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Antifouling Activity of Meroditerpenoids from the Marine Brown Alga *Halidrys siliquosa*

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Nine tetraprenyltoluquinol-related metabolites (**1**−**9**) have been isolated from the organic extract of the temperate brown alga *Halidrys siliquosa* that exhibits antifouling properties. The planar structure of compounds **1**−**9** was elucidated on the basis of extensive spectroscopic analysis and by comparison of the data with those of related metabolites. Antifouling and toxicity tests were conducted on these compounds: the most active (compounds **2**, **6**, and **9**) inhibited both the growth of four strains of bacteria (MICs < 2.5 μg/mL) and settlement of cyprids of *Balanus amphitrite* (EC_{50} < 5 μg/mL), the latter at nontoxic concentrations (LC_{50} > 5 μg/mL).

Microbial biofilms and marine fouling organisms, such as barnacles, can cause substantial technical and economical problems on man-made surfaces submerged in seawater. Due to new regulations on toxic antifouling compounds, agents that are effective against biofouling and are environmentally benign are urgently needed. A number of cobiocides are currently under environmental and legislative scrutiny, and restrictions on where they can be used will continue to grow, especially in Europe.[1]

Marine organisms are a rich source of bioactive substances, and many of them are able to stay free from biofouling. Natural marine antifouling compounds are among the most promising alternatives to the chemicals commonly used in antifouling coatings.[2−4] Sessile organisms such as sponges, soft corals, and seaweeds are known to elaborate chemical defense mechanisms against predation and epibiont growth. The metabolites excreted might repel or inhibit fouling organisms[5] and can act enzymatically by dissolving the adhesives, interfering with the metabolism of the fouling organisms, inhibiting the attachment, metamorphosis, or growth, promoting negative chemotaxis, altering the surface of the organisms, or finally acting as biocides.[6,7]

In the course of our continuing search for bioactive metabolites from marine algae, this report describes the chemical and biological investigations of *Halidrys siliquosa* (L.) Lyngb. (family Sargassaceae, class Phaeophyceae). This brown alga, found along NE Atlantic shores, had previously been shown to contain meroditerpenes[8] as well as phlorotannins[9−11] and arsenosugars.[12] Herein, we describe the structure elucidation and bioactivity of five new compounds [one chromene (**1**) and four linear meroditerpenoids (**2**−**5**)] together with four known metabolites (**6**−**9**). Screening of new natural products with antifouling activities is usually conducted by a bioassay on the cosmopolitan barnacle *Balanus amphitrite*. In this study, these compounds were tested on both *B. amphitrite* and marine bacteria (*Cobetia marina*, *Marinobacterium stanieri*, *Vibrio fischeri*, and *Pseudoalteromonas haloplanktis*). It is of high importance to evaluate the bioactivity of new compounds against a large diversity of fouling organisms, as agents with a broad spectrum activity are expected for antifouling purposes.

Results and Discussion

H. siliquosa was collected by hand from Saint-Guénolé (Brittany) on the French Atlantic coast in May 2004. After extraction with $CHCl_3$/MeOH (2:1 then 1:2, v/v), the resulting crude extracts were

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Table 1. ^{13}C NMR Data for Compounds **1**−**5**, **8**, and **9** (100 MHz, C_6D_6)

