $9CH_2$, 4CH, and 5C), which supported the presence of an epoxide (δ 64.3 (C-7) and δ 60.5 (C-8)), two trisubstituted olefins (δ 134.4 (C-4) and δ 129.2 (C-3); δ 135.2 (C-12) and δ 124.8 (C-11)), a terminal olefin (δ 148.0 (C-15) and δ 110.7 (C-17)), and an acetate group (δ 170.8 (C-21) and δ 21.0 (C-22)). The ^1H and ^{13}C NMR data of **2** (Table 1) were very similar to those of **1**. The main differences were the signals ascribed to C-3, C-4, and C-18, which in **2** resonated at δ 129.2, 134.4, and 68.1, respectively and in **1** at δ 125.4, 139.3, and 66.6. Furthermore, compound **2** displayed two extra resonances at δ 170.8 and 21.0, assigned to an acetate group. Acetylation of the primary hydroxyl group present in **1** at C-18 accounted for the spectral differences. Further detailed analyses of $^1H-^1H$ COSY, HMQC, and HMBC data, as well as other spectral features, allowed us to assign the complete planar structure of this compound.

Compound **3** (knightal) was isolated as an optically active colorless oil with the molecular formula $C_{20}H_{30}O_2$ deduced by HRESIMS and DEPT spectra. In the IR spectrum bands due to epoxide (1228 cm^{-1}) and aldehyde (1727 cm^{-1}) groups were clearly seen. The ^1H and ^{13}C NMR spectra exhibited characteristic signals of a cembrene diterpene, which were very similar to those shown by compound **1**, i.e., two olefinic methyl group substituents, one on a trisubstituted double bond and the other on a disubstituted double bond [δ_H 1.59, s; δ_C 15.4, and δ_H 1.69, br s; δ_C 18.9], one methyl group substituent of an epoxide [δ_H 1.14, s; δ_C 17.2], an epoxymethine [δ_H 2.65, dd, J = 7.3, 4.1 Hz; δ_C 63.8], a terminal olefin [δ_H 4.64, br s and δ_H 4.78, br s; δ_C 111.8], and two olefinic protons, one at δ 5.21 (br t, J = 6.6 Hz) linked to the trisubstituted olefin [δ 143.3 (C-4) and 153.4 (C-3)] and the other at δ 6.49 (dd, J = 9.8, 5.0 Hz) linked to the second trisubstituted olefin [δ 134.6 (C-12) and 125.5 (C-11)] located next to a carbonyl group [δ_H 9.33, s; δ_C 195.1]. The main differences of **3** when compared to **1** were

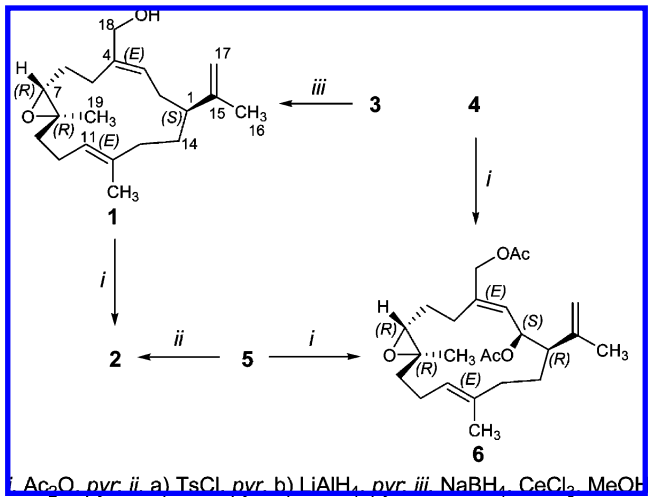

Figure 2. Chemical correlations of compounds **1**−**6**.

the ^{13}C NMR signals ascribed to C-3, C-4, and C-18, which in **3** resonated at δ_C 153.4, 143.3, and 195.1, respectively, and in **1** at δ_C 125.4, 139.3, and 66.6. Additionally in **3** the methylene signals of C-18 [δ_H 4.03, d, J = 12.6 Hz and δ_H 4.09, d, J = 12.6 Hz; δ_C 66.6] are replaced by a signal ascribed to an aldehyde [δ_H 9.33, s; δ_C 195.1]. Further analysis of $^1H-^1H$ COSY, HSQC, and HMBC data confirmed the above-mentioned spectral features and allowed us to assign the complete planar structure of **3**.

Compounds **4** and **5** showed molecular formulas of $C_{20}H_{32}O_3$ and $C_{22}H_{34}O_4$, respectively. The NMR data of **4** agreed with published data for (−)-asperdiol previously isolated from *Eunicea asperula*[14] and from *Eunicea succinea*,[15] and the NMR data of **5** coincided with (−)-asperdiol acetate isolated from *E. succinea*.[15]

The full assignments of NMR data for **1**−**3** are shown in Table 1. The absolute configuration of the three chiral centers at C-1, C-7, and C-8 in **1**−**3**, as well as at C-1, C-2, C-7, and C-8 in **5** and **6**, was established by a combination of chemical and modified Mosher's methods, by extensive NOE studies, and by comparison of the optical rotation of **4** with published data,[14] as described below.

First of all, we established the chemical correlations (Figure 2) of **1**−**5** and conversion of **4** and **5** into compound **6**. Asperdiol acetate **5** was converted to **2** by reduction with $LiAlH_4$ in CH_2Cl_2 and previous derivatization of the C-2 hydroxy group with tosyl chloride, and **1** was transformed to **2** by acetylation. Compound **3** was converted to **1** by Luche reduction,[16] and di- and monoacetylation of **4** and **5**, respectively, afforded in both cases compound **6** (Figure 2). The diacetylation of **6** was evident from its HRESIMS spectrum (molecular formula of $C_{24}H_{36}O_5$ and the ions observed at m/z 344 [M − $CH_3COOH]^+$, m/z 284 [M − $2CH_3COOH]^+$) and from its NMR data (two methyl groups at δ_H 2.06 (6H, s), two methyl groups at δ_C 21.0 and 21.2, and two carbonyls at δ_C 170.3 and 170.6. Complete ^{13}C NMR assignments for **6** are presented in Table 2.