position	**1** δ_C, mult.[a]	**2** δ_C, mult.	**3** δ_C, mult.	**4** δ_C, mult.	**5** δ_C, mult.	**8** δ_C, mult.	**9** δ_C, mult.
1	123.3, CH	30.7, CH_2	30.9, CH_2	30.8, CH_2	30.8, CH_2	30.9, CH_2	31.0, CH_2
2	130.4, CH	127.6, CH	127.4, CH	127.5, CH	127.3, CH	127.3, CH	127.5, CH
3	77.4, qC	131.7, qC	132.2, qC	132.1, qC	132.2, qC	132.2, qC	132.1, qC
4	54.2, CH_2	55.1, CH_2	55.3, CH_2	55.3, CH_2	55.1, CH_2	55.2, CH_2	55.3, CH_2
5	197.1, qC	197.9, qC	197.9, qC	197.9, qC	197.5, qC	197.9, qC	198.0, qC
6	124.6, CH	123.5, CH	122.7, CH	122.7, CH	123.3, CH	122.6, CH	122.7, CH
7	157.8, qC	159.1, qC	158.2, qC	157.8, qC	159.5, qC	157.7, qC	158.7, qC
8	41.3, CH_2	33.5, CH_2	41.0, CH_2	40.9, CH_2	33.8, CH_2	41.2, CH_2	41.4, CH_2
9	25.5, CH_2	25.8, CH_2	25.4, CH_2	25.1, CH_2	26.1, CH_2	25.4, CH_2	25.6, CH_2
10	29.7, CH_2	33.9, CH_2	32.3, CH_2	33.6, CH_2	30.2, CH_2	29.7, CH_2	34.1, CH_2
11	36.9, CH	41.2, CH	41.2, CH	41.5, CH	36.7, CH	36.6, CH	36.5, CH
12	81.1, CH	214.6, qC	213.7, qC	214.0, qC	81.1, CH	81.1, CH	78.7, CH
13	200.9, qC	75.0, CH	73.6, CH	74.9, CH	201.2, qC	201.0, qC	200.9, qC
14	119.8, CH	122.5, CH	122.6, CH	122.4, CH	120.1, CH	119.8, CH	119.5, CH
15	158.6, qC	138.8, qC	138.6, qC	138.8, qC	158.2, qC	158.6, qC	158.8, qC
16	27.5, CH_3	25.7, CH_3	25.7, CH_3	25.7, CH_3	27.5, CH_3	27.5, CH_3	27.5, CH_3
17	21.2, CH_3	18.5, CH_3	18.4, CH_3	18.4, CH_3	21.2, CH_3	21.2, CH_3	21.1, CH_3
18	17.6, CH_3	16.8, CH_3	17.7, CH_3	16.5, CH_3	17.5, CH_3	17.5, CH_3	13.0, CH_3
19	19.2, CH_3	25.1, CH_3	19.2, CH_3	19.2, CH_3	25.1, CH_3	19.2, CH_3	19.4, CH_3
20	26.3, CH_3	16.7, CH_3	16.7, CH_3	16.7, CH_3	16.7, CH_3	16.7, CH_3	16.7, CH_3
1′	144.9, qC	147.3, qC	147.4, qC	147.4, qC	147.3, qC	147.4, qC	147.4, qC
2′	121.7, qC	127.9, qC	128.0, qC	127.8, qC	127.8, qC	128.1, qC	127.9, qC
3′	109.4, CH	113.5, CH	113.6, CH	113.6, CH	113.5, CH	113.5, CH	113.6, CH
4′	154.1, qC	153.9, qC	154.0, qC	154.0, qC	154.0, qC	154.0, qC	153.9, qC
5′	116.9, CH	114.4, CH	114.5, CH	114.5, CH	114.5, CH	114.5, CH	114.5, CH
6′	126.6, qC	126.2, qC	126.3, qC	126.2, qC	126.2, qC	126.4, qC	126.2, qC
7′	16.0, CH_3	16.7, CH_3	16.6, CH_3	16.6, CH_3	16.6, CH_3	16.6, CH_3	16.6, CH_3
4′-OMe	55.2, CH_3	55.2, CH_3	55.3, CH_3	55. 2, CH_3	55.2, CH_3	55.2, CH_3	55.2, CH_3

[a] Multiplicities inferred from DEPT and HSQC experiments.

Table 2. ^1H NMR Data of Compounds **1**−**5**, **8**, and **9** (400 MHz, C_6D_6, *J* in Hz)