Subsequently, we proceeded to open the epoxide ring in compound **6** (Figure 3) by using $CDCl_3-I_2-HCl$ for 36 h (this reaction usually proceeds with retention of configuration of C-7 and C-8, and the hydroxy group is located at C-7).[17,18] The usual workup afforded a mixture of products, which were purified by column chromatography and reversed-phase HPLC to afford compounds **7** (6.0%), **8** (11.0%), **9** (4.0%), and **10** (41.0%) and starting material **6** (38.0%).

Compounds **7**−**10** were identified by HRESIMS and 1D- and 2D-NMR spectroscopy. In addition to the characteristic signals due to the cembrane-type diterpene skeleton, all of the NMR spectra of **7**−**10** revealed an extra signal attributable to a hydroxy-bearing methine group at C-7, resulting from the opening of the epoxide ring. At the same time, the characteristic signals of the epoxide C-7,8 (δ_H 2.74, dd, H-7; δ_C 64.4 (C-7) and δ_C 60.5 (C-8)) were no longer observable. The complete structures of these compounds (Figure 3) could be deduced by $^1H-^1H$ COSY, HMQC, and HMBC experiments, and their configurations at C-1, C-2, C-7, and C-8 were determined as discussed below. Assignments of ^1H and ^{13}C NMR signals for **7**−**10** are listed in Table 2.

Compound **7** had a molecular formula of $C_{24}H_{36}O_5$. ^1H and ^{13}C NMR data (Table 2) furthermore exhibited signals for a hydroxymethine (δ_H 4.17, dd, J = 8.7, 4.7 Hz; δ_C 77.0) and for an exomethylene (δ_H 5.03, 5.06, s, 1H each; δ_C 111.0, CH_2). These signals are evidence of the epoxide ring-opening with the hydroxy group attached to C-7 and of the formation of a $\Delta^{8,19}$ exocyclic double bond, as deduced from $^1H-^1H$ COSY, HMQC, and HMBC experiments.

For compound **8** a $C_{24}H_{36}O_5$ molecular formula was inferred. Similarly to **7** the NMR data of **8** established the presence of a hydroxymethine (δ_H 4.04, dd, J = 10.0, 2.9 Hz; δ_C 77.0) at C-7 and a vinyl methyl (δ_H 1.64, δ_C 11.6) at C-8. Furthermore, the principle of steric compression[19] and comparison of NMR signals with literature data[15,20] revealed the presence of a trisubstituted *E* double bond between C-8 and C-9 (δ_H 5.20 (H-9); δ_C 124.2 (C-9) and δ_C 131.6 (C-8)), as shown in Figure 3.

Compound **9** had a molecular formula of $C_{24}H_{36}O_5$ as deduced from HRESIMS and ^{13}C NMR data. The NMR data of **9** were very similar to **8**, only differing in the chemical shifts for CH_3-19 and for CH-7. In **9** the CH_3-19 resonated at δ_C 17.4 and CH-7 at δ_C 73.7, indicating a *Z* configuration between C-8 and C-9.[19,20] Again, $^1H-^1H$ COSY, HMQC, and HMBC experiments allowed us to establish the structure of **9**, as shown in Figure 3.

The HRESIMS and NMR data established a molecular formula of $C_{24}H_{37}ClO_5$ for compound **10**. The presence of chlorine was evident in its HRESIMS due to the intensity of an adduct ion pair with (3:1) intensities at m/z 463.2218 $[M + Na]^+$ and m/z 465.2205 $[M + 2 + Na]^+$. Furthermore, the electron impact mass spectrum of **10** showed an ion at m/z 404 $[M - HCl]^+$, which together with the chemical shift of C-8 (δ_C 80.4), compared with its counterpart in **6** (δ_C 60.5), clearly supported the presence of chlorine at C-8. The NMR data also revealed the presence of a hydroxymethine (δ 3.50, dd, J = 9.4, 3.3 Hz; δ_C 76.0) located at C-7. 2D NMR particularly $^1H-^1H$ COSY, HMQC, and HMBC experiments allowed us to determine the complete structure of **10**, as shown in Figure 3.

The absolute configuration at C-7 of compounds **7**−**10** was established by using Mosher's ester method with MPA as derivatization reagent.[21,22] The selected ^1H NMR data for the (*S*)- and (*R*)-MPA esters of **7**−**10** (**7s**,**r**−**10s**,**r**) are summarized in Table 3. The data clearly established the 7*R* configuration of **7**−**10**. Hence, the absolute configuration of the C-7 chiral center of **6** as well as of **1**−**3** and **5** was determined to be *R*.

The absolute configuration of **4** was assigned as 1*R*,2*S*,7*R*,8*R* by comparison of its specific rotation with published data,[14] which implies the same absolute configuration at C-1, C-7, and C-8 for **1**−**3** and the same configuration at C-1, C-2, C-7, and C-8 of **5** and **6** due to the chemical correlations established among those compounds.

However, it is noteworthy to mention that the results of applying Mosher's ester method[21,22] at C-2 in **5** also established a 2*S* configuration in **5**, which in turns means the same configuration at C-2 for compounds **6**−**10**. In addition, the configurations of C-1 and C-8 in **6** were also determined on the basis of NOE correlations observed in a ROESY experiment, which established a *trans* relationship between CH_3-19 and H-7, allowing us to assign the *R* configuration at C-8, taking into account that we had determined the *R* configuration for C-7 using Mosher's method. In the case of C-1, the NOE correlations indicated a *syn* configuration for H-3 and H-1, which together with the *S* configuration for C-2 and with

Table 2. NMR Spectroscopic Data[a] (400 MHz, $CDCl_3$) for Compounds **6**−**10**