position	**1**	**2**	**3**	**4**	**5**	**8**	**9**
1	6.11 (br d, 10.0)	3.32 (br d, 7.5)	3.30 (d, 7.0)	3.31 (t, 7.0)	3.28 (d, 7.5)	3.30 (t, 7.0)	3.31 (t, 7.5)
2	5.73 (d, 10.0)	5.31 (t, 7.5)	5.32 (t, 7.0)	5.34 (t, 7.0)	5.27 (t, 7.5)	5.30 (t, 7.0)	5.33 (t, 7.0)
4a	2.79 (d, 14.5)	2.81 (s)	2.89 (s)	2.89 (s)	2.80 (s)	2.86 (s)	2.90 (s)
4b	2.67 (d, 14.5)						
6	5.83 (s)	5.79 (br s)	5.88 (s)	5.89 (br s)	5.78 (br s)	5.84 (s)	5.98 (s)
8a	1.66 (t, 7.5)	2.61 (m)	1.73 (t, 7.5)	1.71 (t, 7.5)	2.66 (m)	1.72 (t, 7.0)	1.86 (t, 7.0)
8b		2.37 (m)			2.37 (m)		
9	1.11 (m)	1.27 (m)	1.13 (m)	1.15 (m)	1.27 (m)	1.11 (m)	1.31 (m)
10a	1.41 (m)	1.69 (m)	1.69 (m)	1.46 (m)	1.69 (m)	1.32 (m)	1.56 (m)
10b	1.05 (m)	1.30 (m)	1.11 (m)	1.10 (m)	1.28 (m)	1.15 (m)	1.29 (m)
11	1.72 (m)	2.67 (m)	2.41 (m)	2.46 (m)	1.94 (m)	1.75 (m)	1.74 (m)
12	3.93 (br s)				3.97 (br s)	3.93 (m)	4.07 (m)
13		5.00 (d, 9.5)	4.82 (d, 9.5)	4.82 (d, 9.5)			
14	5.72 (s)	5.05 (d, 9.5)	4.94 (d, 9.5)	4.95 (d, 9.5)	5.85 (br s)	5.74 (br s)	5.76 (s)
16	1.42 (s)	1.52 (s)	1.49 (s)	1.49 (s)	1.47 (s)	1.43 (s)	1.42 (s)
17	2.07 (s)	1.65 (s)	1.48 (s)	1.53 (s)	2.09 (s)	2.06 (s)	2.06 (s)
18	1.11 (d, 7.0)	0.98 (d, 7.0)	0.80 (d, 7.0)	0.92 (d, 7.0)	1.19 (d, 7.0)	1.11 (d, 7,0)	0.71 (d, 7.0)
19	2.05 (s)	1.48 (s)	2.08 (s)	2.08 (s)	1.49 (s)	2.05 (s)	2.17 (s)
20	1.58 (s)	1.66 (s)	1.66 (s)	1.67 (s)	1.64 (s)	1.67 (s)	1.68 (s)
3′	6.39 (d, 3.0)	6.69 (d, 3.0)	6.70 (d, 3.0)	6.71 (d, 3.0)	6.70 (d, 3.0)	6.70 (d, 3.0)	6.71 (d, 3.0)
5′	6.65 (d, 3.0)	6.63 (d, 3.0)	6.64 (d, 3.0)	6.64 (d, 3.0)	6.64 (d, 3.0)	6.65 (d, 3.0)	6.65 (d, 3.0)
7′	2.22 (s)	2.19 (s)	2.19 (s)	2.19 (s)	2.18 (s)	2.20 (s)	2.21 (s)
4′-OMe	3.35 (s)	3.44 (s)	3.45 (s)	3.45 (s)	3.44 (s)	3.45 (s)	3.44 (s)

concentrated and an initial fractionation was accomplished by normal-phase column chromatography (CC) (EtOAc/*n*-hexane). Subsequent iterative C_{18} reversed-phase HPLC resulted in the isolation of the five new compounds **1**−**5** in addition to the four known meroditerpenoids **6**−**9**.

Compound **1** was isolated as an optically active yellow oil. Its molecular formula $C_{28}H_{38}O_5$ was established by HREIMS (*m*/*z* 454.2722), indicating 10 degrees of unsaturation. The ^{13}C NMR data of **1** in C_6D_6 (Table 1) contained a total of 28 carbon signals, which were identified by the assistance of DEPT spectra as seven methyls, four sp^3 methylenes, one sp^3 methine, two oxygenated sp^3 carbons (one methyne and one quaternary), six sp^2 methines, and eight sp^2 quaternary carbons including those of two ketone carbonyls. The ^1H NMR spectrum (Table 2) measured in C_6D_6 exhibited the presence of two *meta*-coupled aromatic protons (δ_H 6.65 and 6.39), two strongly coupled olefinic protons (δ_H 6.11 and 5.73), one methoxy group (δ_H 3.35), and five singlet (δ_H 2.22, 2.07, 2.05, 1.58, and 1.42) and one doublet (δ_H 1.11) methyl. The proton sequence from H-1 to H-2 and the gHMBC correlations from H_3-7′ to C-1′, C-5′, and C-6′; H-5′ to C-6′, C-1′, C-4′, and C-3′; H-3′ to C-5′, C-4′, C-2′, C-1′, and C-1; and H_3-20 to C-2, C-3, and C-4 allowed the proposal of the chromenol fragment **a** (Figure 1). The deshielded nature of the signals associated with H_2-4, H-6, and H_3-19 revealed the proximity of these atoms to a conjugated carbonyl function (C-5, δ_C 197.1). Long-range gHMBC couplings from H_2-4 to C-5 and C-6; H-6 to C-4, C-5, C-7, C-8, and C-19; and H_3-19 to C-6, C-7, and C-8 allowed inclusion of the carbonyl-containing residue **c** in the already defined fragment **a**. The geometry of the

double bond at C-6 was assigned to be *E* on the basis of (i) the upfield ^{13}C NMR chemical shift of the vinyl methyl carbon at δ_C 19.2 (C-19)[13,14] and (ii) the NOE correlation between H-6 and H_2-8. The two last degrees of unsaturation were attributed, using the as yet unassigned carbon atoms, to a double bond and a supplementary conjugated ketone in an acyclic isoprenoid side chain. Combined analysis of 2D NMR data located this carbonyl and the remaining hydroxyl group at C-13 and C-12, respectively, thus completing the partial structure of **1** with the fragment **e**. The absolute configuration of the asymmetric center at C-12 was assigned as *R* by application of the modified Mosher's ester method[15] (Experimental Section). Thus, the structure of **1** was defined to be a chromene derivative containing cyclized hydroquinone and tetraprenyl moieties.

The second isolated compound (**2**) was an optically active pale yellow oil. The HREIMS of **2** established the molecular formula $C_{28}H_{40}O_5$, implying nine degrees of unsaturation. An initial examination of the ^{13}C NMR data revealed two carbonyl carbons, 12 carbons in the aromatic/olefinic region of the spectrum, and 14 sp^3 carbons. These data were in agreement with a structure that could consist of an aromatic ring joined to a diterpenoid side chain containing three olefinic bonds and two carbonyl groups. Analysis of the NMR data revealed that the aromatic part of **2** was a disubstituted hydroquinone. The ^1H NMR spectrum presented two *meta*-coupled aromatic signals at δ_H 6.69 (H-3′) and 6.63 (H-5′) that showed correlations with (i) the aromatic carbon signals at δ_C 113.5 (C-3′) and 114.4 (C-5′), respectively, in the gHSQC; (ii) a benzylic methylene group (δ_H 3.32, H_2-1), a methyl (δ_H 2.19, H_3-7′), and a methoxyl (δ_H 3.44, H_3-OMe) by $^1H-^1H$ gCOSY; and (iii) all the aromatic carbons in addition to C-1 and C-7′, respectively, by gHMBC. All these data, in addition to further gHMBC correlations between H_2-1 and C-1′, C-2′, C-3′, C-2, and C-3 and between H-2 and C-2′, C-1, C-4, and C-20, were consistent with an isoprene unit being attached to an *O*-methyltoluquinol moiety (fragment **b**). The *E* geometry of the Δ^2 double bond was confirmed by spatial correlations between H_2-1/H_3-20 and H-2/H_2-4 on the basis of the gNOESY experiment. The structure of the second isoprenoid unit was determined to be quite similar to that of compound **1** (fragment **d** instead of **c**). Differences displayed in the NMR data could be attributed to modification of the geometry of the double bond Δ^6. Specifically, the ^1H NMR signal of H_3-19 shifted from δ_H 2.05 in **1** to 1.48 in **2**, the ^{13}C chemical shift of C-19 shifted from δ_C 19.2 in **1** to 25.1 in **2**, and gNOESY key interactions between H_3-19 and H-6 were consistent with the change of the configuration of the Δ^6 double bond from *E* in **1** to *Z* in **2**. The constitution of the rest of the side chain was elucidated initially by ^1H COSY correlations from H_2-8 to H_2-9 and H_2-10, H_2-10 to H-11, H-11 to H_3-18, H-13 to H-14, and H-14 to H_3-16 and H_3-17, and by cross-peaks in the gHMBC spectrum from H_2-8 to C-10, H_2-9 to C-11, H_2-10 to C-11 and C-12, H-11 to C-18 and C-13, H-13 to C-14 and C-15, and H-14 to C15, C-16, and C-17. Thus, by the analysis of these 2D NMR data, the connectivity from C-8 to C-16 was fully established and the localization of a hydroxyl group at C-13 and a ketone at C-12 was confirmed. The planar structure of **2** was completed by the fragment **f**.

Compound **3**, molecular formula $C_{28}H_{40}O_5$ (HREIMS), was obtained as an optically active oil. Comparison of ^{13}C and ^1H NMR data (Tables 1 and 2) for **3** with those of **2** showed slight differences and pointed out the same *O*-methyltoluquinol structure (fragment **b**) of these two isomers. The most significant changes in the NMR spectra appeared for signals of carbons and protons of the second isoprenoid unit: the ^1H signal of H-19 shifted from δ_H 1.48 for **2** to 2.08 for **3**, and in the ^{13}C NMR spectrum, the signal of C-19 moved from δ_C 25.1 in **2** to 19.2 in **3**. These shifts, as well as NOE correlations between H-6 and H_2-8, clearly showed **3** to have an *E* geometry of the Δ^6 carbon−carbon double bond (fragment **c**), as for **1**, instead of *Z* for **2** (fragment **d**). For the two last

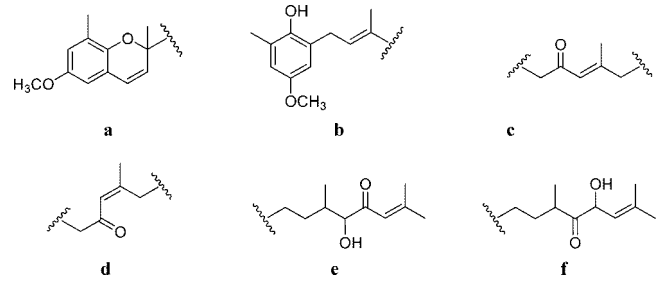


Figure 1. Partial structures **a**−**f** for compounds **1**−**5**, **8**, and **9** as deduced by 2D NMR data.