	6		**7**		**8**		**9**		**10**	
position	δ_C, mult.[b]	δ_H, (*J* in Hz)	δ_C, mult.[b]	δ_H, (*J* in Hz)	δ_C, mult.[b]	δ_H, (*J* in Hz)	δ_C, mult.[b]	δ_H, (*J* in Hz)	δ_C, mult.[b]	δ_H, (*J* in Hz)
1	48.0, CH	2.19, m	47.9, CH	2.07, m	47.2, CH	2.08, m	48.4, CH	2.18, m	48.1, CH	1.99, m
2	71.0, CH	5.71, dd (8.4, 4.3)	69.9, CH	5.64, dd (8.8, 3.7)	70.9, CH	5.60, m	70.9, CH	5.60, dd (7.7, 3.4)	69.0, CH	5.70, dd (9.3, 3.3)
3	127.8, CH	5.38, br d (8.3)	127.6, CH	5.30, br d (8.7)	128.2, CH	5.18, m	128.0, CH	5.32, br d (7.7)	127.1, CH	5.41, br d (9.4)
4	135.8, qC		135.9, qC		136.4, qC		139.7, qC		137.4, qC	
5a	25.9, CH_2	2.48, m	25.6, CH_2	2.34, m	24.2, CH_2	2.00, m	27.1, CH_2	2.22, m	23.5, CH_2	2.38, m
5b		2.30, m		1.87, m						1.85, m
6a	26.3, CH_2	1.86, m	33.8, CH_2	1.77, m	32.3, CH_2	1.83, m	35.4, CH_2	1.90, m	32.8, CH_2	1.64, m
6b		1.48, m		1.48, m		1.41, m				
7	64.4, CH	2.74, dd (6.2, 4.7)	77.0, CH	4.17, dd (8.7, 4.7)	77.0, CH	4.04, dd (10.0, 2.9)	73.7, CH	4.50, m	76.0, CH	3.50, dd (9.4, 3.3)
8	60.5, qC		145.2, qC		131.6, qC		133.5, qC		80.4, qC	
9a	37.0, CH_2	1.94, m	26.6, CH_2	1.69, m	124.2, CH	5.20, m	124.3, CH	5.09, dd (8.3, 2.4)	43.0, CH_2	2.24, m
9b		1.45, m		1.57, m						1.97, m
10a	24.0, CH_2	2.20, m	24.1, CH_2	2.43, m	26.4, CH_2	2.95, m	26.2, CH_2	2.98, m	25.6, CH_2	2.53, m
10b				2.22, m		2.57, m		2.55, m		2.39, m
11	125.7, CH	5.17, br t (6.6)	125.5, CH	5.44, br t (6.8)	125.7, CH	5.62, m	125.7, CH	5.52, br t (8.4)	125.5, CH	5.33, br t (7.8)
12	134.6, qC		134.1, qC		134.8, qC		136.3, qC		136.4, qC	
13a	35.7, CH_2	2.15, m	35.6, CH_2	2.06, m	35.8, CH_2	2.04, m	36.3, CH_2	2.08, m	35.7, CH_2	2.16, m
13b		2.01, m								2.03, m
14a	27.0, CH_2	1.60, m	30.4, CH_2	2.30, m	26.7, CH_2	1.71, m	26.7, CH_2	1.61, m	27.0, CH_2	1.58, m
14b				2.11, m		1.59, m				
15	144.9, qC		148.7, qC		145.2, qC		144.1, qC		145.1, qC	
16	21.4, CH_3	1.74, s	21.8, CH_3	1.73, s	21.8, CH_3	1.71, br s	21.6, CH_3	1.75, br s	21.8, CH_3	1.76, br s
17a	114.1, CH_2	4.84, br s	113.9, CH_2	4.80, br s	113.8, CH_2	4.77, br s	114.8, CH_2	4.83, br s	114.0, CH_2	4.84, br s
17b		4.63, br s		4.65, br s		4.60, br s		4.62, br s		4.69, br s
18a	66.7, CH_2	4.51, d (13.0)	67.1, CH_2	4.53, d (12.8)	66.9, CH_2	4.50, d (12.5)	67.1, CH_2	4.50, d (13.2)	67.4, CH_2	4.51, d (13.2)
18b		4.46, d (13.0)		4.44, d (12.8)		4.41, d (12.5)		4.45, d (13.2)		4.46, d (13.2)
19a	16.9, CH_3	1.19, s	111.0, CH_2	5.06, br s	11.6, CH_3	1.64, br s	17.4, CH_3	1.64, br s	14.7, CH_3	1.56, br s
19b				5.03, br s						
20	15.5, CH_3	1.60, s	14.9, CH_3	1.58, s	15.1, CH_3	1.66, br s	15.7, CH_3	1.72, br s	27.4, CH_3	1.71, br s
21	170.6, qC		170.4, qC		170.7, qC		170.7, qC		170.7, qC	
22	21.2, CH_3	2.06, s	21.0, CH_3	2.05, s	21.2, CH_3	2.07, s	21.2, CH_3	2.08, s	21.2, CH_3	2.04, s
23	170.3, qC		170.7, qC		170.3, qC		170.5, qC		170.3, qC	
24	21.0, CH_3	2.06, s	21.2, CH_3	2.06, s	21.0, CH_3	2.05, s	21.0, CH_3	2.07, s	21.0, CH_3	2.05, s

[a] Full assigments were done by DEPT, HMQC, and HMBC experiments. [b] Multiplicity deduced by DEPT.

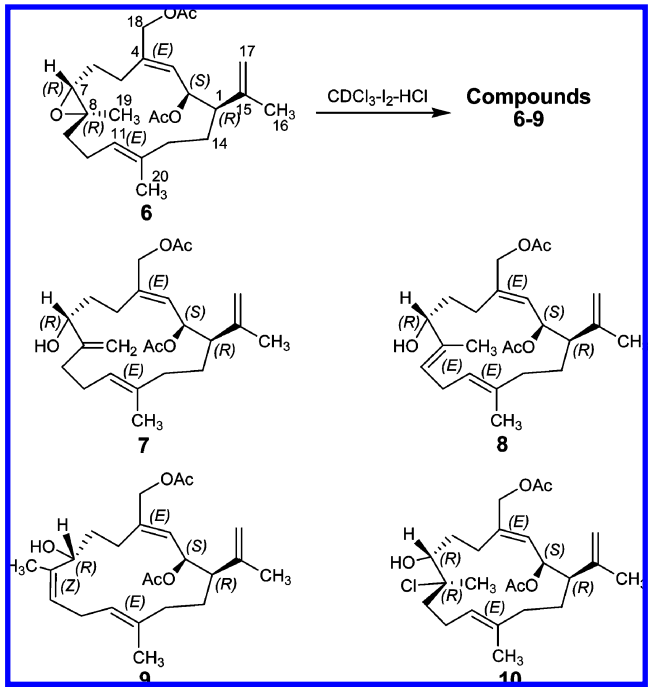

Figure 3. Acid-catalyzed epoxide ring-opening reaction in compound **6**.

the value of the coupling constant (J_{1-2}= 4.3 Hz) for H-1 and H-2 and the energy-optimized model of **6**, clearly indicated the *R* configuration for C-1 in **6**. On the basis of the above results and considering the chemical correlations established for compounds **1**−**6**, the absolute configuration of C-1 in compounds **1**−**5** and **7**−**10** and of C-8 in compounds **1**−**5** described here must be the same as in **6**. The absolute configurations determined by NOE and Mosher's method for **1**−**6** were consistent with those obtained by comparison of the optical rotation of **4** with (−)-asperdiol and with the chemical correlations here established among **1**−**6**.