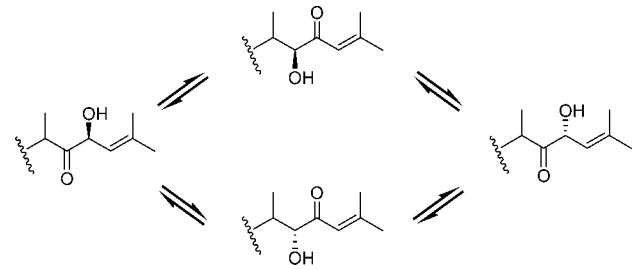


Figure 2. Possible tautomeric acyloin isomerization for compounds **1**−**5**, **8**, and **9**.

isoprenoid units, a complete analysis of the 2D NMR data indicated that **3** and **2** shared the same planar structure (fragment **f**).

Compound **4**, isolated as an optically active oil, has the molecular formula $C_{28}H_{40}O_5$ (HREIMS). Close comparison of its spectroscopic data with those of **2** and **3** led us to conclude that **3** and **4** were diastereoisomers. From a biosynthetic point of view, it seemed more likely that the stereochemistry variation occurs for the hydroxyl group rather than for the secondary methyl. In addition, the hypothesis that **3** and **4** could be epimers at C-13 was supported by the possible occurrence of a tautomeric acyloin-type isomerization (Figure 2).

Compound **5** was an optically active oil, with the molecular formula $C_{28}H_{40}O_5$ deduced by MS and NMR spectrometries. Comparison of its NMR data with those of **1** and **2** clearly showed it to have a planar structure constituted by fragments **b**, **d**, and **e**. Surprisingly, NMR data and the $[\alpha]_D$ value of compound **5** did not match with those of a compound already described by Higgs and Mulheirn,[8] which shows the same planar chemical structure. As discussed previously in the case of **3** and **4**, these two compounds are probably epimers at C-12.

The two metabolites **6** and **7** (geranylgeranyltoluquinol) were identical to compounds previously reported from *Cystoseira elegans*[16] and *Stypopodium zonale*,[17] two Phaeophyceae species belonging to the Sargassaceae and the Dictyotaceae families, respectively.

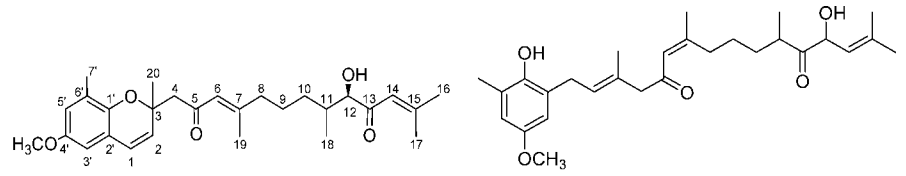
The remaining meroditerpenoids **8** and **9** were characterized as 12′-hydroxy-5,13′-dioxoisohalidrols. These compounds, previously reported from a specimen of *H. siliquosa* collected from the coast of Scotland (UK),[8] showed data in agreement with a probable epimeric relationship at C-12. For these two compounds, all NMR measurements and assignments were recorded, and this revealed that the original data (recorded in CD_3OD for ^{13}C) contained incorrect assignments. Tables 1 and 2 report the completely assigned ^{13}C and ^1H NMR data for **8** and **9** in C_6D_6.

Concerning the absolute configuration of the carbon C-12 (or C-13) of compounds **2**−**5**, **8**, and **9**, the modified Mosher's esterification method was conducted after methylation of the free phenoxy group, but the results were equivocal. The same methodology used by Iwashima et al.[18] with related compounds was also attempted in order to determine simultaneously the absolute

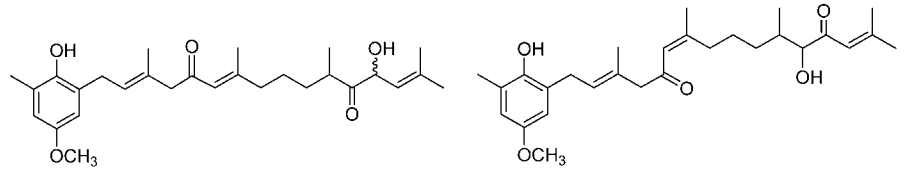
Table 3. Results of the Biological Assays for Compounds **1**−**6**, **8**, and **9** and for the Crude Extract (CE)

	Cobetia marina MIC (μg/mL)	*Marinobacterium stanieri* MIC (μg/mL)	*Vibrio fischeri* MIC (μg/mL)	*Pseudoalteromonas haloplanktis* MIC (μg/mL)	antisettlement assays EC_{50} (μg/mL)	toxicity tests LC_{50} (μg/mL)
1	>100	>100	>100	>100	>100	>100
2	0.5	1	0.5	1	1	5
3	5	5	5	10	>100	>100
4	>100	>100	>100	>100	>100	>100
5	>100	>100	>100	>100	>100	>100
6	1	1	0.5	1	5	10
8	10	10	25	10	>100	>100
9	2.5	1	2.5	1	4	>100
CE	5	5	5	10	5	33

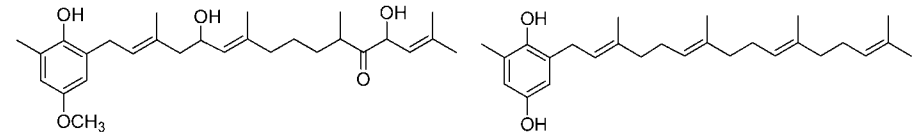
Chart 1



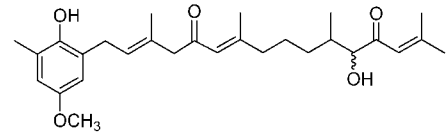
1 **2**



3 and **4** **5**



6 **7** (Geranylgeranyltoluquinol)



8 and **9**

configuration of the stereocenters at C-8 and C-12 or at C-8 and C-13. Unfortunately, this method did not give clear results.

From a chemotaxonomical perspective, it is interesting to note that several *Sargassum* species (e.g., *S. siliquastrum*,[19] *S. autumnale*,[20] or *S. micracanthum*[18]) have the ability to produce structurally similar metabolites to those biosynthesized by *H. siliquosa*. This similarity is in agreement with recent taxonomic works in this field: *H. siliquosa*, which was included originally in the Cystoseiraceae family, is now merged, as well as all the Cystoseiracean species, into the larger Sargassaceae family.[21,22]

Compounds **1**−**6**, **8**, and **9** and the crude extract (CE) were tested for their potential biological activities against important species involved in the colonization of marine surfaces (Table 3). The crude extract gave a good level of bioactivity against all of the organisms screened. Among the compounds tested, **2**, **6**, and **9** were active against the growth of the four strains of bacteria and in antisettlement assays at nontoxic concentrations. For these three compounds, the effect of increasing concentration on settlement was determined: the inhibition of settlement was complete at 5 μg/mL for **2**, 25 μg/mL for **6**, and 100 μg/mL for **9**. We also examined whether settlement inhibition was reversible following transfer to clean seawater. After 24 h, settlement was not significantly different from the control (data not shown).

It is interesting to note that the most effective compound (**2**) is the major constituent of the crude extract. However, in order to fully understand the ecological roles of this compound, it will be necessary to determine its location and measure the amounts released by the alga.[23,24] The surface localization of metabolites could indicate their potential to be used in surface-mediated interactions.[24] The next step will be to determine whether a bioactive substance retains activity following incorporation into a paint matrix and to run immersion tests in various geographical locations.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a Perkin-Elmer 343 polarimeter, using a 10 cm microcell. IR spectra were recorded with a Jasco model J-410 FT-IR spectrometer as KBr plates (films). Mass spectra were carried out at 70 eV with a Varian MAT 311 double-focusing mass spectrometer with reversed Nier-

Johnson BE geometry. NMR spectra were recorded on a Bruker Avance 400 MHz instrument. All chemical shifts were referenced to the residual solvent peak.

Plant Material. *Halidrys siliquosa* was collected in May 2004 in France (Saint-Guénolé, Brittany, France; 47°47′ N, 4°23′ W). A specimen of the alga has been identified by Prof. B. de Reviers (Muséum National d'Histoire Naturelle (MNHN), Paris, France) by comparison to the reference herbarium, and a voucher specimen (No. PC0111849) was deposited in the Herbarium of MNHN.