For compound **10** the epoxide ring-opening resulted in introduction of chlorine at C-8. Its configuration was determined using NOE correlations in a phase-sensitive NOESY experiment. It was found that the methyl signal H_3-19 showed NOE correlations with H_2-6 but not with H-7, which means a *syn* orientation for H_3-19 and H_2-6. In the same way H-7 correlates with H_2-9, indicating the same orientation for H-7 and H_2-9. These data clearly showed a *trans* relationship between CH_3-19 and H-7, like in the compounds **7**−**9**. However, as we had established the *R* configuration for C-7, the configuration of C-8 should also be *R*. This result suggests that the addition of chlorine in C-8 was made by S_N1 substitution.

Thus, on the basis of the overall results the structures of the natural (**1**−**5**) and semisynthetic (**6**−**10**) diterpenes here described are as follows: (−)-(1*S*,7*R*,8*R*,3*E*,11*E*)-7,8-epoxycembra-3,11,15(17)-trien-18-ol, **1** (knightol), acetate of (−)-(1*S*,7*R*,8*R*,3*E*,11*E*)-7,8-epoxycembra-3,11,15(17)-trien-18-ol, **2** (knightol acetate), (−)-(1*S*,7*R*,8*R*,3*E*,11*E*)-7,8-epoxycembra-3,11,15(17)-trien-18-al, **3** (knightal), (−)-(1*R*,2*S*,7*R*,8*R*)-asperdiol, **4**, (−)-(1*R*,2*S*,7*R*,8*R*)-asperdiol acetate, **5**, (−)-(1*R*,2*S*,7*R*,8*R*)-2,18-diacetoxyasperdiol, **6**, (−)-(1*R*,2*S*,7*R*,3*E*,11*E*)-2,18-diacetoxycembra-3,8(19),11,15(17)-tetraen-7-ol, **7**, (−)-(1*R*,2*S*,7*R*,3*E*,8*E*,11*E*)-2,18-diacetoxycembra-3,8,11,15(17)-tetraen-7-ol, **8**, (−)-(1*R*,2*S*,7*R*,3*E*,8*Z*,11*E*)-2,18-diacetoxycembra-3,8,11,15(17)-tetraen-7-ol, **9**, and (−)-

Table 3. Selected ^{1}H NMR Spectroscopic Data (400 MHz, $CDCl_3$) for the MPA Esters **7r**,**s**−**10r**,**s**

no.	**7r**	**7s**	Δ (δ**7r** − δ**7s**)	**8r**	**8s**	Δ (δ**8r** − δ**8s**)	**9r**	**9s**	Δ (δ**9r** − δ**9s**)	**10r**	**10s**	Δ (δ**10r** − δ**10s**)
H-2	5.58, dd, J = 8.8, 3.7	5.56, dd, J = 8.8, 3.6	+0.02	5.55, dd, J = 7.6, 4.2	5.54, dd, J = 7.8, 4.1	+0.01	5.53, dd, J = 7.5, 3.3	5.48, dd, J = 7.2, 3.2	+0.05	5.65, dd, J = 8.9, 3.2	5.56, dd, J = 9.0, 3.2	+0.09
H-3	5.30, d, J = 8.8	5.26, d, J = 8.7	+0.04	5.19, d, J = 7.8	5.16, d, J = 7.8	+0.03	5.32, d. J = 7.6	5.25, d, J = 7.5	+0.07	5.41, d, J = 8.8	5.26, d, J = 9.0	+0.15
H-5	2.31, td	2.26, m	+0.05	2.00, m	1.96, m	+0.04	1.59, m	1.55, m	+0.04	1.73, m	1.51, m	+0.22
H-6	1.80, m	1.67, m	+0.13	1.44, m	1.38, m	+0.06	1.28, m	1.22, m	+0.06	1.67, m	1.43, m	+0.24
H-6′	1.47, m	1.39, m	+0.08	1.87, m	1.71, m	+0.16	2.02, m	1.96, m	+0.06	2.04, m	1.88, m	+0.16
H-7												
H-9	1.98, m	2.14, m	−0.16	5.13, dd	5.19, dd	−0.06	5.07, dd, J = 8.8, 2.2	5.08, dd, J = 8.8, 2.0	−0.01	1.83, m	1.92, m	−0.09
H-10	2.07, m	2.20, m	−0.13	2.48, m	2.57, m	−0.09	3.15, m	3.22, m	−0.07	1.59, m	1.61, m	−0.02
H-10′	2.15, m	2.27, m	−0.12	2.72, m	2.87, m	−0.15	2.47, m	2.52, m	−0.05			
H-11	5.32, t, J = 6.4	5.37, t, J = 6.6	−0.05	5.37, t. J = 7.6	5.58, t, J = 7.8	−0.21	5.47, t, J = 4.3	5.56, t, J = 7.4	−0.09	5.39, t, J = 7.3	5.40, t, J = 7.5	−0.01
H-18	4.45, s	4.27, d, J = 12.8	+0.18	4.40, d, J = 12.8	4.33, d, J = 13.0	+0.07	4.46, d, J = 13.4	4.30, s	+0.16	4.53, d, J = 12.6	4.08, d, J = 13.4	+0.45
H-18′	4.45, s	4.32, d, J=13.0	+0.13	4.45, d, J = 12.8	4.34, d, J = 13.0	+0.11	4.43, d, J = 13.5	4.30, s	+0.13	4.41, d, J = 12.4	4.02, d, J = 13.4	+0.39
H-19	4.89, s	5.04, s	−0.15	1.37, s	1.59, s	−0.22	1.28, s	1.59, s	−0.31	0.99, s	1.52, s	−0.53
H-20	1.54, s	1.56, s	−0.02	1.58, s	1.64, s	−0.06	1.66, s	1.66, s	0.00	1.58, s	1.58, s	0.00