Extraction and Purification. The dried blades and thalli (450 g dry wt) of *H. siliquosa* were crushed in a mill and then extracted at room temperature with $CHCl_3$/MeOH (2:1 then 1:2, v/v) to produce after filtration 17.8 g of crude extract. This extract was then partitioned in a mixture of MeOH/$CHCl_3$/H_2O (4:3:1, v/v/v) to yield 13.5 g of organic phase. A portion of the organic extract (9 g) was subjected to normal-phase CC over silica gel (Si60, 40−63 μm, Merck) using step-gradient elution from *n*-hexane/EtOAc (9:1, v/v) to EtOAc and then from EtOAc to MeOH, to yield 25 fractions each of 150 mL. ^{1}H NMR investigations and TLC analysis of these fractions indicated fractions 6 and 7 (eluted with *n*-hexane/AcOEt, 6:4), fractions 8 and 9 (eluted with *n*-hexane/AcOEt, 5:5), and fraction 10 (eluted with *n*-hexane/AcOEt, 4:6) to be of further interest. Fractions 6 and 7 were subjected to further purification on reversed-phase HPLC (Merck Purospher Star RP-18e 5 μm; 10 × 250 mm; 2 mL/min) eluting with MeCN/H_2O (5:1) to yield, respectively, **7** (4 mg) and **1** (8 mg). From fractions 8 and 9, compounds **2** (76 mg), **5** (11 mg), **3** (14 mg), **4** (9 mg), **9** (12 mg), and **8** (9 mg) were purified by reversed-phase HPLC (eluent MeCN/H_2O, 4:1). Fraction 10 was purified by reversed-phase HPLC with MeCN/H_2O (7:3) as eluent to afford **6** (6 mg).

Compound 1: yellow oil; $[\alpha]^{25}_D$ −11 (*c* 0.1, MeOH); UV (EtOH) λ_{max} (log ϵ) 270 (3.7), 320 (3.5) nm; IR (film) ν_{max} 3420, 2895, 1712, 1680, 1438 cm^{-1}; ^{13}C NMR (100 MHz, C_6D_6), see Table 1; ^{1}H NMR (400 MHz, C_6D_6), see Table 2; HRMS *m*/*z* 454.2722 $[M]^+$ (calcd for 454.2719).

Compound 2: clear yellow oil; $[\alpha]^{25}_D$ −74 (*c* 0.1, MeOH); UV (EtOH) λ_{max} (log ϵ) 240 (4.4), 292 (3.2) nm; IR (film) ν_{max} 3461, 2969, 2929, 2850, 1707, 1684, 1613, 1484, 1445, 1383, 1200 cm^{-1}; ^{13}C NMR (100 MHz, C_6D_6), see Table 1; ^{1}H NMR (400 MHz, C_6D_6), see Table 2; HRMS *m*/*z* 456.2871 $[M]^+$ (calcd for 456.2876).

Compound 3: clear yellow oil; $[\alpha]^{25}_D$ +40 (*c* 0.1, MeOH); UV (EtOH) λ_{max} (log ϵ) 242 (4.2), 270 (2.9) nm; IR (film) ν_{max} 3398, 2925, 1737, 1610, 1437, 1385 cm^{-1}; ^{13}C NMR (100 MHz, C_6D_6), see Table 1; ^{1}H NMR (400 MHz, C_6D_6), see Table 2; HRMS *m*/*z* 456.2869 $[M]^+$ (calcd for 456.2876).

Compound 4: clear yellow oil; $[\alpha]^{25}_D$ −83 (*c* 0.1, MeOH); UV (EtOH) λ_{max} (log ϵ) 239 (4.3), 270 (3.3) nm; IR (film) ν_{max} 3399, 2926, 2855, 1737, 1678, 1612, 1439, 1384 cm^{-1}; ^{13}C NMR (100 MHz, C_6D_6), see Table 1; ^{1}H NMR (400 MHz, C_6D_6), see Table 2; HRMS *m*/*z* 456.2869 $[M]^+$ (calcd for 456.2876).

Compound 5: clear yellow oil; $[\alpha]^{25}_D$ −3 (*c* 0.1, MeOH); UV (EtOH) λ_{max} (log ϵ) 242 (3.9), 292 (2.8) nm; IR (film) ν_{max} 3461, 2939, 1681, 1617, 1485, 1444, 1382 cm^{-1}; ^{13}C NMR (100 MHz, C_6D_6), see Table 1; ^{1}H NMR (400 MHz, C_6D_6), see Table 2; HRMS *m*/*z* 456.2874 $[M]^+$ (calcd for 456.2876).

Compounds 6 and 7. Data were in agreement with those reported in the literature for these compounds.[16,17]

Compound 8: clear yellow oil; $[\alpha]^{25}_D$ −12 (*c* 0.1, MeOH); IR (film) ν_{max} 3399, 2925, 2854, 1736, 1618, 1438, 1384, 1240 cm^{-1}; ^{13}C NMR (100 MHz, C_6D_6), see Table 1; ^{1}H NMR (400 MHz, C_6D_6), see Table 2; HRMS *m*/*z* 479.2783 $[M + Na]^+$ (calcd for 479.2773).

Compound 9: clear yellow oil; $[\alpha]^{25}_D$ +25 (*c* 0.1, MeOH); IR (film) ν_{max} 3453, 2929, 1711, 1682, 1617, 1482, 1383, 1202 cm^{-1}; ^{13}C NMR (100 MHz, C_6D_6), see Table 1; ^{1}H NMR (400 MHz, C_6D_6), see Table 2; HRMS *m*/*z* 479.2777 $[M + Na]^+$ (calcd for 479.2773).