Table 4. Antimicrobial Activity of Compounds **1**−**10**

	antimicrobial activity[b]					
compound[a]	*Ochrobactrum pseudogringnonense* (Gram −) 4-4DEP	*Ochrobactrum pseudogringnonense* (Gram −) 21-6PIN	*Alteromonas* sp. (Gram −) 11-6DEP	*Oceanobacillus iheyensis* (Gram +) 31-C	*Bacillus* sp. (Gram +) 10-6DEP	*Kocuria* sp. (Gram +) 10-4DEP
1	−	+	+	+	+++	+++
2	−	+	−	+	+	−
3	−	+	+	+	++	++
4	−	++	−	−	+	−
5	−	+++	−	−	++	−
6	+	+++	−	+	++	−
7	+	+	−	+	++	+
8	+	++	+	+	++	+
9	+	−	−	−	+	−
10	+	−	−	+	++	−
Cu_2O	+++	++	+++	+++		+++

[a] Concentration for pure compounds 30 μg per disk and positive control Cu_2O 600 μg per disk. [b] Activity was measured by the diameter of the inhibition zone: +++ high activity (≥10 mm); ++ medium activity (8−10 mm); + Low activity (6−8 mm); − no zone of inhibition.

(1*R*,2*S*,7*R*,8*R*,3*E*,11*E*)-8-chloro-2,18-diacetoxycembra-3,11,15(17)-trien-7-ol, **10**. This is the first time that **1**−**3** have been found in nature and also the first time that semisynthetic compounds **6**−**10** are reported in the chemical literature.

Antifouling activities of the natural (**1**−**5**) and semisynthetic (**6**−**10**) compounds were evaluated using six marine bacteria (Table 4): *Ochrobactrum pseudogringnonense* (Gram −) 4-4DEP, *O. pseudogringnonense* (Gram −) 21-6PIN, *Alteromonas* sp. (Gram −) 11-6DEP, *Oceanobacillus iheyensis* (Gram +) 31-C, *Bacillus* sp. (Gram +) 10-6DEP, and *Kocuria* sp. (Gram +) 10-4DEP (all isolated from the surface of a heavily fouled marine sponge, *Aplysina lacunosa*[10]). During the first steps of fouling, microbial communities such as certain bacteria develop rapidly, and they are often involved in the process of regulating either microfouling or macrofouling, by promoting or inhibiting colonization. Thus, many authors often use, in model biassays, the inhibition of the growth of marine bacteria associated with marine surfaces as an indicator of the antifouling potential.[10,23] Most of the assayed compounds exhibited high activity against isolates of Gram (+) bacteria (Table 4), and it is remarkable that the semisynthetic compounds (**6**−**10**), unlike the natural compounds, inhibited as well the growth of Gram (−) bacteria. Compound **1** has a wide antibacterial activity and the best potency (halo >10 mm) of all compounds tested, although it was more specific against Gram (+) bacteria. Compounds **2**−**5** exhibited activity against isolates of *O. pseudogringnonense* (21-6PIN) and *Bacillus* sp. (10-6DEP), with **5** showing higher inhibition halos. The compound **3** showed a wide activity, but it was more powerful against Gram (+) bacteria. Semisynthetic compounds **6**−**10** showed activity against most of tested strains, including *O. pseudogringnonense* (4-4DEP), which was resistant to the natural compounds. Compound **6** exhibited a wide spectrum, showing activity against four of six marine bacteria, although **6** just differs from **5** in the acetate group at C-2. In the case of **8** and **9**, the former showed a wide spectrum of activity unlike **9**. This suggests that an *E* double bond between C-7 and C-8 favors the activity against Gram (+) and Gram (−) bacteria.

MICs for all compounds were determined using *O. pseudogringnonense* (Gram −) and *Bacillus* sp. (Gram +) bacteria, finding knightol as the most active compound, with MICs of 8 and 2 μg/disk, respectively. Knightol acetate and knightal showed MICs values of 8 μg/disk against the Gram (−) and 30 and 16 μg/disk against the Gram (+) bacteria. In the case of compound **4** a noteworthy activity against only *Bacillus* sp. (4 μg/disk) was observed. Compounds **5**, **6**, and **8** all showed MIC values of 30 μg/disk against the Gram (+) and Gram (−) bacteria. The remaining compounds showed MICs of >30 μg/disk.

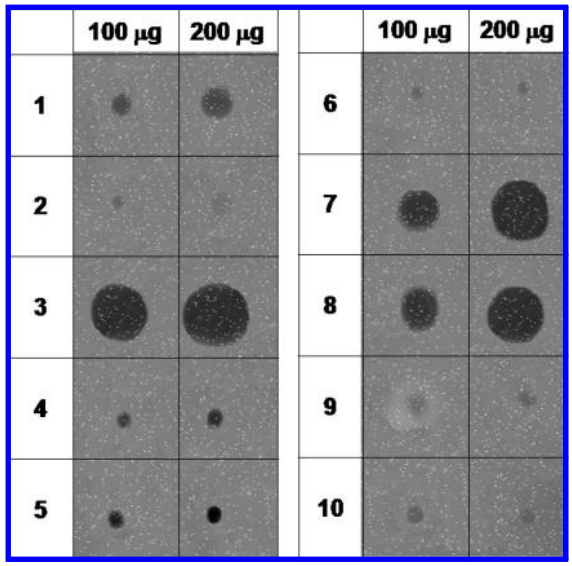

Figure 4. Anti-quorum sensing assay of **1**−**10** using inhibition of bioluminescence in a model bacterial system (*Escherichia coli* ps40−*Pseudomonas putida* IsoF).

Anti-quorum sensing (QS) activities of **1**−**10** were evaluated with biosensor systems using *Escherichia coli* pS401 and *Pseudomonas putida* IsoF wild type.[24] Inhibition of bioluminescence of *E. coli* pS401, normally induced with acyl-homoserinelactone (AHL) of *P. putida*, could be observed for compounds **1**, **3**, **7**, and **8**, although for **1** the inhibition zone was weak (Figure 4). Results presented here indicate that these compounds interfere with QS, which has an essential role in biofilm maturation, therefore affecting subsequent association of other species to the surface, which possibly could prevent later steps in the process of fouling.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a Polartronic E, Schmidt+Haensch polarimeter. IR spectra were recorded on a Perkin-Elmer FT-IR Paragon 500, series 1000 spectrophotometer. ^{1}H and ^{13}C NMR (one- and two-dimensional) spectra were recorded on a Bruker Avance 400 (400 MHz for ^{1}H and 100 MHz for ^{13}C) spectrometer in $CDCl_3$ solution, with TMS as internal standard. The chemical shifts are given in δ and coupling constants in Hz. ROESY experiments were carried out on a Bruker Avance 2, 500.13 MHz using a mixing time of 1.8 s. GC-MS was performed on a Shimadzu GC-17A CGMS-QP5050A gas chromatograph equipped with an DB-1 silica column (25 m × 0.25 mm i.d; 0.25 μm) kept at 300 °C and He (UAP) as carrier gas at 1 mL/min. Split injection 1:10 and 1 μL injection volumes were used. Temperatures of injector and interface were fixed at 300 °C. For the electronic impact mass spectrometry, the ion source was held at 70 eV and the filament at 60 μA. HRESIMS was performed on an APEX III FT/MS with an ESI sound. Preparative HPLC was conducted with a Merck-Hitachi instrument with a UV/vis L-4250 detector (detected at 210 nm) using a LichroCART RP-18 (250 × 10 mm i.d., 10 μm) column, with MeOH−H_2O (85:15 v/v) as eluent at a flow rate of 3 mL/min. Pictures of assays were taken with BioRad software GelDoc 2.1.

Animal Material. The octocoral *Eunicea knighti* was collected at Santa Marta Bay, Colombian Caribbean, in May 2007 by scuba diving at a depth of 12 m. The fresh coral colonies were immediately frozen after collection and maintained frozen until the extraction. Animals were identified by Prof. Dr. S. Zea, and voucher specimens were deposited at the invertebrate collection of ICN (Instituto de Ciencias Naturales de la Universidad Nacional de Colombia) coded as ICN-MHN-CO No 0106.

Extraction and Isolation of Compounds. The octocoral (200 g) was cut into small pieces, weighed, and repeatedly extracted with a MeOH−CH_2Cl_2 (1:1 v/v) mixture. The resulting extract was filtered and concentrated by rotary evaporation, obtaining a dark green oily extract (8 g), which was partitioned between CH_2Cl_2−H_2O (1:1 v/v). The organic fraction (6.5 g) thus obtained was subjected to silica gel vacuum column chromatography eluting with 500 mL solvents of increasing polarity (benzene (100%), benzene−EtOAc (80:20 v/v), benzene−EtOAc (50:50 v/v), EtOAc (100%), EtOAc−MeOH (80:20 v/v), and EtOAc−MeOH (50:50 v/v)) to obtain six fractions. The fractions were assayed for antifouling activity, with 2, 3, and 4 being the most active.

Fraction 2 (eluted with benzene−EtOAc, 80:20 v/v) was further subjected to silica gel column chromatography, eluting with benzene−EtOAc mixtures of increasing polarity (benzene−EtOAc 100:0 v/v to 50:50 v/v). The fraction eluted with benzene−EtOAc (80:20 v/v) was concentrated and finally separated by preparative reversed-phase HPLC to give pure **2** (60 mg). In the same way the fraction eluted with benzene−EtOAc (90:10 v/v) was concentrated and finally separated by preparative reversed-phase HPLC to give pure **3** (18 mg). Fraction 3, eluted with benzene−EtOAc (50:50 v/v), was subjected to silica gel column chromatography eluting with a benzene−EtOAc mixture of increasing polarity (benzene−EtOAc, 100:0 v/v to 25:75 v/v); the fraction eluted with benzene−EtOAc (70:30 v/v) was concentrated and finally separated by preparative reversed-phase HPLC to give pure **1** (20 mg) and **5** (80 mg). Finally, fraction 4 eluted with EtOAc was separated by preparative reversed-phase HPLC to afford pure **4** (400 mg).

Knightol, 1: colorless oil; $[\alpha]_D^{25}$ −32.0 (*c* 2.5, $CHCl_3$); IR (CH_2Cl_2) ν_{max} 3420, 1643, 1224, 736 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; EIMS *m/z* 304 $[M]^+$ (1), 289 (1), 273 (2), 255 (2), 201 (6), 187 (7), 159 (12), 133 (27), 121 (35), 107 (49), 93 (62), 79 (64), 67 (79), 55 (82), 41 (100); HRESIMS *m/z* 327.2301 $[M + Na]^+$ (calcd for $C_{20}H_{32}O_2Na$, 327.2294).

Knightol acetate, 2: colorless oil; $[\alpha]_D^{25}$ −60.0 (*c* 2.5, $CHCl_3$); IR (CH_2Cl_2) ν_{max} 3069, 1742, 1228, 735 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; EIMS *m/z* 346 $[M]^+$ (1), 304 (1), 286 (2), 271 (4), 253 (6), 201 (6), 187 (11), 159 (19), 133 (36), 119 (40), 107 (51), 93 (70), 79 (82), 67 (99), 55 (85), 41 (100); HRESIMS *m/z* 369.2406 $[M + Na]^+$ (calcd for $C_{22}H_{34}O_3Na$, 369.2400).

Knightal, 3: colorless oil; $[\alpha]_D^{25}$ +7.0 (*c* 2.9, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 229 (3.39) nm; IR (CH_2Cl_2) ν_{max} 3075, 1727, 1228, 1024 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; EIMS *m/z* 302 $[M]^+$ (1)], 274 (2), 255 (2), 201 (6), 187 (7), 159 (12), 133 (27), 121 (35), 107 (49), 93 (62), 79 (64), 67 (79), 55 (82), 41 (100); HRESIMS *m/z* 325.2149 $[M + Na]^+$ (calcd for $C_{20}H_{30}O_2Na$, 325.2194).