Preparation of *S*- and *R*-MTPA Ester Derivatives of Compound 1. A solution of pure compound (4.1 mg) was divided into two parts, then transferred into clean NMR tubes and dried under the stream of N_2 gas. Deuterated pyridine (0.6 mL) and (*S*)-(−)-MTPACl (6 μL) or (*R*)-(+)-MTPACl (6 μL) were added successively to the NMR tube under a N_2 gas stream, and the tube was carefully shaken to mix the sample and MTPA chloride evenly. The reaction NMR tubes were monitored by ^{1}H NMR: the reaction was completed in approximately 5 h for the (*S*)-MTPA ester **1*S*** and 3 days for the (*R*)-MTPA ester **1*R***.

Selected signals of **1*S***: ^{1}H NMR (C_5D_5N, 400 MHz) δ_H 6.812 (H-5′), 6.673 (H-3′), 6.460 (H-1), 6.321 (H-14), 6.161 (H-6), 5.973 (H-2), 5.469 (H-12), 2.283 (H-7′), 2.209 (H-17), 2.152 (H-19), 1.777 (H-16), 1.662 (H-20), 1.127 (H-18). Selected signals of **1*R***: ^{1}H NMR (C_5D_5N, 400 MHz) δ_H 6.805 (H-5′), 6.666 (H-3′), 6.459 (H-1), 6.398 (H-14), 6.141 (H-6), 5.969 (H-2), 5.489 (H-12), 2.282 (H-7′), 2.219 (H-17), 2.148 (H-19), 1.818 (H-16), 1.661 (H-20), 1.013 (H-18). $\Delta\delta_H$(**1*S*** − **1*R***): H-5′, +0.002 ppm; H-3′, +0.002 ppm; H-1, +0.001 ppm; H-14, −0.077 ppm; H-6, +0.020 ppm; H-2, +0.004 ppm; H-12, −0.020 ppm; H-7′, +0.001 ppm; H-17, −0.010 ppm; H-19, +0.004 ppm; H-16, −0.041 ppm; H-20, +0.001 ppm; H-18, +0.114 ppm.

Antimicrobial Assays. The compounds were tested for inhibitory activity against four strains of marine bacteria: *Cobetia marina* (ATTC 25374), *Marinobacterium stanieri* (ATCC 27130), *Vibrio fischeri* (ATCC 7744), and *Pseudoalteromonas haloplanktis* (ATCC 14393). The experiments were performed as previously described by Maréchal et al.[25] Compounds (at concentrations of 0.5, 1, 2.5, 5, 10, 25, 50, and 100 μg/mL) were incubated with the bacteria (2 × 10^8 cells/mL) in 96-well plates (Merck) in MHB medium (Mueller Hinton broth, Sigma), supplemented with NaCl (15 g/L), at 25 °C for 24 h. Each treatment and the seawater control were replicated six times. Minimum inhibitory concentrations (MICs), compared to the seawater control, were determined by the microtiter broth dilution method.[26]

Larval Bioassays. Larval Culture. Adult barnacles were collected from the pier pilings at the Duke Marine populations, North Carolina. They were maintained at 22 °C, with aeration (14 h light/10 h dark cycle), and fed on a daily diet of *Artemia salina* nauplii (7 nauplii/mL).[27] Release of larvae was obtained as previously detailed by Hellio et al.[28] Stage-II nauplii were used for toxicity assays. After 4 days, cyprids were collected by filtration.[27]

Settlement Assays. Settlement tests were conducted in 24-well microplates (Iwaki). Compounds were dissolved in 2 mL of seawater in the following concentrations: 0.5, 1, 2.5, 5, 10, 25, 50, and 100 μg/mL. Then, 10 to 15 cyprids were added to wells. Each test was performed in six replicates. Test plates were incubated at 28 °C (in darkness), and results were recorded after 24 h incubation. Each larva was examined under a dissecting microscope and its condition recorded (dead, settled or swimming).[29] Results are presented as 24 h EC_{50} values (=concentration of compound leading to 50% inhibition of settlement in comparison with the seawater control), which were determined using Sigma Plot 8.0.

Toxicity Tests. Toxicity tests were conducted on nauplii according to Wu et al.[30] Ten to fifteen stage-II nauplii were added to 2 mL of solution in the wells of a 24-well (Iwaki) plate. Compounds were tested at the same concentrations as for the settlement assays, with six replicates of each treatment and the control.[27] The number of swimming and dead nauplii was recorded after 24 h exposure to the compounds. The data are expressed as a 24 h LC_{50} (=concentration of extract that produces 50% of mortality in comparison with the control), which was determined using Sigma Plot 8.0.

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