Asperdiol, 4: white needles; $[\alpha]_D^{25}$ −96.3 (*c* 2.7, $CHCl_3$); IR, NMR, and MS data are consistent with literature values.[14,15]

Asperdiol acetate, 5: colorless oil; $[\alpha]_D^{25}$ −60.0 (*c* 2.7, $CHCl_3$); IR, NMR, and MS data are consistent with literature values.[15]

Conversion of Knightol (1) into Knightol Acetate (2). Compound **1** (5.0 mg) was treated with acetic anhydride (0.3 mL) in dry pyridine (1 mL) for about 24 h under stirring at room temperature. The reaction was worked up by addition of water (1 mL) and EtOAc (2 mL). The organic phase was washed sequentially with 2 M H_2SO_4, saturated $NaHCO_3$, and brine. After drying (Na_2SO_4) and removal of the solvent the residue was purified by column chromatography (SiO_2, benzene−EtOAc (9:1)) to obtain a product identical to **2** (5.2 mg). Colorless oil; $[\alpha]_D^{25}$ −58.0 (*c* 2.5, $CHCl_3$); ^{1}H NMR data match those in Table 1.

Conversion of Compound 5 into Compound 2. Tosylation of **5** (7.0 mg) was carried out using *p*-TsCl (4.0 mg) in dry pyridine (1 mL) under stirring, for 6 h at room temperature. Ice and H_2O were added to the reaction mixture and extracted with CH_2Cl_2. The organic layer was separated and concentrated by rotary evaporation. The residue was treated with $LiAlH_4$ (1.0 mg) in dry THF (1 mL) at room temperature. After 10 h, the mixture was treated dropwise with a saturated Na_2SO_4 solution. The final mixture was filtered and purified by column chromatography (benzene−EtOAc (9:1 v/v)) to obtain a pure compound identical to **2** (5.1 mg). Colorless oil; $[\alpha]_D^{25}$ −63.0 (*c* 2.3, $CHCl_3$); ^{1}H NMR data match those in Table 1.

Conversion of Compound 3 into Compound 1. Compound **3** (2 mg) was dissolved in dry MeOH (1 mL), and subsequently $CeCl_3$ (2 mg) and $NaBH_4$ (0,4 mg) were added to the solution, which was left under stirring for 7 min at room temperature. The reaction mixture was added to a NH_4Cl (5%) solution under ice, extracted with chloroform (2 × 5 mL), and filtered through silica gel to obtain pure **1** (1.7 mg). Colorless oil; $[\alpha]_D^{28}$ −40.0 (*c* 2.0, $CHCl_3$); ^{1}H NMR data match those in Table 1.

Conversion of Compounds 4 and 5 into Compound 6. (−)-Asperdiol (**4**) (100 mg) and asperdiol acetate (**5**) (5 mg) were treated

separately with acetic anhydride (1 mL) in dry pyridine (2 mL) for 24 h under stirring, at room temperature. In both cases the products were purified by column chromatography (benzene−EtOAc (9:1 v/v)) to obtain pure diacetylated compound **6** (120 mg from **4** and 5.5 mg from **5**). Colorless oil; $[\alpha]_D^{25}$ −80.0 (*c* 4.0, $CHCl_3$); IR (CH_2Cl_2) ν_{max} 3069, 1739, 1232, 736 cm^{-1}; ^1H and ^{13}C NMR data, see Table 2; EIMS *m/z* 404 $[M]^+$ (1), 386 (1), 362 (1), 300 (6), 271 (14), 177 (5), 152 (1), 121 (24), 109 (33), 95 (55), 81 (100) 67 (87); HRESIMS *m/z* 427.2467 $[M + Na]^+$ (calcd for $C_{24}H_{36}O_5Na$, 427.2460).

Formation of Semisynthetic Compounds 6−10. Compound **6** (174 mg) was dissolved in $CDCl_3$ (6 mL), and an iodine crystal was added. A 250 μL portion of acidified $CDCl_3$ ($HCl_{(g)}$ was bubbled into 5 mL of $CDCl_3$ for 30 s) was added to the mixture. After 36 h, the reaction mixture was dried and chromatographed on a silica gel column, eluted with benzene−EtOAc (90:10 v/v), to yield **7** (11 mg, 6.0%), a mixture of **8** and **9** (30 mg, 17.0%), and **10** (71 mg, 41.0%). The mixture of **8** and **9** was subjected to HPLC RP-18, MeOH−H_2O (50:50 v/v), to obtain pure **8** (*E* isomer) (19 mg, 11.0%) and pure **9** (*Z* isomer) (7 mg, 4.0%).

(−)-(1*R*,2*S*,7*R*,3*E*,11*E*)-2,18-Diacetoxycembra-3,8(19),11,15(17)-tetraen-7-ol (7): colorless oil; $[\alpha]_D^{25}$ −61.5 (*c* 1.3, $CHCl_3$); IR (CH_2Cl_2) ν_{max} 3436, 1732, 1024, 736 cm^{-1}; ^1H and ^{13}C NMR data, see Table 2; EIMS *m/z* 404 $[M]^+$ (1), 386 (1), 362 (1), 344 (2), 302 (3), 284 (12), 241 (12), 201 (15), 187 (18), 145 (42), 119 (55), 107 (67), 93 (83), 81 (100) 67 (91); HRESIMS *m/z* 427.2456 $[M + Na]^+$ (calcd for $C_{24}H_{36}O_5Na$, 427.2454).

(−)-(1*R*,2*S*,7*R*,3*E*,8*E*,11*E*)-2,18-Diacetoxycembra-3,8,11,15(17)-tetraen-7-ol (8): colorless oil; $[\alpha]_D^{25}$ −160.0 (*c* 1.0, $CHCl_3$); IR (CH_2Cl_2) ν_{max} 3455, 1739, 1234, 736 cm^{-1}; ^1H and ^{13}C NMR data, see Table 2; EIMS *m/z* 404 $[M]^+$ (1), 380 (1), 345 (4), 300 (6), 271 (14), 241 (8), 201 (15), 187 (18), 145 (41), 119 (67), 107 (81), 93 (89), 81 (99) 67 (73), 55 (100); HRESIMS *m/z* 427.2459 $[M + Na]^+$ (calcd for $C_{24}H_{36}O_5Na$, 427.2454).

(−)-(1*R*,2*S*,7*R*,3*E*,8*Z*,11*E*)-2,18-Diacetoxycembra-3,8,11,15(17)-tetraen-7-ol (9): colorless oil; $[\alpha]_D^{25}$ −55.9 (*c* 1.4, $CHCl_3$); IR (CH_2Cl_2) ν_{max} 3436, 1730, 1025, 736 cm^{-1}; ^1H and ^{13}C NMR data, see Table 2; EIMS *m/z* 404 $[M]^+$ (1), 375 (1), 344 (1), 301 (3), 269 (4), 241 (2), 201 (5), 187 (5), 145 (31), 119 (17), 107 (21), 93 (27), 81 (59) 67 (32), 55 (100); HRESIMS *m/z* 427.2457 $[M + Na]^+$ (calcd for $C_{24}H_{36}O_5Na$, 427.2454).

(−)-(1*R*,2*S*,7*R*,8*R*,3*E*,11*E*)-8-Chloro-2,18-diacetoxycembra-3,11,15(17)-trien-7-ol (10): colorless oil; $[\alpha]_D^{25}$ −66.2 (*c* 2.7, $CHCl_3$); IR (CH_2Cl_2) ν_{max} 3457, 1730, 1372, 1235, 737 cm^{-1}; ^1H and ^{13}C NMR data, see Table 2; EIMS *m/z* 442/440 $[M]^+$ (1), 404 (1), 380 (2), 345 (3), 320 (16), 307 (10), 285 (18), 241 (7), 201 (12), 187 (14), 147 (30), 121 (48), 107 (64), 93 (63), 81 (100) 67 (77), 55 (74); HRESIMS *m/z* 465.2205/463.2218 $[M + Na]^+$ (calcd for $C_{24}H_{37}ClO_5Na$, 465.2198/463.2221).

Preparation of the *R*- and *S*-MPA Esters of Compounds 7−10. In order to determine the absolute configuration at C-7, the ester derivates of **7−10** were prepared by treating separately compounds **7−10** (2.0 mg) with *R*- and *S*-MPA (1.2 mg) in dry methylene chloride (1 mL), in the presence of DCC (3.4 mg) and DMAP (catalytic amount) for 24 h at room temperature.[20,21] Each reaction mixture was filtered and the ester purified by reversed-phase HPLC (LichroCART RP-18). For selected ^1H NMR data for derivatives of **7− 10**, see Table 3.

Preparation of the *R*- and *S*-MPA Esters of Compound 5. To determine the absolute configuration at C-2, the *R*- and *S*-MPA ester derivatives of **5** were prepared in the same way as described for compounds **7−10**.

Evaluation of Antibacterial Activities. Antibacterial tests of the extract, fractions, and pure compounds were performed using marine bacteria associated with a heavily fouled surface,[10] by disk-diffusion technique in agar Petri dishes. Whatman filter paper disks (5.2 mm diameter) were initially sterilized at 10×10^4 Pa pressure for 15 min. A sample (300 μg for extracts and fractions and 30 μg for pure compounds) dissolved in CH_2Cl_2 was loaded on the disks after filtration and allowed to dry at room temperature during 3 h. Disks were then placed on agar dishes plated with 100 μL of bacterial culture grown in liquid marine agar overnight (10^6 cfu/mL, 0.5 Mac Farlan). Three Gram (+) and three Gram (−) bacterial isolates were used. Those isolates were preliminary identified by 16Sr RNA gene sequences.[10] Following incubation for 24 and 48 h at 26 °C, the activity was evaluated by measuring the diameter (*D,* in mm) of the inhibition zones around the disk. Control tests with the solvents were performed for every assay and showed no inhibition of the microbial growth. In addition, the biocide Cu_2O (600 μg/disk) was used as positive control and CH_2Cl_2 was used as solvent control. All the inhibition assays were carried out in triplicate.

MICs (minimal inhibitory concentrations) were determined for all compounds using the disk diffusion technique in agar Petri dishes (NCCLS),[25] against two representative bacteria, Gram (−) and Gram (+), *O. pseudogringnonense* (21-6PIN) and *Bacillus* sp. (10-6DEP), respectively. Those strains were selected because they were susceptible to every tested compound in the initial antibacterial tests. The quantities per disk tested were 30, 16, 8, 4, 2, 1, and 0.5 μg. All compounds were delivered onto the disks using MeOH as solvent. Methanol alone was used also as negative control, and the assay was performed in triplicate.[26]

Antiquorum Sensing (QS) Activity Test. The standard disk-diffusion assay was done for all compounds following the parameters of the NCCLS[22] as described before. Inhibition of bioluminescence of the biosensor *Escherichia coli* pS401 induced with acyl-homoserinelactone (AHL) of *Pseudomonas putida* IsoF wild type[24] was taken as a positive anti-QS result only when modifications in the bacterial growth pattern around the disks were not observed. *E. coli* pSB401 and *P. putida* IsoF were grown in Luria−Bertani (LB) broth (1% tryptone, 0.5% yeast extract, 0.5% NaCl). Fifteen milliliters of each culture were mixed with LB agar in order to have a solidified agar (0.8%) plate. The mixture was immediately poured over the surface of prewarmed LB agar plates. Ten and 20 μL of a 10 mg/mL solution of each compounds were pipetted onto sterile paper disks, placed on the solidified agar. The plates were incubated overnight at 37 °C and examined for luminescence production. Bioluminescence was detected using a digitized image, and QS inhibition was considered when bioluminescence was inhibited, but bacterial growth around the disk was not affected.

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Supporting Information Available: ^1H and ^{13}C NMR spectra of compounds **1−10**, figures of COSY and HMBC correlations for compounds **2** and **3**, $\Delta\delta_{R-S}$ values for the C-2 MPA derivatives of **5** in $CDCl_3$, and energy-optimized model of compounds **3** and **6**. This material is available free of charge via the Internet at http://pubs.acs.org.

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